Bioaccumulation of metals in mullet from the Mhlathuze Estuary and the effects of Cu and Pb on the uptake kinetics, haematology and acid-base balance of *Liza dumerelii*.

by

Hendrick Mabutho Mduduzi Mzimela

submitted to the **FACULTY OF SCIENCE AND AGRICULTURE**
in fulfillment of the requirements for the degree of
MASTERS OF SCIENCE
IN THE DEPARTMENT OF ZOOLOGY,
UNIVERSITY OF ZULULAND.

Supervisor : Dr V. Wepener
Co-supervisor :Prof D.P. Cyrus

January 2001

TABLE OF CONTENTS

CHAPTER 4

SPATIAL AND TEMPORAL ACCUMULATION OF AL, CR, CU, FE, MN, PB AND ZN IN WATER, SEDIMENT AND BIOTA OF THE MHLATHUZE ESTUARY

CHAPTER 5

THE EFFECTS OF CU AND PB ON THE HAEMATOLOGY AND ACID-BASE BALANCE OF *L. DUMERELII*

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

ACKNOWLEDGEMENTS

This thesis is dedicated to my mother, Mrs G.F. Mzimela who instilled in me the love for education.

Many thanks go to the following people and organisations:

My supervisor, Dr V. Wepener and my co-supervisor Prof. D.P. Cyrus for their unending support, advice and encouragement during the course of the study.

The staff from Zoology Department and Coastal Research Unit in particular, Alf Viljoen, Gerhard Cilliers, Louise Bakkes, Fiona Mackay, Steve Weerts and Leon Vivier.

Dr R. Owen for reviewing my script and grammar correction.

Jack Iosifov and Mandla Mhlongo for their assistance with the construction of experimental layouts.

Mr J.H.Ntuli from the African languages for Isizulu translation of the executive summary.

All my family, my wife, kids and friends for their patience and assistance.

Coastal Research Unit of Zululand, Zoology Department, University of Zululand, for their support with equipment, assistance with research and financial support.

National Research Fund (NRF) for funding the research.

Last but not least, thanks to the Almighty for making everything possible.

SUMMARY

Mhlathuze Estuary came into being when the original Richards Bay was divided into the northern part, which was dredged to form the habour and the southern part, which was reserved for the preservation of natural resources as a sanctuary. This sanctuary was however disturbed during development. The disturbance took the form of canalisation and redirection of Mhlathuze River and cutting of the new mouth after the berm wall was erected. These were drastic changes, which had a profound influence on the sanctuary (estuary). Resultant changes included siltation of the estuary and loss of water residence time.

The construction of the harbour was in line and enhanced the increase of industries in the Richards Bay area. The industries manufacture a number of metal products and also utilise other metal ores in the manufacturing process. The high population growth and concentration of industries in the area have a potential to pollute the sanctuary and the nearby harbour. Research was undertaken to assess the amount of metal pollution in the Mhlathuze Estuary and their possible effects on the biota.

In order for meaningful assessment of ecosystem health to be realised, the cause and also the effects of metals were investigated. Metals are non-biodegradable pollutants. Their persistence can result in them taken up by biota to concentrations far above ambient levels. This is termed bioaccumulation. The accumulation of metals in biota can result in stress, which can in turn induce changes in physiological parameters and other metabolic processes. The need to understand and predict the stress conditions, which metals will pose to fish, and extrapolate the effects of pollutants from laboratory to populations levels, have necessitated the search for physiological and biochemical indicators of health and sublethal toxicant effects. To determine the effects of these pollutants on the structure and functions of metabolic processes, it is important to monitor the bioaccumulation of such metals in the field and to expose the fish to environmental relevant concentrations of the same metal under controlled laboratory conditions.

In this study, this was achieved by conducting field observation and sampling, that involved the collection of water, sediment, fish (*Liza dumerelii*), benthic invertebrates (*Paratylodiplax blephariskios* and *Apseudes digitalis*) and submerged macrophytes (*Zostera capensis*) and analysing for metal accumulation. Seven metals, i.e. Al, Cr. Cu, Fe, Mn, Pb and Zn were analysed in the abovementioned abiotic and biotic compartments following standard nitric/perchloric acid digestion techniques and using a Varian AA50 atomic absorption spectrophotometer.

The study found elevated metal concentrations in the water and sediment samples from the Mhlathuze Estuary. High metal accumulation in water and tissues were found during summer months, which coincided with high rainfall and floods. The highest metal concentrations in the sediment were found during periods of low flow e.g. in winter and spring. A significant correlation was found between metals in water and fish liver tissue. Further significant correlations were recorded between metals in sediment and benthic invertebrates.

Metals occur in nature as ions and compounds. In order to understand their effects on biota, the effects of metal mixtures have to be investigated. In a metal mixture, the effects of metals can be additive, synergistic or antagonistic. Laboratory investigations involved the determination of effects of copper (Cu), lead (Pb) and their mixture on the haematology and acid-base balance of a mullet species, *Liza dumerelii*. Changes in physiological parameters measured, must be directly affected by the exposure to a toxicant, and the changes must not be because of other factors such as handling stress. Experiments were, therefore conducted in controlled environmental conditions to maintain constant temperatures and photoperiods.

Haematological and acid-base balance evaluation of fish blood provides valuable information concerning the response of fish to changes in the external media, and could be used to diagnose abnormal functioning of physiological mechanisms in fish. Haematological parameters measured were, red blood cell count (RBC), white blood cell count (WBC), haematocrit (Hct), haemoglobin (Hb), mean cell volume (MCV), mean cellular haemoglobin concentration (MCHC), mean cellular haemoglobin (MCH), blood glucose, and plasma lactate. Acid-base balance parameters measured were

bicarbonate (HCO_3^-), pH, PCO_2, the plasma ions, sodium (Na), potassium (K), chloride (Cl), and osmolarity.

In the study of the effects of metals on physiology, the combination of Cu and Pb was found to induce more physiological stress as compared to individual metals. Copper binds to ligands in the gill lammellae of fish where it can disrupt the Na and Cl balance. Lead on the other hand inhibits delta aminolevulinic acid dehydratase, an enzyme responsible for haemoglobin synthesis thus decreasing the potential for red blood cells to carry oxygen.

During stress the organism responds by either increasing or decreasing the particular parameter as a compensatory measure. The fish responded to the metal stress by increasing the RBC's, blood glucose concentration and haemoglobin. This was an attempt by the fish to increase the respiration potential, which was decreased by both the disruption of gill lamellae by Cu, and the inhibition of haemoglobin synthesis by Pb. Bicarbonates and PCO_2 levels were also increased. The HCO_3^- increase was probably due to increase in lactic acid that acidified the blood or, it was caused by the ß - adregenic stimulation of red blood cells, which also slows the process of CO_2 elimination from the blood.

The amount of metal accumulation in the Mhlathuze Estuary raises some concerns. While previous studies of metal accumulation in the water, sediment and fish from the Mhlathuze Estuary are comparable to this study, there seems to be a gradual increase in the amounts of metals from both the biotic and abiotic components of the Mhlathuze Estuary. The existence of metals in the water bodies as individual metals or as mixtures, as it was also shown in the study, has an effect on the physiology of the fish communities.

ISIFINYEZO

Umfudlana ophambuka eMhlathuze wadaleka ngenkathi i-Richards Bay ihlukana kabili. Yayehlukaniselwa ukumba ichweba lemikhumbi. Kwadaleka ingxenye yasenyakatho neyaseningizimu. Enye ingxenye yaba ichweba enye yaba eyokulondoloza imvelo. Lengxenye yemvelo yabuye yonakala ngenkathi kuqhutshwa intuthuko kulendawo. Ukonakala kwaba ukuvulwa komfula ube umsele omkhulu nokuphambuka kwendlela yomfula uMhlathuze. Lezizinguquko zaba nomthelela kulomchachazo onjekuzika kwamanzi enhlabathini nokuncipha kwesikhathi esihlalwa amanzi ngesikhathi ulwandle lungenisa.

Ukwakhiwa kwecheba kwakusemthethweni ngoba kwakuzodala izikhungo zomsebenzi eRichards Bay. Lezizikhungo zikhiqiza okusansimbi zibuye zikusebenzise okusansimbi ekwakheni umkhiqizo. Ukwanda kwesibalo sabantu nokwanda kwezikhungo zokukhiqiza kwadala ukungcola komchachazo nechweba elaliseduze. Uphenyo lwenziwa ukuzama ukuthola ukuthi insimbi leyo ingcolisa kangakanani umchachazo nechweba lelo.

Kwabuye kwabhekwa isisusa nemithelela yalensimbi ukuze kubonakale kahle ukuthikamezeka kwempilo yemvelo. Insimbi izingcolisi ezingathikazisi kakhulu empilweni mvelo. Ukuphikelela kwezingcolisi kungadala ukuba konakale imvelo emazingeni aphezulu ngokuba insimbi itholakale seyinqwabelene ezilwaneni ezidla ezinye. Ukwanda kwensimbi ezilwaneni nasezitshalweni ingcindezelo engadala ushintsho kwezomzimba neziphathelene nomgudu yokudla.

Isidingo sokuqonda nokusola isimo insimbi engasifaka ezinhlanzini nendlela izingcolisi ezingahlukumeza ngazo imvelo nasemalabhorathi, sekudale ukuba kuhlolwe ukusebenza kwamabhayokhemikhali emizimbeni nasempilweni. Ukuze sibheke izinga lamandla ezingcolisi ezakhiweni nasekusebenzeni kwemigudu yokudla kufanelwe kuqashelwe ukunqwabelana kokusansimbi ngokuhlalisa imvelo emalabhorathi anesimo esifana naleso sasendle.

Kulolucwaningo, lokhu kwazuzwa ngokuphenya ezindaweni nokucaphuna amasampula amanzi, inhlabathi, izinhlanzi, izilwanyana ezingenamgogodla kanye nezihlahla ezizike emanzini. Ukunqwabelana kwensimbi kwahlolwa kulezizinto esezibaliwe. Okusansimbi okunjenge Al, Cr, Cu, Fe, Mn, Pb kanye ne Zn okwakuphuma emanzini nasezilwaneni nezitshalo kwahlolwa kusetshenziswa indlela eqondile yokugaya ngenitric neperchloric esidi. Ukunqwabelana kwensimbi esisindweni esomile kwatholakala ngokusebenzisa iVarian AA50 spectrophotometry.

Okusansimbi kutholakala emvelweni kuyingxube nama-ayoni. Ukuqonda umthelela wakho ezilwaneni kanye nasezihlahleni, kufanele kuhlolwe nomthelela wezinsimbi eziyingxube. Ensimbini eyingxube, umthelela wokusansimbi ungaba yisenezeli noma iphikisane bese uyancipha. Izinguquko kweziphathelene nomzimba ezikaliwe kufanele zithintwe ukuveza kwesidakisi, izinguquko zingasuswa ukuphathwa kwengcindezelo.

Ucwaningo lwaselabhorathi lwalumayelana nomthelela wekhopha nomthofi, nengxube yakho egazini kanye nezingxenye zegazi kanye nokulingana kwe esidi kwinhlanzi egama layo liyi *Liza dumerelii*. Ukusetshenziswa kwegazi lenhlanzi kwi himatoloji kuveza ulwazi olubalulekile ngokwenziwa inhlanzi ezinguqukweni zangaphandle okungasetshenziswa ukuhlola indlela yokusebenza kwenhlanzi. Amazinga ehimatholoji akalwayo kwakuyizinhlayiya zegazi ezibomvu RBC, kwakungamasosha WBC, ihimathokhrithi, ihimoglobhini, umthamo wamaseli MCV ushukela osegazini nomantshu. Ukulinganiswa kwamazinga e-esidi kwaku-yi Bhayikhabhonethi, ama-ayoni omantshu, isodiyamu, iphotasiyamu kanye klorayidi.

Isifundo sathola ukuthi izinga liphakeme lokusansimbi emanzini nasenzikeni yoMhlathuze. Izinga lokusansimbi emanzini latholakala liphezulu ngezikhathi zasehlobo lapho lina khona izimvula ezinkulu. Inzika yokusansimbi ephezulu yatholokala ngesikhathi amanzi emancane njengasebusika nasentwasahlobo.

Esifundweni somthelela wensimbi emzimbeni inhlanganisela yekhopha (Cu) nomthofi (Pb) kwatholakala ukuthi isusa ingcindezelo yokomzimba kunezinhlalwana zokusansimbi. Ikhopha sengathi ibopha amaliganda kuziphefumulo zenhlanzi lapho-ke ithikameza ukulingana kwe Na^+ ne Cl^-. Umthofi ngakolunye uhlangothi usebenza ku

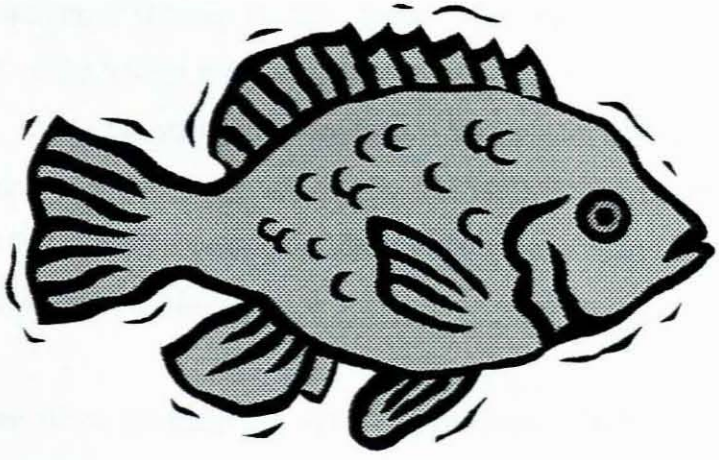
enzayimi esebenza ukuphehla ihimoglobhini. Umthofi ukhathaza uhlise leenzayimi bese kwehla amandla egazi okuthwala i-oksijini.

Ngesikhathi sengcindezelo isilokazana siphendula ngokukhuphula noma ngokwehlisa izinga ukuzama ukuzivikela kwingcindezelo. Inhlanzi yaphendula kungcindezelo esansimbi ngokwnyusa izinhlayiya zegazi ezibomvu noshukela wegazi kanye nehimoglobhini. Lokhu kwakuwukuzama kwenhlanzi ukukhuphula izinga lokuphefumula elehliswe ukuthikaziseka kweziphefumuli yikhopha nokuvimbeka kokuphehlwa kwehimiglobhini yi Pb.

Amabhayikhabhonethi kanye ne PCO_2 nakho kwakhuphuka. Lokhu kukhuphuka kwebhayikhabhonithi kwadalwa ukenyuka kwe-lakthiki esidi edala ubu-esidi egazini.noma kwadalwa ukuvimbeleka kokwakheka kwamasotsha abomvu yi B-adregenic ebuye yehlise ukukhishwa kwesikhutha (CO_2) egazini.

Izinga lokuqoqeka kokosansimbi kulomchachazo woMhlathuze kuyethusa. Nakuba ucwaningo oluphambili lokusansimbi emanzini nasezitshalweni emchachazweni woMhlathuze kuqhathaniseka nalokhu, kubonakala sengathi kukhona ukwnyuka kokusansimbi kuzo zonke izindawo zemvelo kulomchachazo.

Chapter 1



CHAPTER 1

General Introduction

1.1 Introduction

There is extensive, world-wide literature to indicate that the accumulation of heavy metals in estuaries is having an increasingly negative impact on the physiology of organisms inhabiting these ecosystems (Moore & Ramamoorthy, 1984; Mance, 1987). This is also the case in South Africa (S.A). An increasing amount of attention has been focussed on the effects of pollutants on aquatic systems in general and estuarine organisms in particular (Hemens & Connell, 1975; Watling & Watling, 1982a, 1982b).

There are very few, if any, pristine estuaries in S.A. A number of them have, to a certain degree, been affected by human impacts. In a semi arid country like S.A., the water resources are continually challenged (Davies & Day, 1998). There are high population densities and industries around water bodies that necessitate a high water demand. A major part (about 80%) of S.A. is, by world standards, dry land. South Africa receives an average annual rainfall of 464 mm. Of that, only about 7 % (32 mm) is converted to runoff into rivers (Davies & Day, 1998). Efforts must be made to conserve water and keep any contaminant or pollutant entering water to a minimum.

In aquatic systems there are different types of pollutants. They include organic pollution, (eutrophication) acidification, thermal pollution, radioactivity, heavy metals, and organochlorides (James & Evison, 1979; Mance, 1987; Mason, 1991). Pollutants like heavy metals, organochlorides, and polychlorinated hydrocarbons are toxic to biota resident in aquatic systems (Mance, 1987; Phillips & Rainbow, 1993). Heavy metals are those metals that are classified as Class B elements (Whitton, 1975; Hellawell, 1986; Sorensen, 1991) and have a density above 5. They are approximately 40 metals classified as heavy metals. They include essential metals like manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and molybdenum (Mo) and non-essential elements like cadmium (Cd), mercury (Hg) and lead (Pb) (Whitton, 1975). They are not easily broken down and are therefore very persistent pollutants (Mason, 1991).

Metals in aquatic systems occur either as dissolved forms in the water or as the form bound in particulate matter in the sediments (Abel, 1989; Allen & Hansen, 1996). The ratio of water-borne to sediment metals depends to a large extent on the chemistry of the element in aquatic condition and the general properties of the particular aquatic system (McIntosh, 1991). The toxic metals are usually found dissolved in water. Sediment, however, represents a pool of metals from where they can speciate to toxic forms or be available to organisms resident in the sediment (Sly & Hart, 1989; Novotny, 1995). It is therefore important that metal concentration in water and sediments be measured and related to body concentrations and ultimately effects on living organisms.

In estuaries, the degree to which pollutants accumulate is dependent upon inputs, flushing time of the estuary and the availability and nature of sediments, which may scavenge and concentrate substances from solutions by absorption or chelation (James & Evison, 1979; Reish, 1988). While dissolved metals are the main source of bioaccumulation in aquatic organisms and plants (Heath, 1987; Soule, 1988), the potential for bioaccumulation is greater in sediments as greater proportion of metals exists in sediment than in dissolved form (Soule, 1988; Sorensen, 1991). These elevated metal concentrations, coupled with relative greater availability of pollutants to organisms in diluted media than seawater, contribute to concentrations of pollutants within organisms living in estuaries (Reish, 1988).

Some metals are needed by the organisms in trace amounts for their natural physiological processes (Pinder & Giesy, 1981). Both plants and animals for normal functioning of their metabolic processes (Conn & Stumpf, 1976; Pinder & Giesy, 1981), require metals, such as Cu, Zn, Fe, and Mn. Iron is associated with numerous enzymes like peroxidase, catalase and cytochrome oxidase. It forms the metal part of at least two plant cytochromes that function in the transfer of electrons during photosynthesis (Enserink, Diepevieen & Leeuven, 1991). Manganese stimulates plant growth in plankton by activating enzyme systems. Zinc is a metal constituent of dehydrogenase, a photosynthesising agent (Whitton, 1975; Moore & Ramamoorthy, 1984).

When the concentrations of trace metals exceed tolerable levels, these heavy metals become toxic to the biota. Fish, which are present in water bodies, may obtain these metals by

diffusion through the gills and skin surfaces (Heath, 1987; Sorensen, 1991; Phillips & Rainbow, 1993), or in natural food (Moore & Ramamoorthy, 1984; Villegas-Navaro & Villereo- Trevino, 1989). These metals can accumulate in fish tissues to levels higher than ambient concentration through bioaccumulation (bioconcentration). Submerged plants (Wolfe, Thayer & Adams, 1976; Lingby & Brix, 1989) and invertebrates (Cheung & Wong, 1992; Rosenberg & Resh, 1993) are able to utilise these metals directly or indirectly from the sediment. Such metals can be transferred from plants and invertebrate fauna and be accumulated in fish via several pathways in the food chain. This is called biomagnification (James & Evison, 1979; Heath, 1987).

1.2 Estuaries

The geomorphology of the coastal region of S.A. has a profound influence on the type of estuarine environments (Whitfield, 1998). A majority of the estuaries are often sheltered extensions of the marine environment and are thus important to marine species seeking sheltered environments. These species enter as post larval or juveniles and remain until first maturity (Wallace & Van der Elst, 1975; Day, 1982; Cyrus & Blaber, 1987). Lower reaches of rivers and estuaries also attract many industries and mining activity because of their proximity to the export and import facilities harbours offer and the amount of water they contain. Agricultural activities also depend on fresh water from lower reaches of rivers for irrigation. All these activities put stress on the estuarine environment in terms of water quality and quantity.

Most KwaZulu-Natal (KZN) estuaries have a steep gradient. During summer, when heavy rains fall, the velocity increases carrying silt organic matter and pollutants from the upper reaches in the catchment into the estuary (Whitfield, 1998). This contributes much to the pollution found in estuaries. During winter the flow in estuaries may be greatly reduced. The metal ions may then be adsorbed onto particulate matter and be incorporated into sediments where they may remain indefinitely (Mason, 1991; Phillips & Rainbow, 1993).

Estuaries are environments that are very dynamic in nature. They vary in chemical, physical and biotic characteristics, temporally and also spatially (Cyrus, 1991). Environmental factors

such as temperature, salinity, acidity and oxygen fluctuate in an estuarine environment (Mance, 1987; McIntosh, 1991). These fluctuations exert stresses on organisms living in estuaries. These factors may also influence the toxicity, availability, and accumulation of pollutants in aquatic organisms. Oxygen varies from highly oxygenated waters in areas of active wave action to partial or complete deoxygenation in muddy substrates (Novotna, 1995). There is inconsistency in the effects of temperature on metal accumulation (Mance, 1987). Generally an increase in temperature increases the rate of metal uptake (Mance, 1987; Phillips & Rainbow, 1993).

Metal uptake rates decrease with increasing salinity. The consequence of this in estuaries is that seasonal variations in freshwater flows will expose biota to seasonal variations in salinity, which will have a direct effect on metal uptake and concentrations in tissues (James & Evison, 1979; Mance, 1987). Mantoura *et al.* (1978) quoted by Phillips and Rainbow (1993) estimated a 25 % increase in dissolved Zn^{2+} ions when the salinity decreased from 32 ‰ to 15 ‰.

1.3 Rationale of this study

The decision by the state to create a deepwater harbour at Richards Bay, saw the division of the original estuary into the northern harbour and southern "sanctuary" area (Begg, 1978). This provided a compromise in that the southern portion was to serve as sanctuary for plant, animal life and their respective habitats (Day, 1982). This included diverting the Mhlathuze River course into the sanctuary area to form the Mhlathuze Estuary.

There has been a substantial industrial development in the Richards Bay area due to easy access to the sea brought about by the harbour (Cyrus, 1991; Connell, 1996). Dune mining, north of the town exploits the metal rich sand dunes to mine metals including titanium, zircon, and rutile. Included in the harbour terminals is the dry bulk metal terminal that handles the export and import of metal commodities. These commodities include chrome sand, copper concentrates, manganese ore, ferro alloys, alumina powder and aluminium (Cyrus & Wepener, 1998). These activities, together with stormwater runoff, have a potential to pollute the harbour and estuary (Cyrus & Wepener, 1998)

As estuarine environments are already stressed, these additional activities increase the pressures on organisms living in estuarine areas (Cyrus, 1991). It is thus necessary to determine and quantify the potential effects of these activities on the water quality and biota. Additionally, the lower catchment of the Mhlathuze River services large scale and subsistence agricultural developments, which could also contribute to input of pollutants into the estuary (Cyrus & Wepener, 1998).

The estuary plays an important ecological role in acting as a nursery area for many commercially important fish and prawn species (Cyrus & Blaber, 1987). The estuary is of significance from an avifaunal point of view on both regional as well as a national level. Cyrus (1999) reported the estuary as holding the second largest number of water-associated birds after St Lucia along the entire KZN Coast. There are 99 species of water-associated birds found in the estuary (Cyrus, 1999). They include species like the African Fish Eagle, various waders, Caspian tern, White pelican, and Pinkbacked pelican. Some of these birds utilise the system mainly for feeding both in summer and winter and others like the terns and gulls also utilise the estuary for breeding (Cyrus, 1999).

Ecological surveys on KZN estuaries only started in 1947 (Connell, 1996) by which time man had contributed much to their pollution and many estuaries were already in a degraded state. Generally, the accumulation of metals in the biota in the Mhlathuze Estuary and the harbour is not well documented. The Council for Scientific and Industrial Research (CSIR) and the National Institute of Water Research (NIWR) commissioned a study (Hemens & Connell, 1975; Hemens, Connell, McClurg, Simpson, Warwick, & Muller, 1976) the aim of which was to investigate the conditions in the sanctuary after the cutting of the new sanctuary mouth. Accumulation of metals in water, sediment, fish, and invertebrates was investigated. The research, however, had very little information on metal contamination, and accumulation in aquatic biota (Hemens & Connell, 1975).

In order to assess the extent of metal contamination in the estuary, research was conducted on the bioaccumulation of selected heavy metals in the water, sediment, and biota. A general scan of metals was conducted on water and sediment from the Mhlathuze Estuary. The metals selected from the scanning process were aluminium (Al), chromium (Cr), copper (Cu), iron

(Fe), manganese (Mn), and zinc (Zn). The metals that were scanned incorporated those metals that are produced in the surrounding industries and are utilised in the processing of products at these industries.

Laboratory bioassays were initiated to investigate the effects of Cu and Pb on the haematology and acid-base balance of a fish bio-indicator, *Liza dumerelii*. Copper is the essential metal responsible for many physiological processes in the animal body (Phillips and Rainbow, 1993). The toxicity of copper in aquatic environments is mostly attributed to ionic forms especially the Cu^{2+} (McIntosh, 1991). Lead has not been recorded as having any essential function in living organisms. It is however toxic to fish at sublethal concentrations (Somero, Chow, Yancey & Snyder, 1977; Sorensen, 1991). These bioassays were therefore to investigate the effects of an essential and a non-essential metal on the haematology and acid base balance of *L. dumerelii*.

1.4 Aims

In view of the lack of information on metal contamination and accumulation in the biota of the Mhlathuze Estuary, the aims of the study were to determine:

- The extent of metal contamination in the Mhlathuze Estuary.
    - The spatial and temporal variations of selected heavy metals in the water and sediment.
    - The temporal bioaccumulation of selected heavy metals in whole fish and in the muscle, gill and liver in *L. dumerelii*.
    - Temporal variation of heavy metals in submerged macrophytes and invertebrates.
- The sublethal effects of Cu and Pb on the physiology of *L. dumerelii*.

- The effects of an essential (Cu) and non-essential (Pb) metals on the haematology of *L. dumerelii*.
- The effects of essential (Cu) and non-essential (Pb) metals on the acid-base balance of *L. dumerelii*.

1.5 References

Abel, P.D. (1989). **Water Pollution Biology.** Ellis Horwood, Chichester. 231pp.

Allen, H.E. & Hansen, D.J. (1996). The importance of trace metal speciation to water quality criteria. **Water Environment Research** 68(1):42-54

Begg, G. (1978). **Estuaries of Natal.** Town and Regional Planning Report V41. 657pp.

Cheung, Y.H. & Wong, M.H. (1992). Comparison of trace metals content of sediment and mussels collected within and outside Tolo Harbour, Hong Kong. **Environmental Management** 16:743-751.

Conn, E.E. & Stumpf, P.K. (1976). **Outlines of Biochemistry.** John Wiley and Sons, New York. 629pp.

Connell, A.D. (1996). Environmental Studies in the Richards Bay Offshore Outfalls Region. Report 10, CSIR, KwaZulu Natal. 199pp.

Cyrus, D.P. (1991). Fish conservation in South African estuaries: Pressures, problems and prospects. **South African Journal of Aquatic Sciences** 17(1/2):19-27.

Cyrus, D.P. (1999). A review of the aquatic avifauna of the Mhlathuze Estuary. **South African Journal of Aquatic Sciences** 24(1/2):131-140.

Cyrus, D.P. & Blaber, S.J.M. (1987). The influence of turbidity on juvenile marine fishes in estuaries, Part 1. **Journal of Experimental Marine Biology and Ecology** 109:53-70.

Cyrus, D.P. & Wepener, V. (1998). The Impact of Dredger Spoil on Selected Components of the Mhlathuze Estuary Biota. Report prepared for Portnet Richards Bay, CRUZ Investigational Report No 52. 60pp.

Davies, B. & Day, J. (1998). **Vanishing Waters.** University of Cape Town Press, Cape Town. 487pp.

Day, J.H. (1982). **The Ecology of Southern African Estuaries.** A.A. Balkema, Rotterdam. 411pp.

Enserink, E.J., Maas-Diepeveen, J.L. & Van Leeuven, C.J. (1991). Combined effects of metals: An ecotoxicological evaluation. **Water Research** (6):679-687.

Heath, A.G. (1987). **Water Pollution and Fish Physiology.** CRC Press, Boca Raton, Florida. 245pp.

Hemens, J. & Connell, A.D. (1975). Richards Bay: Southern Bay Conservation Area. Progress Report No 29, Thirty Second Steering Committee Meeting, CSIR/NIWR. 250pp.

Hemens, J., Connell, A.D., McClurg, T.P., Simpson, D.E., Warwick, R.J. & Muller, M.S. (1976). Richards Bay Sanctuary Area: Report on Field Surveys in October 1975 and February 1976. Progress Report No 31, Thirty Second Steering Committee Meeting, CSIR/NIWR. 146pp.

Hellawell, J.M. (1986). **Biological Indicators of Freshwater Pollution and Environmental Management.** Elsevier Applied Science, London. 454pp.

James, A. & Evison, L.E. (1979). **Biological Indicators of Water Quality.** Wiley Interscience, New York. 692pp.

Lingby, J.E. & Brix, H. (1989). Heavy metals in eelgrass (*Zostera marina)* during growth and decomposition. **Hydrobiologia** 176/177:189-196.

Mance, G. (1987). **Pollution Threat of Heavy Metals in Aquatic Environments.** Elsevier App. Science, England. 372pp.

Mason, C.F. (1991). **Biology of Freshwater Pollution.** Longman Scientific and Technical, New York. 347pp.

McIntosh, A.W. (1991). Trace Metals in Freshwater Sediments: A Review of the Literature and an Assessment of Research Needs. In: **Metal Ecotoxicology.** M.C. Newman & A.W. McIntosh (Eds.), Lewis Publishers, Michigan. 368pp.

Moore, J.W. & Ramamoorthy, S. (1984). **Heavy Metals in Natural Waters. Applied Monitoring and Impact Assessment**. Springer-Verlag, New York. 268 pp.

Novotny, V. (1995). Diffuse Sources of Pollution by Toxic Metals and Impacts on Receiving waters. In: **Heavy Metals Problems and Solutions.** W. Salomon, U. Forstner & S. Mader (Eds.), Springer-Verlag, Berlin. 412pp.

Phillips, D.H. & Rainbow, P.S. (1993). **Biomonitoring of Trace Aquatic Contaminants.** Chapman and Hall, London. 256pp.

Pinder, J.E. & Giesy, J.P. (1981). Frequency distribution of essential and non-essential elements in largemouth bass *Micropterus salmoides.* **Ecology** 62(2):456-468.

Reish, D.J. (1988). The Use of Toxicity Testing in Marine Environmental Research. In: **Marine Organisms as Indicators.** D.F. Soule & G.S Kleppel (Eds), Springer-Verlag. New York. 342pp.

Rosenberg, D.M. & Resh, V. (1993). **Freshwater Biomonitoring and Benthic Macro-Invertebrates.** Chapman & Hall, New York. 488pp.

Sly, P.G. & Hart, B.T. (1989). **Sediment/Water Interactions.** Proceedings of the 4th Interim Symposium, Kluwer Academic Publishers, London. 701pp.

Somero, G.N., Chow, T.J., Yancey, P.H. & Snyder, C.B. (1977). Lead accumulation rates in tissues of the estuarine teleost fish, *Gillichthys mirabilis*: Salinity and temperature effects. **Archives of Environmental Contamination and Toxicology** 6:337-348.

Sorensen, E.M.B. (1991). **Metal Poisoning in Fish.** CRC Press, Boca Raton, Florida. 374pp.

Soule, D.F. (1988). Marine organisms as Indicators: Reality or Wishful Thinking? In: **Marine Organisms as Indicators.** D.F. Soule & G.S. Kleppel (Eds.), Springer-Verlag, New York. 342pp.

Villegas-Navarro, A. & Villereal-Trevino, C.M. (1989). Uptake of zinc and copper and lead in Texas cichlid, *Cichhasoma cyanoguttum.* **Bulletin of Environmental Contamination & Toxicology** 42:761-768.

Wallace, J.H. & Van der Elst, R.P. (1975). **The Estuarine Fishes of the East-Coast of Africa V5: Ecology, Estuarine Dependence and Status.** South African Association for Marine Biological Research, Oceanographic Research Institute Investigational Report 42. 63pp.

Watling, R.J. & Watling, H.R. (1982a). Metal surveys in South African estuaries I. Swartkops River. **Water SA** 8(1):26-35.

Watling, R.J. & Watling, H.R. (1982b). Metal surveys in South African estuaries II. Knysna River. **Water SA** 8(1):36-44.

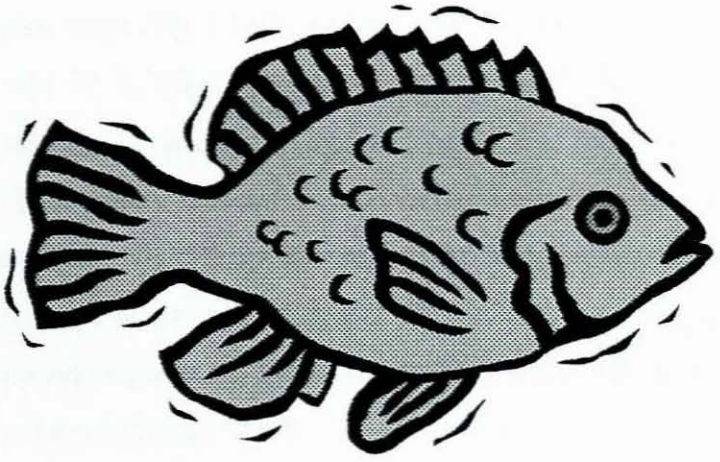
Whitfield, A.K. (1998). **The Biology and Ecology of Fishes in Southern African Estuaries.** JLB Smith Institute of Ichthyology, Ichthyological Monograph Number 10. 223pp.

Whitton, B.A. (1975). **River Ecology.** Blackwell Scientific Publishers, Oxford. 725pp.

Wolfe, D.A., Thayer, G.W. & Adams, S.M. (1976). Manganese, Iron, Copper and Zinc in Eelgrass, (*Zostera marina)* Community. In: **Radioecology and Energy Resource.** C.E.

Cushing (Ed.), Proceeds of the National Symposium on Radioecology, 4th Oregon State University Corvallis, Dowden Hutchinson and Ross, Stroudsburg.

Chapter 2



CHAPTER 2

Situational analysis

2.1 Preview of the Mhlathuze Estuarine system

2.1.1 Historical aspects

Richards Bay formerly, known as Umhlathuze lagoon, is situated on the KZN north coast (28° 48′S, 32° 05′ E). It was discovered by Portuguese mariners and later named Richards Bay after Sir Frederick Richards who landed a small force of men in the Bay during the Anglo-Zulu war in 1897 (Begg, 1978; Hay, 1992).

The history of the Richards Bay Harbour and Mhlathuze Estuary is well described by Begg (1978) and Day (1982). The estuary was shallow and was fed by the Mhlathuze, Mzingazi and the Mtantathweni rivers (Fig 2.1A). The average depth was about 0,9m. Salinity ranged between 12,5 ‰ and 35 ‰ (Day, 1982). High turbidity levels were reported to provide protection for penaeid prawns and mullet against predation as they were abundant in the open bay (Day, 1982). The estuary was rich in zooplankton owing to the relatively small mouth and longer residence time. High freshwater inflow also provided a greater amount of available nutrients. The tidal range was restricted due to the shallows and the partially blocked mouth of the system (estimated height at 0,35 m). The average water level in the bay was more than 1 meter higher than the sea (Begg, 1978).

Extensive beds of *Zostera capensis* (eelgrass) which supported one of the richest and most diverse benthic and fish communities on the Natal coast were found in the estuary (Day, 1982; Adams, Bates & Riddin, 1998). The reed *Phragmites australis* surrounded the estuary. A number of hygrophyllous grasses could be found on the sedge land and on the floodplains. The white mangroves *Aviccenia marina,* black *Brugiuera gymnorrhiza,* and red *Rhizophora macronata* were present on edges of the estuary. They were however less extensive than at present.

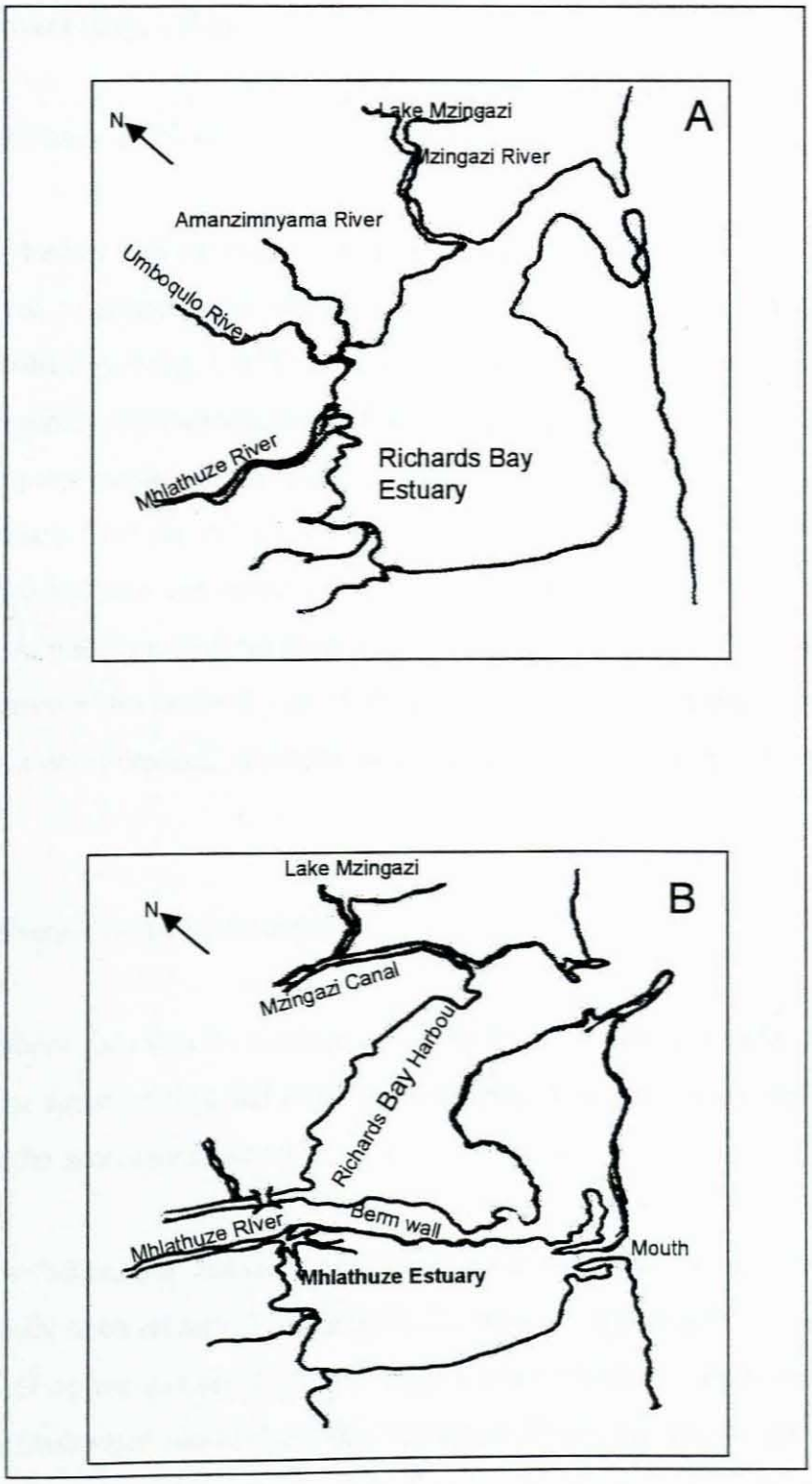


Figure 2.1 Diagram of Richards Bay before (Fig 2.1A) and after harbour developments (Fig 2.1 B).

Papyrus swamps on the floodplains acted as the sponge for the silt that was carried by the flowing rivers (Day, 1982).

2.1.2 Harbour development

In 1976, the bay was developed into a deep water harbour. A five kilometer berm wall was constructed to separate the original bay into the southern and northern parts. The division was described by Begg, (1978) as "Natal's great environmental experiment and an exercise in interdisciplinary communications." The northern part was dredged to form a harbour and the southern part was declared a nature reserve (sanctuary) (Fig 2.1B). Since the berm separated the sanctuary from the original mouth, a new mouth had to be cut and the Mhlathuze River was redirected into the sanctuary, that is now the Mhlathuze Estuary. Tidal gates were installed in the berm wall between the harbour and the estuary. These were for passage of water between the sanctuary and harbour in case the new mouth closed (Day, 1982). The gates have never worked, although overtopping has been observed at high spring tides.

2.1.3 Present status of the estuary

The southern part was to function as a nursing and feeding ground, and provide a suitable habitat for aquatic fishes and other invertebrates. Very little work has been done to actually evaluate the sanctuary function.

The new Mhlathuze Estuary, associated with the river canal could be regarded as a permanently open estuary (Whitfield, 1992). It is a large estuary by S.A. standards, covering an area of approximately 11.5 km^2 with a total shoreline length of 30 km. The estuary receives freshwater runoff from the Mhlathuze River that enters the estuary on the north-western side. The Mtantathweni River drains the nearby Lake Cubhu which flows into the south-western embayment of the estuary (Wepener & Vermeulen, 1999). Sugar cane is cultivated on the flood plains and there has been an increase of informal settlements around the area, especially in the northern side increasing the subsistence farming in the vicinity of the southern and eastern shore (Cyrus, 1999)

The estuary supports one of the biggest areas of mangroves in South Africa (Adams *et al.*, 1998). The mangroves are dominated by the white mangrove, *A. marina* with patches of black mangroves *B. gymnorrhiza* and the red mangroves, *R. mucronata,* occurring on the southern part of the estuary (Adams *et al.,* 1998). Submerged on the eastern part of the estuary is the eelgrass, *Z. capensis*. These *Z. capensis* beds were reduced from 63 ha to 5 ha during the division of the original bay and any efforts by the regional parks board to reintroduce it have had limited success (Day, 1982). *Zostera* traps nutrients and recycles minerals and also improves the water quality by increasing the depth of the oxidised microzone (Adams *et al.,* 1998). *Zostera* also provides shelter for juvenile fish and invertebrates. Mackay & Cyrus (1999) found that the *Zostera* area was the greatest in species richness when compared to other habitats in the estuary. The eelgrass cannot however establish itself because of the unstable substrate and turbidities caused by fine sediment present in the area. Other macrophytes that are present in the estuary include *Phragmites australis* and *Ruppia cirrhosa.*

Before the development of the harbour, water from the Mhlathuze River was filtered through an extensive vegetated delta before entering the estuary. The alteration of Mhlathuze River through canalisation caused substantial sediment deposition, which in turn initiated morphometric variations within the system (Day, 1982; Huizenga & Van Niekerk, 1998). This deposition also altered the relative depth of the water at different areas within the system resulting in diverse habitats. Wepener & Vermeulen (1999) found that water in the Mhlathuze Estuary was highly turbid particularly in the basin becoming clearer towards the mouth. Due to the shallow nature of the estuary, and the strong wind that prevails in the vicinity, the soft bottom sediments are brought into suspension fairly easily, with potential deleterious effects to biota. The authors found the estuary to be having low salinity gradients with marine conditions prevailing. The only area where greater salinity gradients (some freshwater inflow) were recorded is where the Mhlathuze and Mthantathweni rivers flow into the estuary.

Substrata differed from coarse sand to fine sand and mud in different areas of the Mhlathuze Estuary. Sand and mud dominated the bottom substrate of the embayment. The substratum in the mouth and along the Mhlathuze River watercourse was dominated by coarse sand (Huizenga & Van Niekerk, 1998). This area is greatly influenced by tidal action.

The faunal composition in the muddy areas of the estuary was poor in both species diversity and richness when compared to the fauna of the original embayment (Hemens, McClurg, Simpson, & Warwick, 1971; Hemens & Connell, 1975; Hemens *et al.*, 1976). According to Mackay & Cyrus (1999), crustaceans of marine origin and a few taxa associated with the freshwater environment presently dominate the estuarine benthic fauna. The benthic community was dominated by the burrowing crab, *Paratylodiplax blephariskios* and the tanaid, *Apseudes digitalis*. *Paratylodiplax blephariskios* has been dominant since 1970 (Hemens *et al.*, 1971) and it forms the greatest portion of benthic biomass (Mackay & Cyrus, 1999). Mackay & Cyrus (1999) reported that the crustacean tanaid, *A. digitals,* formed the most important component of the benthic fauna in terms of numbers.

The ichthyofauna of the Mhlathuze Estuary has been comparatively well studied (Cyrus, Wepener, Mackay, Cilliers, Weerts & Viljoen, 2000). It was characterised by a large number species mostly of marine origin. A survey by Weerts & Cyrus (1999) categorised the ichthyofauna into various estuarine dependent categories (EDC) outlined by Whitfield (1992, 1998) as follows:

- EDC I - species that reside in the estuary and utilise the area for breeding.
- EDC II - marine species that breed at sea with juveniles showing varying degrees of dependence on estuaries.
- EDC III - euryhaline marine species.
- EDC IV - use the estuary as a transit route between marine and freshwater environments.
- EDC V - euryhaline freshwater species.

Weerts & Cyrus (1999) recorded 133 species from 52 families. The number included about five species listed as rare or endangered in the fish Red data list (Skelton, 1987). Of the 88 estuarine dependent species recorded by Weerts & Cyrus (2000) 37 species (42.1%) fitted the Estuarine dependent category (EDC II) described by Whitfield (1998). Other species included 20 (22.7 %) estuarine breeders (EDC I), 20 (22.7 %) marine estuarine dependent species (EDC III), 6 (6.8 %) fresh water species (EDC IV) and 5 (5.7 %) catadromous species (EDC V).

Mhlathuze Estuary supports different groups of avifauna, some of which are vulnerable and rare (Cyrus, 1999). A total number of 99 species of water-associated birds were reported, of which 11 species appear in the Red Data book (Brookes, 1984). Of the generally recognised avifauna groups, the estuary is well represented (Cyrus, 1999). Resident species constitute the majority in terms of abundance. These include birds like the African Fish Eagle. They utilise the estuarine areas for feeding. Summer fauna included different types of waders. The group that utilises the estuary during non-breeding period in winter includes Caspian terns, white pelican, Pinkbacked pelicans and flamingos. Species such as seagulls and the redwinged pratincole use the estuary extensively for breeding (Cyrus, 1999).

2.1.4 Trace metal surveys in the Mhlathuze Estuary

Millard & Harrison (1954) conducted the earliest biological research in the bay. It consisted of two summer and two winter sampling surveys between 1948 and 1951. It was not until 1970 that the metal survey was undertaken by Hemens *et al.* (1971). Sediment characteristics and physico-chemical conditions were investigated at sixteen stations on a quarterly basis beginning in November 1969. This was prior to harbour developments.

Hemens & Connell (1975) conducted the first survey of metal accumulation of the Mhlathuze Estuary. The survey was initiated by the CSIR in collaboration with NIWR to monitor the effects of the new mouth after opening the outlet to the sea from the sanctuary. This study was conducted during the autumn, winter and spring of 1976 (Hemens & Connell, 1975). Water, sediment, and biological material were analysed for metal accumulation. The concentrations of Hg , Cd, Pb, Zn, Cu and Fe were found to be comparable to those of other KZN estuaries.

2.2 Sources of metal pollution in the Mhlathuze Estuary

There are a number of potential sources of pollution in the estuary. These are differentiated into point and diffuse sources.

2.2.1 Point sources

Industrial point source effluent originates from industries such as Mondi Paper and Pulp and Felixton sugar mills a few kilometers upstream from the estuary. The cargo dock of the harbour is used to ship a number of metals and metal ores. Anti-fouling paint that is used on ships is another potential source of metals in the harbour. These metals enter the estuary by overtopping through the non-functional tidal gates separating the harbour from the estuary, particularly during high spring tide.

2.2.2 Diffuse sources

Discharges from storm water drains from the Richards Bay Coal Terminal (RBCT) in the harbour contribute to the contaminants input into the harbour. Pesticides and fertilisers from agricultural activities such as sugarcane plantations in the catchment of the Mhlathuze River also form part of the diffuse source of pollution into the estuary.

2.3 Description of the sampling sites

Five sampling sites were selected in the Mhlathuze Estuary. The different sampling sites were chosen to represent all habitat types of the estuary. The positions of the sampling sites are presented in Figure 2.2. Site 1 is situated in eelgrass beds in a muddy sand substrate with a maximum depth at high tide of 1 m. Site 2 is situated opposite the KZN Wildlife boma in the main river channel and is characterised by fine sand and a maximum depth of 1.5 m. Site 3 is situatd in the main channel of the estuary mouth with medium to coarse sandy substratum. The depth at this site is approximately 1.5 m. Site 4 is situated in open water in the western part of the estuary embayment, and the sediment consists of mud and the maximum depth is approximately 1 m. Site 5 is situated in the Mhlathuze River approximately 4 km upstream from the mouth with bottom sediments of coarse sand and a maximum depth of 2 m.

Water and sediment for metal analyses were collected from sites 1, 2, 3, 4, and 5. Submerged macrophytes were collected from site 1, whole fish samples were collected from site 2, and fish tissue samples were collected from site 1 and site 4.

2.4 References

Adams, J.B., Bates, G.C. & Riddin, T. (1998). Freshwater Requirements of the Mhlathuze Estuary: Vegetation. Report Submitted to the Institute for Water Research, on behalf of the Department of Water Affairs and Forestry. 30pp.

Begg, G. (1978). **Estuaries of Natal.** Town and Regional Planning Report V41. 657pp.

Brookes, R.K. (1984). **South African Red Data Book – Birds.** South African Progress Report 97. 213pp.

Cyrus, D.P. (1999). A review of the aquatic avifauna of the Mhlathuze Estuary. **South African Journal of Aquatic Sciences** 24(1/2):131-140.

Cyrus, D.P., Wepener, V., Mackay, C.F., Cilliers, P.M., Weerts, S.P. & Viljoen, A. (2000). The Effects of Intrabasin Transfer on the Hydrochemistry, Benthic Invertebrates & Ichthyofauna of the Mhlathuze Estuary & Lake Nsezi. WRC Report 772/1/99. 255pp.

Day, J.H. (1982). Summaries of Current Knowledge of 43 Estuaries in S.A. In: **Estuarine Ecology: with Particular Reference to Southern Africa.** J.H. Day (Ed.), A.A. Balkemia, Rotterdam. 411pp.

Hay, D. (1992). Development of a Conceptual Basis for the Environmental Management of the Port of Richards Bay. Institute of Natural Resources Institutional Report 79. 39pp.

Hemens, J., McClurg, T. P. Simpson, D. E. & Warwick R. J. (1971). Detailed Survey of Richards Bay. Progress Report No. 21, Twentieth Steering Committee Meeting, CSIR/NIWR.

Hemens, J., & Connell, A.D. (1975). Richards Bay: Southern Bay Conservation Area. Progress Report No 29, Thirty Second Steering Committee Meeting, CSIR/NIWR. 250pp.

Hemens, J. Simpson, D.E & Warwick, R.J. (1976). Richards Bay Sanctuary Area. Report on Fields Survey in May and June 1976. Progress Report *CSIR/NIWR* Thirty Third Steering Committee Meeting Marine Disposal of effluents 32. 146pp.

Huizenga, P. & Van Niekerk, L. (1998). Freshwater Requirements of the Mhlathuze Estuary: Physical Aspects. Report submitted to the Institute for Water Research on behalf of the Department of Water Affairs and Forestry. 12pp.

Mackay C.F. & Cyrus, D.P. (1999) A review of the macrobenthos of the Mhlathuze Estuary. **South African Journal of Aquatic Sciences** 24(1/2):111-129.

Millard, N.A.H. & Harrison, A.D. (1954). The ecology of South African estuaries: Part V Richards Bay. **Transaction of Royal Society of Southern Africa** 34(1):157-179.

Skelton, P.H. (1987). **South African Red Data Book-Fishes.** South African National Scientific Programmes Report No 137, Foundation for Research development CSRI. Pretoria. 199pp.

Weerts, S.P. & Cyrus, D.P. (1999). A review of the ichthyofauna of the Mhlathuze Estuary. **South African Journal of Aquatic Sciences** 24(1/2):100-110.

Wepener, V. & Vermeulen, L.A. (1999). Comments on the water quality of the Mhlathuze Estuary in relation to determining the ecological integrity class. **South African Journal of Aquatic Sciences** 24(1/2):87-98.

Whitfield, A.K. (1992). A characterisation of Southern African estuarine systems. **South African Journal of Aquatic Sciences** 18(1/2):89-102.

Whitfield, A.K. (1998). **Biology and Ecology of Fishes in Southern African Estuaries.** Ichthyological Monographs of the JLB Smith Institute of Ichthyology 2. 223pp.

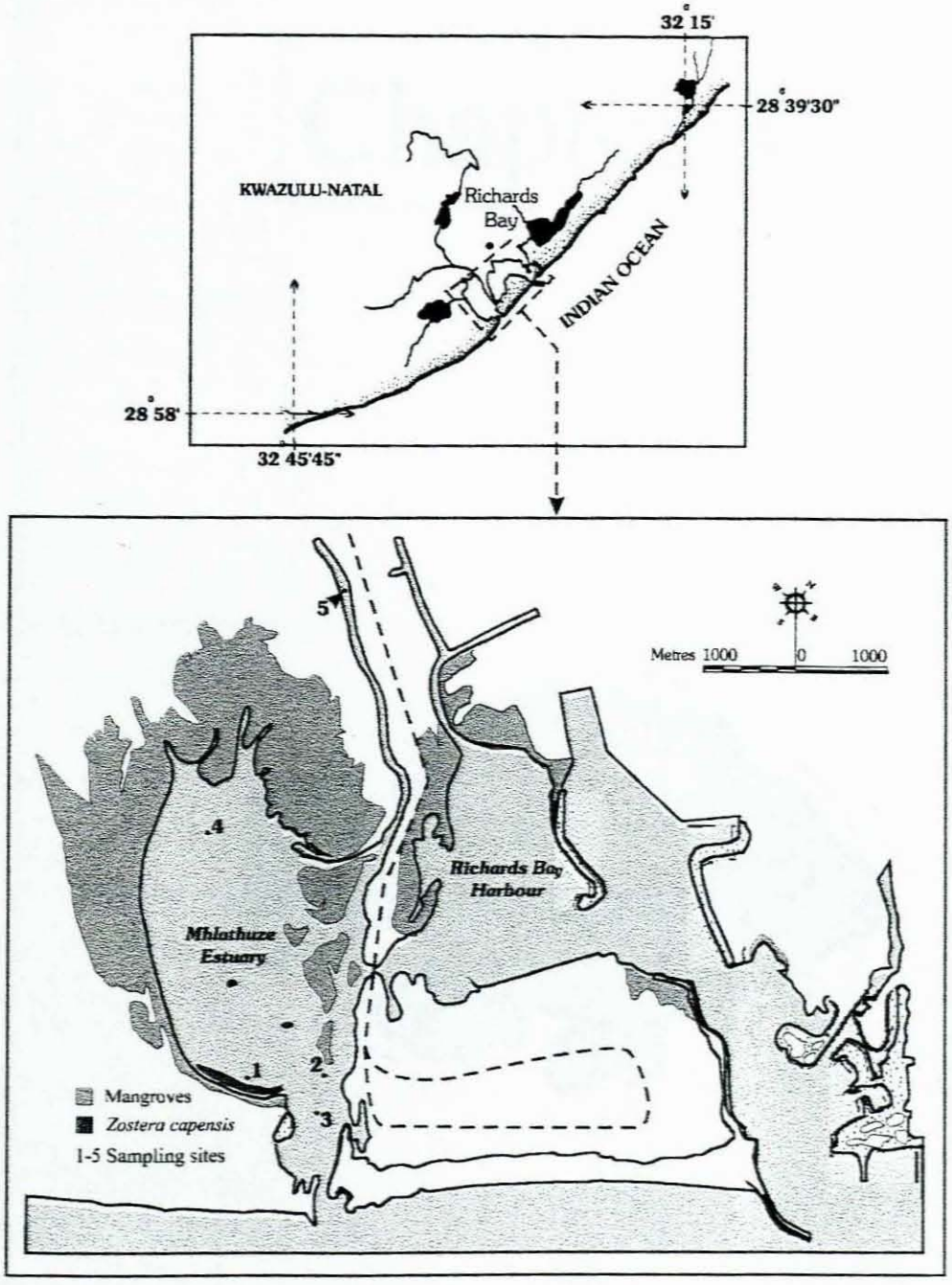
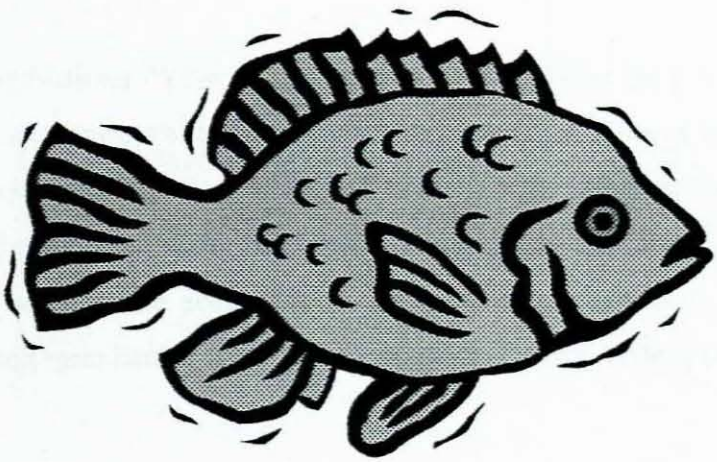


Figure 2.2 Diagram of the Mhlathuze Estuary showing the location of the sampling sites.

Chapter 3



CHAPTER 3

Biomonitoring

3.1 Introduction

Water quality monitoring has previously focused on physical and chemical measurement (Cairns, 1982; Hellawell, 1986). Water quality was measured according to acceptable human standards established by different health departments. Management had to comply with such engineering methodologies (Hols, 1996). There has been increased recognition of the use of indicators to complement the interpretation of physical and chemical measurement (Buikema, Niederlehner & Cairns, 1982). The paradigm shift from direct toxicity to humans, to far more subtle effects that pollutant chemicals exert on natural biota (Heath, 1987), had a great effect on the emergence of biomonitoring, as opposed to chemical tests (Forbes & Forbes, 1993). That has enhanced the assessment of management with respect to aquatic ecosystems.

South Africa's new National Water Act, Act 36 of 1998 specifies the role of biomonitoring in the protection and assessment of the country's water resources. One of its requirements is the establishment of a national information system to include the information required to monitor the quantity and quality of all water resources. It emphasises the role of water, as a resource, and the need for water to be accessible to all humans, as well as to maintain the biotic functioning and long term health of all aquatic systems (Davies & Day, 1998).

3.2 History of biomonitoring

Toxicology was developed in the 1800's. Scientists wanted to know the effect of chemicals on human population health. It was only in the late 1960's that ecotoxicology became a coherent subject area. That was when chemical monitoring was extended to biological, non-human organisms such as fish and invertebrates (Moriarty, 1991; Forbes & Forbes, 1993).

Biomonitoring is defined by Abel, (1989) as a relatively new field, which looks at the fate and effects of chemicals in the environment, estimates the role of such activity to the health

of the ecosystem and design management strategies. It merges the field of toxicology and ecology and assesses toxic chemicals in ecosystems. It however goes beyond looking at individual organisms or sub-organism level (Moriarty, 1991). It attempts to quantify the human induced disturbances in the environment, predict their outcome and supply management strategies for the rehabilitation of disturbed ecosystems.

3.3 What is biomonitoring

Biomonitoring or biological monitoring can be described as the use of biological responses or indicators to determine the effects of change in environmental conditions (Roux, Van Vliet & Van Veelen, 1993). It involves the gathering of data in both the laboratory and field to assess or determine whether regulatory (health of ecosystem) standards and criteria are being met in aquatic environments (Hols, 1996).

The integration of different fields like physiology, chemistry, and ecology, makes it easier to elucidate the relationship between chemicals in the environment and their effects on organisms and their metabolic systems (Moriarty, 1991). It also contributes to understanding of general ecosystem integrity and enables the effects of disturbances in the environment to be better predicted. This forms the basis of the field of ecotoxicology.

Many reasons have been given explaining the shortfall of chemical monitoring in defining the integrity of ecosystems and providing information on the state of aquatic environments. Some of these are the failure of chemical measurements to provide the cause and effect chemicals have on ecosystems and on individual organisms (Hellawell, 1986; Forbes & Forbes, 1993). With chemical monitoring, it is not possible to explain some important disturbances like habitat degradation. Another reason is that the information gained from chemical monitoring only provides the condition at time of sampling (Roux *et al.*, 1993) and consequently cannot be used to predict long term events.

Moriarty (1990) gives the following reasons why reliance cannot be placed on the measurement of pollutants alone:

- It is often impracticable to measure all contaminants.

- Routine analytical techniques may be too insensitive.
- The biological significance of pollutants may not be fully known at the level at which they are measured.
- Combination of pollutants may interact.
- Regular chemical measurement may miss out occasional, significant high values.

Ecosystems are complex systems. These complexities have compelled most studies to deal with separate components. Biomonitoring attempts to deal with toxic effects of pollutants on the entire ecosystems in an integral context (Phillips & Rainbow, 1993). Ecosystem studies are generally incomplete due to the complex nature of these systems and do not allow the whole system to be investigated. In biomonitoring studies, the monitoring exercises are separated into realistic components to investigate different biological levels of organisations for the purpose of pollution determination. These are then integrated or assembled to give a better understanding of the environment (Forbes & Forbes, 1993; Chapman, 1997).

Biological monitoring is a valuable tool that can be used to understand the impacts of chemicals not only on individual organisms but also on entire ecosystems (Moriarty, 1991; Roux *et al.*, 1993). It can assist in the establishment of regional and national baseline of chemical, physical and biological resource characteristics of aquatic systems (Wepener, Fleischer & Vivier, 1995).

3.4 Types of biomonitoring

Biomonitoring can be divided into field biomonitoring and laboratory based toxicity evaluation (effluent and ambient biomonitoring). Effluent and ambient biomonitoring measures the effects of toxicants by observation of organisms under controlled conditions. Bioassays are experiments in which the potency of the chemical to an organism is measured. Toxicity tests measure the effects of a chemical or factor on a living organism (Reish, 1988).

Roux *et al* (1993) gives the different types of biomonitoring as:

- Bioassessment of fish and macro-invertebrates.
- Bioassay of water and sediment (acute and chronic).

- Fish health assessment.
- Bioaccumulation of trace metals and organic compounds.

3.4.1 Bioaccumulation

Bioaccumulation is the net uptake and retention of a substance by an organism over a period of time. The uptake is either from water or from food. Bioaccumulation can be by direct uptake (a process called bioconcentration) or accumulation can be by uptake up the food chain, a condition called biomagnification. According to Chapman (1997) the reasons why bioaccumulation is measured are:

- To determine the specific bioavailability of a contaminant.
- To identify possible causative agents of toxicity.
- To relate body burdens to food chain accumulation.
- To assess or predict effects of chronic low levels exposures.

Measurement of bioaccumulation is through bioconcentration factor (BCF) or bioaccumulation factor (BAF). The BCF is the accumulation from water only whereas the BAF involves the uptake from water, sediment and diet.

The following are some of the problems related to bioaccumulation. Bioaccumulation does not identify the toxicity of the metal or the metal state. The difficulty and variability of some of the bioaccumulation measurement makes it to be unreliable. It is difficult to predict at which concentration the accumulated contaminant has an effect on the specific organism. Bioaccumulation, however, is still widely used as an effective tool in the prediction of impacts and in the determination of causative agents (Chapman, 1997).

In this study, field biomonitoring involved quantifying the spatial and temporal accumulation of Al, Cr, Cu, Mn, Fe, Zn and Pb in the water and sediments in the Mhlathuze estuary. The bioaccumulation of the metals in the biota was assessed through using fish tissues analysis (gill, liver and axial muscle), whole fish, benthic invertebrates, and submerged macrophytes. The reasons for selecting these bio-indicators are discussed in section 3.6.

3.4.2 *Laboratory based biomonitoring*

Toxicity tests are tests that are conducted under laboratory conditions. They range from single species test to tests on components of whole systems. They differ with respect to their level of complexity. These tests are divided into acute and chronic tests. The most frequently used is the single species acute tests. Toxicity tests require knowledge on the basic biology of the species as to enable effective planning of the test and interpret the effects (Mason, 1991; Phillips & Rainbow, 1993).

Acute toxicity

The most commonly used tests are the lethality tests where organisms are exposed to pollutants until death that is used to calculate their LC_{50} (Hellawell, 1986; Mance, 1987). The test ranges between 24 hours and 96 hours. The most common is the 96-hour LC_{50}. The duration is usually determined by starvation, as animals are not fed during the test. While single species toxicity tests have shortcomings they will however continue to be used for comparing pollutant effects and subsequent management. Only the integration of the acute test with chronic and ecosystems tests will make meaningful predictions of pollutants in ecosystems possible (Phillips & Rainbow, 1993).

Sublethal toxicity

Sublethal toxicity can be defined as an effect, due to some man-induced change in the environment, which does not necessarily cause death of the organism. The effects may result, however, in some alterations in one of the biological process that could lead to inability of the organism or its offspring to function normally (Heath, 1987; Mance, 1987; Soule, 1988). The tests can be short, conducted on part of the life cycle of the test organism or can continue throughout the life cycle (Buikema *et al.*, 1982). Measured endpoints could include the inability to feed, grow, and reproduce or a change in behaviour. These tests can be short (24-96 hours) or can be of longer duration e.g. 21 days, 3 months. Although different types of organisms have been used in these tests as single species and multiple species tests, there is still a need for development of specific methods for different species (Cairns, Dickson & Westlake, 1982; Hellawell, 1986; Rosenberg & Resh, 1993). Since most tests are long term,

they are better recommended for aquatic resource protection. It is however important that multiple species tests be emphasised to give better interpretation at ecosystem level (Mason, 1991). In this study, laboratory bioassay investigated effects of Cu and Pb, and the mixture of Cu and Pb on the haematology and acid base balance of an estuarine fish, *L. dumerelii.*

3.5 Choice of indicator species

Different authors give different definitions of bio-indicators (Soule, 1988; Hellawell, 1986). Phillips & Rainbow (1993) differentiate between indicators and monitors. These authors define the bio-indicators as those species and populations that are employed by virtue of their presence and absence in the system or the change in densities. According to the authors, indicators are used in the field and monitors are used in the laboratory.

Biological monitors are those organisms used through toxicity test and accumulation to measure contaminant. Criteria given by Phillips & Rainbow (1993) for the choice of bio-monitors and or bio-indicators are that they should be:

- Sessile or sedentary in order to be representative of the study area.
- Abundant in the study area.
- Hardy, tolerating wide ranges of contaminant concentrations and physicochemical variables.
- Active accumulators of relevant trace metals.

The choice of which species to use in toxicity tests also depend upon many criteria such as (Mance, 1987; Reish, 1988):

- Availability of the species.
- Ability of the species to live under laboratory conditions.
- Convenient size.
- Available data on the biology of the species.
- Ecological significance and economic value.

The effects of heavy metal contamination on aquatic biota are highly variable. The

accumulation and distribution potential of metals in aquatic environments is known to depend on a number of factors which include physical, chemical and biological factors (Mance, 1987; Phillips & Rainbow, 1993). Biologically, the type of organism is important in determining how the metals will be accumulated. However most research in estuarine and fresh water systems deals with the accumulation of metals in the water sediment and only in selected organisms.

In integrated environmental monitoring it is important that organisms are selected at different trophic levels and that these are investigated at different levels of organisation (Cairns *et al.*, 1982). Indicators selected in this study were fish, benthic invertebrates, and submerged macrophytes. These indicators were chosen to represent comprehensive variety of species. The following three sections provide the rationale behind the selection of specific genera as bio-indicators in this study.

3.5.1 Fish

Fish have widely been used as indicators of contamination in aquatic environments. This is because of their ease of collection and their ecological and economic importance (Stephens, Hose & Love, 1988; James & Evison, 1989). Fish biology is also generally well understood. Mullet are widespread estuarine and coastal fish species, which form an important and component of estuarine biological community. They feed at the lowest trophic level. The juveniles are sources of food to a number of estuarine birds and the adult fish provide a protein source to human populations around estuaries. For example, the mullet genera *Mugil* and *Liza* (Family Mugilidae) are cosmopolitan in distribution. These species are euryhaline and spawn at sea. Between May and August the juveniles follow the turbidity gradients towards estuaries (Wallace & Van der Elst, 1975). These tropical, subtropical or temperate species are iliophagous feeders feeding by sucking up surface layer of the substrate or by grazing on submerged rocks and plant surface (Cyrus & Blaber, 1987). Although their common feed is the small gastropod, *Assiminea bifasciata*, they are capable of feeding on a wide range of food particles (Wallace & Van der Elst, 1975).

Their exposure to heavy metals in estuarine environments can take the form of uptake through contaminated sediment and waterborne metals taken up through the gills. Little information is available on the uptake and accumulation of metals by mullet species. Mullets

have been found to accumulate metals (Plaskett & Potter, 1979; Sultana & Rio, 1998). Sultana & Rio (1998) found increased metals accumulated in the liver, and also there was less metal accumulation in muscle tissue of the flathead mullet, *Mugil cephalus.*

3.5.2 Benthic invertebrates

A high load of metals in estuaries is brought about by urbanisation, industrial development and numerous wastewater discharges. These metals precipitate and settle out often causing severe sediment contamination (Chen & Wong, 1992). Monitoring of bottom substrates may be enhanced by concurrent sediment and benthic invertebrate analysis.

Benthic invertebrates are often used in monitoring due to their relative lack of mobility and their low trophic positions. They are mostly sedentary and are thus less able to avoid potentially harmful conditions (Rosenberg & Resh, 1993). They are also closely coupled with the pelagic food web, constituting a link of transport of contaminants to higher trophic levels (Smith, Bernstein & Cimberg, 1988).

The use of benthic invertebrates as a bio-indicator of contamination in estuaries is well described (e.g. Jackson & Resh, 1989; Cheung, Neller, Chu, Tam, Wong, Wong, & Wong, 1997). Because of their infaunal, epibenthic and burrowing habitats, benthic invertebrates are directly exposed to accumulated pollutants in the substratum they inhabit. The invertebrate species used in this study are the tanaid, *A. digitalis* and the burrowing crab, *P. blephariskios*, (formerly *Tylodiplax)*. Both these species are benthic species found in the muddy substrate of Mhlathuze Estuary. They are able to survive in substrates that have muddy constituents with large amount of decaying organic matter and low oxygen tensions (Mackay & Cyrus, 1999).

Apseudes digitalis is found in large numbers in the Mhlathuze Estuary in the area of the *Z. capensis* located on the southern part of the estuary. The species is not an active burrower. It is an epibenthic species that feeds on the detritus. The adult female keeps the eggs in a brood pouch and the juveniles develop directly from the eggs in the brood pouch. This species is the most important benthic invertebrate in terms of numbers in the Mhlathuze Estuary (Mackay & Cyrus, 1999).

Paratylodiplax blephariskios is a sub-tidal crab. In the Mhlathuze Estuary, it is found in the muddy substrata of the marine embayment. It is an active burrower living in burrows in the muddy substrates. Its biology is not well understood. The juveniles develop from larval stages which probably recruit from the sea (R. Owen, pers com[1]). They have a salinity range of about 3-60 ‰ and they are prey for different carnivorous benthic feeding species like grunter and birds. *Paratylodiplax blephariskios* is a benthic species that forms the highest component of benthic invertebrate's biomass in the Mhlathuze Estuary (Mackay & Cyrus, 1999).

Benthic invertebrates can accumulate metals to very high levels. The epibenthic species, such as *A. digitalis* can take up metals from the overlaying water while the burrowing species like *P. blephariskios* can accumulate metals from both the overlaying and pore water. Both these species can also take up metals from the bulk of the sediment through feeding.

3.5.3 Submerged macrophytes

The importance of aquatic plants as biological indicators of pollution is the fact that they are stationary and easily detected (Hellawell, 1986). Their response to pollution is highly variable, and their responses to heavy metal are not well documented. They can however help in assessing environmental damage, as they are a highly productive, nutrient source and have a potential to exert a significant role in heavy metal cycling (Moore & Ramamoorthy, 1984; Lyngby & Brix, 1989).

Zostera capensis is an aquatic plant that is found submerged in water column. It is known to accumulate metals, with the roots showing a greater heavy metals absorption capacity than the rest of the plant (Lyngby & Brix, 1989). Wolfe *et al.* (1976) also showed the roots to be the important area in the cycling of metals in *Z. marina*. The authors found high accumulation of Mn and Fe metals.

3.6 References

Abel, P.D. (1989). **Water Pollution Biology.** Ellis Horwood, Chichester. 231pp.

[1] Dr. R.K. Owen – Department of Zoology, University of Zululand.

Buikema, A.L., Niederlehner, B.R. & Cairns, J. Jr. (1982). Biological monitoring Part IV. **Water Research** 16:239-262.

Cairns, J. Jr, (1982). **Biological Monitoring in Water Pollution.** Pergamon Press, New York. 355 pp.

Cairns, J.Jr, Dickson, K.L. & Westlake, G.F. (1982). **Biological Monitoring of Water and Effluent Quality.** American Society of Testing and Materials, Philadelphia. 246pp.

Chapman P.M. (1997). Is bioaccumulation useful for predicting impacts? **Marine Pollution Bulletin** 34(5):282-283.

Cheung, Y.H. & Wong, M.H. (1992). Comparison of trace metals content of sediment and mussels collected within and outside Tolo Harbour, Hong Kong. **Environmental Management** 16:743-751.

Cheung, Y.K., Neller, K.H., Chu, N.F.Y., Tam, C.K., Wong, Y.K., Wong, Y.S. & Wong, M.H. (1997). Assessment of toxicity using different trophic organisms**. Archives of Environmental Contamination and Toxicology** 32:260-267.

Cyrus, D.P. & Blaber, S.J.M. (1987). The influence of turbidity on juvenile marine fishes in estuaries Part 1. **Journal of Experimental Marine Biology and Ecology** 109:53-70.

Davies, B. & Day, J. (1998). **Vanishing Waters.** University of Cape Town Press, Cape Town. 487pp.

Forbes, V.E. & Forbes, T.L. (1993). **Ecotoxicology in Theory and Practice.** Chapman & Hall, London. 247pp.

Heath, A.G. (1987). **Water Pollution and Fish Physiology.** CRC Press Inc. Boca Raton, Florida. 245pp.

Hellawell, J.M. (1986). **Biological Indicators of Water Pollution and Environmental Management.** Elsevier Science, London. 546pp.

Hols, D.R. (1996). National Biomonitoring Programme for Riverine Ecosystems. Framework Document Report 1 Institute for Water Quality Studies Pretoria. 176pp.

Jackson, J.K. and Resh, V.H. (1989). Sequential decision plans, benthic macroinvertebrates. **Environmental Management** 13(4):455-468.

James, A. & Evison, L.E. (1979). **Biological Indicators of Water Quality.** Wiley Interscience, New York. 692pp.

Lingby, J.E. and Brix, H. (1989). Heavy metals in eelgrass (*Zostera marina)* during growth and decomposition. **Hydrobiologia** 176/177:189-196.

Mackay, C.F. & Cyrus, D.P. (1999). A review of the macrobenthos of the Mhlathuze Estuary. **South African Journal of Aquatic Sciences** 24(1/2):111-129.

Mance, G. (1987). **Pollution Threat of Heavy Metals in Aquatic Environments.** Elsevier Applied Science, England. 372pp.

Mason, C.F. (1991). **Biology of Freshwater Pollution.** Longman Scientific and Technical, London. 347pp.

Moore, J.W. & Ramamoorthy, S. (1984). **Heavy Metals in Natural Waters.** Applied Monitoring and Impact Assessment Springer-Verlag, New York. 268pp.

Moriarty, F. (1991). **Ecotoxicology: The Study of Pollutants in Ecosystems.** Academic Press, London. 289pp.

Phillips, D.J.H. & Rainbow, P.S. (1993). **Biomonitoring of Trace Aquatic Contaminants.** Chapman and Hall, London. 256pp.

Plaskett, D. & Potter, I.C. (1979). Heavy metal concentrations in the muscle tissue of 12 species of teleost from Cockburn Sound, Western Australia**. Australian Marine and Freshwater Research** 30:607-616.

Reish, D.J. (1988). The Use of Toxicity Testing in Marine Environmental Research. In: **Marine Organisms as Indicators.** D.F. Soule & G.S. Kleppel (Eds.), Springer Verlag, New York. 342pp.

Rosenberg, D.M. & Resh, V. (1993). **Freshwater Biomonitoring and Benthic Macro-Invertebrates.** Chapman and Hall, New York. 488pp.

Roux, D.J., van Vliet, H.R. & van Veelen, M. (1993). Towards integrated water quality monitoring : Assessment of ecosystem health. **Water S.A.** 19(4):275-280.

Smith, R.W., Bernstein, B.B. & Cimberg, R.L. (1988). Community Environmental Relationships in the Benthos: Application of Multivariate Analytical Techniques. In: **Marine Organisms as Indicators.** D.F. Soule & G.S. Kleppel (Eds.), Springer-Verlag, New York. 342pp.

Soule, D.F. (1988). Marine Organisms as Indicators: Reality or Wishful Thinking? In: **Marine Organisms as Indicators.** D.F. Soule & G.S. Kleppel (Eds.), Springer-Verlag, New York. 342pp.

Stephens, J.S.Jr., Hose, J.E. & Love, M.S. (1988). Fish Assemblages as Indicators of Environmental Changes in Nearshore Environments. In: **Marine Organisms as Indicators.** D.F. Soule & G.S. Kleppel (Eds.), Springer-Verlag, New York. 342pp.

Sultana, R. & Rio, D.P. (1998). Bioaccumulation pattens of zinc, copper, lead and cadium in grey mullet *Mugil cephalus* (L) from harbour waters of Visakhapatnam, India. **Bulletin of Environmental Contamination and Toxicology** 60:949-955.

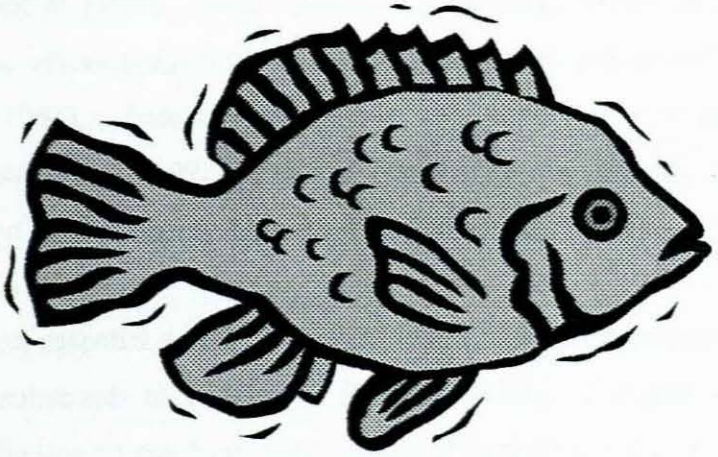
Wallace, J.H. & Van der Elst, R. (1975). **The Estuarine Fishes of the East Coast of Africa**

V5: Ecology, Estuarine Dependence and Status. Oceanographic Research Institute Report 42. 63pp.

Wepener, V., Fleischer, L. & Vivier, L. (1995). Biomonitoring: A literature Survey. Document prepared for Richards Bay Minerals, CRUZ University of Zululand. 103pp.

Wolfe, D.A., Thayer, G.W. & Adams, S.M. (1976). Manganese, Iron, Copper and Zinc in Eelgrass, (*Zostera marina)* community. In: **Radioecology and Energy Resource.** C.E. Cushing (Ed), Proceeds of the National Symposium on Radioecology, 4th Oregon State University Corvallis, Dowden Hutchinson and Ross Stroudsburg.

Chapter 4



CHAPTER 4

Spatial and temporal accumulation of Al, Cr, Cu, Fe, Mn, Pb and Zn in water, sediment and biota of the Mhlathuze Estuary

4.1 Introduction

Natural waters contain a complex mixture of heavy metals. These are derived from both the geological weathering processes and anthropogenic sources (Moore & Ramamoorthy, 1984; Mason, 1991). Heavy metals are classified as metals with a density above five and they number approximately 40 (Whitton, 1975). They include the essential metals, e.g. Cr, Cu, Mn, Fe, and Zn and non-essential elements e.g. Ag, Cd, Hg, and Pb. In most instances the former dominates (Pinder & Giesy, 1981). Since the essential metals are regulated, they have lower toxicity than non-essentials metals (Whitton, 1975). When the concentrations in external media exceed tolerable levels, the essential metals become toxic (Mance, 1987; Miller, Muntkittrick & Dixon, 1992) whereas non-essential metals are toxic at low levels since they have no essential function and regulation therefore does not take place (Mason, 1991; Sorensen, 1991). Essential metals play an important role in plant and animal metabolism (Miller *et al.*, 1992) and are responsible for growth stimulation, enzyme activation, transport of oxygen, etc. (Moore & Ramamoorthy, 1984).

Aluminium is a non-essential metal. However, it can replace magnesium in acting as a link between enzyme substrates in vertebrates (Bowen, 1966). Trivalent Al species (Al^{3+}) at acidic pH values (below 5) can have very serious effects on the growth of plants and other soil organisms. Even at very low concentrations (<2.7µg/l) it can result in the elimination of ATP induced transmembrane potential (Kennedy, 1994). Chromium is an essential metal needed for insulin uptake by tissues (Farmer, Ashfield & Samant, 1979; Sorensen, 1991). It can also activate phosphoglucomutase in vertebrate lungs (Bowen, 1966).

Copper forms a constituent of many enzymes and glycoproteins e.g. amine, oxidase, cerulosplasmic, and cytochrome oxidase (Moore & Ramamoorthy, 1984; Mc Dowell, 1992). Copper promotes Fe absorption from the gastrointestinal system and is involved in the transfer of Fe from the tissue into plasma. It is also a constituent of erythrocuprein in erythrocytes and is necessary for haemoglobin synthesis. Copper forms an active centre for

the invertebrate blood pigment, haemocyanin (Moore & Ramamoorthy, 1984). Iron is an essential constituent of many enzymatic and other cellular processes. Oxidative metabolism in all organisms and photosynthesis in plants involves cytochrome, which contains Fe (Horne & Goldman, 1994). Iron forms a constituent of haemoglobin, a blood pigment, and several other intracellular enzymes systems.

Both invertebrates and vertebrates need manganese as a co-factor in several enzyme systems and it is needed by vertebrates for the functioning of ovaries and testes (Whitton, 1975). Manganese resembles magnesium in activating a number of phosphotransferases and decarboxylases enzymes in the Krebs cycle (Bowen, 1966). Zinc is a constituent of many important enzymes, which include carbonic anhydratase. This enzyme plays a role in binding carbon dioxide in teleost cells. Carbonic anhydratase is also important in maintaining acid-base balance in renal tubule cells (Schimdt-Nielson, 1990). Zinc is also a cofactor in carboxypeptidase serving a key role in protein digestion.

Lead is a non-essential metal and is toxic in aquatic environments and toxicity is attributed to the ionic form (Sorensen, 1991). The acute toxicity of lead ranges from 0.1 to 10 mg/l for several species with variation due to water quality and presence of different competing elements. Acute toxicity also differs with different stages of life cycle with the embryos more sensitive followed by juvenile (Sorensen, 1991). Sublethal toxicity of Pb in fish causes haematological, neuronal and muscular pigment alteration, coagulation of surface mucus and other effects. Haematological disturbances include direct erythrocytic injury, resulting in fragile or distorted erythrocytes, and basophyl stripping (Johansson-Sjobeck & Larsson, 1979). Tewari, Gill & Pant (1987) observed the inactivation of delta aminolevulinic acid dehydratase, which resulted in decreased haemoglobin formation. Neuronal and muscular abnormalities include muscle spasm, paralysis and loss of equilibrium (Davies, Goetll, Sinley & Smith, 1976).

Heavy metals occur either as freely dissolved ions or in the form of precipitates in water bodies. The soluble form of the heavy metals is considered as biologically available to organisms and therefore the toxic form. Divalent ions such as Cu^{2+} and Pb^{2+} are considered the most toxic in aquatic systems (Sorensen, 1991; Allen & Hansen, 1996). Physico-chemical factors are important in transforming the species of metals in water bodies (Engel & Sunda, 1981; McIntosh, 1991). The presence of organic matter, organic fluvic and humic

acids help to bind the metal ions thus reducing toxicity of metals in aquatic systems (Moore & Ramamoorthy, 1984; Allen & Hansen, 1996). The measurement of metals in aquatic systems is important in assessing the contribution of the water-borne metal concentrations to the accumulated metals in aquatic organisms (Phillips & Rainbow, 1993; Chapman, 1997). The ratio of water-borne to sediment bound metals depends to a large extent on the chemistry of the element in aquatic condition and the general properties of the particular aquatic system (Reish, 1988). It is therefore important that metal concentrations both in water and sediments be measured and related to bioaccumulation in biological tissue (Cross & Sunda, 1982).

Most studies concentrate on metals suspended in water and neglected the sediment bound metals (Cheung & Wong, 1992; Novotny, 1995). This is because water-borne metals are the main source of toxicity and bioaccumulation and is easy to quantify in the laboratory (Rosenberg & Resh, 1993; Roux *et al.*, 1993). Metals in sediment are bound to particulate matter (Sly & Hart, 1989) and they are generally not biologically available. Sediment, therefore, acts as a reservoir of metals and a large proportion of metals are found bound on them (Mason, 1991; Chapman, 1997). The decrease in the dissolved form of metals coupled with the physico-chemical factors of the water body can cause the metals in the sediment to change in speciation and become bioavailable (Moore & Rammoorthy, 1984). Benthic organisms utilize sediment for feeding and are therefore exposed to metals in sediment. Quantifying sediment bound metal is therefore necessary to predict the amount of metal that can be available to organisms in aquatic systems (Phillips & Rainbow, 1993; Cheung *et al.*, 1997).

Metals enter the organisms by several routes. They may enter the body through the gills causing damage to the gill membranes and disturbing ion and osmoregulation (Dallinger, Prosi, Segner & Back, 1987). This in turn will affect the respiratory gas exchange, acid-base balance and excretion of waste products (Spry & Wood, 1985). They can also enter through the mouth with food and be absorbed via the gastro-intestinal tract (Villegas Navarro & Villerea Trevino, 1989). In most fish species, gills are the major metal uptake sites (Taylor, Maddock & Mance, 1985), whereas in benthophagic species the intestine is the major uptake site (Cheung *et al.*, 1997; Chapman, 1985). The permeability of the skin also allows metals dissolved in water to be taken up through the skin (Heath, 1987).

Several authors have shown experimentally that copepods and mussels have high bioaccumulation ability. Metal levels in invertebrates can reach values in order of magnitudes higher than ambient concentrations. Hernandez-Hernandez, Medina, Ansuategui & Canesa (1990) found the mussel, *Mytilus gallaprocincialis* and the crab, *Carcinus meditteraneus* to accumulate high levels of Cd, Cr, and Pb in digestive tract liver as well as gills. Invertebrates, particularly those residing in sediment have a potential to accumulate metals from overlaying water, pore water and from the sediment. Chapman (1985) found high levels of metals in intestine of benthic invertebrates and this was attributed to sediment ingested with food during feeding. Metal uptake and accumulation for these organisms are influenced by the type of habitat i.e. whether epifaunal or infaunal (Cheung *et al.,* 1997). As sediments act as a sink for metals in estuarine environment, benthic invertebrates can be used to predict the possible uptake of different metals associated with sediments.

Submerged macrophytes provide detritus, which is utilised by a variety of consumer organisms (Driftmeyer *et al.*, 1980). As detritus decompose the metal content is increased causing trace metal cycling in the aquatic environment (Driftmeyer & Rublee, 1981). Trace metals have been reported to accumulate in aquatic macrophytes like *Z. marina* (Driftmeyer *et al.,* 1980; Lingby & Brix, 1989). It was found that different parts of eelgrass contain significantly different concentration of trace metals. The roots are the major sites for metal accumulation followed by the older leaf blades. Possible routes of metal uptake in *Z. marina* are from the sediment via the roots (Driftmeyer *et al.*, 1980). Metals are also taken up from water by the leaves (Lingby & Brix, 1989). Eelgrass also provide a habitat for a diverse community of epiphytic and benthic algae, infaunal and epifaunal invertebrates, fishes and benthic fauna living in detritus rich sediments (Wolfe *et al.,* 1976). As such, metal bioaccumulation could affect different levels of organisation in estuarine ecosystems.

Most research has focused on the direct effects of metals in aquatic organisms resulting from concentration from water (Buikema *et al.*, 1982). There is, however, a realization that there is also a risk arising from the progressive accumulation of pollutants. It is believed that many pollutants pass through succeeding trophic levels and accumulated in high concentrations in the tissues of longer living specimens such as predators (Abel, 1989; Chapman, 1997).

The uptake and excretion rate and ultimate tissue burden can be related to the external environmental concentrations of metals (Cross & Sunda, 1982). Elimination of the pollutant

may occur through outward diffusion, through the renal or gastrointestinal excretion or by the metabolic breakdown (Heath, 1987). Metals may be deposited and stored in various tissues of the body such that with prolonged exposure the concentration in the tissues is higher than the concentration in water and sediment, a condition called bioaccumulation (Buikema *et al.*, 1982, Chapman, 1997). Bioconcentration refers to the accumulation directly from water. Besides the direct uptake from the environment, metals can be taken up through the trophic route. By occupying the higher niche in the food chain, the concentration of metals in the organism can be amplified. This is called biomagnification (Phillips & Rainbow, 1993). These two processes can occur simultaneously in an organism (Heath, 1987).

In the present study an attempt has been made to study the temporal and spatial distribution of metals in the Mhlathuze Estuary. Metal accumulation in various fish tissue is used as a measure of contaminant exposure. Metal concentrations were analysed in gills, muscle and liver tissues. Gills are highly specialized and are exposed part of the body surface, separating the outside environment from the internal body cavity by only one layer of cells (Dallinger *et al.*, 1987). They provide the relatively easy access through which pollutant can enter the body of fish (Heath, 1987) and damage of gills can therefore interfere with gaseous exchange and ionic regulation in fish (Harrison, 1990). Muscle tissue is most commonly chosen for bioaccumulation because of the implication it carries for human consumption and health risk (Plaskett & Potter, 1979; Abel, 1989). The measurement of metals in liver is mostly recommended because of its ability to concentrate mainly contaminants to higher levels, as it is the site for detoxification and plays a part in storage, distribution or transformation of metals (Mance, 1987). It is also an important site for pathological effects induced by contaminants. Fish, therefore, provide a unique system for study of metal accumulation from both direct ingestion and through being at a high trophic level.

The increasing need for accuracy in predictions of toxicity in aquatic organisms has led to techniques being devised, which permit experiments to be related to the natural environment in a more meaningful way (Jackson & Resh, 1989). This increased the list of organisms, which can be used as bio-indicators of contamination in aquatic environments. The integration of different types of organisms and environmental factors allow us to easily interpret the state of the aquatic environments (Canfield, Kemble, Brumbaugh, Dwyer, Ingersoll & Fairchild, 1994). Therefore, use of aquatic macrophytes and benthic invertebrates can assist in understanding the uptake and accumulation in other organisms, the

rate of speciation of certain metals and the relationships between sediment bound and waterborne metals (Rosenberg & Resh, 1993; Cheung *et al.*, 1997).

The objective of this chapter was therefore to investigate the bioaccumulation of metals in selected fish tissues and whole fish (*L. dumerelli*), benthic invertebrates, *(P. blephariskios* and *A. digitalis)* and submerged macrophytes, *(Z. capensis)* on a spatial and temporal scale. Furthermore, the relationships between metal concentrations in water, sediment and in biota sampled from Mhlathuze Estuary were investigated.

4.2 Materials and Methods

4.2.1 Sampling protocol

Water, sediment, and biological samples were collected from the Mhlathuze Estuary on a quarterly basis over eight seasons from April 1996 to December 1997. The position of the sampling sites is presented in Figure 4.1.

4.2.2 Water samples

The following surface water variables were determined *in situ* at each site using a "Surveyor 3 Hydrolab" connected to an "H20" water quality multiprobe: pH, water temperature, dissolved oxygen and percentage oxygen saturation, turbidity, and salinity. A second set of water samples was collected and the following water quality determinants were analysed by the analytical laboratory of Mhlathuze Water Scientific Services: orthophosphates, sulphates and fluorides.

Two surface water samples were collected at each site and one of the samples was filtered through a 0.45µm cellulose acetate filter. The unfiltered sample represents total metal concentrations whereas the filtered sample represents dissolved metal concentrations. The samples were frozen until they could be subjected to metal concentration analysis in the laboratory. The samples were thawed in the laboratory and pre-concentration was carried out by acidifying 250 ml water with 10 ml of 55% nitric acid and 5 ml perchloric acid in

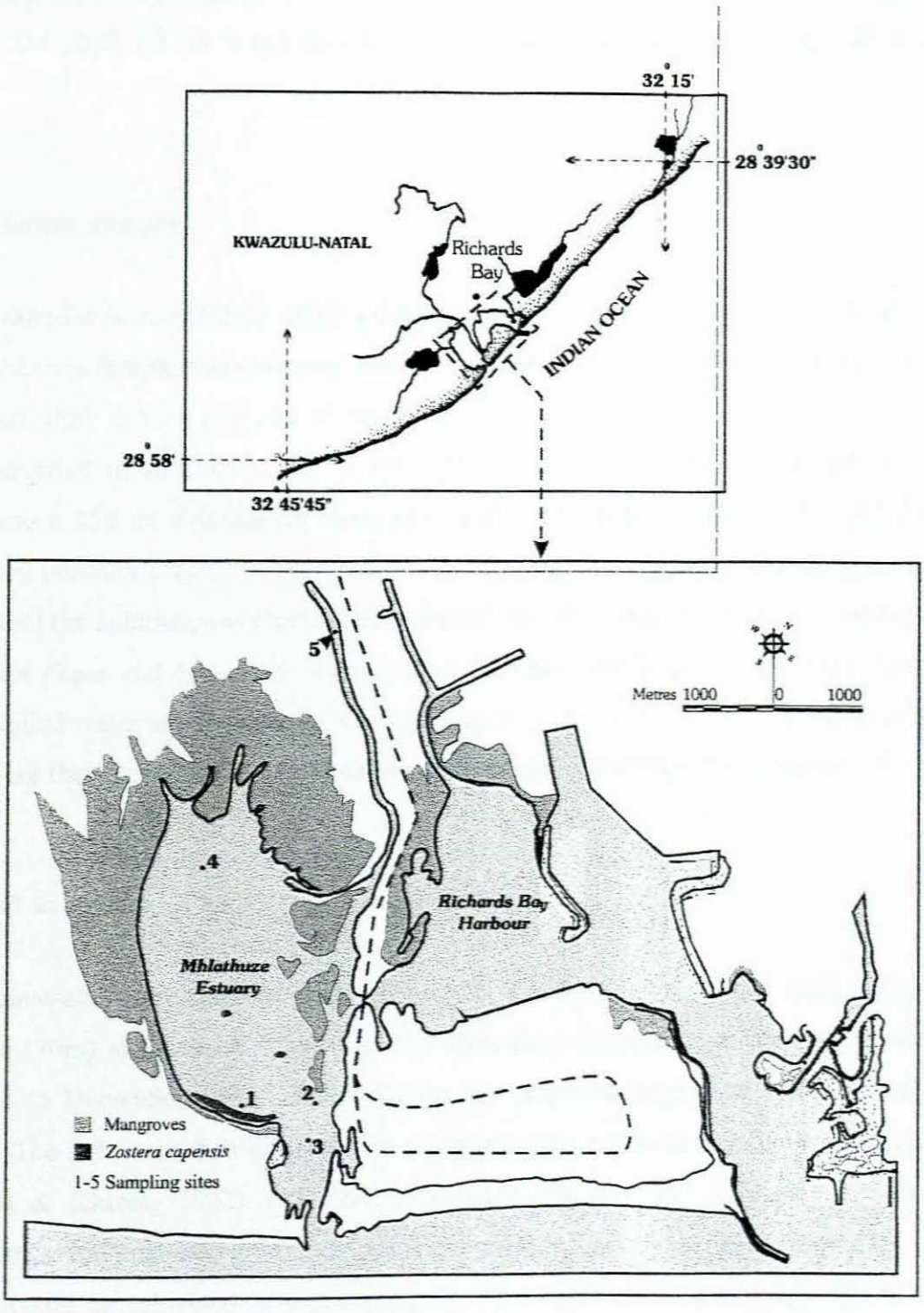


Fig. 4.1 Diagram of the Mhlathuze Estuary showing the location of the sampling sites.

Erlenmeyer flasks and evaporating to 5 ml on a hotplate (APHA 1987). Samples were made up to 50 ml with doubly distilled water. Prior to use all glassware were soaked in a 2M Contrad soap solution (MERCK Chemicals) for 24h, rinsed in doubly distilled water, acid washed in 1M HCL for 24 h and rinsed again in doubly distilled water (Giesy & Wiener, 1977).

4.2.3 Sediment samples

Sediment samples were obtained using a Zabalocki-type Eckman grab. The top 5 cm of the centre of the core sample was collected and stored in pre-cleaned plastic containers. Samples were frozen until further analysis in the laboratory. In the laboratory the samples were thawed and dried in an oven at 60° C for a period of 24 h. One gram of sediment was weighed into a 250 ml Erlenmeyer flask and 10 ml concentrated nitric (55%) and five ml concentrated perchloric acid (70%) were added, after which, digestion was performed on a hot plate until the solution was clear. Each solution was then filtered using 0.45 µm cellulose acetate filter paper 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 metal analyses.

4.2.4 Fish samples

Fish (*L. dumerelii*) were sampled with gill nets (2, 3 and 4 inch stretched mesh size) and a large seine (70m) at localities 1 and 4 in the Mhlathuze Estuary on a quarterly basis from April 1996 to December 1997. After capture, the standard length and mass of fish were recorded. The fish were then dissected on a polyethylene work-surface using stainless steel tools (Heit & Klusek, 1982) and wearing surgical gloves. The following tissues were removed for metal analysis: gills, liver and axial muscle. All the samples were kept frozen until they could be subjected to metal analysis. Small fish of approximately 80 mm were collected with small seine nets at site 2 for whole fish metal analysis. The samples were frozen in plastic bottles for further analysis.

4.2.5 *Laboratory procedures*

The tissue samples were thawed and wet mass and dry mass (following drying at 60°C for 24 hr) of all samples were recorded in order to calculate the percentage of moisture of each sample. Ten ml concentrated nitric acid (55%) and 5 ml concentrated perchloric acid (70%) were added to dry samples in a 250ml Erlenmeyer flask. Digestion was performed on an hot plate until solutions were clear (Van Loon, 1980). Digestion and filtration was conducted as described in section 4.2.3.

4.2.6 *Benthic invertebrates and aquatic macrophytes.*

Benthic invertebrates

Benthic invertebrates were sampled from the sites 1 (*A. digitals*) and 4 (*P. blephariskios*) of the Mhlathuze Estuary using a Zabalocki-type Eckman grab. These organisms were separated from the rest of the benthic invertebrates in the sample. They were thoroughly rinsed with water to remove excess sediment. Because of the small size, individual organisms had to be pooled to obtain the required mass for metal analyses.

Aquatic macrophytes

Aquatic macrophyte (*Z. capensis*) leaf blades were collected from site 1 in the Mhlathuze Estuary. They were placed in pre-cleaned plastic containers and frozen pending analysis.

Laboratory procedures

The aquatic macrophytes and benthic invertebrate samples were thawed and thoroughly rinsed with doubly distilled water to remove excess sediment. Dry mass of all samples was determined and digestion was carried out as described in section 4.2.3.

4.2.7 Atomic Absorption Spectrophotometer analysis of samples

Aluminum, Cr, Cu, Fe, Mn and Zn in total sediments and sediment fractions were measured by flame furnace atomic absorption spectrophotometry using a Varian SpectrAA 50B spectrophotometer fitted with a deuterium arc background corrector. Calibration was carried out using matrix matched calibration standards. Analytical accuracy was determined using Standard Reference Material (SRM) of the National Bureau of Standards: standard for trace elements in water (SRM 1643c), Buffalo River sediment (SRM 2704), and bovine liver (SRM 1577a). Recoveries were within 10% of the certified values.

The metal concentrations in water were calculated as follows

$$\text{Metal concentrations } (\mu g/l) = \text{AAS reading } (\mu g/ml) \times 1000$$

The metal concentration in the sediment, tissue, whole fish, benthic invertebrates and aquatic macrophytes were calculated as follows:

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

4.2.8 Statistical Analyses

Statistical analyses of the data were performed with standard ANOVA using Tukey's multiple comparison test to measure ad hoc significance differences (Zar, 1984). Significance was regarded at the $P<0.05$ significance level. Following ANOVA procedures, means were ranked for the development of Spearman's rank correlation matrix.

4.3 Results

4.3.1 Physico-chemical parameters

The selected physico-chemical variables of the Mhlathuze Estuary are summarised in Table 4.1. Temperatures remained constant at all sites during the quarterly surveys reflecting the natural seasonal temperature fluctuations. Only the water temperatures at site 4 seemed consistently different to those measured at each of the other sampling sites during the particular survey. Turbidity showed seasonal fluctuations with Spring and Summer showing elevated turbidities. Salinity remained constant for all sites during the quarterly surveys with the exception of Summer 1997 where the salinity at all sites was below 6 ‰. Salinity measured at site 5 was consistently lower that other sites during a particular survey. Dissolved oxygen was also constant for all sites with site 3 showing higher dissolved oxygen values. This was also the case for sulfate and fluoride. Only the sulfate measured at site 5 seemed consistently different to those measured at each of the other sampling sites during the particular survey. Sulfates sampled during Summer 1997 sampling trip showed decreased concentrations when compared to those measured in other seasons. Nutrients (nitrates and orthophosphates) did not display any clear trends during the quarterly sampling surveys.

4.3.2 Metal concentrations in water

Metal concentrations of the surface water samples are presented in Tables 4.2 (total metal), 4.3 (dissolved metal) and 4.4 (suspended matter).

Aluminium

Total, dissolved and suspended Al concentrations in water are presented in Tables 4.2 to 4.4. The highest total and dissolved Al concentrations in water from all sites were recorded during Summer 1997, followed by Summer 1996. The lowest total Al concentrations were recorded during Autumn 1997. The lowest concentrations of Al dissolved in water at sites 1 and 2 were recorded during Spring 1997 followed by Winter 1997 and at sites 3, 4 and 5 the lowest Al concentrations in water was recorded during Winter 1997 followed by Spring 1997. No single site showed consistently different concentrations throughout sampling period. In order to evaluate the significance of temporal concentrations of Al in water samples, the concentrations

of the different sites were pooled. Analysis of variance (ANOVA – Tukey's Multiple comparison) revealed that Al concentrations (Fig 4.2 A and C) collected during Summer 1997 were significantly higher than the other surveys ($P<0.05$). However, the concentrations of suspended matter were significantly lower during the 1997 sampling period than the 1996 period (Fig 4.2).

Table 4.1 Quarterly physico-chemical values recorded at five sites in the Mhlathuze Estuary for the sampling period from April 1996 to December 1997. Historical data in the original estuary before the construction of the harbour are represented by A and after the construction of the harbour are presented by B.

Season	Site	Temp (°C)	Turbidity (NTU)	O2 (mg/l)	O2 (%)	Salinity (‰)	pH	PO_4 (mg/l)	NO_3 (mg/l)	SO_4 (mg/l)
Historical data A[1]		21	6	7.58	101	28	8.1	0.018	0.0008	NA
Historical data B[2]		NA	NA	NA	NA	32.8	NA	0.017	0.067	NA
Autumn 1996	1	21.00	3.7	7.07	98.5	36.5	7.87	0.23	ND	2583
Autumn 1996	2	20.09	4.6	6.91	96.9	36.5	7.92	0.09	ND	2667
Autumn 1996	3	22.95	BDL	6.84	94.3	36.7	7.79	0.24	ND	2639
Autumn 1996	4	19.91	31.7	6.34	88.2	29.2	7.72	0.16	ND	2222
Autumn 1996	5	19.86	BDL	0.00	89.4	0.8	7.91	0.25	0.80	107
Winter 1997	1	19.09	5.0	8.53	119.5	34.0	4.19	BDL	ND	2600
Winter 1997	2	20.15	19.6	7.42	98.8	30.4	5.40	BDL	ND	3100
Winter 1997	3	17.36	4.4	7.82	108.2	34.5	8.78	BDL	ND	3000
Winter 1997	4	16.31	6.0	7.41	96.8	30.5	7.56	BDL	ND	2600
Winter 1997	5	21.45	6.5	8.31	88.6	8.1	7.49	BDL	ND	410
Spring 1996	1	21.03	6.0	7.63	108.2	34.4	7.82	0.18	ND	3860
Spring 1996	2	20.96	26.0	7.66	109.5	34.4	7.86	0.12	ND	3700
Spring 1996	3	21.88	24.0	7.74	110.3	34.4	7.88	BDL	ND	3140
Spring 1996	4	24.12	5.0	7.48	107.2	34.2	7.78	BDL	ND	3860
Spring 1996	5	25.47	14.0	8.01	108.1	22.2	7.67	BDL	ND	2420
Summer 1996	1	23.62	8.0.	7.74	115.3	34.6	8.58	0.10	ND	3300
Summer 1996	2	24.00	5.0	7.88	115.2	34.9	8.44	0.17	ND	2700
Summer 1996	3	25.84	4.0	8.23	12.2	34.9	8.49	0.17	ND	3150
Summer 1996	4	30.41	15.0	7.57	114.8	34.0	8.52	0.20	ND	2850
Summer 1996	5	24.58	28.0	7.94	114.7	14.2	8.44	ND	ND	ND
Autumn 1997	1	22.10	16.0	6.94	101.4	32.3	8.45	BDL	ND	4000
Autumn 1997	2	24.05	12.0	64.80	90.9	32.9	8.40	0.21	ND	3500
Autumn 1997	3	23.80	18.0	7.46	108.1	32.5	8.52	0.09	ND	2900
Autumn 1997	4	23.20	14.0	6.42	88.2	24.2	8.32	0.19	ND	2200
Autumn 1997	5	19.51	26.0	7.50	86.6	0.3	8.25	0.45	0.50	32
Winter 1997	1	19.18	15.0	5.84	105.6	34.2	8.40	0.21	0.07	2881
Winter 1997	2	19.16	22.0	8.01	105.7	35.1	8.40	0.21	0.07	2959
Winter 1997	3	19.04	20.0	8.12	105.1	35.1	8.40	0.20	0.06	3026
Winter 1997	4	14.76	24.0	8.22	94.8	6.5	7.74	0.18	0.06	189
Winter 1997	5	20.22	46.0	9.11	91.2	0.4	7.27	0.11	0.49	24
Spring 1997	1	21.15	14.0	7.62	103.8	35.0	8.82	0.17	BDL	2989
Spring 1997	2	20.03	28.0	7.45	98.4	26.5	8.64	0.13	0.14	1806
Spring 1997	3	18.71	17.5	20.30	102.0	35.3	8.80	0.22	0.12	3137
Spring 1997	4	21.72	10.0	7.03	89.8	29.4	8.36	0.13	0.11	2285
Spring 1997	5	21.42	29.0	21.42	78.4	0.3	8.68	0.06	0.21	32
Summer 1997	1	22.67	20.0	7.16	84.2	4.1	7.70	0.08	0.28	370
Summer 1997	2	22.66	25.0	6.58	76.1	0.2	7.43	0.06	0.46	185
Summer 1997	3	21.76	17.0	7.37	86.5	5.5	7.66	0.10	0.36	477
Summer 1997	4	22.48	45.5	7.36	85.4	0.7	7.78	BDL	0.35	46
Summer 1997	5	22.61	10.00	5.10	59.70	0.20	7.19	0.07	0.28	12

BDL= Below detection limit. ND= No data
[1]Hemens *et al.* (1971) and [2]Hemens *et al.* (1976a)

Chromium

Total dissolved and suspended Cr concentrations in water are presented in Tables 4.2 to 4.4. The highest total, dissolved and suspended Cr concentrations in water from all sites were recorded during Summer 1997. The lowest suspended Cr concentrations were recorded in Winter 1996 and the lowest dissolved Cr concentrations from all sites were recorded in Summer 1996. No definite spatial trends were noticeable. In order to evaluate the significance of temporal concentrations of Cr in water samples, the concentrations of the different sites were pooled. Analyses of variance revealed that Cr concentrations in (Fig 4.3) water collected during Summer 1997 were significantly higher than other surveys ($P<0.05$).

Table 4.2 Seasonal concentrations (µg/l) of selected total heavy metals in water samples from selected sites in the Mhlathuze Estuary for the period April 1996 to December 1997.

Season	Site	Al	Cr	Cu	Fe	Mn	Pb	Zn
Autumn 1996	1	460	12	4.4	440	36	154	62
Autumn 1996	2	3574	126	32	2626	142	142	80
Autumn 1996	3	240	6	26	300	26	104	52
Autumn 1996	4	1360	10	34	820	56	130	68
Autumn 1996	5	180	BDL	6	ND	14	36	36
Winter 1996	1	540	10	52	620	52	152	50
Winter 1996	2	1050	30	70	1200	126	280	80
Winter 1996	3	400	12	48	420	44	170	52
Winter 1996	4	620	8	30	500	38	118	48
Winter 1996	5	440	BDL	22	520	70	52	36
Spring 1996	1	1660	20	72	1700	106	204	76
Spring 1996	2	2060	22	48	2200	82	210	96
Spring 1996	3	1080	8	42	920	54	176	64
Spring 1996	4	1900	18	58	1300	76	192	74
Spring 1996	5	1160	10	42	780	136	138	58
Summer 1996	1	340	22	74	360	54	40	68
Summer 1996	2	260	28	76	360	50	242	66
Summer 1996	3	140	28	54	480	62	224	60
Summer 1996	4	560	16	60	920	96	286	66
Summer 1996	5	540	8	40	340	52	126	44
Autumn 1997	1	120	24	64	420	70	246	58
Autumn 1997	2	360	14	58	500	74	236	68
Autumn 1997	3	120	24	74	360	64	242	54
Autumn 1997	4	120	12	48	200	38	174	40
Autumn 1997	5	80	4	42	260	48	166	44
Winter 1997	1	320	22	54	540	70	174	62
Winter 1997	2	500	28	62	460	70	228	66
Winter 1997	3	1060	28	68	1560	98	264	76
Winter 1997	4	680	8	6	1456	44	42	30
Winter 1997	5	600	BDL	6	1260	72	30	34
Spring 1997	1	140	80	88	740	98	236	76
Spring 1997	2	160	20	50	360	74	448	52
Spring 1997	3	540	30	54	440	74	542	60
Spring 1997	4	674	74	70	660	92	530	530
Spring 1997	5	1240	50	6	420	74	48	36
Summer 1997	1	12800	110	16	9040	136	144	82
Summer 1997	2	26200	226	14	23500	266	82	112
Summer 1997	3	12900	126	20	10500	136	198	86
Summer 1997	4	40600	230	ND	27100	432	72	100
Summer 1997	5	16800	132	10	17100	196	34	72

BDL-Below detectable limit. ND-No data

Table 4.3 Seasonal concentrations (µg/l) of selected dissolved heavy metals in water samples from selected sites in the Mhlathuze Estuary for the period April 1996 to December 1997.

Season	Site	Al	Cr	Cu	Fe	Mn	Pb	Zn
Autumn 1996	1	528	52	58	640	46	140	80
Autumn 1996	2	828	46	46	976	60	152	98
Autumn 1996	3	310	6	6	360	18	26	ND
Autumn 1996	4	298	34	44	320	42	124	70
Autumn 1996	5	194	12	22	220	26	18	44
Winter 1996	1	320	38	BDL	360	38	110	64
Winter 1996	2	476	90	46	650	76	260	136
Winter 1996	3	472	56	BDL	440	50	176	78
Winter 1996	4	402	58	72	380	50	146	76
Winter 1996	5	248	24	20	420	60	86	76
Spring 1996	1	238	58	92	320	36	154	96
Spring 1996	2	316	68	106	360	42	186	132
Spring 1996	3	450	62	98	460	52	192	90
Spring 1996	4	308	58	88	360	44	156	ND
Spring 1996	5	282	34	40	420	198	100	58
Summer 1996	1	636	8	6	440	12	28	44
Summer 1996	2	296	8	BDL	300	6	BDL	30
Summer 1996	3	1188	16	8	440	14	32	58
Summer 1996	4	1704	26	8	1200	30	20	ND
Summer 1996	5	1412	14	8	1000	54	8	46
Autumn 1997	1	342	10	6	340	10	8	34
Autumn 1997	2	866	66	BDL	360	54	BDL	50
Autumn 1997	3	288	14	102	54	18	190	84
Autumn 1997	4	282	8	BDL	240	10	BDL	40
Autumn 1997	7	714	6	BDL	880	26	20	46
Winter 1997	1	270	70	78	580	68	208	102
Winter 1997	2	310	74	110	420	58	200	88
Winter 1997	3	108	72	100	500	76	214	96
Winter 1997	4	144	10	6	240	22	34	32
Winter 1997	5	116	8	8	ND	54	18	68
Spring 1997	1	160	34	72	400	66	644	62
Spring 1997	2	156	74	96	500	70	198	90
Spring 1997	3	140	38	64	400	66	584	60
Spring 1997	4	160	32	66	480	60	562	78
Spring 1997	5	120	34	BDL	300	24	38	40
Summer 1997	1	2568	98	16	2160	66	48	70
Summer 1997	2	3508	106	18	2500	44	40	64
Summer 1997	3	4966	106	24	5040	72	74	86
Summer 1997	4	9074	164	18	7320	62	BDL	104
Summer 1997	5	4428	136	12	3500	48	12	100

BDL-Below detectable limits. ND-No data

Table 4.4 Seasonal concentrations (µg/l) of selected heavy metals in suspended particles in water samples from selected sites in the Mhlathuze Estuary for the period April 1996 to December 1997.

Season	Site	Al	Cr	Cu	Fe	Mn	Pb	Zn
Autumn 1996	1	22	10	BDL	180	6	20	22
Autumn 1996	2	52	26	6	826	26	42	52
Autumn 1996	3	200	10	6	260	10	20	24
Autumn 1996	4	48	18	BDL	2880	118	28	48
Autumn 1996	5	22	12	BDL	1960	32	BDL	22
Winter 1996	1	26	14	BDL	460	12	22	26
Winter 1996	2	60	26	6	900	46	46	60
Winter 1996	3	16	14	BDL	260	12	10	16
Winter 1996	4	22	14	BDL	760	28	22	22
Winter 1996	5	32	10	BDL	660	44	16	32
Spring 1996	1	28	20	BDL	580	34	24	28
Spring 1996	2	12	BDL	BDL	2860	30	BDL	12
Spring 1996	3	32	24	BDL	1760	12	18	32
Spring 1996	4	22	18	BDL	1620	44	26	22
Spring 1996	5	22	10	BDL	1040	188	18	22
Summer 1996	1	94	78	BDL	620	62	216	94
Summer 1996	2	110	62	92	540	66	178	110
Summer 1996	3	112	74	BDL	1980	124	254	112
Summer 1996	4	104	72	BDL	680	66	180	164
Summer 1996	5	134	40	BDL	420	58	138	134
Autumn 1997	1	104	48	BDL	580	64	148	104
Autumn 1997	2	134	52	36	720	96	144	134
Autumn 1997	3	112	60	50	940	88	202	112
Autumn 1997	4	156	62	BDL	720	136	182	156
Autumn 1997	5	94	30	26	520	48	116	94
Winter 1997	1	42	8	BDL	780	18	30	42
Winter 1997	2	34	16	BDL	1640	34	18	34
Winter 1997	3	26	12	BDL	1300	24	20	26
Winter 1997	4	32	12	BDL	1720	30	24	32
Winter 1997	5	46	10	BDL	ND	34	24	40
Spring 1997	1	58	58	BDL	1600	34	8	56
Spring 1997	2	130	114	BDL	2200	56	4	130
Spring 1997	3	60	60	BDL	1480	24	12	60
Spring 1997	4	48	48	BDL	760	32	8	48
Spring 1997	5	62	62	BDL	2300	52	4	62
Summer 1997	1	64	122	10	7540	64	4	64
Summer 1997	2	70	112	6	35100	132	30	70
Summer 1997	3	54	150	6	8560	56	BDL	54
Summer 1997	4	116	186	20	ND	520	30	116
Summer 1997	5	62	146	6	7480	108	BDL	62

BDL- Below detectable limits. ND- No data

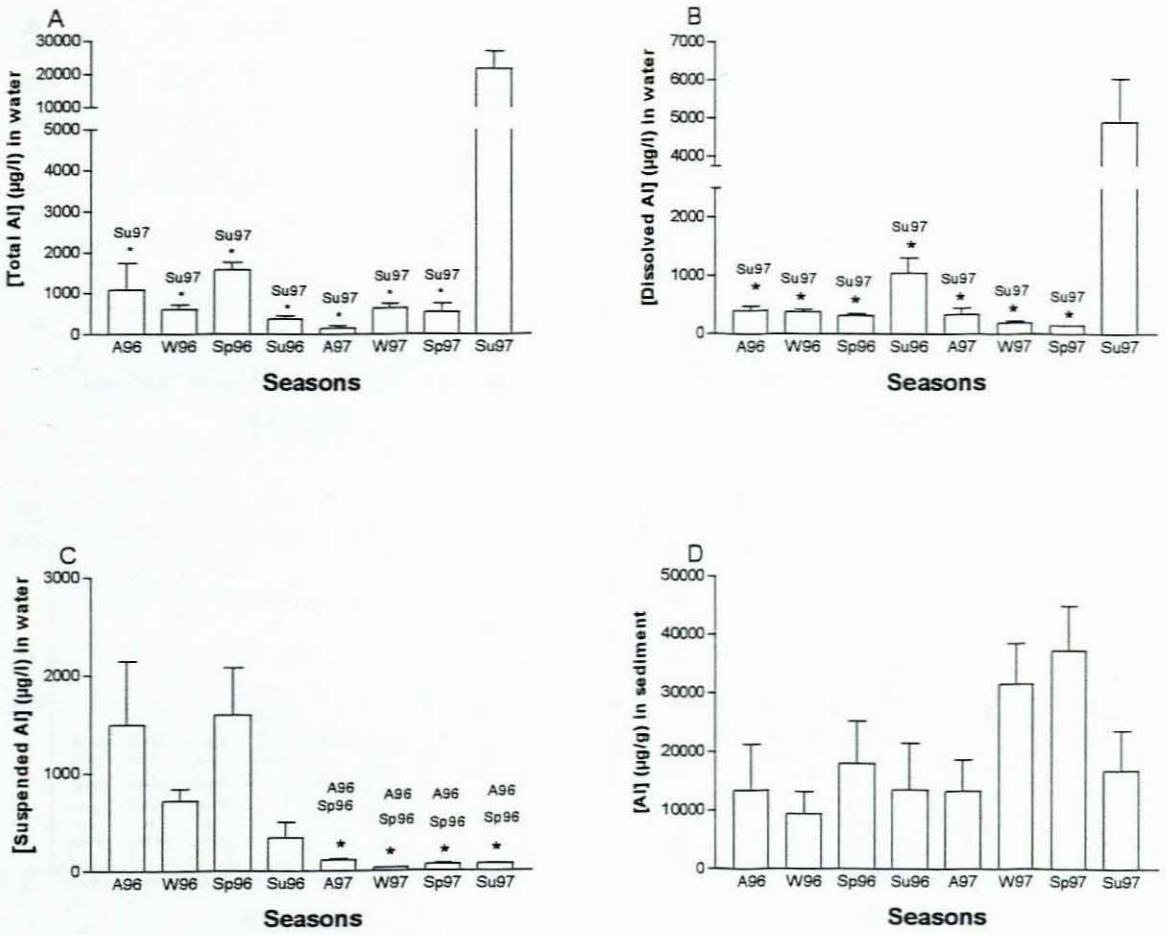


Fig. 4.2 Concentrations (mean ± standard error) of total (A), dissolved (B) and suspended (C) Al concentrations in water samples for the period April 1996 to December 1997. Mean quarterly Al concentration in sediment for the same period are presented in D. Asterisks indicate significant differences at $P<0.05$ (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

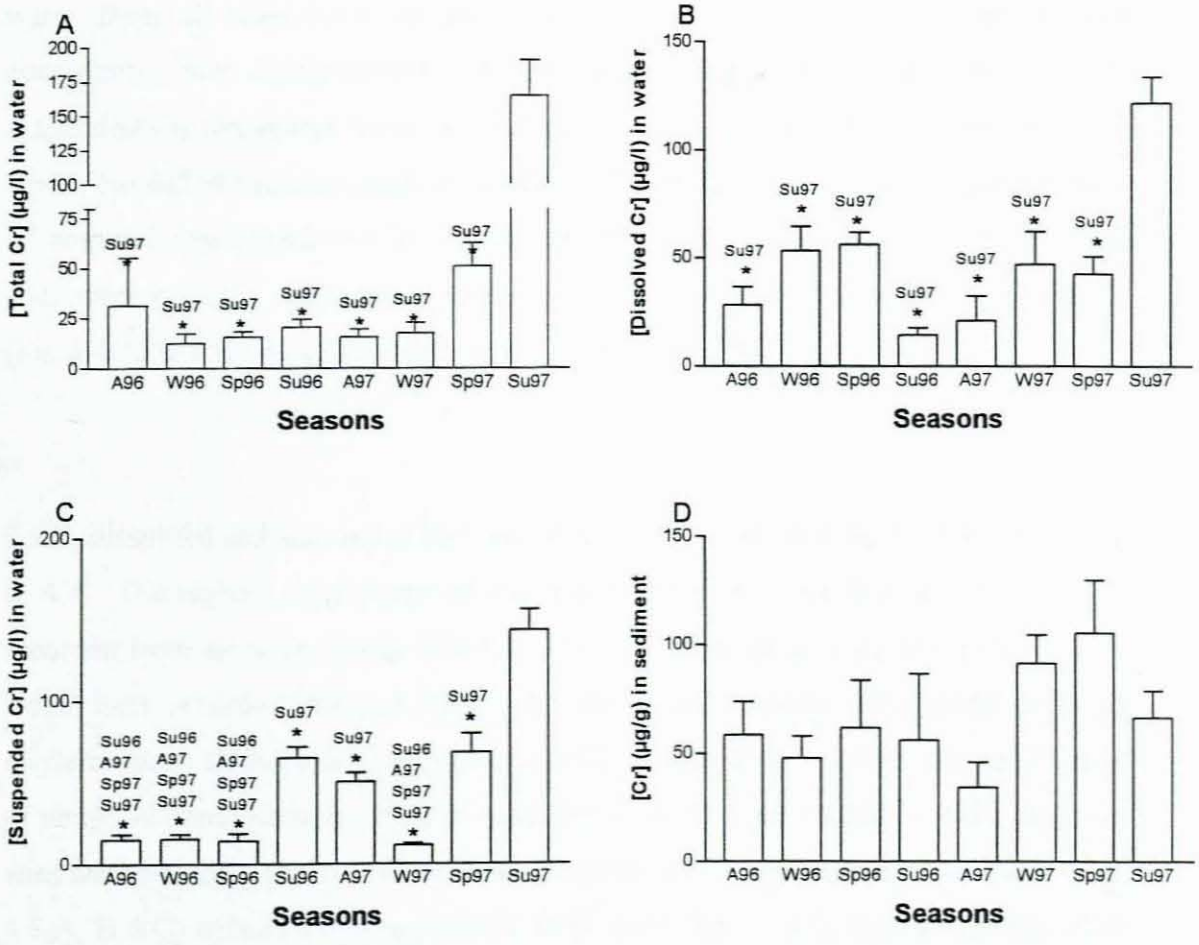


Fig. 4.3 Concentrations (mean ± standard error) of total (A) dissolved (B) and suspended (C) Cr concentrations in water samples for the period April 1996 to December 1997. Mean quarterly Al concentration in sediment for the same period are presented in D. Asterisks indicate significant differences at $P<0.05$.(Tukey's multiple Range, ANOVA) with the season concerned listed above it.

Copper

Total, dissolved and suspended Cu concentrations in water are presented in Tables 4.2 to 4.4. The highest total Cu concentrations in water from all sites were recorded during Summer 1996 followed by autumn 1997. The lowest total Cu concentrations of Cr were recorded during Summer 1997. The lowest dissolved Cu concentrations in water from all sites were recorded during summer 1996. Site seven showed consistently low concentrations of Cu throughout the sampling period. The suspended Cu concentrations in water were very low usually falling below detection limits. No definite spatial trends were noticeable. In order to evaluate the significance of temporal concentrations of Cu in water samples, the concentrations of the different sites were pooled. Analyses of variance revealed no significant difference in total (Fig 4.4 A) in Cu concentrations in water between seasons.

Iron

Total, dissolved and suspended Fe concentrations in water are presented in Tables 4.2 to 4.4. The highest total dissolved and suspended Fe concentrations in water were recorded from all sites during Summer 1997. The lowest total Fe concentrations in water were recorded Autumn 1997. No single site showed consistently different concentrations throughout the sampling period. In order to evaluate the significance of temporal concentrations of Fe in water samples, the concentrations of the different sites were pooled. Analyses of variance revealed that Fe concentrations in water (Fig. 4.5 A, B &C) collected during Summer 1997 were significantly higher than the other surveys ($P<0.05$).

Manganese

Total, dissolved and suspended Mn concentrations in water are presented in Tables 4.2 to 4.4. The highest total Mn concentrations in water from all sites were recorded during Summer 1997. The lowest suspended Mn concentrations in water were recorded during Autumn 1997 followed by Summer 1996. The lowest concentrations of Mn from all sites were recorded during Summer 1996. No definite spatial trends were noticeable. In order to evaluate the significance of temporal concentrations of Mn in water samples, the concentrations of the different sites were pooled. Analyses

of variance revealed that Mn concentrations in water (Fig. 4.6 A and B) collected during Summer 1997 were significantly higher than the other surveys ($P<0.05$).

Lead

Total dissolved and suspended Pb concentrations in water are presented in Tables 4.2 to 4.4. The highest total Pb concentrations in water from sites 1,2 3 and 4 were recorded during Spring 1997. The highest dissolved Pb concentrations in water were recorded at site 2 during winter 1996. The lowest total Pb concentrations in water were recorded at site 7 during Winter 1997. The lowest suspended Pb concentrations in water were recorded at site, 2, 3,4 and 7 during Spring 1997. The lowest suspended Pb in water was recorded during Summer 1997. The lowest dissolved Pb concentrations in water were recorded at site 2 during Summer 1996. Site 7 showed consistently low total concentrations of Pb throughout the sampling period. No definite spatial trends were noticeable. In order to evaluate the significance of temporal concentrations of Pb in water samples, the concentrations of the different sites were pooled. Analyses of variance revealed that Pb concentrations in water (Fig. 4.7B) collected during Summer 1996 and Autumn 1997 were significantly higher than other surveys ($P<0.05$)

Zinc

Total suspended and dissolved Zn concentrations in water are presented in Tables 4.2 to 4.4. The highest total Zn concentration was recorded during Spring 1997 and the lowest total Zn concentration was recorded during Winter 1997. The highest suspended Zn was recorded during Spring 1996 and the lowest particulate Zn was recorded during Summer 1996. The highest dissolved Zn concentration was recorded during Winter 1996 and the lowest dissolved Zn concentration was recorded during Winter 1997. No single site showed consistently different concentrations throughout the sampling period. In order to evaluate the significance of temporal concentrations of Zn in water samples, the concentrations of the different sites were pooled. Analyses of variance revealed that Zn concentrations in water (Fig. 4.8C) collected during Summer 96 and Autumn 97 was significantly higher than the other surveys ($P<0.05$).

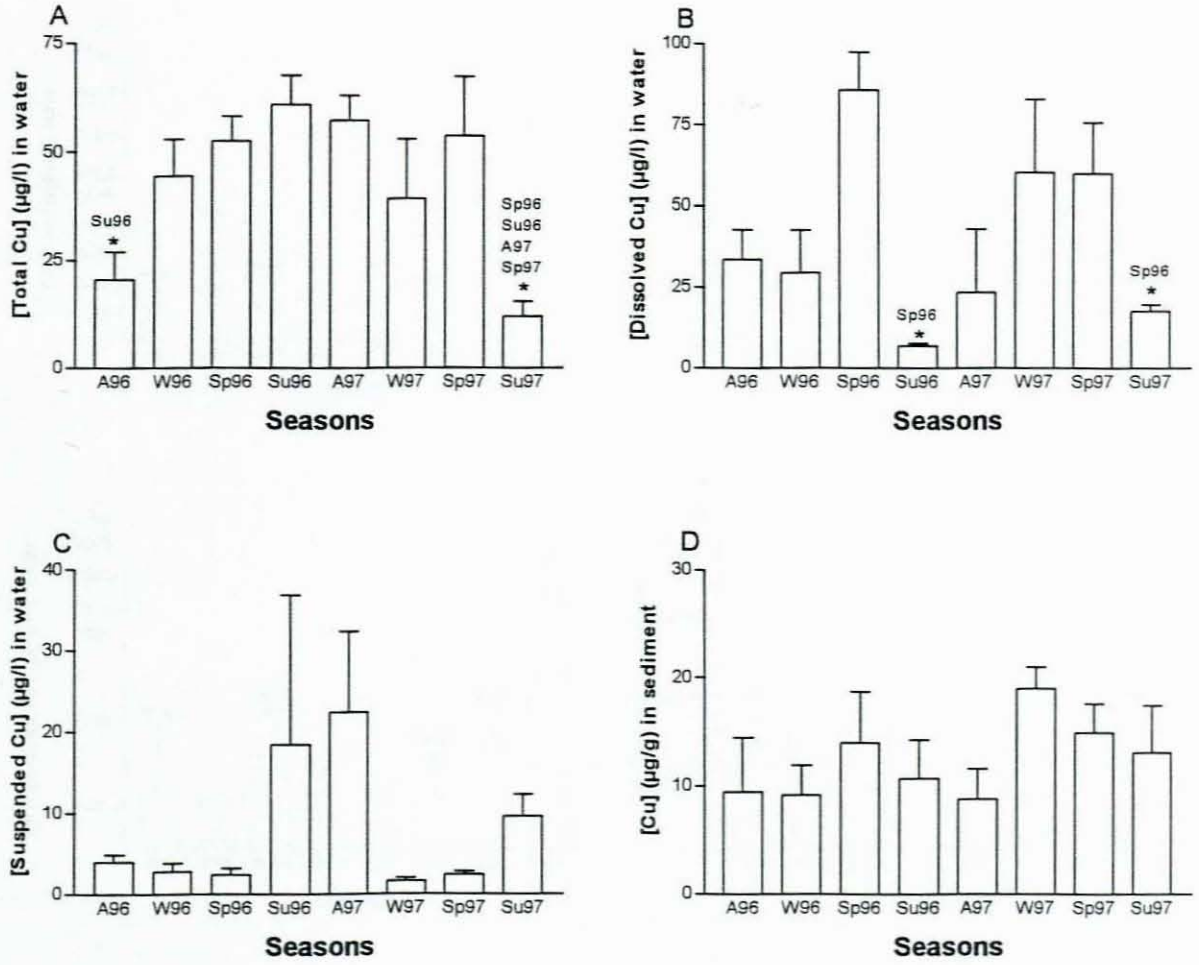


Fig. 4.4 Concentrations (mean ± standard error) of total (A), dissolved (B) and suspended (C) Cu concentrations in water samples for the period April 1996 to December 1997. Mean quarterly Al concentration in sediment for the same period are presented in D. Asterisks indicate significant differences at $P<0.05$ (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

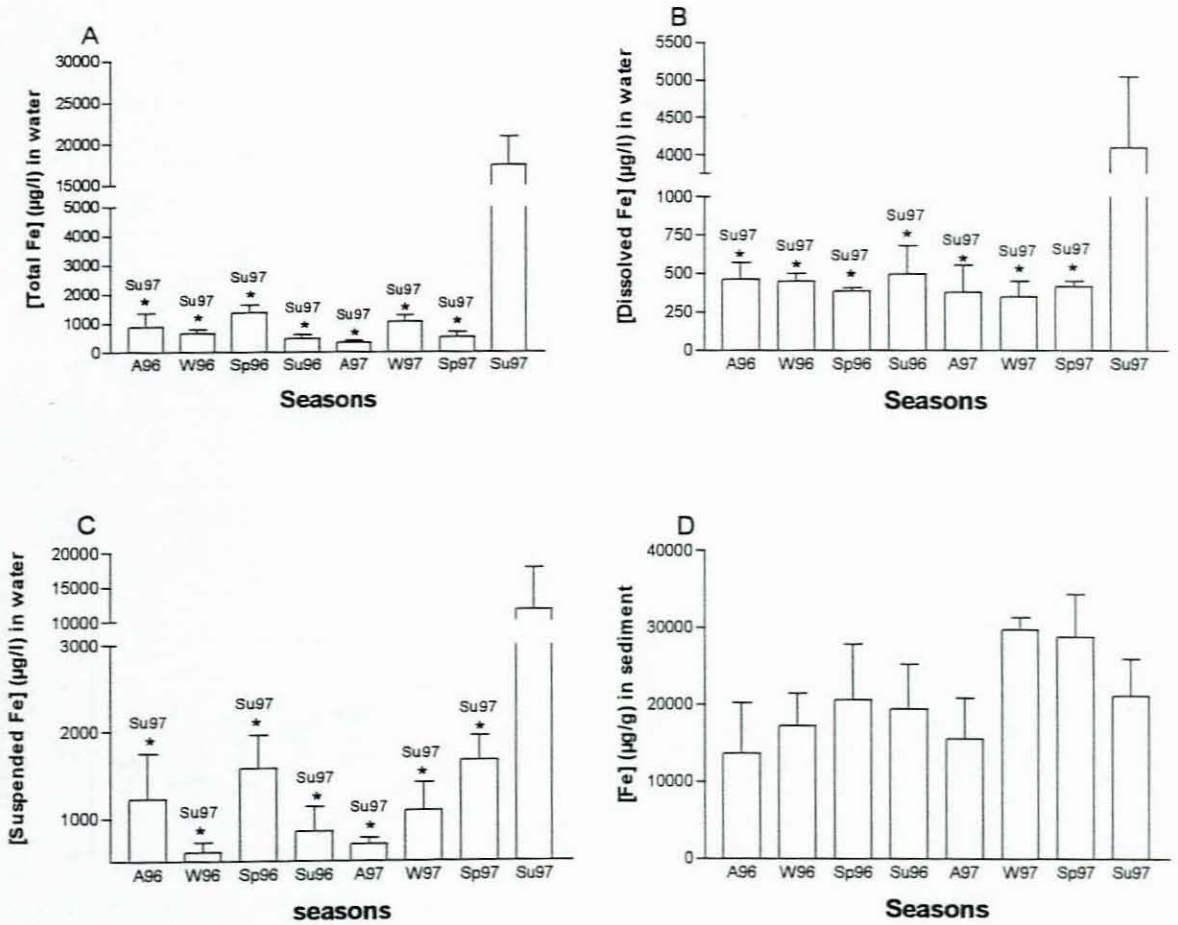


Fig. 4.5 Concentrations (mean ± standard error) of total (A), dissolved (B) and suspended (C) Fe concentrations in water samples for the period April 1996 to December 1997. Mean quarterly Al concentration in sediment for the same period are presented in D. Asterisks indicate significant differences ($P<0.05$ ANOVA Tukey's multiple Range, ANOVA) with the season concerned listed above it.

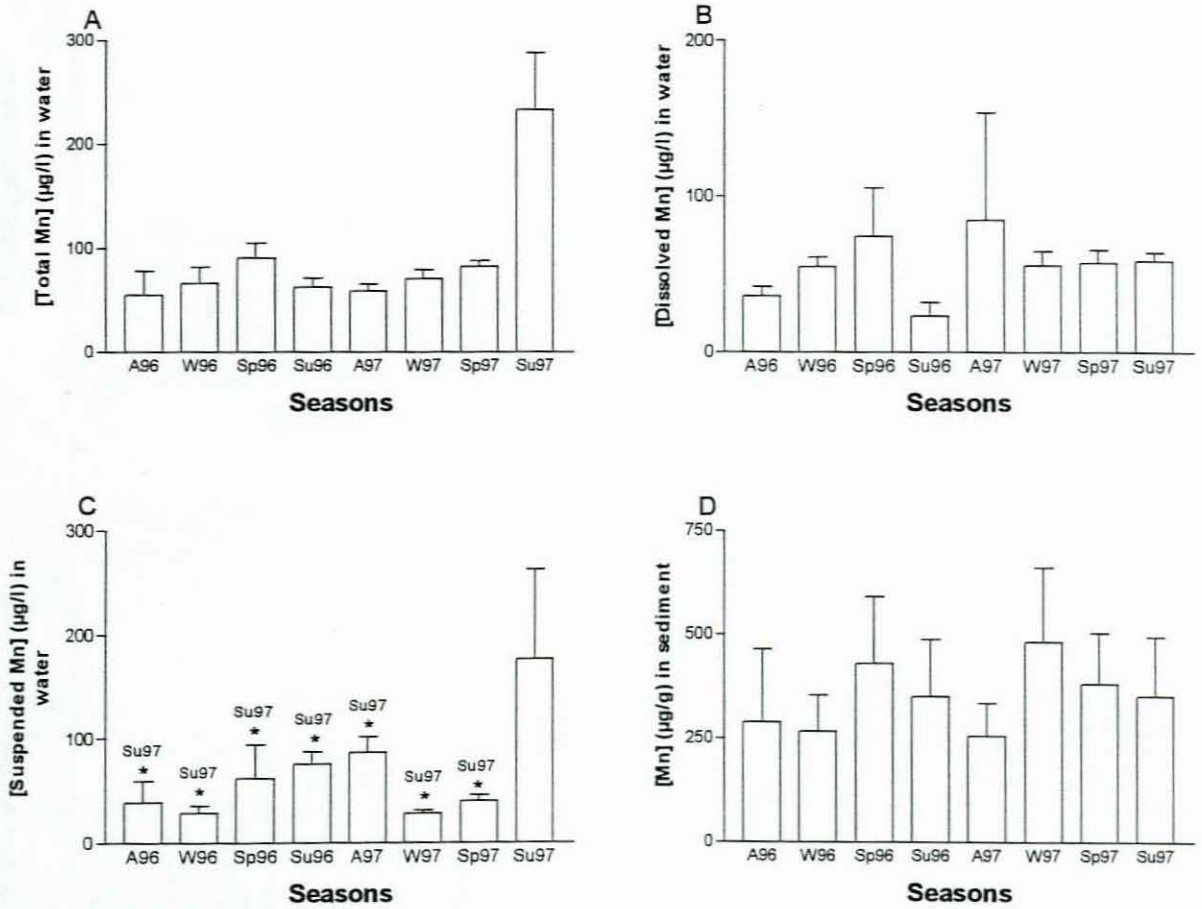


Fig. 4.6 Concentrations (mean ± standard error) of total (A), dissolved (B) and suspended (C) Mn concentrations in water samples for the period April 1996 to December 1997. Mean quarterly Al concentration in sediment for the same period are presented in D. Asterisks indicate significant differences at $P<0.05$ (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

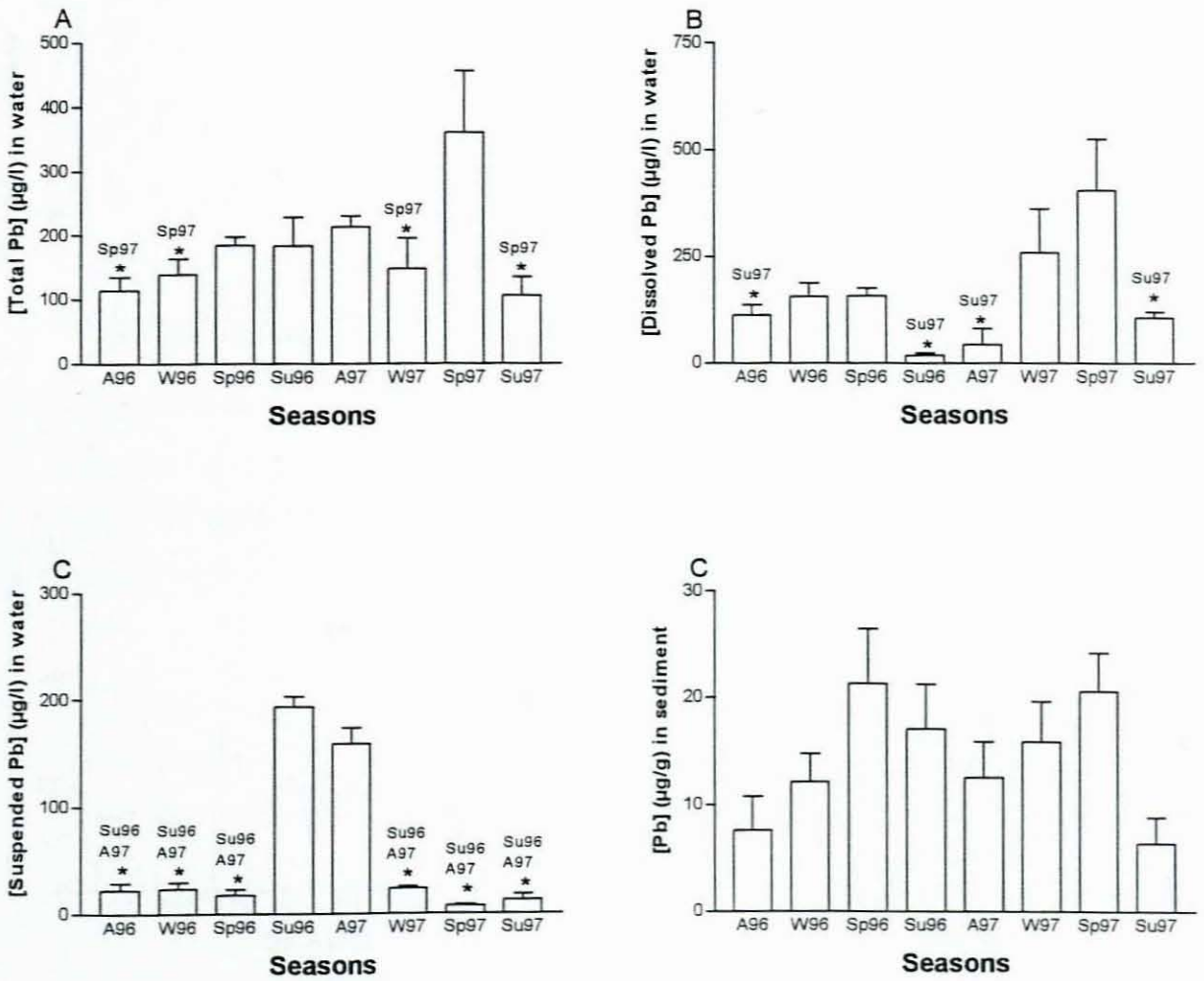


Fig. 4.7 Concentrations (mean ± standard error) of total (A), dissolved (B) and suspended (C) Pb concentrations in water samples for the period April 1996 to December 1997. Mean quarterly Al concentration in sediment for the same period are presented in D. Asterisks indicate significant differences at $P<0.05$ (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

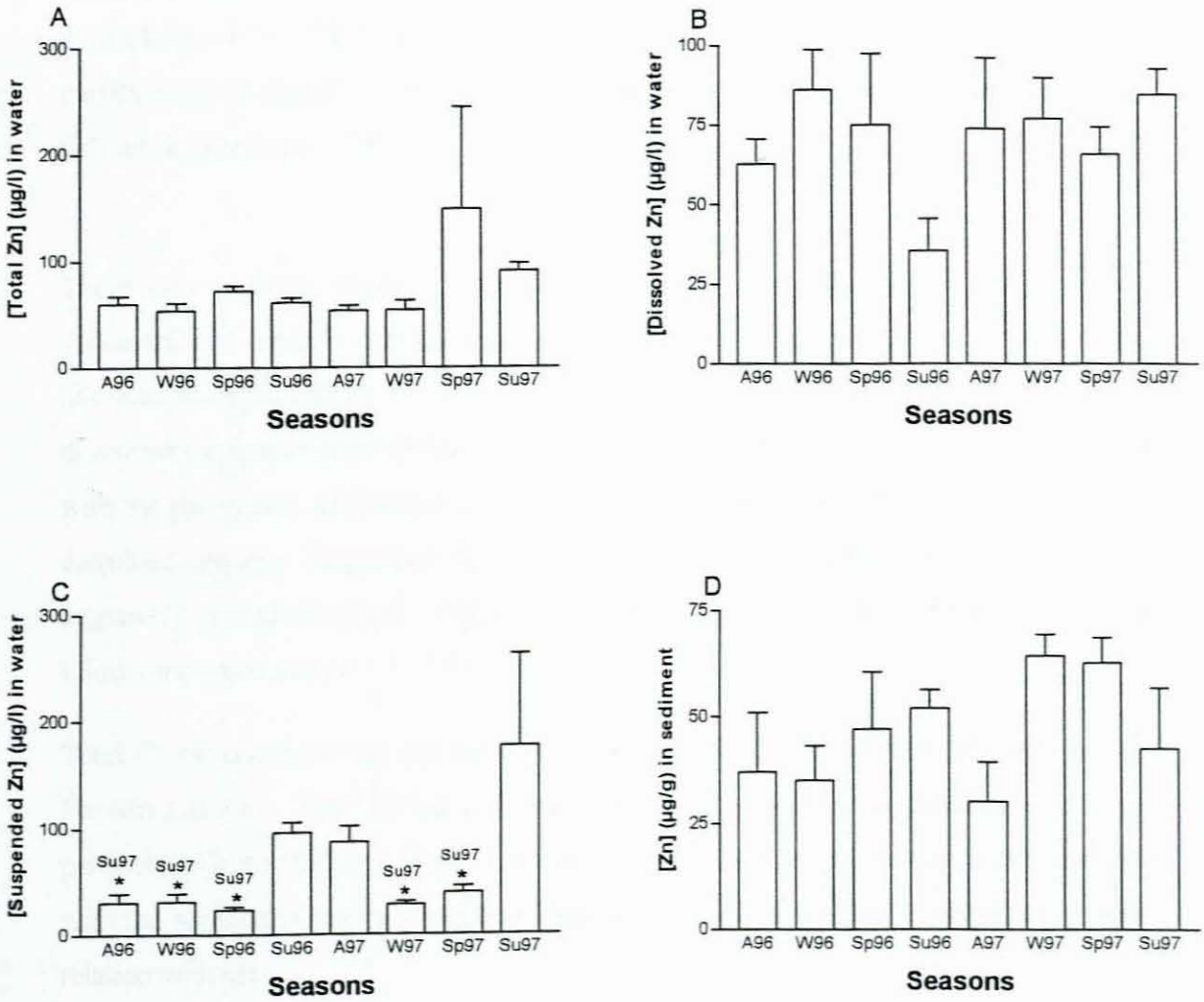


Fig. 4.8 Concentrations (mean ± standard error) of total (A), dissolved (B) and suspended (C) Zn concentrations in water samples for the period April 1996 to December 1997. Mean quarterly Al concentration in sediment for the same period are presented in D. Asterisks indicate significant differences at $P<0.05$ (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

Relationships between physico-chemical properties and metal concentrations in water

In order to assess the relationships between the partitioning of metals in the different water phases and the physical processes driving them in the estuary, a Spearman's Rank Correlation Analyses was performed on the pooled physico-chemical and metal data (Table 4.5). Physical components were selected based on their importance in partitioning of metals in estuaries as reported in the literature (Baeyens, Elskens, Gillian & Goeyens, 1998).

There was a strong negative correlation (-0.58) between total Al and pH. Total Al concentrations seem to increase in high turbidity areas. The dissolved fraction of Al does not seem to depend on salinity and turbidity and seems to be dependent on the presence of dissolved oxygen in areas of low pH. At low pH total Al seems to exist as dissolved Al with the particulate Al forming a majority component in areas with high salinity and low dissolved oxygen. Suspended Al seems to be strongly related to salinity (0.36) and negatively related dissolved oxygen (-0.37) suggesting an increase in suspended Al in as salinity increases and very low dissolved oxygen.

Total Cr did not show any correlation with salinity. This was also true of metals such as Fe, Mn and Zn. Total Cr has a strong relationship with both the dissolved (0.51) and particulate (0.66) fractions. Both the particulate and the dissolved were related to turbidity with the suspended having a negative relationship with salinity but a significant positive relation with pH.

Total Cu showed a tendency of decreasing with increase in turbidity. This was also true of Pb. Total Cu was positively correlated with salinity and pH. Dissolved Cu also had a positive correlation with salinity. This implies that the concentrations of total Cu together with the production of dissolved Cu increases with the increase in salinity.

There was a strong correlation between suspended and total (0.51) Fe. A strong negative correlation between total Fe and pH was also observed. It suggests an increase in total Fe as the pH decreases. Suspended fraction was strongly related to turbidity (0.62) suggesting the production of particulate Fe in highly turbid areas with lower pH levels. The relationship between total Mn and turbidity and salinity was very, whereas there was a strong relationship between dissolved and total Mn concentrations.

Table 4.5 Spearman's rank correlation coefficients between metal concentrations (total, dissolved and suspended) and selected physico-chemical parameters (turbidity, salinity, organic content of sediments, dissolved oxygen and pH) in the Mhlathuze Estuary. Means from all the stations were ranked prior to analyses and statistical significance was at P<0.05 (indicated by *).

Aluminium	Turbidity	Salinity	Organic	Dissolved O_2	pH	Total	Suspended	Dissolved
Turbidity	1.00							
Salinity	-0.64*	1.00						
Organic	0.02	-0.27*	1.00					
Dissolved O_2	0.06	0.11	0.10	1.00				
pH	-0.18*	0.45*	-0.04	0.22*	1.00			
Total	0.23*	-0.24*	-0.19	-0.11	-0.58*	1.00		
Suspended	-0.28*	0.36*	-0.21*	-0.37*	-0.15	0.117	1.00	
Dissolved	0.05	0.03	-0.32*	0.13	-0.22*	0.44*	0.35*	1.00
Chromium								
Turbidity	1.00							
Salinity	-0.70*	1.00						
Organic	-0.02	-0.16	1.00					
Dissolved O_2	0.01	0.10	-0.04	1.00				
pH	-0.19*	0.40*	-0.01	0.11	1.00			
Total	-0.02	0.08	-0.28*	-0.17	0.19	1.00		
Suspended	0.24*	-0.29*	0.01	-0.34	0.33*	0.66*	1.00	
Dissolved	0.25*	-0.05	-0.29*	-0.02	-0.23*	0.51*	0.26*	1.00
Copper								
Turbidity	1.00							
Salinity	-0.53*	1.00						
Organic	-0.10	-0.26*	1.00					
Dissolved O_2	-0.07	0.12	-0.05	1.00				
pH	-0.10	0.34*	-0.06	0.28*	1.00			
Total	-0.20*	0.44*	0.17	0.14	0.55*	1.00		
Suspended	-0.07	0.12	-0.46*	-0.16	-0.27*	-0.12	1.00	
Dissolved	0.07	0.30*	-0.06	-0.09	-0.11	0.29*	0.11	1.00
Manganese								
Turbidity	1.00							
Salinity	-0.61*	1.00						
Organic	-0.06	-0.26*	1.00					
Dissolved O_2	0.01	0.17	-0.10	1.00				
pH	-0.19*	0.46*	-0.09	0.19	1.00			
Total	0.11	0.03	-0.41*	-0.06	0-.01	1.00		
Suspended	0.48*	-0.51*	0.17	-0.36*	-0.10	0.13	1.00	
Dissolved	0.11	0.03	-0.23*	-0.09	-00.03	.52*	-0.13	1.00
Iron								
Turbidity	1.00							
Salinity	-0.52*	1.00						
Organic	-0.22*	-0.22	1.00					
Dissolved O_2	-0.04	0.22*	-0.20*	1.00				
pH	-0.11	0.43*	-0.04	0.27*	1.00			
Total	0.11	0-.09	-0.30*	0.08	-0.50*	1.00		
Suspended	0.62*	0-.42*	-0.23*	-0.13	-0.43*	0.508*	1.00	
Dissolved	-0.07	0.20*	-0.52*	-0.12	0.08	0.36*	0.05	1.00
Lead								
Turbidity	1.00							
Salinity	-0.60*	1.00						
Organic	-0.05	0-.27*	1.00					
Dissolved O_2	0.06	0.18*	-0.13	1.00				
pH	-0.18*	0.44*	-0.02	0.16	1.00			
Total	-0.20*	0.44*	0.05	0.13	0.58*	1.00		
Suspended	-0.11	0.14	0.07	-0.28*	0.27*	-0.00	1.00	
Dissolved	-0.16	0.35*	-0.18*	0.19*	-0.06	0.41*	-0.63*	1.00
Zinc								
Turbidity	1.00							
Salinity	-0.62*	1.00						
Organic	0.03	-0.29*	1.00					
Dissolved O_2	0.06	0.13	-0.12	1.00				
PH	-0.20*	0.43*	-0.02	0.24*	1.00			
Total	0.01	0.10	-0.22*	0.32*	-0.11	1.00		
Suspended	0.20*	-0.34*	0.08	-0.39*	-0.04	0.21*	1.00	
Dissolved	0.12	0.01	0.04	-0.23*	0.12	0.16	0.15	1.00

The suspended fraction had a strong relationship with turbidity (0.48) suggesting its production in highly turbid and low salinity (-0.51) areas. Except for the negative correlation with organic matter, there seems to be no correlation of both total and dissolved fractions with any environmental variables.

In Pb, the relationship between salinity (0.44) and both total and dissolved Pb was very strong. This suggested increasing concentrations of total Pb with increased salinities. This favours the occurrence of total Pb in areas of high dissolved oxygen and salinity. The good relationship between dissolved and total lead suggests that dissolved phase is controlling the concentration of total Pb. The suspended fraction was positively correlated to pH.

Total Zn had a good correlation with dissolved oxygen suggesting an increase in total Zn with an increase in dissolved oxygen. There was also a positive correlation between suspended Zn and total Zn. The suspended Zn concentrations increase with an increase in turbidity. The negative correlation between suspended Zn with salinity together with dissolved oxygen suggested a decrease in the concentrations of suspended Zn as salinity and dissolved oxygen increase. Dissolved Zn increased as pH increased, whereas it decreased with a decrease in dissolved oxygen.

4.3.3 Sediment

Seasonal and spatial organic content (mean ± standard error) of sediment samples from the Mhlathuze Estuary are presented in Table 4.6. Mean organic content was highest during Summer 1996 and was lowest during Autumn 1996. The highest mean organic content was recorded at site 4 with the lowest levels measured at site 3. Seasonal and temporal total metal concentrations in sediment collected from the Mhlathuze Estuary are presented in Table 4.7.

Aluminium

Aluminium concentrations in sediment are presented in Table 4.7 and Fig. 4.2D. The highest sediment Al concentrations were recorded at site 2 during Spring 1997. The lowest sediment concentrations were recorded at site 1 during Summer 1997. No definite spatial trends were noticeable. In order to evaluate the significance of temporal concentrations of Cr in sediment samples, the concentrations of the different

sites were pooled. Analyses of variance revealed no significant differences in Al concentrations in sediment between seasons.

Table 4.6 Temporal and spatial organic content of the sediments from the Mhlathuze Estuary. Organic content is expressed as percentage (%) of total sediment and presented in mean ± standard error.

Temporal	Organic content (%)
Autumn 1996	2.55± 0.97
Winter 1996	5.43± 3.27
Spring 1996	6.19± 2.77
Summer 1996	7.55± 2.74
Autumn 1997	6.25± 2.82
Winter 1997	6.13± 1.07
Spring 1997	6.09± 1.83
Summer 1997	4.46± 1.21
Spatial	
Site 1	5.12± 1.18
Site 2	4.55± 1.05
Site 3	2.51± 0.76
Site 4	11.17± 1.73
Site 5	4.53± 1.93

Chromium

Chromium concentrations in sediment are presented in Table 4.7 and Fig. 4.3 D. The highest Cr concentrations in sediment were recorded at site 4 during Spring 1997. The lowest Cr concentrations in sediment were recorded at site 7 during Autumn 1997. No definite spatial trends were noticeable. In order to evaluate the significance of temporal concentrations of Cr in sediment samples, the concentrations of the different sites were pooled. Analyses of variance revealed no significant differences in Cr concentrations in sediment between seasons.

Copper

Copper concentrations in sediment are presented in Table 4.7 and Fig. 4.4D. The highest Cu concentrations in sediment were recorded at site 4 during Spring 1996. The lowest Cu concentration in sediment was recorded at site 7 during Autumn 1996. No definite spatial trends were noticeable. In order to evaluate the significance of temporal concentrations of Cu in sediment samples, the concentrations of the different sites were pooled. Analyses of variance revealed no significant differences in Cu concentrations in sediment between seasons.

Table 4.7 Seasonal concentrations (μg/g) of selected heavy metals in sediment samples from selected sites in the Mhlathuze Estuary for the period April 1996 to December 1997.

Season	Site	Al	Cr	Cu	Fe	Mn	Pb	Zn
Autumn 1996	1	12804.88	62.8	9.15	16219.51	166.46	9.76	51.83
Autumn 1996	2	NO SAMPLE COLLECTED						
Autumn 1996	3	2500	30.43	2.72	3260.87	83.15	2.17	15.76
Autumn 1996	4	35718.75	100	23.75	31250	815	15.63	68.75
Autumn 1996	5	2833.33	40	2.22	4055.56	88.33	2.78	11.67
Winter 1996	1	17800	70	10.67	22466.67	218.67	17.33	45.33
Winter 1996	2	2957.75	59.86	15.49	25281.69	507.04	15.49	51.41
Winter 1996	3	2848.84	29.65	2.33	6656.98	97.67	9.88	16.86
Winter 1996	4	NO SAMPLE COLLECTED						
Winter 1996	5	14011.63	31.98	8.14	14709.3	243.6	5.81	26.74
Spring 1996	1	38114.75	121.31	21.31	33852.46	276.72	31.15	77.87
Spring 1996	2	24935.06	13.64	5.19	4318.18	165.58	7.79	17.53
Spring 1996	3	2783.51	15.46	2.58	3659.79	90.21	5.15	18.04
Spring 1996	4	ND	100	27.34	37578.13	957.81	27.34	77.34
Spring 1996	5	24054.05	58.78	13.51	23918.92	639.19	18.92	44.59
Summer 1996	1	5822.37	137.5	12.5	22368.42	292.76	23.03	62.5
Summer 1996	2	37636.36	ND	ND	34727.27	753.64	25.45	42.73
Summer 1996	3	4519.23	21.15	15.38	9198.72	125	8.97	48.08
Summer 1996	4	5979.73	66.22	14.86	11655.41	228.38	10.81	54.73
Summer 1996	5	NO SAMPLE COLLECTED						
Autumn 1997	1	9178.08	28.77	7.53	15616.44	554.11	10.27	39.04
Autumn 1997	2	2640.45	20.22	BDL	3089.89	86.52	3.37	15.73
Autumn 1997	3	21931.82	27.27	15.34	23693.18	207.39	21.59	15.34
Autumn 1997	4	29078.95	80.26	15.13	30592.11	200.66	18.42	62.50
Autumn 1997	5	4096.39	14.46	4.22	5030.12	231.93	9.04	18.07
Winter 1997	1	27982.46	131.58	17.54	30526.32	272.81	12.28	65.79
Winter 1997	2	21904.76	54.76	16.67	27301.59	243.65	8.73	61.9
Winter 1997	3	21904..76	73.02	15.87	26111.11	271.43	8.73	58.73
Winter 1997	4	59016.39	107.38	27.05	35081.97	1186.07	26.23	82.79
Winter 1997	5	27641.51	90.57	17.92	30188.68	442.45	23.58	52.83
Spring 1997	1	62337.66	87.01	11.69	18863.64	155.19	26.62	51.95
Spring 1997	2	44411.76	92.65	11.76	20257.35	218.38	27.94	55.88
Spring 1997	3	23000	83.13	11.88	21250.0	374.38	11.88	61.88
Spring 1997	4	37368.42	201.75	25.44	37280.7	846.49	24.56	85.96
Spring 1997	5	19533.33	64.0	14.0	46666.67	318.67	12.0	58.0
Summer 1997	1	2318.84	34.06	6.52	25144.93	236.96	4.35	43.48
Summer 1997	2	13928.57	55.71	7.86	16035.71	182.86	9.29	ND
Summer 1997	3	2938.14	53.61	4.12	6855.67	73.2	4.12	30.41
Summer 1997	4	36666.67	100	25.44	35526.32	885.96	ND	86.84
Summer 1997	5	28445.95	89.19	21.62	22770.27	384.46	14.19	52.03

BDL-Below detectable limits. ND- No data

Iron

Iron concentrations in sediment are presented in Table 4.7 and Fig. 4.5 D. The highest Fe concentrations in sediment were recorded at site 7 during spring 1997. The lowest Fe concentrations were recorded at site 3 during Autumn 1996. Site 3 showed consistently low concentrations of iron in sediment during 1996 period. In order to evaluate the significance of temporal concentrations of Fe in sediment samples, the concentrations of the different sites were pooled. Analyses of variance revealed no significant differences in Fe concentrations in sediment between seasons.

Manganese

Manganese concentrations in sediment are presented in Table 4.6 and Fig. 4.6D. The highest Mn concentration in sediment was recorded at site 4 during Winter 1997. The lowest concentrations of Mn in sediment were recorded at site 3 during Summer 1997. Site 3 showed consistently lower concentrations of Mn in sediment during 1996 period. No definite spatial trends were noticeable. In order to evaluate the significance of temporal concentrations of Mn in sediment samples, the concentrations of the different sites were pooled. Analyses of variance revealed no significant differences in Mn concentrations in sediment between seasons.

Lead

Lead concentrations in sediment are presented in Table 4.7 and Fig. 4.7D. The highest Pb concentration in sediment was recorded at site 1 during Spring 1996. The lowest Pb concentrations in sediment were recorded at site 3 during Autumn 1996. No definite spatial trends were noticeable. In order to evaluate the significance of temporal concentrations of Pb in sediment samples, the concentrations of the different sites were pooled. Analyses of variance revealed no significant differences in Pb concentrations in sediment between seasons.

Zinc

Zinc concentrations in sediment are presented in Table 4.7 and Fig. 4.8 D. The highest Zn concentrations in sediment were recorded at site 4 during Summer 1997. The lowest Zn concentrations were recorded at site 7 during Autumn 1996. No definite spatial trends were noticeable. In order to evaluate the significance of temporal concentrations of Zn in sediment samples, the concentrations of the different sites were pooled. Analyses of variance revealed no significant differences in Zn concentrations in sediment between seasons.

4.3.4 Fish

Table 4.8 represents the mean length and mass of fish collected in the Mhlathuze Estuary for tissue and whole body metal analyses. Fish for tissue analyses ranged in length from 163

mm to 189 mm. The mass ranged from 69 g to 125 g. Fish for whole body metal analyses ranged in length from 96 mm to 104 mm. The mass ranged from 0.7 g to 5.74 g.

Table 4.8 Mean ± standard error of fish standard length (mm) and mass (g) collected in the Mhlathuze Estuary for tissue and whole body analyses for the period April 1996-December 1997.

	Tissue fish		Whole fish	
Season	Mean length (mm)	Mean mass (g)	Mean length (mm)	Mean mass (g)
Autumn 1996	189± 4.07	116.65± 4.98	96± 3.71	4.53± 0.51
Winter 1996	163.33± 8.82	68.69± 12.01	88.0± 6.63	4.70± 0.93
Spring 1996	178.13± 6.88	113.62± 40.42	97.5± 17.50	5.42± 2.57
Summer 1996	176.67± 6.01	134.33± 14.91	91.0± 12.29	2.82± 0.35
Autumn 1997	186.67± 3.73	125.0± 7.23	100.0± 10.0	7.20± 0.24
Winter 1997	181.0± 3.14	124.87± 4.17	86.0± 2.45	0.47± 0.07
Spring 1997	187.0± 3.66	113.00± 3.86	130. ± 10.0	5.74± 0.48
Summer 1997	186.0± 4.81	112.87± 11.20	104.0± 2.45	1.74± 0.17

Aluminium

The analysis of variance (ANOVA-Tukey's Multiple Range test) revealed no significant seasonal differences in Al concentrations in the gill tissues (Fig. 4.9A). There were significant seasonal differences in the liver Al (Fig. 4.9B) concentrations ($P<0.05$). No significant seasonal differences were found in Al concentrations in the muscle tissue (Fig. 4.9C). The highest gill Al concentrations were measured during Spring 1996. The highest liver Al concentrations were measured during Winter 1997 and the highest muscle concentrations were measured during Spring 1996. The lowest gill Al concentrations were measured during Autumn 1997. The lowest liver concentrations were measured during Summer 1997 and the lowest muscle Al concentrations were measured during Summer 1996. The order of accumulation was gill>liver >muscle. ANOVA of Al concentrations in whole fish revealed that concentrations during Winter 1997 were significantly lower ($P<0.05$) than all the other surveys (Fig. 4.9D). The highest Al concentrations were recorded during Spring 1996.

Chromium

ANOVA revealed no significant seasonal differences in gill Cr concentrations. There were significant seasonal differences ($P<0.05$) in the liver Cr concentrations (Fig. 4.10B) whereas no seasonal differences were found in the muscle tissue (Fig. 4 10C).

The highest gill Cr concentrations were recorded during Autumn 1996. The highest liver concentrations were recorded during Summer 1997 and the highest muscle Cr concentrations were measured during Autumn 1997. Tissue distribution of Cr was in the order of gill>liver>muscle. ANOVA revealed significant seasonal differences in Cr concentrations in whole body analysis (Fig. 4.10 D). The highest Cr concentrations were recorded during Spring 1997 and the lowest Cr concentrations were recorded during Autumn 1997.

Copper

Significant seasonal differences ($P<0.05$) in the liver Cu concentrations (Fig. 4.11B) were recorded with the highest Cu concentrations found in liver samples from Summer 1997. The highest gill and muscle Cu concentrations were recorded during Winter 1996. The gill concentrations during Winter 1996 were significantly higher than the other sampling seasons (Fig. 4.11A). The lowest gill and muscle concentrations were measured in Winter 1997, and the lowest liver concentrations in Summer 1996. The order of accumulation in tissues was liver>gill>muscle. ANOVA revealed no significant seasonal differences in Cu concentrations in whole fish (Fig. 4.11D). The highest Cu concentrations were recorded during Summer 1996 and the lowest concentrations were recorded during Summer 1997.

Iron

There were no significant seasonal differences in the gill and muscle Fe concentrations, whereas significant seasonal differences ($P<0.05$) were recorded in liver Fe concentrations. The highest Fe gill concentrations were recorded during Spring 1996 (Fig. 4.12A), whereas the highest liver (Fig. 4.12B) and muscle (Fig. 4.12C) concentrations were measured during Autumn 1996. The lowest gill and muscle Fe concentration were recorded during Autumn 97 and lowest liver Fe concentrations were measured during Summer 1997. The order of accumulation was gill>liver>muscle. There were significant seasonal differences in whole fish Fe concentrations (Fig. 4.12D). The highest Fe concentrations were recorded during Summer 1996 and the lowest Fe concentrations were recorded during Winter 1997.

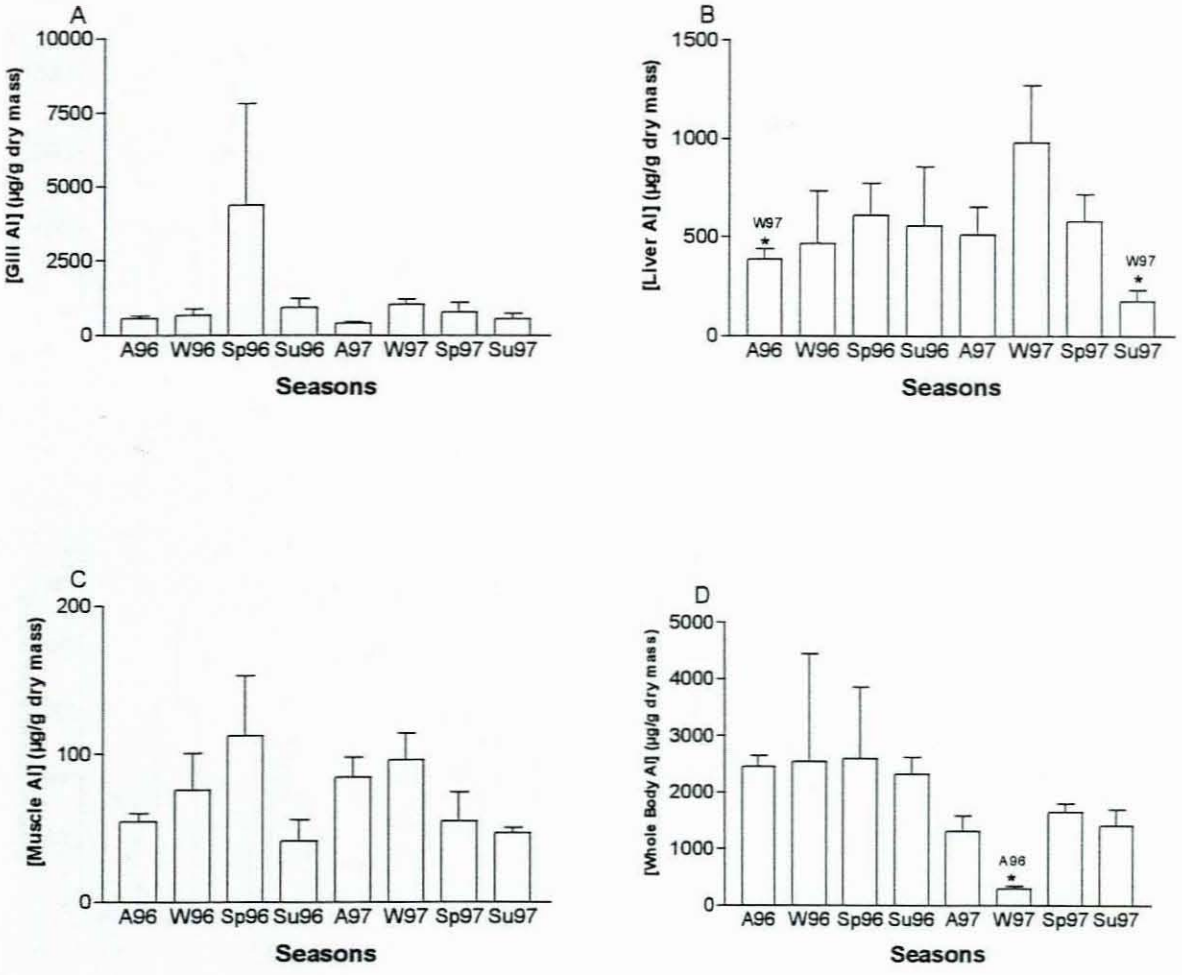


Fig. 4.9 Concentrations (mean ± standard error) of Al in gill, (A), liver (B), muscle (C) and whole fish (D) for the period April 1996 to December 1997. Fish tissues were sampled from site 4 and whole fish samples were collected from site 2. Concentrations expressed in terms of µg/g dry mass. Asterisks indicate significant differences at $P \leq 0.05$ (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

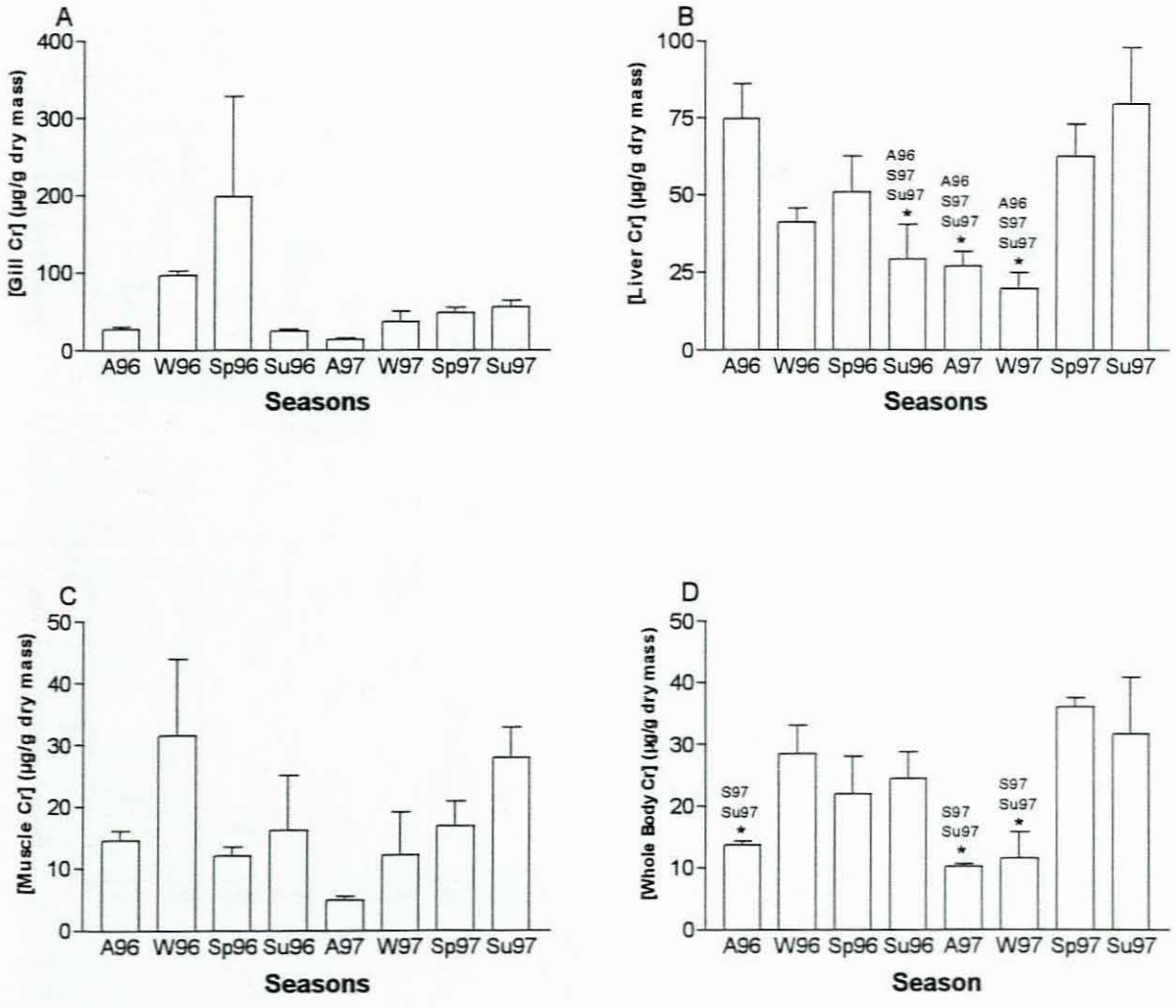


Fig. 4.10 Concentrations (mean ± standard error) of Cr in gill, (A), Liver (B), muscle (C) and whole fish (D) for the period April 1996 to December 1997. Fish tissues were sampled from site 4 and whole fish samples were collected from site 2. Concentrations expressed in terms of µg/g dry mass. Asterisks indicated significant differences at P≤0.05 (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

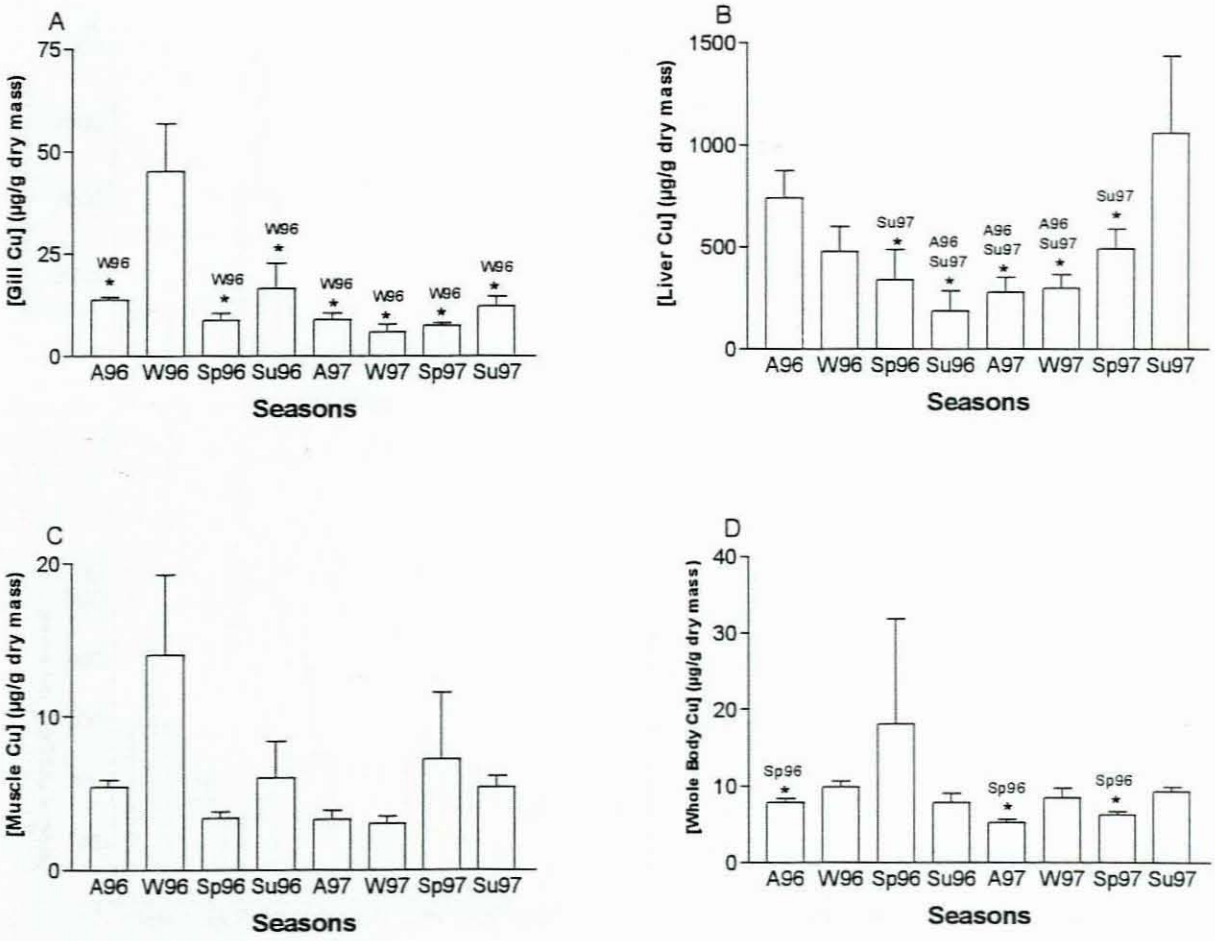


Fig. 4.11 Concentrations (mean ± standard error) of Cu in gill, (A), Liver (B), muscle (C) and whole fish (D) for the period April 1996 to December 1997. Fish tissues were sampled from site 4 and whole fish samples were collected from site 2. Concentrations expressed in terms of µg/g dry mass. Asterisks indicated significant differences at P≤0.05 (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

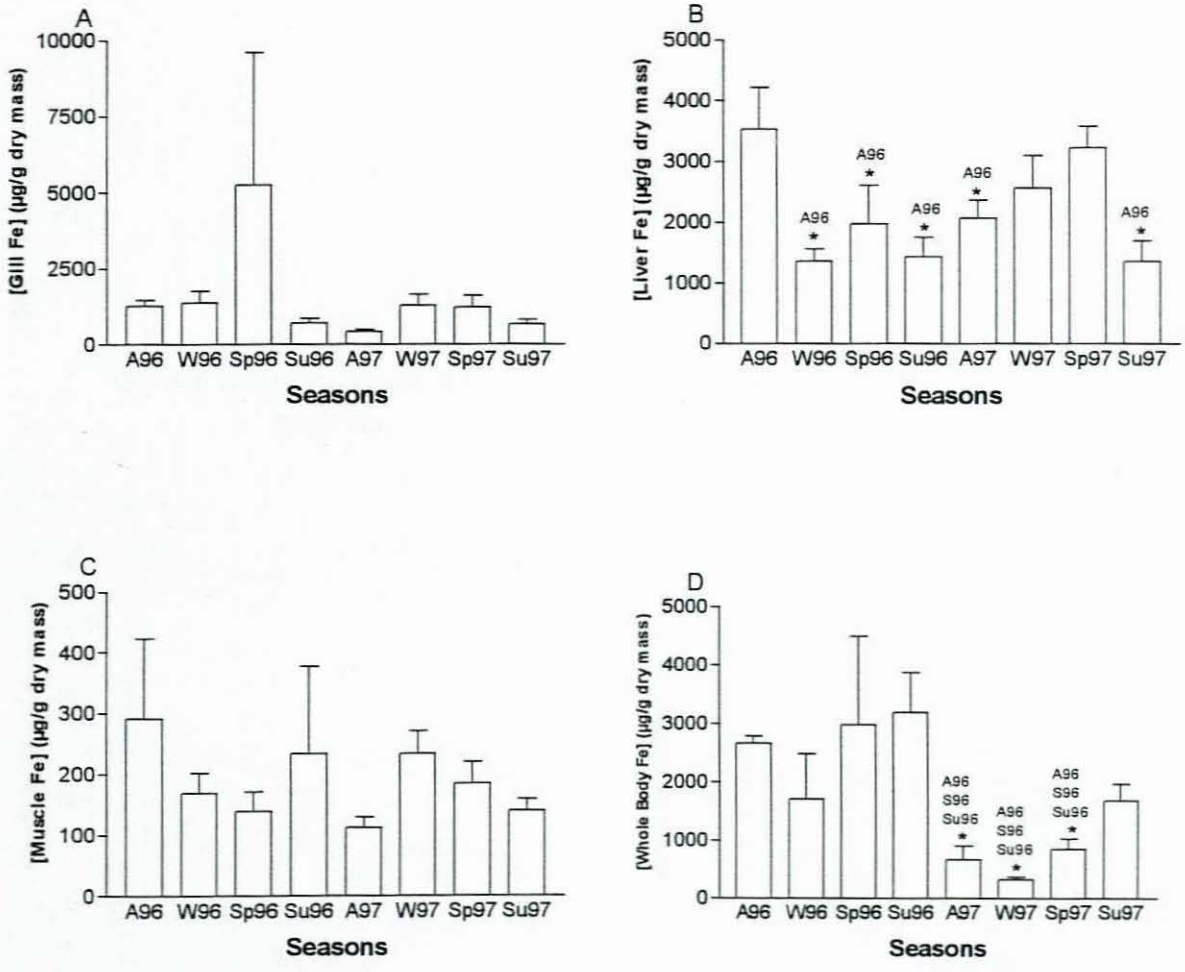


Fig. 4.12 Concentrations (mean ± standard error) of Fe in gill, (A), Liver (B), muscle (C) and whole fish (D) for the period April 1996 to December 1997. Fish tissues were sampled from site 4 and whole fish samples were collected from site 2. Concentrations expressed in terms of µg/g dry mass. Asterisks indicated significant differences at $P \leq 0.05$ (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

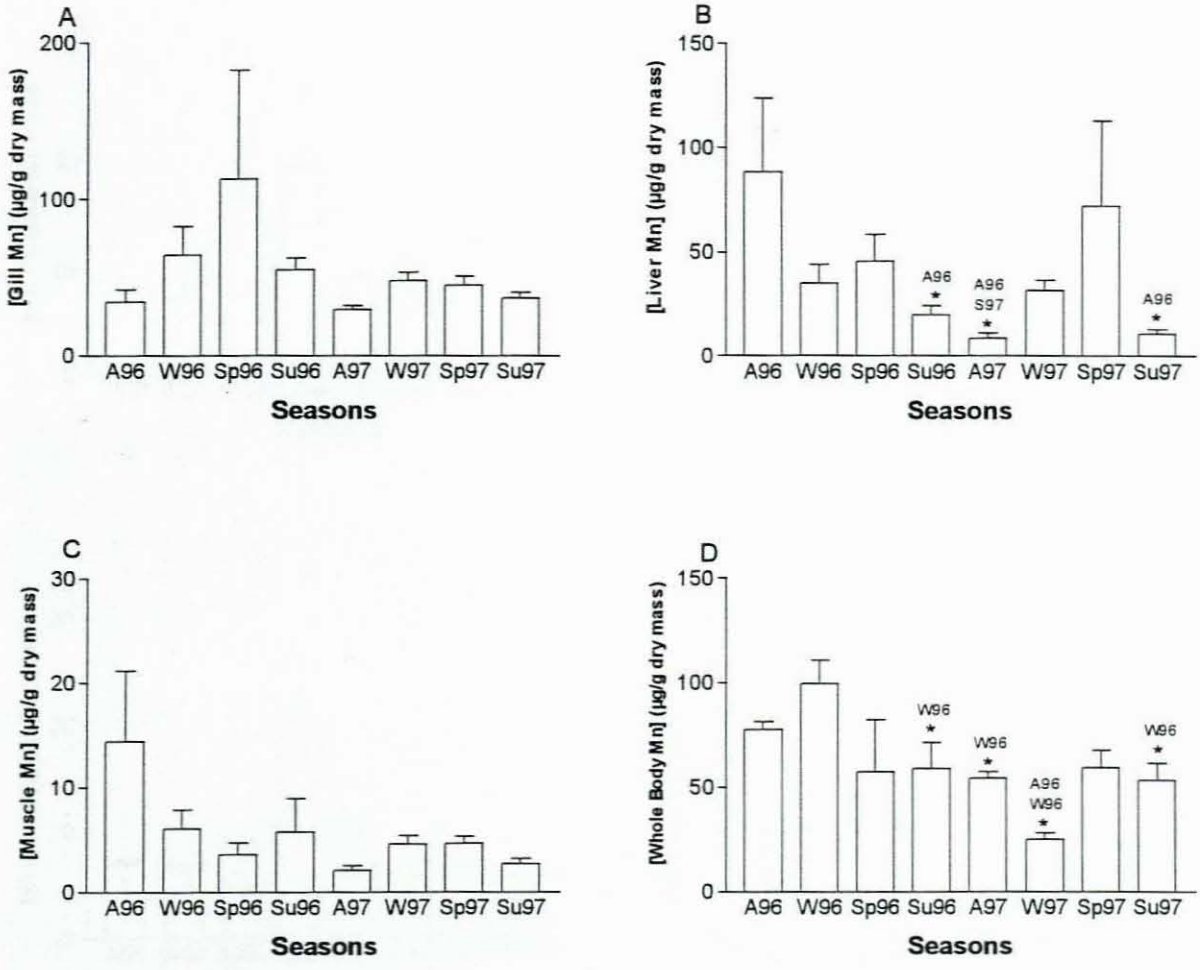


Fig. 4.13 Concentrations (mean ± standard error) of Mn in gill, (A), Liver (B), muscle (C) and whole fish (D) for the period April 1996 to December 1997. Fish tissues were sampled from site 4 and whole fish samples were collected from site 2. Concentrations expressed in terms of µg/g dry mass. Asterisks indicated significant differences at P≤0.05 (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

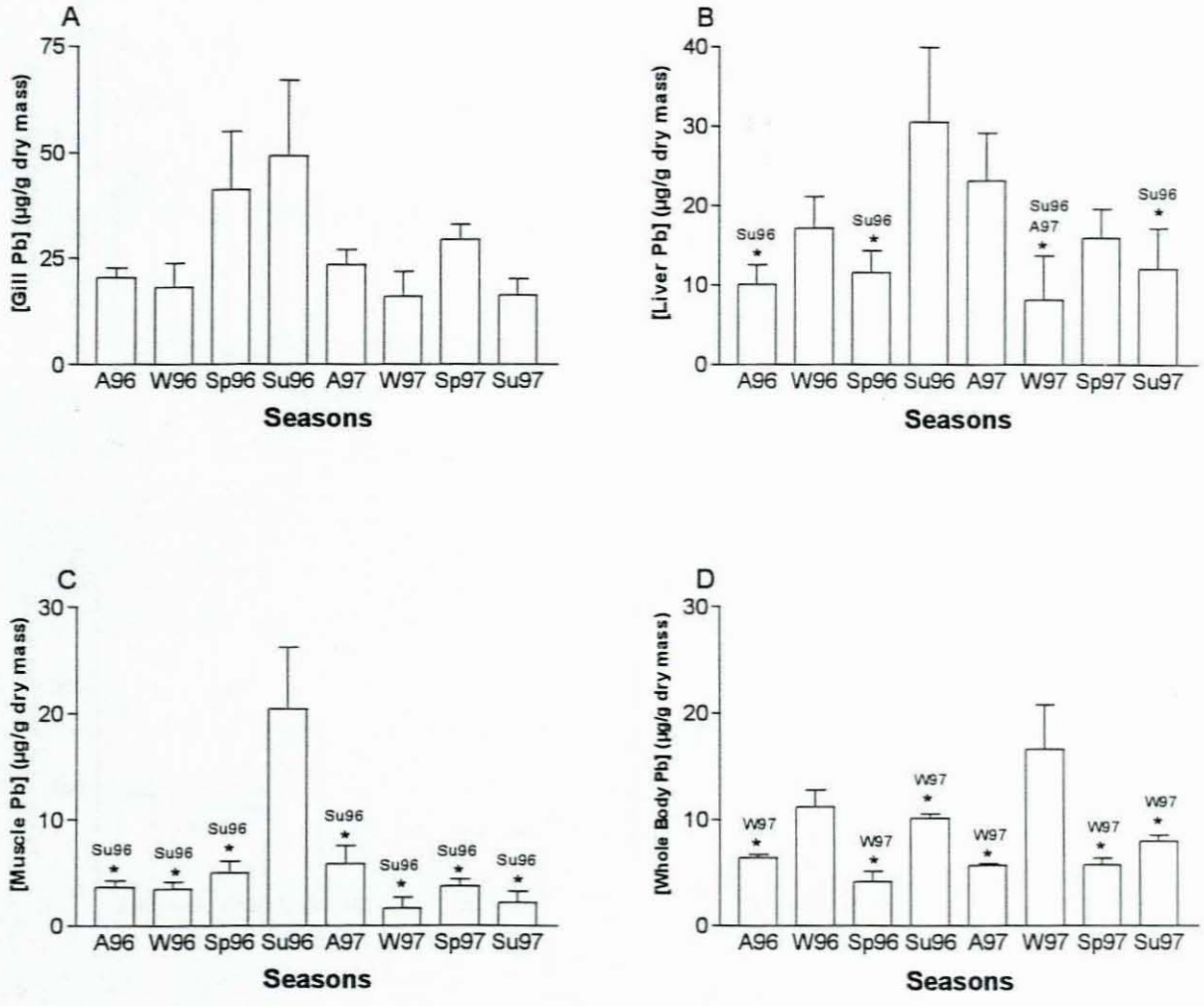


Fig. 4.14 Concentrations (mean ± standard error) of Pb in gill, (A), liver (B), muscle (C) and whole fish (D) for the period April 1996 to December 1997. Fish tissues were sampled from site 4 and whole fish samples were collected from site 2. Concentrations expressed in terms of µg/g dry mass. Asterisks indicated significant differences at $P \leq 0.05$ (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

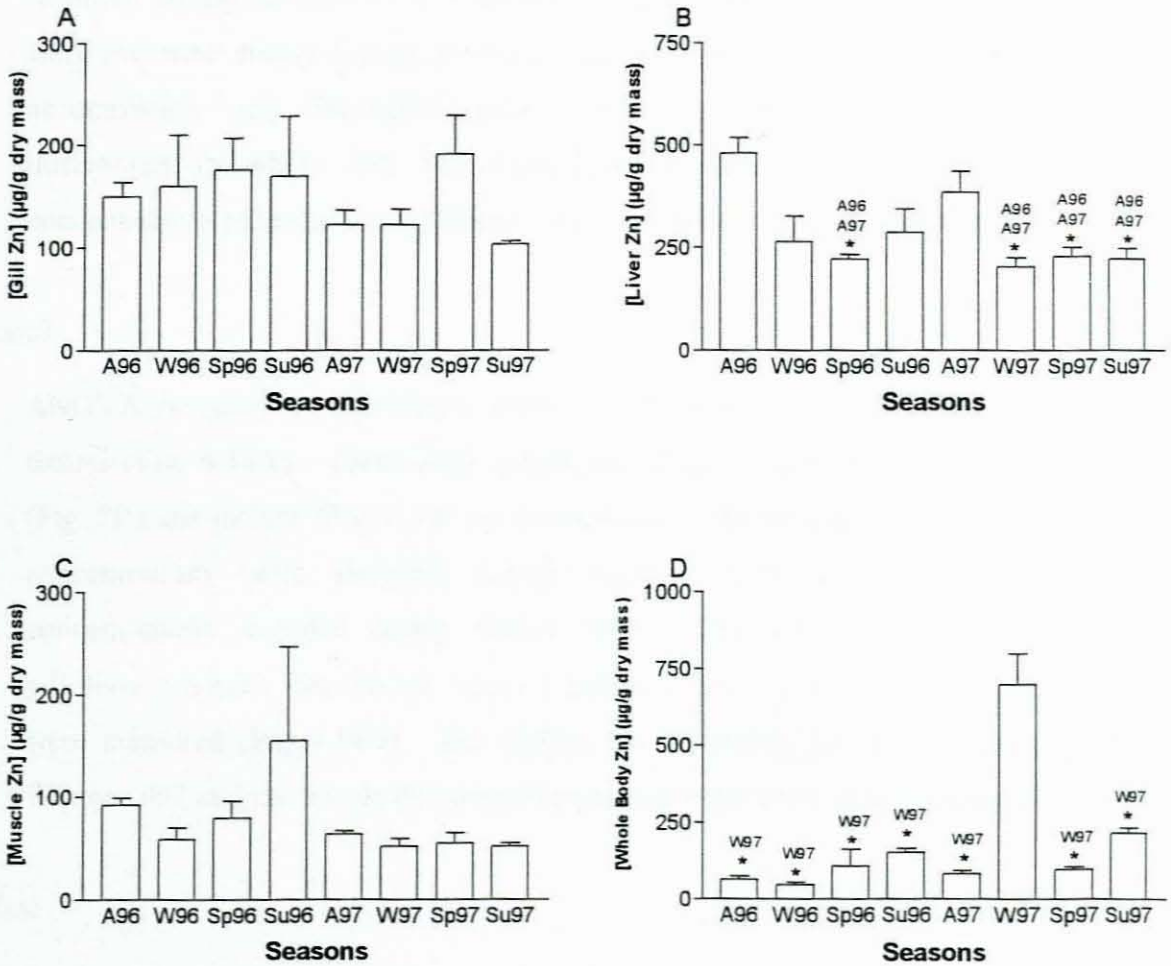


Fig. 4.15 Concentrations (mean ± standard error) of Zn in gill, (A), Liver (B), muscle (C) and whole fish (D) for the period April 1996 to December 1997. Fish tissues were sampled from site 4 and whole fish samples were collected from site 2. Concentrations expressed in terms of µg/g dry mass. Asterisks indicated significant differences at P≤0.05 (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

Manganese

Significant seasonal differences ($P<0.05$) were observed in gill Mn concentrations (Fig. 4.13A). No seasonal differences were found in liver and muscle Mn concentrations (Fig. 4.13B and Fig. 4.13C). The highest gill Mn concentrations were recorded during Autumn 1996, whereas the highest liver and muscle concentrations were recorded during Spring 1996 and Autumn 1996 respectively. The order of accumulation was liver>gill>muscle. ANOVA revealed significant seasonal differences in whole fish Mn concentrations (Fig. 4.13D) with the highest concentrations recorded during Winter 1996 and the lowest during Winter 1997.

Lead

ANOVA revealed no significant seasonal differences in Pb concentrations in gill tissues (Fig. 4.14A). There were significant ($P<0.05$) seasonal differences in liver (Fig. 7B) and muscle (Fig. 4.14C) concentrations. The highest gill, liver and muscle concentrations were recorded during Summer 1996 and the lowest tissue concentrations recorded during Winter 1997. The order of accumulation was gill>liver >muscle. Significant seasonal differences in whole body Pb concentrations were measured (Fig 4.14D). The highest Pb concentrations were recorded during Winter 1997 and the lowest Pb concentrations were recorded during Spring 1996.

Zinc

No significant seasonal differences were recorded in gill (Fig. 4.15A) and muscle (Fig. 8C) concentration. There were significant seasonal differences ($P<0.05$) in the Zn concentrations in the liver (Figure 4.15B). The highest gill concentrations were recorded during Spring 1997. The highest liver Zn concentrations were recorded during Autumn 1996 and the highest muscle concentrations were recorded during Summer 1997. The lowest gill and muscle Zn concentrations were measured during Summer 1997 and the lowest liver concentrations during Winter 1997. The order of accumulation was liver>gill>muscle. ANOVA revealed that Zn concentrations in whole fish collected during Winter 1997 were significantly higher than the other surveys (Fig. 4.15D). The lowest Zn concentrations were recorded during Winter 1996.

General results

The concentrations of Cu and Mn were the highest in the liver whereas the rest of the metals showed highest concentrations in gill tissue. With the exception of Cu, significant seasonal differences (P<0,05) were observed in the liver (Fig.4.9-4.15). Gill tissue displayed significant seasonal differences (P<0.05) in Cu concentrations. Lead was the only metal that showed significant seasonal (P<0.05) differences in accumulation in muscle tissue. Lead was highest in all tissue types during Summer 1996 and it was lowest during Winter 1997. The rest of the metals did not show any definite trends.

4.3.5 Benthic invertebrates

Concentrations of metals in two benthic invertebrates, *P. blephariskios* and *A. digitalis* are presented in Figures 4.16 and 4.17.

Aluminium

Analysis of variance revealed no significant seasonal difference in the Al concentrations in *P. blephariskios* and *A. digitalis* (Fig. 4.16A). The highest concentrations in *P. blephariskios* were recorded during Summer 1996 and the lowest during Spring 1997. The highest Al concentrations in *A. digitalis* were recorded during Summer 1997 and the lowest during Spring 1996.

Chromium

Analysis of variance revealed no significant seasonal differences in the *P. blephariskios* Cr concentrations (Fig. 4.16B), whereas significant seasonal differences in *A. digitalis* were recorded. The highest concentrations in *P. blephariskios* were recorded in Winter 1996 and the lowest during Spring 1997. The highest Cr concentrations in *A. digitalis* were recorded during Spring 1996 and the lowest during Spring 1997.

Copper

The Cu concentrations in *P. blephariskios* collected during Autumn 1996 were significantly higher (P<0.05) than the other surveys (Fig. 4.16C). The lowest

concentrations of Cu were recorded during Spring 1997. Significant seasonal differences in *A. digitalis* Cu concentrations were recorded with the highest concentrations found during Spring 1996 and the lowest during Spring 1997.

Iron

Analysis of variance revealed no significant seasonal differences in the *P. blephariskios* Fe concentrations (Fig. 4.16G), whereas significant seasonal differences in *A. digitalis* were recorded. The highest concentrations in *P. blephariskios* were recorded in Summer 1996 and the lowest during Spring 1997. The highest Cr concentrations in *A. digitalis* were recorded during Summer 1996 and the lowest during Spring 1997.

Manganese

There were no significant seasonal differences in *P. blephariskios* Mn concentrations (Fig. 4.17A). The highest concentrations of Mn were recorded during Summer 1996 and the lowest during Spring 1997. Significant seasonal differences ($P<0.05$) in *A. digitalis* Mn concentrations were found with the highest concentrations were recorded during Summer 1997 and the lowest during Spring 1996.

Lead

Analysis of variance showed no significant seasonal differences in *P. blephariskios* Pb concentrations (Fig. 4.17B), whereas significant seasonal differences were found in *A. digitalis*. The highest concentrations in *P. blephariskios* were recorded during Winter 1997 and the lowest during Autumn 1996. *Apseudes digitalis* showed the highest Pb concentrations during Spring 1996 and the lowest during Summer 1997.

Zinc

No significant seasonal patterns in Zn concentrations in *P. blephariskios* were recorded (Fig. 4.17C). The highest Zn concentrations were recorded during Spring 1997 and the lowest during Autumn 1997. In contrast, significant seasonal differences in *A. digitalis* Zn concentrations were found with the highest recorded during Spring 1996 and the lowest during Spring 1997.

The order of metal accumulation in *P. blephariskios* was Fe>Al>Mn>Cr>Zn>Cu>Pb. In *A. digitalis* metal accumulation was in the order of Fe>Al>Mn> Zn>Cr>Cu>Pb

4.3.6 Aquatic macrophytes

Concentrations of metals in leaves and stalks of the marine eelgrass, *Z. capensis* are presented in Figure 4.18. Unfortunately, no replicate samples were collected and concentrations presented were based on one sample of *Z. capensis,* which was collected seasonally. The results show that the highest concentrations of Cr, Cu and Zn in *Z. capensis* were measured during Winter 1996. The other metals showed different seasonal patterns with the highest concentrations of Al measured during Summer 1996, Fe during Spring 1997, Mn during Summer 1997 and Pb during Autumn 1996. The lowest metal concentrations of Cr, Cu, Fe and Pb were measured in *Z. capensis* during Spring 1996, whereas the lowest concentrations of Al, Mn and Zn were measured during Summer 1997, Autumn 1997 and Winter 1996 respectively. Metal bioaccumulation patterns in *Z. capensis* were in the order of Al>Fe>Mn>Cr>Zn>Cu>Pb.

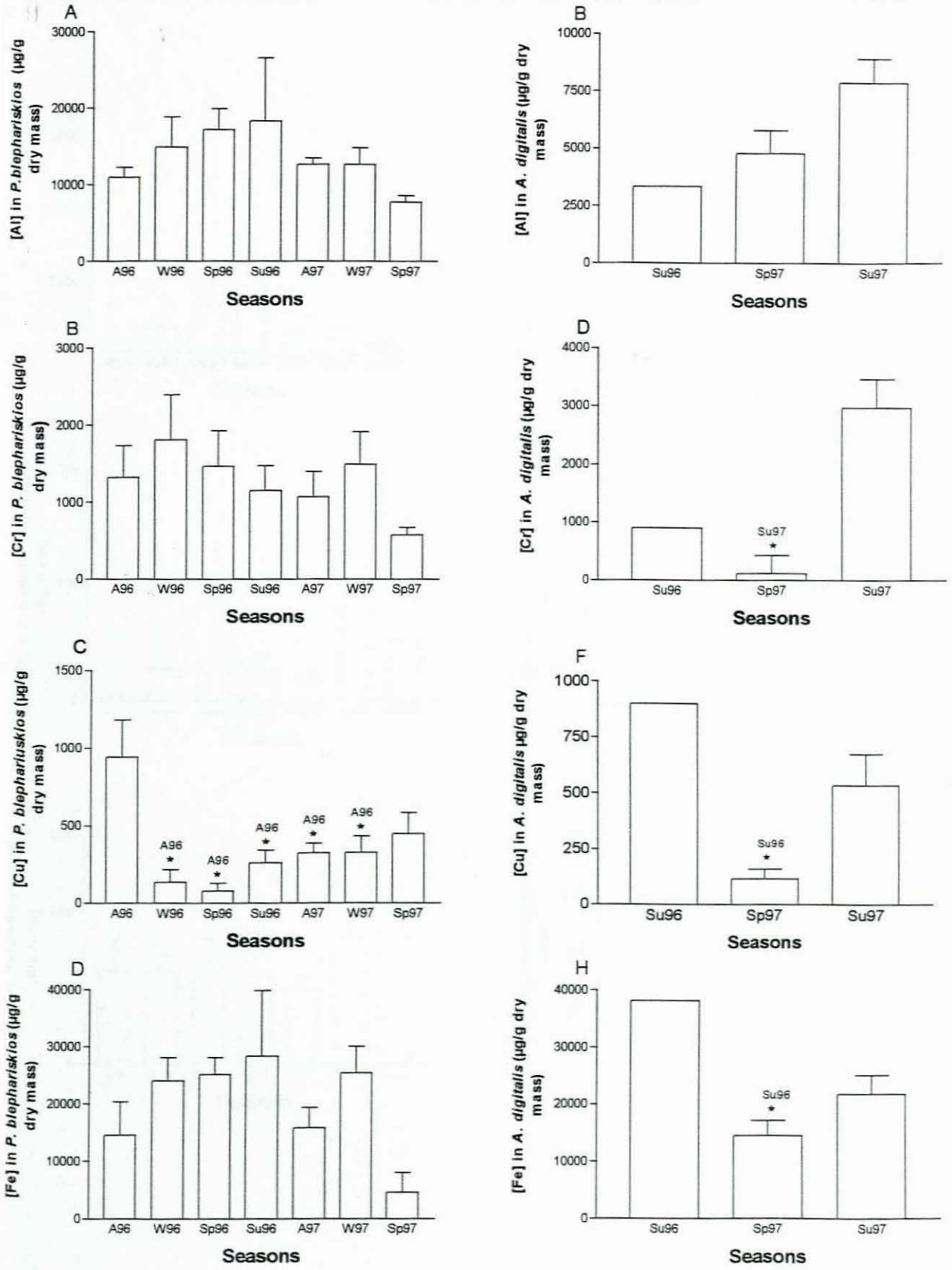


Fig. 4.16 Mean ±standard error of Al in *P. blephariskios* (A), and *A. digitalis* (B), Cr concentrations of *P.blephariskios* (C) and *A. digitalis*, (D), Cu in *P. blephariskios* (E), and *A. digitalis* (F) and Fe concentrations of *P. blephariskios* (G) and *A. digitalis*, (H). Sampling was carried out for the period April 1996 to December 1997. *Paratylodiplax blephariskios* was samples at site 4 and *A. digitalis* was sampled at site 1. Concentrations expressed in terms of µg/g dry mass. Asterisks indicate significant differences at $P<0.05$ (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

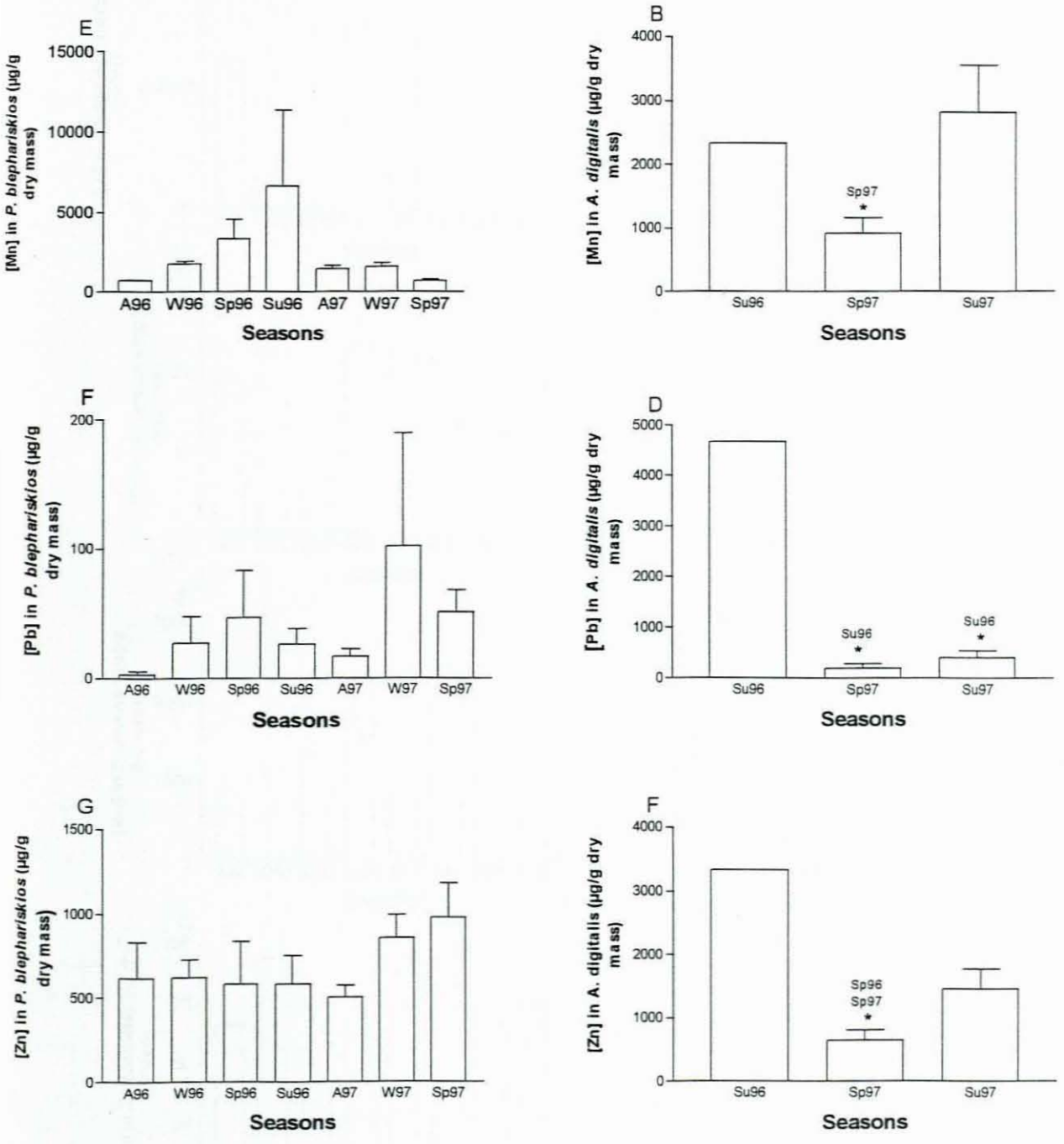


Fig. 4.17 Mean ±standard error of Mn in *P. blephariskios* (A), and *A. digitalis* (B) Pb concentrations of *P.blephariskios* (C) and *A. digitalis*, (D) and Zn concentrations in *P. blephariskios* (E) and A. digitalis (F). Sampling was carried out for the period April 1996 to December 1997. *Paratylodiplax blephariskios* was samples at site 4 and *A. digitalis* was sampled at site 1. Concentrations expressed in terms of µg/g dry mass. Asterisks indicate significant differences at $P<0.05$ (Tukey's multiple Range, ANOVA) with the season concerned listed above it.

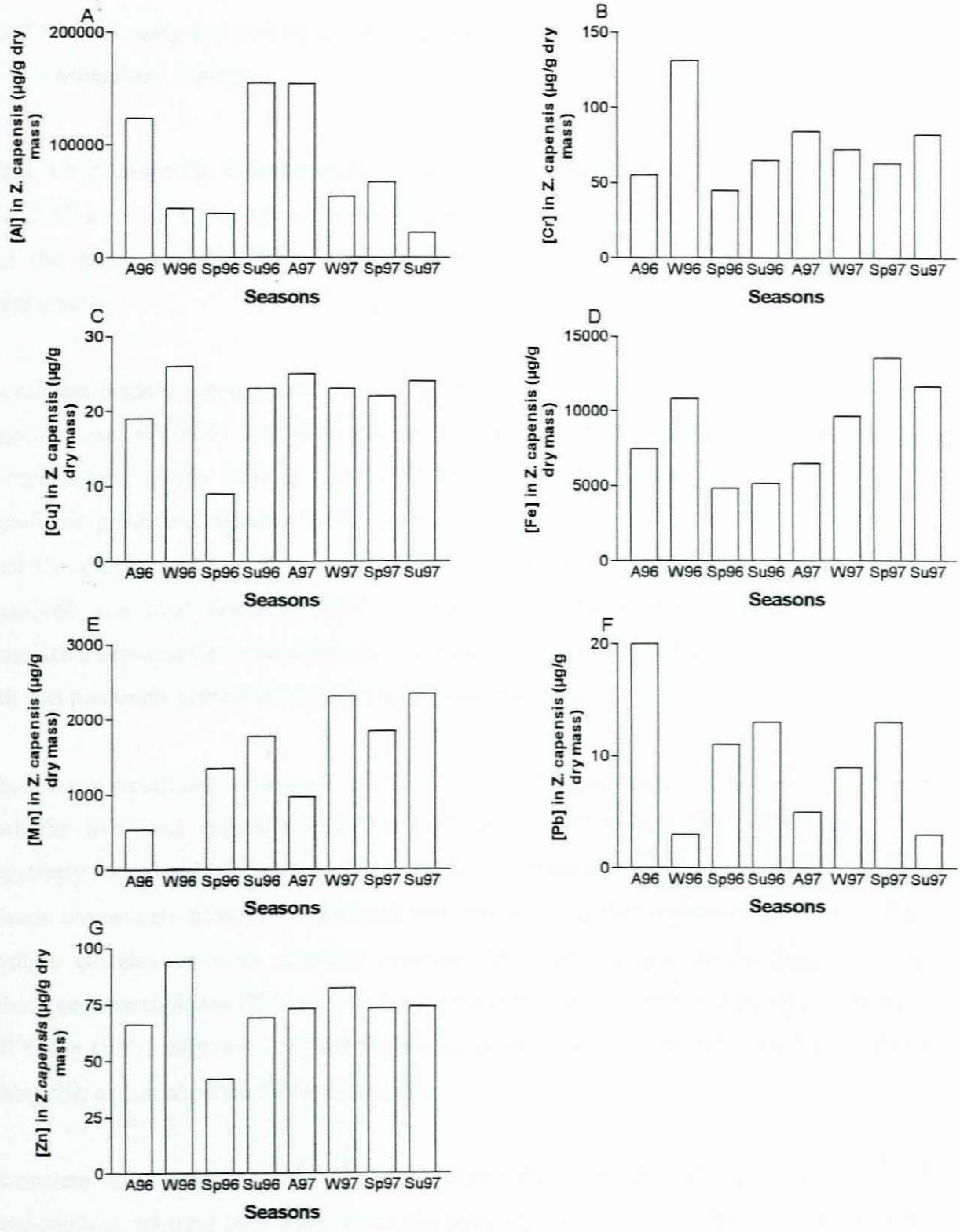


Fig. 4.18 Concentrations of Al (A), Cr (B) Cu(C) and Fe (D) Mn (E), Pb (F) and Zn (G) in *Z. capensis* for the period April 1996 to December 1997. *Zostera capensis* was sampled at site 1. Concentrations are expressed in terms of µg/g dry mass.

4.3.7 Relationship between metal concentrations in biotic and abiotic compartments of the Mhlathuze Estuary

Table 4.5 presents the correlations between metal concentrations in different water phases and Tables 4.9 to 4.11 present the correlations between metal concentrations in sediments and the metal concentrations in whole fish, fish tissue, invertebrates and submerged macrophytes.

Significant negative correlations were recorded between dissolved Al in water and liver and muscle tissue ($P<0.05$) and there was a significant positive correlation between the Al concentrations in the liver and the gill tissue. Spearman rank correlation indicated a significant positive correlation between gill, liver and muscle and dissolved, particulate and total Cr concentrations in the water. All tissues were significantly correlated to both the dissolved and total water ($P<0.05$) concentrations. There was a significant positive correlation between Cr in the sediment and muscle, gill and whole fish. Chromium in whole fish was positively correlated to both the gill and liver tissue.

There was a significant correlation ($P<0.05$) between the particulate Cu and concentrations in both the liver and muscle (Table 4.10). The gill and muscle Cu concentration were negatively correlated ($P<0.05$) to total water concentrations. The Cu concentrations in muscle tissue were positively correlated ($P<0.05$) to whole fish concentrations. Significant positive correlations were recorded between particulate Fe and all the fish tissue and submerged macrophytes (Table 4.10). Sediment concentrations were positively correlated to gill tissue and *A. digitalis*. Further significant positive correlations were recorded between whole fish and *A. digitalis* Fe concentrations.

Particulate concentrations of Mn were negatively correlated to fish tissue and *P. blephariskios*, whereas they were positively correlated to *A. digitalis* and *Z. capensis* (Table 4.11). Significant positive correlations were also recorded between Mn in sediments and the two benthic invertebrate species, liver and muscle tissue, and *A. digitalis* and *Z. capensis*. Dissolved Pb was negatively correlated to Pb in liver and muscle tissue ($P<0.05$), whereas particulate Pb concentrations were positively correlated to whole fish, muscle tissue and *P. blephariskios*. Lead in sediments were positively correlated to muscle concentrations as well

as concentrations in both benthic invertebrate species. A significant negative correlation was found between Pb in sediment and whole fish. A significant positive correlation between total Zn concentrations in the water and the sediment with whole fish concentrations was recorded. Particulate Zn was negatively correlated to gill tissue and *P. blephariskios*. Zinc concentrations in whole fish and *P. blephariskios* were positively correlated to sediment concentrations, whereas concentrations in liver and *Z. capensis* samples were negatively correlated to the sediment ($P<0.05$). There was a significant positive correlation ($P<0.05$) between muscle and the liver tissue.

In order to assess seasonal patterns in metal accumulation in the biotic and abiotic compartments of the Mhlathuze Estuary, non-metric multi-dimensional scaling analyses (NMDS) were performed on all the metal-data from all 8 seasons. The two-dimensional plot (Figure 4.19) of the eight seasons formed two groups at the 90% similarity level. The one group comprised all the seasons whereas the second group only contained season 8 (Summer 1997). The analyses were repeated using the individual metal data sets to assess whether similar seasonal patterns could be observed for all seven metals studied (Figure 4.19). All metals except Pb showed the similar seasonal distributions in the abiotic and biotic compartments.

The NMDS plots of individual metal distribution in the biotic and abiotic compartments showed four distinct patterns at a similarity level of 80% (Figure 4.20). Concentrations of Al, Mn and Fe were distinctly similar (90% similarity level) in sediment, the mud crab (*P. blephariskios*) and eelgrass as well as fish muscle tissue. For both Cr and Zn, sediment concentrations were conspicuously dissimilar to concentrations of these metals in the other compartments. Lead concentrations displayed three distinct groupings of similarity i.e. (i) sediment, (ii) whole fish and total water concentrations, and (iii) rest of the compartments, whereas Cu displayed two groupings, i.e. (i) sediment and fish liver and (ii) rest of the compartments.

Table 4.9 Spearman's rank correlation coefficients for aluminium and chromium concentrations in the abiotic and biotic components of the Mhlathuze Estuary. Means from all the stations were ranked before analyses and statistical significance was at $P<0.05$ (indicated by *). Note that critical values for $P<0.05$ differ between different compartments.

Aluminium	Water [Total]	Water [Dissolved]	Water [Particulate]	Sediment	Whole fish	Gill	Liver	Muscle	*Apseudes digitalsi*	*Paratylodiplax blephariskios*	Zostera capensis
Water [Total]	1.000										
Water [Dissolved]	0.32	1.000									
Water [Particulate]	-0.06	0.65*	1.000								
Sediment	0.48*	-0.20	-0.82*	1.000							
Whole fish	-0.32	-0.15	0.51*	0.21	1.000						
Gill	0.07	-0.20	-0.23	0.22	-0.25	1.000					
Liver	0.19	-0.39*	-0.16	0.14	-0.14	0.31	1.000				
Muscle	0.17	-0.30*	-0.14	0.07	-0.32	0.07	0.27	1.000			
Apseudes digitalis	0.53	0.52	0.23	-0.52	-0.31	0.21	-0.56	-0.82	1.000		
P. blephariskios	0.02	-0.29	-0.26	-0.22	0.13	-0.16	-0.02	-0.34	0.79	1.000	
Zostera capensis	-0.90*	-0.63*	-0.30	0.62*	0.30	-0.30	-0.09	-0.10	0.30	0.79	1.000
Chromium	**Water [Total]**	**Water [Dissolved]**	**Water [Particulate]**	**Sediment**	**Whole fish**	**Gill**	**Liver**	**Muscle**	***Apseudes digitalsi***	***Paratylodiplax blephariskios***	**Zostera capensis**
Water [Total]	1.00										
Water [Dissolved]	0.60*	1.00									
Water [Particulate]	0.76*	0.20	1.00								
Sediment	0.05	0.10	-0.43*	1.00							
Whole fish	0.50*	0.65*	0.61*	0.78*	1.00						
Gill	0.44*	0.53*	0.01	0.49*	0.68*	1.00					
Liver	0.53*	0.51*	0.37*	0.07	0.41*	0.20	1.00				
Muscle	0.56*	0.70*	0.25	0.31*	0.60	0.55*	0.48*	1.00			
Apseudes digitalis	-0.32	-0.32	0.40	0.32	-0.70	-0.52	0.73	-0.70	1.00		
P. blephariskios	-0.27	-0.16	-0.23	-0.19	-0.06	0.03	0.12	0.01	0.70	1.00	
Zostera capensis	-0.22	0.07	0.00	-0.24	-0.03	0.05	-0.31	-0.13	0.84	-0.36	1.00

Table 4.10 Spearman's rank correlation coefficients for copper and iron concentrations in the abiotic and biotic components of the Mhlathuze Estuary. Means from all the stations were ranked before analyses and statistical significance was at $P<0.05$ (indicated by *). Note that critical values for $P<0.05$ differ between different compartments.

Copper	Water [Total]	Water [Dissolved]	Water [Particulate]	Sediment	Whole fish	Gill	Liver	Muscle	*Apseudes digitalsi*	*Paratylodiplax blephariskios*	Zostera capensis
Water [Total]	1.00										
Water [Dissolved]	0.63*	1.00									
Water [Particulate]	-0.34	0.27	1.00								
Sediment	0.29	0.80*	0.13	1.00							
Whole fish	0.01	-0.24	0.26	0.05	1.00						
Gill	0.38	-0.26	0.27	-0.30	0.15	1.00					
Liver	-0.10	0.06	0.48*	-0.21	0.02	0.01	1.00				
Muscle	-0.36*	-0.07	0.50*	0.11	0.42*	0.29	0.13	1.00			
Apseudes digitalis	-0.72*	-0.38	0.58	0.38	-0.23	0.22	0.61	0.14	1.00		
P. blephariskios	-0.37	-0.18	0.27	-0.35*	0.23	0.20	0.28	0.14	-0.63	1.00	
Zostera capensis	-0.80	-0.82*	0.39	-0.75	0.01	0.20	-0.48*	0.15	-0.80	-0.17	1.00
Iron	**Water [Total]**	**Water [Dissolved]**	**Water [Particulate]**	**Sediment**	**Whole fish**	**Gill**	**Liver**	**Muscle**	***Apseudes digitalsi***	***Paratylodiplax blephariskios***	**Zostera capensis**
Water [Total]	1.00										
Water [Dissolved]	0.21	1.00									
Water [Particulate]	0.15	-0.41*	1.00								
Sediment	0.56*	-0.23	0.67*	1.00							
Whole fish	-0.09	-0.08	-0.12	0.07	1.00						
Gill	0.37*	0.00	0.41*	0.34*	-0.28	1.00					
Liver	-0.09	-0.23	0.40*	0.15	-0.17	0.07	1.00				
Muscle	-0.15	-0.20	0.48*	0.29	-0.02	0.23	0.04	1.00			
Apseudes digitalis	0.51	0.15	0.15	0.70*	0.995*	-0.74	0.36	-0.60	1.00		
P. blephariskios	0.31	0.13	0.08	0.30	0.03	0.15	-0.51*	-0.16	0.41	1.00	
Zostera capensis	0.44	0.07	0.75*	-0.32	-0.32	0.45*	0.16	0.40	-0.90	0.41	1.00

Table 4.11 Spearman's rank correlation coefficients for manganese, lead and zinc concentrations in the abiotic and biotic components of the Mhlathuze Estuary. Means from all the stations were ranked before analyses and statistical significance was at $P<0.05$ (indicated by *). Note that critical values for $P<0.05$ differ between different compartments.

Manganese	Water [Total]	Water [Dissolved]	Water [Particulate]	Sediment	Whole fish	Gill	Liver	Muscle	*Apseudes digitalsi*	*Paratylodiplax blephariskios*	Zostera capensis
Water [Total]	1.00										
Water [Dissolved]	0.79*	1.00									
Water [Particulate]	0.21	0.14	1.00								
Sediment	0.08	0.32	0.55*	1.00							
Whole fish	0.33	0.15	0.17	0.03	1.00						
Gill	0.12	0.05	-0.33*	0.22	0.23	1.00					
Liver	-0.12	0.05	-0.43	0.22	0.26	0.19	1.00				
Muscle	0.02	0.08	-0.45*	0.30	0.26	0.17	0.45*	1.00			
Apseudes digitalis	0.80*	-0.17	0.71*	0.74*	0.00	0.20	0.80	-0.40	1.00		
P. blephariskios	-0.28	0.03	-0.36*	0.95*	-0.33	-0.13	-0.64	-0.27	-0.80	1.00	
Zostera capensis	0.81*	0.63	0.78*	0.18	-0.50*	0.08	-0.31	-0.07	1.00*	0.07	1.00
Lead	**Water [Total]**	**Water [Dissolved]**	**Water [Particulate]**	**Sediment**	**Whole fish**	**Gill**	**Liver**	**Muscle**	***Apseudes digitalsi***	***Paratylodiplax blephariskios***	**Zostera capensis**
Water [Total]	1.00										
Water [Dissolved]	0.60*	1.00									
Water [Particulate]	-0.54*	-0.96*	1.00								
Sediment	0.05	0.58*	0.62*	1.00							
Whole fish	-0.39	0.45	0.52*	-0.64*	1.00						
Gill	0.20	0.13	0.13	0.09	0.04	1.00					
Liver	-0.14	-0.37*	0.33	-0.16	0.27	-0.06	1.00				
Muscle	-0.08	-0.44*	0.46*	0.34*	0.00	0.08	0.09	1.00			
Apseudes digitalis	-0.32	0.00	-0.32	1.00*	-0.29	0.22	-0.13	-0.78	1.00		
P. blephariskios	0.39	0.45	0.52*	0.64*	-0.14	-0.15	-0.00	-0.33	0.52	1.00	
Zostera capensis	0.21	0.53	0.17	0.35	-0.18	0.17	-0.13	0.13	-0.80*	-0.02	1.00
Zinc	**Water [Total]**	**Water [Dissolved]**	**Water [Particulate]**	**Sediment**	**Whole fish**	**Gill**	**Liver**	**Muscle**	***Apseudes digitalsi***	***Paratylodiplax blephariskios***	**Zostera capensis**
Water [Total]	1.00										
Water [Dissolved]	0.19	1.00									
Water [Particulate]	0.31	0.80*	1.00								
Sediment	0.60*	0.26	0.06	1.00							
Whole fish	0.41*	0.35	0.22	0.48*	1.00						
Gill	0.05	0.29	-0.43*	-0.25	-0.08	1.00					
Liver	0.06	0.24	0.16	-0.45*	0.24	0.33	1.00				
Muscle	0.02	0.00	0.08	-0.25	0.33	0.02	0.47*	1.00			
Apseudes digitalis	0.29	0.65	0.10	-0.10	-0.71	0.36	0.71	0.16	1.00		
P. blephariskios	0.23	-0.29	-0.46*	0.51*	0.25	0.61	0.07	-0.43	0.29	1.00	
Zostera capensis	0.30	0.34	0.16	-0.48*	-0.14	-0.13	0.29	0.81*	-0.34	-0.23	1.00

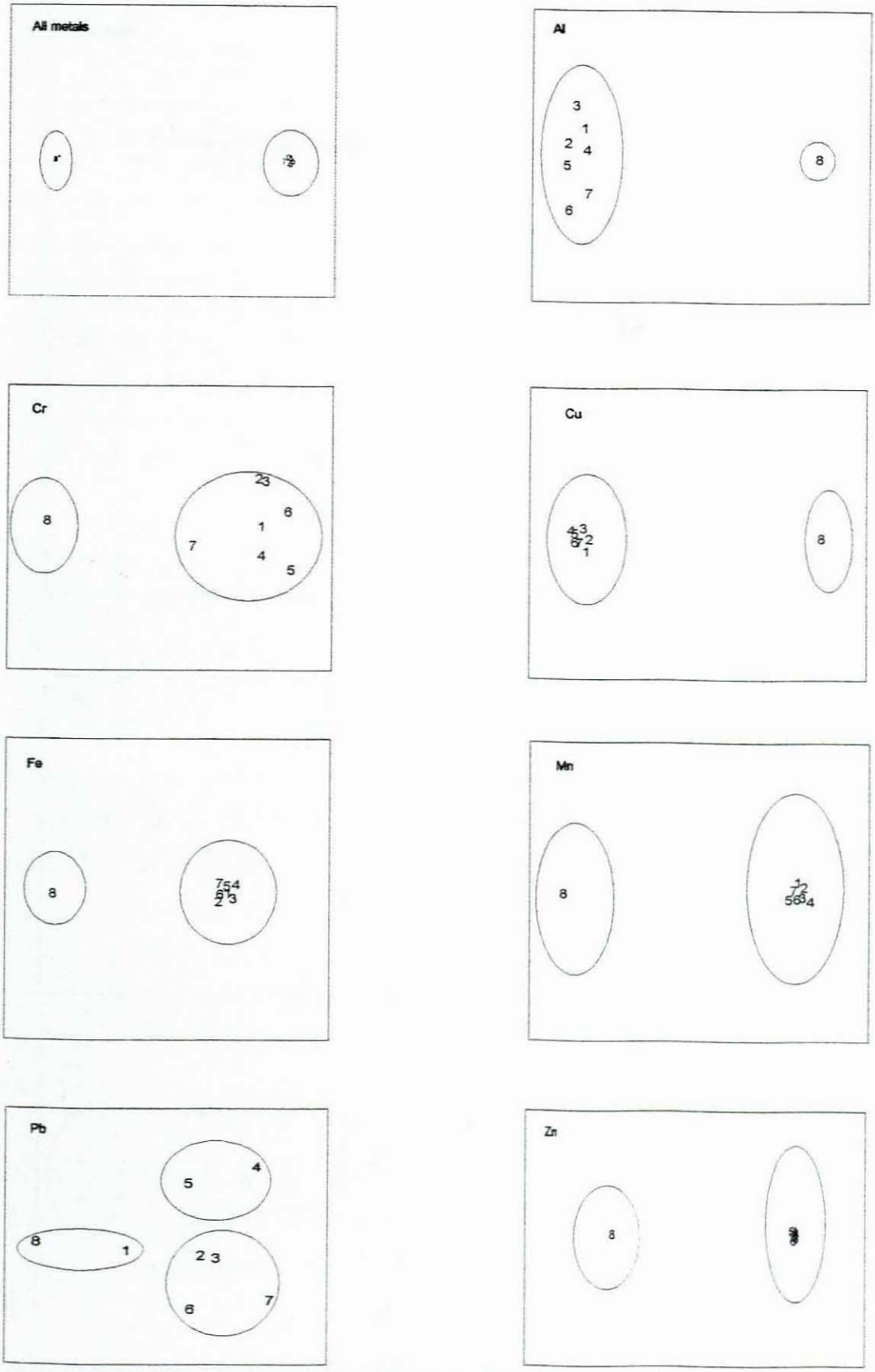


Fig. 4.19 Non-metric dimensional scaling (NMDS) plots of the seasons for all metals combined and individual metals at a similarity level of 90%. Seasons are represented as follows: 1=autumn 96, 2=winter 96, 3=spring 96, 4=summer 96, 5=autumn 97, 6=winter 97, 7=spring 97, 8=summer 97. For all plots stress levels = 0.02.

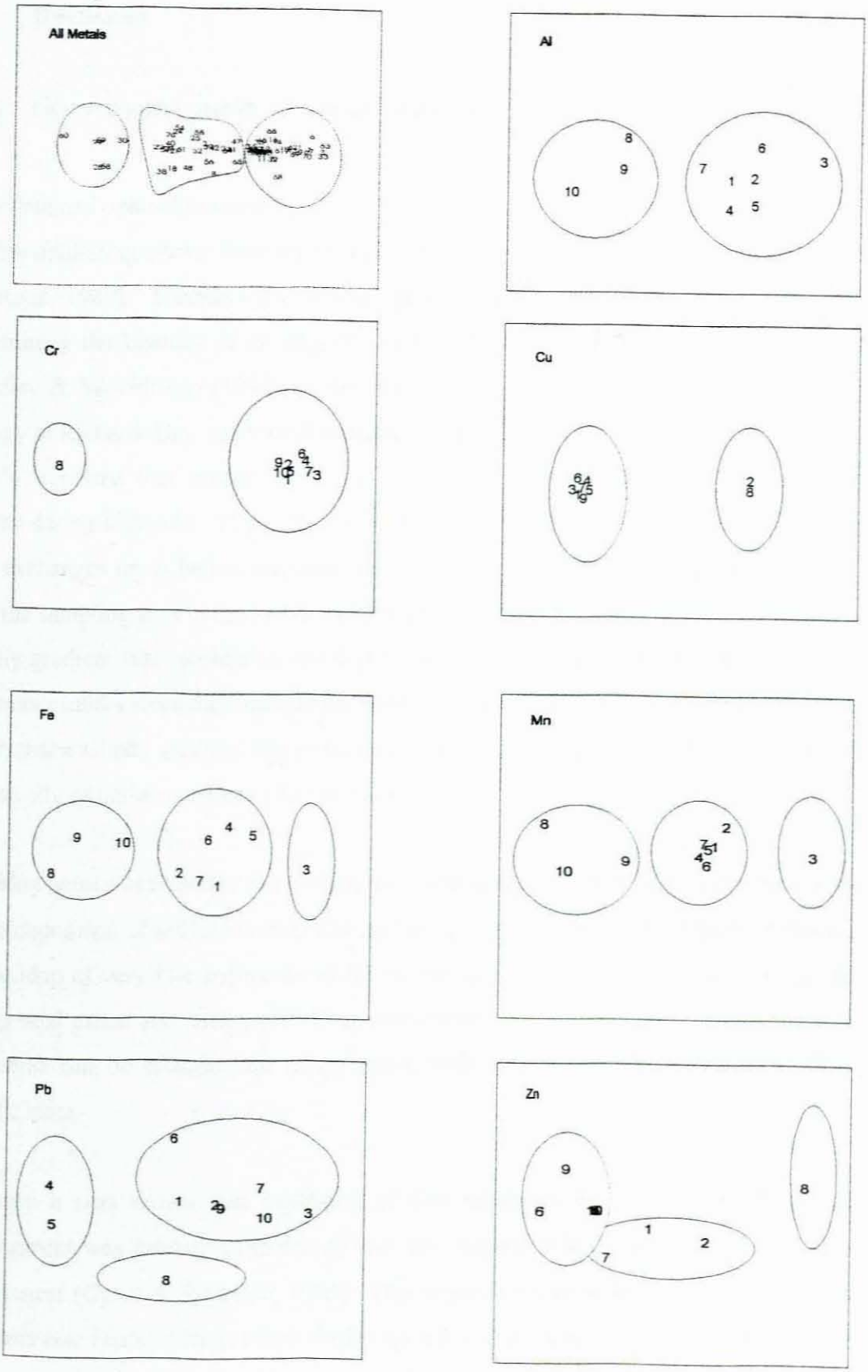


Fig. 4.20 Non-metric dimensional scaling (NMDS) plots of the biotic and abiotic compartments for all metals combined and individual metals at a similarity level of 80%. Samples are represented as follows: 1=gill, 2=liver, 3=muscle, 4=whole fish, 5=total metal in water, 6=dissolved metal in water, 7=particulate metal, 8=*P. blephariskios*, 9=sediment and 10 =*Z. capensis*. For all plots stress levels = 0.02

4.4 Discussion

4.4.1 General water quality of Mhlathuze Estuary

The continued optimal functioning of estuaries relies on the maintenance of the natural dynamism and the oscillating phases imposed on such systems by riverine and marine influences (Whitfield & Bruton, 1989). Therefore the presence of a salinity gradient is regarded as the major factor determining the biomass of an estuarine system (Schlacher & Wooldridge, 1996). Studies by Grindley & Wooldridge (1974) on the plankton and by Hemens *et al.* (1971) on the general biology of Richards Bay, reported fluctuating salinity regimes. Only five years later Hemens *et al.* (1976a) reported that marine conditions (salinities >30 ‰) were experienced throughout the estuary during high tide. This was attributed to the presence of a new mouth, which allowed for tidal exchanges never before recorded in the estuary. The current study obtained similar results, with the sampling sites in the mouth and embayment showing limited salinity gradients. A slight salinity gradient was recorded at site 4 in the upper portion of the embayment. This was due to freshwater inflow from the Mtantatweni River. It was only at site 5, in the canal of the Mhlathuze River, that a salinity gradient was recorded (Table 4.1). During periods of reduced runoff the area of typically estuarine conditions moves further up the river.

Turbidity values increased at site 4 when compared to the historical data. This could be attributed to the deposition of sediments from the catchment in the estuary. The deposition has resulted in the buildup of very fine sediments on the bottom of the estuary. Due to its shallow nature, the strong tidal prism and strong prevailing north-easterly or south westerly winds, the soft bottom sediments can be brought into resuspension, with potential ensuing deleterious effects to the aquatic biota.

Recently it was shown that ingression of fine sediments into the estuary from the marine environment was causing a buildup of very fine sediments in the mouth of the estuary and the embayment (Cyrus & Wepener, 1998). This ingression was attributed to dredging activities in Richards Bay Harbour from which dredge spoil was deposited onto the beach to the north of the estuary mouth and allowed to dissipate through wave action. However, the fine particulate matter was carried south with the near-shore current and ultimately washed into the estuary with the incoming tide. Benthic surveys indicated that the sediment ingression was responsible for shifts in

faunal diversities and abundances in the affected areas. The *Z..capensis* beds were also affected due to a reduction in the euphotic zone and sedimentation. The turbidities recorded in the mouth were comparable to the historical data and reflected the sediment transport and flushing out of the estuary.

The temperatures recorded in the estuary were within the normal seasonal range. The median temperatures of the Mhlathuze Estuary were lower than the temperatures of Nhlabane Estuary (Wepener, 1998). This was due to the estuary mouth remaining open and thereby reflecting the temperature fluctuations caused by changes in flow in the river as opposed to the more constant marine influence on water temperature. The variation in water pH values also reflected the riverine influence at site 5, and to a much lesser extent at site 4. The marine influence at site 3 resulted in the pH remaining stable.

Before the construction of the harbour the main source of nutrients was freshwater drainage of the catchment. Water quality data from the lower reaches of the Mhlathuze River (site 5) clearly indicate that the freshwater-derived nutrient input into the estuary is very low. The contribution of nitrogen by the mangroves was regarded as unlikely (Hemens *et al.*, 1971). During the construction of the harbour in 1975 the nutrient levels increased due to the redistribution of anaerobic silt dredged from several metres below the mud surface (Hemens *et al.*, 1975) but the following year nutrient levels decreased. Hemens *et al.* (1976b) speculated that this was due to the bottom consisting of redeposited silt that had been depleted of its soluble nitrogen, or that the release of nitrogen from dead and decaying plankton had decreased, together with plankton densities, due to the opening of the new mouth. The present study showed that currently there are substantially higher nutrient levels than during the 1970s. It is unlikely that runoff from the catchment could contribute to the high nitrate concentrations measured at site 57 and site 4, since low nutrient levels were recorded in the lower reaches of the river and at site 1 (see Table 4.1). According to Wepener & Vermeulen (1999) the source of the nutrients is possibly related to fixation of nitrogen by blue-green algae in the muds of the vast mangrove areas. Another potential source of nutrients is sewage runoff from the surrounding rural settlements, as indicated by high faecal coliform counts obtained. The low ortho-phosphate concentrations recorded in the estuary were indicative of low input via freshwater inflow from the river, which most probably is the main source of phosphate in the estuary (Wepener & Vermeulen, 1999).

4.4.2 *Metals in water*

Metals in general

Metals in water column generally did not show any significant differences between seasons with the exception of December 1997 where concentrations were significantly different from the rest of the seasons. The increase in metals during Summer 1997 was probably related to metals bound to suspended particles during the high runoff of freshwater during the flood conditions that prevailed during the sampling trip. A general trend of a reduction in metals was observed during June 1997. According to Rainbow (1995) wide variations in water-borne metal concentration within estuaries are normally related to the degree of fresh water contribution or the presence of industrial effluents. One could therefore expect that metal concentrations in estuaries to fluctuate with the sediment bound metals increasing during seasons of low flow and waterborne metals increasing during floods (Nimmo, Willox, Lafrancois, Chapman, Brinkman & Greene, 1998).

The elevated concentrations of Fe, Al, Zn and Cr recorded in the water from the Mhlathuze Estuary are in agreement with the results reported by Vermeulen & Wepener (1999) for metals in water from the adjacent Richards Bay Harbour (Table 4.12). Historical reports from the original Richards Bay Estuary, reported only Zn as being present in water at high concentrations. The study by Hemens *et al.* (1975) commented on the high Zn concentrations as "reason for concern". Their study was however conducted before the completion of the harbour. The current levels measured in this study could therefore be related to subsequent activities in the catchment and adjacent harbour area. Tidal gates, that are no longer in use, serve as a connection between the water in the harbour and the estuary. During high tide water can be observed overtopping over the tidal gates (*pers. observation*) and it is therefore highly likely that metals originating in the harbour will find its way into the estuary. The concentrations of metals from water in this study were much higher than metal concentrations in the water of other estuaries on the east coast of South Africa (Table 4.12). It must be borne in mind that most of these surveys were undertaken between 20 and 30 years ago on and that these concentrations may have increased in the intervening period.

Table 4.12 Comparisons of dissolved metal concentrations in the Mhlathuze Estuary with dissolved metal concentrations in other estuaries on the eastern seaboard of South Africa.

Estuary	Al (µg/l)	Cr (µg/l)	Cu (µg/l)	Fe (µg/l)	Mn (µg/l)	Pb (µg/l)	Zn (µg/l)	Reference:
Mhlathuze	990.0	48.0	39.1	907.0	48.2	130.15	66.5	This study
Knysna	-	0.1	0.2	81	5	0.6	0.3	a
Bietou	-	-	0.1	68	14	2.6	2.0	b
Keurbooms	-	-	0.1	61	6.7	2.5	1.9	b
Kromme	-	-	0.6	100	5.4	0.2	0.5	c
Gamtoos	-	-	0.6	372	41	0.6	1.1	c
Swartkops	-	-	3.9	275	41	1.5	3.6	d
Sundays	-	1.3	3.2	334	18	0.7	2.4	e
Bushmans	-	0.4	1.8	302	10.2	0.2	0.5	f
Kariega	-	0.3	1.5	170	9.5	0.22	0.51	f
Kowie	-	0.2	1.7	254	14.6	0.33	0.63	f
Great Fish	-	2.1	2.4	133	52	1.1	2.1	f
Buffalo	-	0.1	3.1	154	15	78	4.7	g
Blind	-	1.4	3.0	38	3	17	1.2	h
Ihlaza	-	9.1	6.5	36	8	20	34	h
Nahoon	-	0.5	0.5	110	19	64	2.6	h
Quinera	-	2.7	1.5	202	36	12	5.8	h
Kosi Bay	-	-	1.1	-	-	1.0	5.7	i
St Lucia E.	-	-	3.33	68.81	-	39.15	2.31	j
St Lucia E.	-	-	17	2000	-	-	11.7	i
Richards Bay #			3.08			3.94	NR	k
Richards Bay #	-	-	1.70	-	-	1.87	-	l
Richards Bay	-	-	4.0	-	-	4.2	3.8	i
Richards Bay*	504.4	23.6	50.8	782.4	80.7	-	85.4	m
Durban *	-	-	27	800	-	117	287	i
Umzimkhulu	-	-	2.21	-	-	3.5	8.9	i
Umzimvubu	-	-	1.9	460	-	2.9	20.1	i

References a. Watling & Watling, 1982a; b. Watling & Watling, 1982b; c. Watling & Watling, 1982c; d. Watling & Watling, 1982d; e. Watling & Watling, 1982e; f. Watling & Watling, 1983b; g. Watling *et al.*, 1985; h. Watling & Talbot, 1985; i. Cloete *et al.*, 1979; j. Oliff, 1977; k. Connell *et al.*, 1985; l, Hemens et al 1975; m, Vermeulen & Wepener, 1999.

* = Harbour # = Pre Harbour development NR = Not reported

Metals such as Zn and Cr are important constituents of industrial mining and domestic effluents (Rainbow, 1995). High concentrations of these metals may be a direct consequence of anthropogenic contamination from these activities. It is not possible to comment on the high Al levels since there is no historical data (the Hemens *et al.*, 1975 study) against which to measure it. However, it is highly likely that the current levels are due to a combination of anthropogenic sources (i.e. the nearby aluminium smelter complexes, dredging of sediments in Richards Bay Harbour) and natural leaching and weathering processes in the catchment.

Relationships between physico-chemical properties and metal partitioning in water

The explanation of heavy metal transport in an estuary requires a basic understanding of the physical behaviour of the estuary and of the influence individual parts of the system experience or brings to bear on other parts (Baeyens *et al.*, 1998). Estuaries are governed by tidal action at the seaside and by river flow. In the mixing zone of salt and freshwater, strong longitudinal gradients in physico-chemical parameters such as salinity, pH and temperature develop. They affect internal processes such as:

- removal of trace elements from the dissolved phase by adsorption, by precipitation and by co-precipitation of solutes;
- removal of trace elements associated with fine suspended matter, by flocculation and net sedimentation;
- production of trace elements in the dissolved phase by desorption and by solubilisation of particulate matter including the associated metals;
- re-suspension of sediment and subsequent release of their metals; and
- transformation and migration of trace metals species at the sediment /water interface.

These processes as well as external effects, mainly anthropogenic activities such as discharging of pollutants or cooling water, dredging of the channels and in some cases subsequent disposal of the spoilt on the shoals of the river bank, modify the conservative mixing of river-borne and sea-borne solutes and particulate matter (Baeyens *et al.*, 1998). The overall effects of internal and external processes are often unexpected and result in complex distribution patterns, which differ, from one element to the other and from one estuary to the other.

The distribution and behaviour of metals in the Mhlathuze Estuary were elucidated using non-parametric correlation analyses procedures (Spearman's Rank Correlation matrix). From Table 4.5 it was evident that the physico-chemical conditions that were controlling the partitioning of metals in the water column was mainly related to salinity, turbidity, dissolved oxygen and pH. With the exception of Cr, these relationships were significantly related to the particulate phase and a lesser degree the total phases of metals in the water column.

The physical and chemical interaction between the dissolved metal and the particulate suspended metals is usually the process governing the distribution of metals in estuaries. In the area of maximum turbidity the adsorption/desorption processes are in favour of removal of dissolved metals, due to low salinity of the environment and high availability of adsorptive surfaces. Displacement also occurs between marine cations (Mg, Na) and trace metals in marine particles that have low content of trace metals. Downstream, the higher salinity and reduced amount of suspended matter will favour the desorption process. Organic ligands might play a role in the adsorption/desorption equilibrium (Mantoura, Dickson & Riley, 1978). The adsorption/desorption process has a minor effect on the distribution profile of particulate metal in the upper part of the estuary. The presence of maximum turbidity, the resuspension of bottom material and the impact of local industrial discharge are major parameters (Baeyens, Panutrakul, Elskens, Leermakers, Navez & Monteny, 1991; Baeyens *et al.*, 1998).

With the exception of the most abundant elements, Al, Fe and Mn, most heavy metals occur mainly as surface complexes and minor inclusions in minerals so they contribute an insignificant fraction of the total mass of suspended matter and colloids as observed in this study. The more abundant metals Al, Fe and Mn can form a major portion of oxyhydroxide precipitates and silicates, especially in clay minerals (Gaboury & Rozan, 1999) as a result contribute significantly to metal content in suspended matter.

Two distinct patterns in metal partitioning were observed during this study. The concentrations of Cr, Mn, Fe, and Zn in the suspended matter fraction decreased as salinity increased and turbidity decreased. A similar occurrence with Al was noted for total metal concentrations and it could therefore be assumed that the suspended fraction is slightly smaller than the fraction of Cr, Mn, Fe and Zn but that the partitioning is still related to suspended particles. This seaward decrease in particulate metal concentrations is a common characteristic of estuaries (Owens, Ball & Price, 1997). The concentrations of particulate metals have been shown to decrease seawards due to mixing of contaminated river-borne material with relatively cleaner coastal suspended particulate matter, deposition of particulate matter transferred by the river, and also desorption processes (Wollast, 1988; Regnier & Wollast, 1990; GESAMP/UNESCO, 1994; Barak & Nurit,

1997). Copper and Pb concentrations displayed the opposite partitioning pattern with increased salinity and decreased turbidity resulting in increased dissolved metal concentrations. This suggests that flocculation and mobilisation of particulate phases through adsorption or re-suspension in the estuary embayment occurs with resulting desorption process caused by the increased chlorinity and major cations concentrations (Baeyens *et al.*, 1998).

From the above it can be concluded that the behaviour of the selected metals has some similarities. Some metals show similarity of increasing their dissolved phase in high salinity where as others have increased dissolved phases when salinities are low. Those that increase in their particulate phase in high turbidity are those that favour the adsorption process whereas the particulate phase increases by precipitation in areas with lower oxygen. The existence in water of particulate associated metals such as Fe, and Al in the estuary in high concentrations is due to the high silt content, which leads to re-suspension of sediment as a result of the shallow nature of the estuary.

4.4.3 Metals in sediment

According to Allen & Hansen (1996), sediments represent the most concentrated pool of metals in aquatic environments. In estuarine systems these metals are not bio-available except to organisms that are resident in sediments (Williams, Attrill & Nimmo, 1998). Since these organisms are primary food source for bottom feeding fish, the accumulation of metals potentially depends on uptake from food as well as from water (Nott, & Nicolaidou, 1994). Metal concentrations from this study were elevated orders of magnitude above the concentrations recorded in water (Table 4.5) and this supports the findings by Hall & Pulliam (1995) in the Hackensack Estuary in North America.

No significant temporal differences were recorded for metals in the sediment. This may be due to most metals being present in sediment as precipitates or in an un-dissolved state. The highest concentrations of metals in the sediment were Fe, Al and Zn, which coincided with the levels of metals in the particulate matter in the water column. Watling & Watling (1982c, d, e) also reported high concentrations of Fe and Zn from sediments in Gamtoos, Swartkops, and Sundays River estuaries (Table 4.13). Their values were, however lower than this study.

Table 4.13 Comparisons of metal concentrations in the sediments of the Mhlathuze Estuary with sediment concentrations in other estuaries on the eastern seaboard of South Africa.

Estuary	Al (µg/g)	Cr (µg/g)	Cu (µg/g)	Fe (µg/g)	Mn (µg/g)	Pb (µg/g)	Zn (µg/g)	Reference:
Mhlathuze	18677.4	64.4	12.2	20606.9	345.3	13.5	45.6	This study
Knysna	-	21	5	-	40	14	17	a
Bietou	-	2	3	10120	54	2	27	b
Keurbooms	-	1	2	6600	73	1	17	b
Kromme	-	7	1	2653	20	3	4	c
Gamtoos	-	15	5	9180	79	7	16	c
Swartkops	-	5	18	20800	298	31	55	d
Sundays	-	38	16	72	360	18	57	e
Bushmans	-	22	3	7330	49	5	13	f
Kariega	-	27	8	16048	131	10.7	27	f
Kowie	-	64	17	32300	530	29.8	96	f
Great Fish	-	13	7	10760	140	10	34	f
Buffalo	-	39	24	31	143	4	37	g
Blind	-	29	29	9	346	14	124	h
Ihlaza	-	7	29	2	35	24	5	h
Nahoon	-	34	15	19	280	17	47	h
Quinera	-	44	9	11	108	14	19	h
St Lucia E.	-	150	61	60000	-	19	72	i
St Lucia E.	-	7	2	3000	-	0.8	3.4	i
Richards Bay #	-		9.9	23640	-	24	98	k
Richards Bay #	-	74.8	24.04	5814	-	17.47	87.16	j
Richards Bay *	31323.4	110.3	19.22	31762.7	411.3	-	95.54	l
Durban *	-	388	57	40000	303	117	287	i
Umzimkhulu	-		10	11000		9	649	i
Umzimvubu	-	27	4.8	1540		0.35	16.4	i

References a. Watling & Watling, 1982a; b. Watling & Watling, 1982b; c. Watling & Watling, 1982c; d. Watling & Watling, 1982d; e. Watling & Watling, 1982e; f. Watling & Watling, 1983b; g. Watling *et al.*, 1985; h. Watling & Talbot, 1985; i. Cloete *et al.*, 1979; j. Oliff, 1977; k. Connell *et al.*, 1985; l. Vermeulen & Wepener, 1999.
* = Harbour # = Pre Harbour developments

The metals in the Mhlathuze Estuary probably exist as particulate matter or precipitated metals. Sediment-bound metals are mainly the result of man-made activities (Fairey, Roberts, Jacobi, Lamerdin, Clark, Downing, Long, Hunt Anderson, Newman, Tjeerdema, Stephenson & Wilson, 1998) and deposition of dredged material from the harbours (Van den Hurk, Eertman & Stronkhorst, 1997). The metals are then precipitated onto and into the sediments. According to Chon Lin, Meng-der & Ming-Tsuen (1998) and Williams *et al.* (1998) high loads of heavy metals are normally concentrated in the fine sediments. It is highly probable that the ingression of particulate bound metals into the Mhlathuze Estuary took place when dredger spoil from the Richards Bay Harbour was deposited on the beach north of the estuary (Wepener & Cyrus, 1997; Mackay & Cyrus, 1999). Wave action and the

near-shore current would have resulted in contaminated fine silt finding its way into the estuary.

The very high levels of Al in sediment may either be from the geological leaching, as the area is known for its high Al metal content or from pollution effects such as dredger spoil (Van den Hurk *et al.*, 1997; Syderman & Jarman, 1998). Virkanen (1998) also reported high Al concentrations (30000 µg/g) in the upper 10 cm of sediment cores from the Bay of Töölönlahti in Finland.

Concentrations of Cu and Pb in sediment were low, ranging from 20 –50 µg/g. These metals exist mostly as dissolved ions hence their low concentrations in sediment. Copper is also known to form complexes with organic matter (Cheung & Wong, 1992). The highest Cu concentrations in the sediment (at site 4) could be attributed to the complexation of Cu with organic ligands since the highest organic content was also recorded at this site (Table 4.6). Petri & Zauke (1993) found Cu and Pb in the Weddell Sea to be tightly bound to the sediment and as such were not bio-available. According to Sunda, Testa & Huntsman (1990) metal complexation decreases toxicity by decreasing free metal ions. Copper is one such metal that is highly complexed by natural ligands. The presence of high organic content in estuaries can therefore decrease toxicity of metals such as Cu (Sunda *et al.*, 1990).

Chromium concentrations were elevated in sediments of the Mhlathuze Estuary. High concentrations of Cr may be a result of contamination from the harbour due to sediment bound metals entering the estuary as discussed in the previous paragraphs. Loring (1979) also reported total Cr levels of 8-241 mg/kg Cr in the sediment of the Gulf of St Lawrence, Canada. It is possible that Cr exist both as dissolved the fraction in pore water and particulate fractions in sediment. This is because the percentage of particulate to dissolved Cr was almost equal and that both the fractions were significantly different between seasons. Tack, Vossius & Verloo (1996) found Cr concentration to occur predominantly in the exchangeable form in the sediment. Studies on estuarine water and sediment samples have shown that dissolved and particulate Cr are present in almost equal quantities with the flocculation processes increasing Cr^{3+} concentrations in the salinities below sea water (Anon 1975).

Fish tissues

The relationship between metal uptake and loss or transformation dictates the particular metal accumulation strategy of an organism (Phillips & Rainbow, 1993). According to Luoma (1983) metal uptake by fish is due to more than one mechanism. During all contamination processes in fish, toxicants come up against biological barriers: the gill epithelium and skin for the direct route, and the wall of the digestive tract for the indirect route (Boudou & Ribeyre, 1989). However, the relative importance of these uptake routes has not clearly been established (Dallinger *et al.*, 1987). According to Brusle (1981) the feeding habits and life style of bottom dwelling fish such as *Liza* spp. is likely to result in accumulation of sediment-associated contaminants. There are three likely pathways for the uptake of sediment-associated metals: (1) fine particles that are re-suspended in the water column, which are taken up via gill and digestive tract, (2) leaching of sedimentary contaminants to water which are accumulated in the fish body via respiration, and (3) direct contact and consumption of the sediment via skin and intestine (Chen & Chen, 1999).

Gills appeared to be the main uptake route for Cr, Mn, Pb and Zn in *L. dumerelii*. These were in accordance with results from studies conducted on other estuarine fish species for Pb (*So et al.*, 1999, Sultana & Rio, 1998), Cr (Taylor *et al.*, 1985; Hernandez-Hernandez, Medina, Ansuategui, & Conessa, 1990), Mn (Bendell-Young & Harvey, 1986; Chen & Chen, 1999) and Zn (Saltes & Bailey; 1984, Sultana & Rio, 1998; Chen & Chen, 1999).

This does, however, not imply that the gills did not take up Cu and Fe. The lower Cu in the gill tissue could be ascribed to the lower binding affinity of gill metallothionein (MT) when compared to liver MT (Noël-Lambot, Gerday, & Disteche, 1978). Gills are not only responsible for metal uptake, but also play a role in excretion of metals. The excretion process is associated with mucus production by the gill tissue (Heath, 1995) following exposure to exogenous metal exposure. Since the gills were the organs that accumulated the highest metal concentrations, it is probable that the increased metal accumulation could be attributed to both the uptake and excretory functions of the tissue. The concentrations of metals reported in gill tissue of *L. dumerelii* were all within the same range when compared to other studies on Cu, Pb and Zn in *Mugil cepha*lus (Sultana & Rio, 1998), Cu, Fe, Mn and

Zn in *L. macrolepis* (Chen & Chen, 1999), Cu in *Siganus oramin* (So, Cheung, & Chan, 1999), and Pb in *Glossogobius giuria* (Singh, Varma, & Munshi, 1990).

Following transportation, metals are distributed in the cytoplasmic compartments of target organs in a variety of ways. Intracellular storage of metals is extremely important since it conditions the toxicological effects (Boudou & Ribeyre, 1989). Lipid soluble contaminants undergo biotransformation but hydrosoluble contaminants such as metals undergo a neutralisation or sequestration process. These processes do not reduce the amounts of metals accumulated, but they withdraw them from the metabolic circuits to a varying degree. Bioaccumulation of metals in muscle and liver tissues was most probably the result of complexation reactions that resulted in precipitation of metals as insoluble salts or granular deposits (Boudou & Ribeyre, 1989).

According to Sultana and Rio (1998), Cu accumulates predominantly in liver tissue. This was also the case during this study. Liver tissue contains high concentrations of MT, which play a role in the sequestration of primarily Cu, Zn, Cd and Hg (Hogstrand & Haux, 1991). Although Zn concentrations in liver tissue were higher than Cr, Mn, and Pb concentrations, they were not as high as the Fe and Cu concentrations. This was probably due to lower MT induction in liver tissue by Zn. Spry and Wood (1989) found that induction of MT in fish liver by Zn was not as responsive as for Cu, and its role in Zn detoxification remains unclear.

According to Dalinger *et al.*, (1987) high liver concentrations are directly related to the blood flow associated with dietary uptake of metals. Hodson & Hilton (1983) attributed high concentrations of selenium in the liver to dietary uptake and subsequent transport to the liver via the intestinal portal system, in contrast to the other tissues, which are supplied directly from the gills. From this study it was apparent that liver predominantly accumulated Cu and Fe and it could possibly be attributed to ingestion of Cu and Fe contaminated sediments. The very high Fe concentrations which were recorded (Fig. 5B) could also be attributed to haemoglobin found in the highly vascularised liver tissue. The other metals (Cr, Mn, and Pb) were most probably stored as insoluble precipitations in the cytosol. Once again the concentrations reported for metals in the liver tissue of *L. dumerellii* were all within the same range when compared to other studies on Cu, Pb and Zn in *M. cephalus* (Sultana & Rio, 1998), Cu, Fe, Mn and Zn in *L. macrolepis* (Chen & Chen 1999), Cu and Zn in *S. oramin* (So

et al., 1999), Pb in *G. giuria* (Singh *et al.* 1990), and Cu and Zn in *L. aurata, M. cephalus* and *M. labrosus* (Hamaza-Chaffai, Roméo, Abed, 1996).

Muscle concentrations of Cu, Cr, Mn and Pb were generally below 30 µg/g. According to Moore & Ramamoorthy (1984) low levels of Pb in skeletal muscle could be attributed to the low binding rate to sulfhydryl groups as well as the low solubility. Aluminium, Fe and Zn concentrations in muscle tissue ranged between 6 and 178 µg/g and were clearly higher than the other metals. The higher concentrations are related to the increased ambient concentrations of these metals. Plaskett and Potter (1979) also found that bioaccumulation of Zn in muscle tissue of the bottom-feeder, *M. cephalus*, was higher than other metals investigated. The metal concentrations in the muscle tissue of *L. dumerelii* from the Mhlatuze Estuary were comparable to levels recorded in muscle tissue of estuarine and marine fish from areas with known anthropogenic influences e.g. Visakhapatnam Harbour on the east coast of India (Sultana & Rio, 1998), Mediterranean coast of Tunisia (Hamaza-Chaffai *et al.*, 1996), Tolo and Victoria harbours in Hong Kong (So *et al.*, 1999), Kaohsiung Harbour in Taiwan (Chen & Chen, 1999), and Sydney Harbour (Gibbs & Miskiewics, 1995). All the concentrations reported in the abovementioned studies were far higher than metal concentrations measured in 12 marine fish species in the unpolluted Cockburn Sound, Australia (Plaskett & Potter, 1979).

Mean concentrations of Al and Fe in whole fish were very high ranging between 450 and 3000 µg/g. The concentrations were however within the same range reported for whole *L. dumerelii* in Richards Bay Harbour (Vermeulen & Wepener, 1999). Whole body concentrations of Cr were much lower than the levels reported for Richards Bay Harbour. The metals Cu, Mn and Pb on the other hand were generally low below 100 µg/g indicating low bioaccumulation. This may be due to regulation of the metals in muscle by homeostatic control (Plaskett & Potter, 1979).

Table 4.14 presents historical bioaccumulation data from before the construction of the harbour (Hemens & Connell 1975, Hemens et al. 1976) and before the construction of a submarine outfall pipeline off the mouth of the harbour (Connell, Mc Clurg & Livingstone, 1985). When compared to the results of the present study it is evident that metal concentrations have increased in fish tissue. It must however be borne in mind that the

Table 4.14 Comparison between historical and present study metal bioaccumulation data in fish tissue from the Mhlathuze Estuary. Data are presented as minimum and maximum concentrations in µg/g (dry mass). ND represents data which are not available.

Species	Tissue	Cr	Cu	Fe	Pb	Zn	Reference
Liza dumerelii	muscle	2.5– 87.5	0.5 – 24.3	69.57 – 1470.59	0.41 – 50.52	33.66 –893.75	This study
	liver	4.48 – 190.91	16.0 – 2961.1	235.29 – 8684.21	2.08. – 55.88	89.55 – 800.0	
Mugil sp.	muscle	ND	0.6 –0.9	9 - 12	0.68 – 0.73	42 - 61	Hemens and Connell 1975
Mugil cephalus	muscle	ND	ND	ND	ND	69 – 74	Hemens *et al.*, 1976
	liver	ND	113 - 226	ND	ND	126 - 173	
Pomadasys commersonni	muscle	1.5 - 2.2	0.9 - 1.8	ND	0.12 - 0.13	9 – 26	Connell *et al.*, 1985
	liver	3 - 27	65 - 246	ND	0.19 – 1.21	16 – 209	
	muscle	ND	0.25	ND	ND	ND	Hemens and Connell 1975
	liver	ND	58.3	657	0.15	42	
	muscle	ND	ND	ND	ND	73	
	liver	ND	40	ND	ND	149	Hemens *et al.*, 1976
Rhabdosargus holubi	muscle	1.3	0.9	ND	0.115	20	Connell *et al.*, 1985
	liver	3.5	11	ND	1.13	67	
	muscle	ND	ND	ND	ND	89	Hemens *et al.*, 1976
	liver	ND	12	ND	ND	178	
Acanthopagrus berda	muscle	1.7	0.5	ND	0.153	13	Connell *et al.*, 1985
	liver	1.8	13	ND	0.56	105	
	muscle	ND	0.05	ND	11.3	ND	Hemens and Connell 1975
Argyrosomus holelopidotus	muscle	ND	0.09	13	1.2	7	Hemens and Connell 1975
	liver	ND	2.5	251	0.4	24	Hemens and Connell 1975
	muscle	ND	8	ND	ND	2.4	Hemens *et al.*, 1976
	liver	ND	10	ND	ND	82	
Phadogarbus sp.	muscle	ND	0.3	4	0.7	56	Hemens and Connell 1975
Elops machnata	muscle	ND	10	ND	ND	50	Hemens *et al.*, 1976
	liver	ND	ND	ND	ND	87	

the route of metal uptake by *A. digitalis*. Wolfe *et al.* (1976) found more accumulated metals in infaunal crustaceans that ingest sediment than the epifauna of the benthic community. While microphagous feeders may ingest many potentially, metal rich particles, they also pass large volumes of water across the permeable body surfaces, which could facilitate further uptake at higher rates (Moore & Rainbow, 1989; Rainbow & White, 1989). It is therefore possible that during this study the gills were the main uptake site for Cu and Zn, while the high metal content (Al, Fe and Mn) in *A. digitalis* may be due to ingested sediment bound metals entering the body via the gastro-intestinal tract. The metals concentrations in *A digitalis* were highly elevated when compared to *P. blephariskios*.

In contrast to *A. digitalis* only three metals, i.e. Al, Cr and Cu differed significantly in *P. blephariskios* between seasons. This is probably related to the particulate metal fractions being prevalent during high flow periods (Summer 1997) in the Mhlathuze Estuary. Devescovi & Lucu (1995) reported seasonal difference in Cu levels in the shore crab, *Carcinus meditteraneus*, when exposed to contaminated sediments. It is however not easy to predict the uptake of metals in *P. blephariskios* because of the lack of information on the biology of the species. However, since *P. blephariskios* is a deposit feeder that burrows in the sediment it is likely that bioaccumulation would be a combination of metals ingested from the sediment as well as metals in the pore waters of the burrows. While there was no evident correlation between concentrations of Al, Cr and Cu in *P. blephariskios* and sediment concentrations, other metals such as Mn, Pb and Zn were positively correlated with the sediment levels, suggesting their increase in the body with the increase in sediment concentrations. This supported the findings by Bat, Raffaeli & Marr (1998) who found that Zn and Cd were inversely related to water concentrations in *Corophium volutator* and attributed this to uptake of these metals from the sediment.

The differences in the type and proportions of metal bioaccumulation in the two invertebrate species in this study could be related to interspecies variation. Invertebrates vary widely in their tolerance. Some accumulate high metal levels while others, such as some species of crustacea are extremely efficient at metal regulation and excretion. *Paratylodiplax blephariskios* in this case seems to be regulating the accumulation of certain metals. While absolute metal concentrations differ between the two species due to subtle differences in habitat, the similarities of profiles suggests that both these species are responding to the same source of metals.

Concentrations of Al, Fe and Mn and Cr were elevated in both invertebrate species reported in this study. These metals coincide with elevated concentrations in the water and sediments of the Mhlathuze Estuary (Fig. 4.2-4.8). The levels of metals in the study were comparable to other invertebrate studies (Lyla & Khan, 1996; Bat *et al.*, 1998; Blackmore, Morton & Huang, 1998). Aluminium burdens in invertebrates in this study were higher than those reported for the oyster, *Crassostrea rizophorae*, from Dominican Republic (Briz, Aquino, De Rodriguez, Fowler & Sericano, 1998). Bioaccumulation of Fe and Mn were consistently the highest during summer surveys in both invertebrates. This is in agreement with other studies by Lyla & Khan (1996) who also reported maximum values in Fe and Mn recorded during summer in hermit crab (*Clibanarius longitarsus*) tissue from the Vellar Estuary (India). Blackmore *et al.* (1998) also reported highest values of these metals in the crustacean, *Tetraclita squamosa*, from the coastal waters of Xiamen in China. It is interesting to note that although Zn concentrations in sediments are the third highest of all the metals measured in this study its bioaccumulation in benthic invertebrates is relatively low. This may be because crustaceans are able to excrete Zn unless it is required for metabolic processes such as egg production. Some species of crab e.g. *Carcinus maenas* are able to excrete excess Zn across the gills (Phillips & Rainbow, 1993).

From this study of accumulation of metals in benthic organisms it is evident that uptake of contaminants by aquatic organisms is depended on the habitat where the organism live. There can be sediment infauna and sediment epifauna species (Forstner, 1997). The route of exposure of metals in benthic organism can therefore be through pore water, overlaying water or by direct sediment injection (Cheung *et al.*, 1997). The mudcrab, *P. blephariskios* is a deposit feeder that burrows in the sediment and is therefore exposed to metals ingested from the sediment as well as metals in the pore waters of the burrows. On the other hand, *A. digitalis* is a suspension feeder, exposed to the bulk of the metal in the sediment and the metals in the overlaying water.

Moreover, it is important to establish direct correlation between the content of the contaminants in the sediment samples and the toxicity of the metals. It is therefore concluded that the combination of bioassays and chemical analysis is a valuable tool for evaluating the environmental risks of sediments (Pedersen, Bjoernestad, Andersen, Kjolholt & Poll, 1997).

4.4.5 Submerged macrophytes

Little has been reported on the toxicity and bioaccumulation of metals in *Zostera capensis*. During this study, *Z. capensis* was sampled at only one site (site 1) in the estuary. Concentrations of Al (39000-154750), Fe (4826-13526), Mn (538-2311) and Cr (45-131) were found to be elevated in *Z. capensis* during the study. These coincide with elevated concentrations of the same in the water and sediments of the Mhlathuze Estuary Fig 4.2-4.8). Of the four mentioned metals, only Al is not an essential metal and it can be assumed that the Fe, Mn and Cr are probably found in high concentrations in *Z. capensis* due to them being required for metabolic processes in plants. Driftmeyer *et al.* (1980) also found high metal concentrations in *Zostera marina* from the Newport River Estuary. The highest concentrations were measured in leaf blades with values being Fe-1400 mg/kg, Mn-98 mg/kg, Zn-86 mg/kg and Cu-6.6 mg/kg.

Results from the literature showed that Mn is one of the metals with the highest bioaccumulation properties in *Z. marina* (Brinkhuis, Pennell & Churchill, 1980; Driftmeyer *et al.*, 1980) and *Cymodocea nodosa* (Nicolaidou & Nott, 1998). This was also recorded in *Z. capensis* during this study and could be attributed to the fact that Mn is more of an essential element in plants than in animals. The above mentioned authors also found that the highest Mn levels were found in leaves of the seagrass, whereas all other metals were the highest in roots.

Based on bioaccumulation patterns in *Z. marina* (Driftmeyer *et al.*, 1980; Driftmeyer & Rublee, 1980; Lingby & Britz, 1989) it can be assumed that this would be the main route of metal bioaccumulation in *Z. capensis*. The aforementioned studies indicated that Al, Cu and Fe were predominantly taken up from the sediment while Mn and Zn accumulation was due to water-borne sources. The metal are then transported and stored on leaf blades. Driftmeyer & Rublee (1980) also reported increased Mn, Fe, Cu and Zn in *Spartina alterniflora* with roots being the main uptake route.

4.4.6 *Relationship between metal concentrations in biotic and abiotic compartments of the Mhlathuze Estuary*

There is an appreciable amount of metal accumulation in the biota of Mhlathuze Estuary with the benthic invertebrates accumulating a high percentage of heavy metals followed by the aquatic macrophyte, *Z. capensis*. It is, however, not easy to trace the origin of most metals and the uptake media because of the complexity of the processes controlling estuarine heavy metal geochemistry. These processes involve water and sediment hydrodynamics, particle water interactions and biological mediation (Williams & Willward, 1998). The high levels of these heavy metals in water and sediments of the Mhlathuze Estuary may therefore be a by-product of both biogeochemical processes and anthropogenic factors.

Accumulation of these metals in water and biota differs for different seasons but generally during seasons of increased freshwater inflow, high concentrations of water-soluble metals were found in water. In winter, the metals that are mostly found in dissolved state are increased in water. Although metals accumulation in sediments is in orders of magnitude more than concentrations in water, there does not seem to be any difference in the accumulation of metals for different seasons in the sediment.

In fish, the gills accumulate most of the metals with a large percentage taken up from water either by passive diffusion or active uptake. The liver however is a storage organ for metals and they are found there in high concentrations. The muscle on the other hand is very low in accumulation. The correlation analyses revealed that for most metals there were significant positive relationships between metals in muscle tissue and other tissues. This has significant implications for on-going bioaccumulation monitoring since it would appear that it would be sufficient to just concentrate on sampling muscle tissue. Similar findings were obtained for freshwater systems (Wepener, 1997).

4.5 Conclusions

There is a high measure of metal accumulation in water, sediment and the biota of the Mhlathuze Estuary when compared to earlier research (Hemens *et al.*, 1975; Hemens & Connell, 1976: Connell *et al.*, 1985). When compared with data from other estuaries on the

eastern coast of South Africa (Watling & Watling, 1982 a,b,c,d,e; Watling & Watling, 1983 a,b) these values are also very high. As the estuary is considered an estuarine embayment with substantial influence from the sea, the water quality of its natural environment can only be compared to the guidelines for coastal marine waters. In addition no water quality guidelines currently exist for the S. A. estuaries. When compared with the water quality guidelines for coastal marine waters, (Table 4.15), the values in Mhlathuze Estuary were found to exceed the guidelines by orders of magnitude.

Table 4.15 Water quality guidelines for coastal marine waters for some metals selected for this study.

Metal	Target value (µg/l)	Mhlathuze Estuary values (µg/l)
Cr	8	48
Cu	5	39.1
Pb	12	130.15
Zn	25	66.5

The importance of the Mhlathuze Estuary on a national scale cannot be over-emphasised. This was evident from a number of studies on the biota of the estuary which formed part of the recent DWAF Estuarine Freshwater requirements (ERF study (i.e. botanical importance - Adams *et al.*, 1998; macrocrustacea – Cyrus *et al.*, 2000; fish – Weerts *et al.*, 1999. Further research and monitoring should be instituted to ascertain the causal effects of such accumulation and the true source of heavy metals. This should be followed by the development of an appropriate management plan for the Mhlathuze Estuary and its catchment.

The study of accumulation of metals in fauna, water and sediment gives an understanding on the level of accumulation in different media and on the rate of uptake and prediction on subsequent accumulation of those metals by the organism over time. It does not however predict the amount of toxicity of a particular metal at that specific load on an individual organism. The alternative approach is to establish a relationship between the levels in the media, accumulation and toxicity is to use a tripartite method (Chapman *et al.*, 1992). The method uses toxicity bioassay and testing in addition to community and medium accumulation.

4.6 References

Abel, P.D. (1989). **Water Pollution Biology.** Ellis Horwood, Chichester. 231pp.

Adams, J.B., Bates, G.C. & Riddin, T. (1998). Freshwater requirements of the Mhlathuze Estuary: Vegetation. Report submitted to the institute for Water Research on behalf of the Department of Water Affairs and Forestry 30pp.

Allen, H.E. & Hansen, D.J. (1996). The importance of trace metal speciation to water quality criteria. **Water Environment Research** 68:42-54.

Anon, J. (1975). Distribution of some trace metals in Poole Harbour, Dorset. **Marine Pollution Bulletin** 6:180-186.

APHA (1987). **Standard Methods for the Examination of Water and Waste Water.** Washington APHA 1268pp.

Baeyens, W., Panutrakul, S., Elkens, M., Leermakers, M., Navez, J. & Monteny, F. (1991). Geochemical processes in muddy and sandy tidal flats. **Geo-Marine Journal** 11:188-193.

Baeyens, W, Elskens, M., Gillian, G. & Goeyens, L. (1998). Biogeochemical behaviour of Cd, Cu, Pb, and Zn in the Scheldt estuary during period 1981-1983. **Hydrobiologia** 366:15-44.

Barak, H. & Nurit, K. (1997). Particulate metals contamination in the Kishon River Estuary, Isreal. **Marine Pollution Bulletin** 34:706-711.

Bat, L., Raffaelli, D. & Marr, I.L. (1998). The accumulation of copper, zinc and cadmium by the amphipod, *Corophium volutator* (Pallas) **Journal of Experimental Marine Biology and Ecology** 223:167-184.

Bendell-Young, L.I. & Harvey, H.H. (1986). Uptake and tissue distribution of manganese in the white sucker (*Catostomus commersoni*) under conditions of low pH. **Hydrobiologia** 133:117-122.

Blackmore, G., Morton, B.V. & Huang, Z.G. (1998). Heavy metals in *Balanus amphitrite* and *Tetraclita squamosa* (Crustacea cirripede) from the coastal waters of Xiamen China. **Marine Pollution Bulletin** 36(1):32-40.

Boudou, A. & Ribeyre, F. (1989). **Aquatic Ecotoxicology: Fundamental concepts and Methodologies.** Vol. I. CRC Press, Inc. Boca Raton, Florida.

Bowen, H.J.M. (1966). **Trace Elements in Biochemistry.** Academic Press, New York. 241pp.

Brinkhuis, B.H., Penello, W.F. & Churchill A.C. (1980). Cadium and Mn flux in eelgrass *Zostera marina*: Metal uptake by leaf and root rhizome tissues. **Marine Biology** 58:187-196.

Briz, S., Aquino, L., De-Rodriguez, R.M., Fowler, A. & Sericano, J.L. (1998). Levels of chlorinated hydrocarbons and trace metals in bivalves and near-shore sediments from Dominican Republic. **Marine Pollution Bulletin** 36(12):971-979.

Brusle, J. 1981–Food and Feeding in Grey Mullet. In **Aquaculture of Grey Mullet.** O.H. Oren, (Ed.) Cambridge University Press Cambridge:185-217.

Buikema, A.L., Niederlehner, B.R. & Cairns, J. Jr. (1982). Biological monitoring Part IV: Toxicity testing. **Water Research** 16:239-262.

Bray, J.R. & Curtis, J.T. (1957). An ordination of the upland forest communities of southern Wisconsin. **Ecological Monographs** 27:235-349.

Canfield, T.J., Kemble, N.E., Brumbaugh, W.G., Dwyer, F.J., Ingersoll, C.G. & Fairchild, F.J. (1994). Use of benthic invertebrate community structure and the sediment quality triad to evaluate metal-contaminated sediment in the upper Clark Fork River, Montana. **Environmental Toxicology and Chemistry** 13(12):1999-2012.

Chapman, P.M. (1985). Effects of gut sediment contents on measurement of metal levels in benthic invertebrates: A cautionary note. **Bulletin of Environmental Contamination and Toxicology** 35:345-347.

Chapman, P.M., Power, F.A. & Burton G.A. (1992). Integrated assessment in aquatic ecosystems. In **Sediment Toxicity Assessment**. G.A Burton (ed) Lewis Publishing, Boca Raton. pp.313-339.

Chapman P.M. (1997). Is bioaccumulation useful for predicting impacts? **Marine Pollution Bulletin** 34(5):282-283.

Chen, M-H. & Chen C-Y. (1999). Bioaccumulation of sediment-bound heavy metals in grey mullet, *Liza macrolepis*. **Marine Pollution Bulletin** 39(1-12): 239-244.

Cheung, Y.H. & Wong, M.H. (1992). Comparison of trace metals content of sediment and mussels collected within and outside Tolo Harbour, Hong Kong. **Environmental Management**. 16:743-751.

Cheung, Y.H., Neller, A., Chu, K.H Tam, N.F.Y., Wong, C.K., Wong, Y.S. & Wong, M.H. (1997). Assessment of sediment toxicity using different trophic organisms. **Archives of Environvironmental Contamination and Toxicology** 32:260-267.

Chon-Lin, L., Meng-Der, F. & Ming-Tsuen, H.S. (1998). Characterisation and distribution of metals in surficial sediments in Southwestern Taiwan. **Marine Pollution Bulletin** 36:464-471.

Clarke, K.R. & Warwick, R.M. (1994). **Changes in Marine Communities and Approach to Statistical Analysis and Interpretation.** Natural Environment Research Council, U.K. 172 pp.

Cloete, C.E. (1979). The transfer of pollutants in two southern hemisphere oceanic systems. Proceeding of the workshop held at Plattenberg Bay, South Africa 23-26 April 1975. South African Scientific Programmes Report No 39.

Connell A.D., Turner, W.D., Gardner, B.D., McClurg, T.P., Livingston, D.J., Carter, J.E. & Gertenbach, W.J.N. (1975). National marine pollution monitoring programme, East Coast Section. Progress Report No 2 March 1974-March 1975, 118pp.Connell, A.D. (1996) Environmental studies in the Richards Bay offshore outfalls region CSIR Report No. 10, Kwazulu Natal. 199pp.

Connell, A.D., McClurg, T.P. & Livingstone D.J. (1985). Environmental Studies at Richards Bay Prior to Discharge of Submarine Outfalls: 1974-1984. National Insitute For Waster Research Durban. 289pp.

Cross, F.A. & Sunda, W.C. (1982). The relationship between chemical speciation and bioavailability of trace metals to marine organisms. Proceedings of the International Symposium on utilization of coastal ecosystems: Planning Pollution and Productivity Nov 21-27.

Cyrus, D.P. & Wepener, V. (1998). The impact of dredger spoil on selected components of the Mhlathuze Estuary biota Report prepared for Portnet Richards Bay. CRUZ investigational Report No 52. 62pp.

Cyrus, D.P., Wepener, V., Mackay, C.F., Weerts, S.P. & Viljoen, A. (2000). The Effects of Intrabasin Transfer on the Hydrochemistry, Benthic Invertebrates and Ichthyofauna of the Mhlathuze Estuary and Lake Nsezi. WRC Report No. 722/1/99. 255pp.

Dallinger, R., Prosi, F., Segner, H. & Back, H. (1987). Contaminated food uptake of heavy metals by fish: A review and a proposal for further research **Oecologia** 73:91-98.

Davies, P.H., Goettl, J.P., Sinley, J.R. & Smith, N.F. (1976). Acute and chronic toxicity of lead to rainbow trout *Salmo gairdneri* in hard and soft water. **Water Research** 10:1099-1021.

Department of Water Affairs and Forestry (1995). South African Water Quality Guidelines for Coastal Marine Waters. Volume 1: Natural environment. 298pp.

Devescovi, M. & Lucu, C. (1995). Seasonal changes of the copper level in shore crabs Carcinus mediterraneus. **Marine Ecology Progress Series** 120(1-3):169-174.

Driftmeyer, J.E. & Rublee, P.A. (1981). Mn Fe, Cu and Zn in *Spartina alterniflora* detritus and microorganisms. **Botanica Marina** 24:251-256.

Driftmeyer, J.E., Thayer, G.W. Cross, F.A. & Zieman, J.C. (1980). Cycling of Mn, Fe, Cu, and Zn by eelgrass, *Zostera marina* L. **American Journal of Botany** 76(7):1089-1096.

Engel, D.W. & Sunda, W.G. (1981). Factors Affecting Trace Metal Uptake and Toxicity to Estuarine Organims: Environmental Parameters. In: **Biological Monitoring of Marine Pollutants.** F.J. Vernberg, A. Calabrese, F.P. Thurberg & W.B. Vernberg (Eds.), Academic Press Inc, New York. 555pp.

Farmer, G.J. Ashfiels, D. & Samant, H.S. (1979). Effects of zinc on juvenile Atlantic salmon *Salmar salar* :Acute toxicity, food intake growth and bioaccumulation. **Environmental Pollution** 19:103-117.

Fairey, R., Robert, C., Jacobi, M. Lamerdin, S., Clark, R., Downing, J. Long, E., Hunt, J., Anderson, B., Newman, J., Jjeerdema, R., Stephenson, M., & Wilson, C. (1998). Assessment of sediment toxicity and chemical concentration in the San Diego Bay Carlifornia. **Environmental Toxicology and Chemistry** 17(8):1570-1581.

Forstner, U. (1995). Contaminated Aquatic Sediments and Waste Sites: Geochemical Engineering. In: **Heavy metals Problems and Solutions.** Salomon, Forstner & Mader (Eds.), Springer Verlag. 412pp.

GESAMP/UNESCO, (1994). Anthropogenic influences on sediment discharge to the coastal zone and environmental consequences. **Reports and Studies** 52:4-30.

Gibbs, P.J.& Miskiewics M.J. (1995) Heavy metals in fish near a major primary treatment sewage plant outfall. **Marine Pollution Bulletin** 30:667-674.

Giesy, J.P. & Wiener, J.G. (1977). Frequency distribution of trace metals concentrations in five freshwater fishes. **Transactions of American Fisheries Society** 106:393-403.

Gaboury, B. & Rozan, T.F. (1999). The influence of size distribution on the particle concentration effect and trace metal partitioning in rivers. **Geochemica et Cosmochimica Acta** 63 (1):113-127.

Grindley, J.R & Wooldridge, T.H. (1974). The plankton of Richards Bay. **Hydrobiological Bulletin** 8(1/2): 202-212.

Devescovi, M. & Lucu, C. (1995). Seasonal changes of the copper level in shore crabs Carcinus mediterraneus. **Marine Ecology Progress Series** 120(1-3):169-174.

Driftmeyer, J.E. & Rublee, P.A. (1981). Mn Fe, Cu and Zn in *Spartina alterniflora* detritus and microorganisms. **Botanica Marina** 24:251-256.

Driftmeyer, J.E., Thayer, G.W. Cross, F.A. & Zieman, J.C. (1980). Cycling of Mn, Fe, Cu, and Zn by eelgrass, *Zostera marina* L. **American Journal of Botany** 76(7):1089-1096.

Engel, D.W. & Sunda, W.G. (1981). Factors Affecting Trace Metal Uptake and Toxicity to Estuarine Organims: Environmental Parameters. In: **Biological Monitoring of Marine Pollutants.** F.J. Vernberg, A. Calabrese, F.P. Thurberg & W.B. Vernberg (Eds.), Academic Press Inc, New York. 555pp.

Farmer, G.J. Ashfiels, D. & Samant, H.S. (1979). Effects of zinc on juvenile Atlantic salmon *Salmar salar* :Acute toxicity, food intake growth and bioaccumulation. **Environmental Pollution** 19:103-117.

Fairey, R., Robert, C., Jacobi, M. Lamerdin, S., Clark, R., Downing, J. Long, E., Hunt, J., Anderson, B., Newman, J., Jjeerdema, R., Stephenson, M., & Wilson, C. (1998). Assessment of sediment toxicity and chemical concentration in the San Diego Bay Carlifornia. **Environmental Toxicology and Chemistry** 17(8):1570-1581.

Forstner, U. (1995). Contaminated Aquatic Sediments and Waste Sites: Geochemical Engineering. In: **Heavy metals Problems and Solutions.** Salomon, Forstner & Mader (Eds.), Springer Verlag. 412pp.

GESAMP/UNESCO, (1994). Anthropogenic influences on sediment discharge to the coastal zone and environmental consequences. **Reports and Studies** 52:4-30.

Gibbs, P.J.& Miskiewics M.J. (1995) Heavy metals in fish near a major primary treatment sewage plant outfall. **Marine Pollution Bulletin** 30:667-674.

Giesy, J.P. & Wiener, J.G. (1977). Frequency distribution of trace metals concentrations in five freshwater fishes. **Transactions of American Fisheries Society** 106:393-403.

Gaboury, B. & Rozan, T.F. (1999). The influence of size distribution on the particle concentration effect and trace metal partitioning in rivers. **Geochemica et Cosmochimica Acta** 63 (1):113-127.

Grindley, J.R & Wooldridge, T.H. (1974). The plankton of Richards Bay. **Hydrobiological Bulletin** 8(1/2): 202-212.

Devescovi, M. & Lucu, C. (1995). Seasonal changes of the copper level in shore crabs Carcinus mediterraneus. **Marine Ecology Progress Series** 120(1-3):169-174.

Driftmeyer, J.E. & Rublee, P.A. (1981). Mn Fe, Cu and Zn in *Spartina alterniflora* detritus and microorganisms. **Botanica Marina** 24:251-256.

Driftmeyer, J.E., Thayer, G.W. Cross, F.A. & Zieman, J.C. (1980). Cycling of Mn, Fe, Cu, and Zn by eelgrass, *Zostera marina* L. **American Journal of Botany** 76(7):1089-1096.

Engel, D.W. & Sunda, W.G. (1981). Factors Affecting Trace Metal Uptake and Toxicity to Estuarine Organims: Environmental Parameters. In: **Biological Monitoring of Marine Pollutants.** F.J. Vernberg, A. Calabrese, F.P. Thurberg & W.B. Vernberg (Eds.), Academic Press Inc, New York. 555pp.

Farmer, G.J. Ashfiels, D. & Samant, H.S. (1979). Effects of zinc on juvenile Atlantic salmon *Salmar salar* :Acute toxicity, food intake growth and bioaccumulation. **Environmental Pollution** 19:103-117.

Fairey, R., Robert, C., Jacobi, M. Lamerdin, S., Clark, R., Downing, J. Long, E., Hunt, J., Anderson, B., Newman, J., Jjeerdema, R., Stephenson, M., & Wilson, C. (1998). Assessment of sediment toxicity and chemical concentration in the San Diego Bay Carlifornia. **Environmental Toxicology and Chemistry** 17(8):1570-1581.

Forstner, U. (1995). Contaminated Aquatic Sediments and Waste Sites: Geochemical Engineering. In: **Heavy metals Problems and Solutions.** Salomon, Forstner & Mader (Eds.), Springer Verlag. 412pp.

GESAMP/UNESCO, (1994). Anthropogenic influences on sediment discharge to the coastal zone and environmental consequences. **Reports and Studies** 52:4-30.

Gibbs, P.J.& Miskiewics M.J. (1995) Heavy metals in fish near a major primary treatment sewage plant outfall. **Marine Pollution Bulletin** 30:667-674.

Giesy, J.P. & Wiener, J.G. (1977). Frequency distribution of trace metals concentrations in five freshwater fishes. **Transactions of American Fisheries Society** 106:393-403.

Gaboury, B. & Rozan, T.F. (1999). The influence of size distribution on the particle concentration effect and trace metal partitioning in rivers. **Geochemica et Cosmochimica Acta** 63 (1):113-127.

Grindley, J.R & Wooldridge, T.H. (1974). The plankton of Richards Bay. **Hydrobiological Bulletin** 8(1/2): 202-212.

Hall, W.S. & Pulliam, G.W. (1995). An assessment of metals in an estuarine wetlands ecosystem. **Archives of Environmental Contamination and Toxicology** 29(2):164-173.

Hamaza-Chaffai, A., Roméo, M. & El Abed, A. (1996). Heavy metals in different fishes from the middle eastern coast of Tunisia. **Bulletin of Environmental Contamination and Toxicology** 56:766-773.

Harrison, R.M. (1990). Treatment of Toxic Waste. In: **Pollution: Causes, Effects and Control.** R.M. Harrison (Ed.) Chapman and Hall, London. 393pp.

Heath, A.G. (1987). **Water Pollution and Fish Physiology.** CRC Press Inc. Boca Raton, Florida. 245pp.

Heath, A.G. (1995). **Water pollution and Fish Physiology.** Lewis, Boca Raton. 359pp.

Heit, M. & Klusek, C.S. (1982). The effects of dissecting tools on the trace metals concentrations of fish and mussel tissues. **Science of Total Environment** 24:129-134.

Hemens, J., T.P. McClurg, T.P., Simpson, D.J. & Warwick, R.J. (1971). Detailed survey of Richards Bay. Progress Report No. 21. Twentieth Steering Committee Meeting. CSIR/NIWR. 49pp.

Hemens, J. & A.D. Connell (1975). Richards Bay: Southern Bay Conservation Area. Progress Report No 29. 250pp.

Hemens, J., Connell, A.D., McClurg, T.P., Simpson, D.E., Warwick, R.J. &. Muller. M.S (1976a). Richards Bay Sanctuary Area: Report on field surveys in October 1975 and February 1976. Progress Report No 31. Thirty-Second Steering Committee Meeting. CSIR/NIWR. 146pp.

Hemens, J., D.E. Simpson, & R.J. Warwick. (1976b). Richards Bay Sanctuary Area: Report on field surveys in May and June 1976. Progress Report No 32. Thirty-Third Steering Committee Meeting. CSIR/NIWR. 149pp.

Hernández, F.H., Medina, J., Ansuátegui, J. & Conesa, M. (1990). Heavy metals in some marine organisms from the Mediterranean Sea (Castellon, Spain): Metal accumulation in different tissues. **Scientae Marina** 54(2):113-129.

Hodson, P.V. & Hilton J,W. (1983). The nutritional requirements and toxicity to fish of dietary and waterborne selenium. **Ecology Bulletin** 35:335-340.

Horne, A.J. & Goldman, C.R. (1994). **Limniology.** Mc Graw-Hill Inc, New York. 576pp.

Hogstrand, C. & Haux, C. (1991). Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionein. **Comparative Biochemistry and Physiology** 100C(1/2):137-141.

Jackson, J.K. & Resh, V.H. (1989). Sequential decision plans, benthic macroinvertebrates and biological monitoring programs. **Environmental Management** 13(4):455-468.

Johansson-Sjöbeck, M. & Larsson, A. (1979). Effects of inorganic lead on delta aminolevulinic acid dehydratase (ALA-D) activity and haematological variables in the rainbow trout, *Salmo gaidneri.* **Archives of Environmental Contamination and Toxicology** 8:419-428.

Kennedy, I.R. (1994). **Acid Rain and Acid Soils.** Gilliard Printers, Great-Britain. 254pp.

Lawrence, A.J. & Poulter, C. (1998). Development of a sub-lethal pollution bioassay using the estuarine amphipod *Gammarus duebeni.* **Water Research** 32(3):569-578.

Lingby, J.E. & Brix, H. (1989). Heavy metals in eelgrass *(Zostera marina)* during growth and decomposition. **Hydrobiologia** 176/177:189-196.

Loring, D.H. (1979). Geochemistry of cobalt nickel chromium and vanadium in the sediment of the estuary and open gulf of St Lawrence. **Canadian Journal of Earth Sciences** 16:1196-1209.

Luoma, S.N. (1983). Bioavailability of trace metals to aquatic organisms-a review. **Science of Total Environment** 28:1-22.

Luoma, S.N..&. Carter J.L.(1991). Effects of trace metals on aquatic benthos. In**: Metal Ecotoxicology: Concepts and Applications.** M.C. Newman, & A.W. McIntosh (Eds.) Michigan; Lewis Publishers. 399pp.

Lyla, P.S. & Khan, S.A. (1996). Heavy metals iron and manganese in the estuarine hermit crab *Clibanarius longitarsus* (De Haan) of Vellar Estuary. **Journal of Ecotoxicology and Environmental Monitoring** 6(1):21-28.

Mackay, C..F. & Cyrus, D.P. (1999). A review of the macrobenthic fauna of the Mhlathuze Estuary: Setting the ecological Reserve**. South African Journal of Aquatic Sciences** 24 (1/2) 111-129.

Mance, G. (1987). **Pollution Threat of Heavy Metals in Aquatic Environments.** Elsevier Science, London. 372pp.

Mantoura, R.F.C., Dickson, A. & Riley, J.P. (1978). The complexation of metal with bunic materials in natural waters. **Estuarine Coastal and Marine Science** 6:378-408.

Mason, C.F (1991). **Biology of Freshwater Pollution.** Longman Scientific and Technical New York. 347pp.

McDowell, L. R. (1992). **Minerals in Animals and Human Nutrition.** Academic Press, San Diego. 524pp.

McIntosh, A.W. (1991). Trace Metals in Freshwater Sediments: A Review of the Literature and an Assessment of Research Needs. In: **Metal Ecotoxicology.** M.C. Newman & A.W. McIntosh (Eds.), Lewis Publishers, Michigan. 368pp.

Miller, P.A., Munkittrick, L.R. & Dixon, D.C. (1992). Relationship between the concentration of copper and zinc in water, sediment, benthic invertebrates and tissues of white sucker *Catostomus commersoni* at metal contaminated sites. **Canadian Journal of Aquatic Sciences** 49:978-984.

Moore, P.G. & Rainbow, P.S. (1987). Copper and zinc in an ecological series of Talitroiden Amphipod (Crustacea). **Oecologia** 73:120-126.

Nicolaidou A. & Nott J.A. (1998). Metals in sediment, seagrass and gastropod near a nickel smelter in Greece Possible interactions. **Marine Pollution Bulletin** 36(5): 360-365.

Nimmo, D.R., Willox, M.J., Lafrancois, T.D., Chapman, P.L., Brinkman, S.F. & Greene, J.C. (1998). The effects of metal mining on boundary waters of Yellowstone National Park, USA. **Environmental Management** 22(6):913-926.

Noël-Lambot, F., Gerday, C.H. & Disteche, A. (1978). Distribution of Cd, Cu and Zn in liver and gills of the eel *Anguilla anguilla* with special reference to metallothioneins. **Comparative Biochemistry and Physiology** 61C:171-187.

Nott, J.A. & Nicolaidou, A. (1994). Variable transfer of detoxified metals from snails to hermit crabs in marine food chains. **Marine Biology** 120(3): 369-377.

Novotny, V. (1995). Diffuse Sources of Pollution by Toxic Metals and Impacts on Receiving waters. In: **Heavy Metals Problems and Solutions.** W. Salomon, U. Forstner & S. Mader (Eds.), Springer-Verlag, Berlin. 412pp.

Oliff, W.D. (1977). Environmental pollution studies, C: Estuarine surveys, III: Richards Bay southern sanctuary. Progress report, CSIR/NIWR 34th steering committee meeting: Marine disposal of effluents (33):57-59.

Owens, R.E., Balls, P.W., & Price, N.B. (1997). Physicochemical processes and their effects on composition of suspended particulate material in estuaries: Implication for monitoring and modelling. **Marine Pollution Bulletin** 34 (1):51-60.

Pedersen, F., Bjoernestad, E. Andersen, H.V., Kjolholt, J. & Poll, C. (1998). Characterisation of sediment from Copenhagen Harbour by use of biotests. **Water Science and Technology** 37(6-7) 435-451.

Petri G. & Zauke G.P. (1993). Trace metal in crustacea in antartic ocean. **Ambio Stockholm** 22(8):529-536.

Pinder, J.E. & Giesy, J.P. (1981). Frequency distribution of the concentrations of essential and non-essential elements in largemouth bass, *Micropterus salmoides*. **Ecology** 62(2):456-468.

Plaskett, D. & Potter, J.C. (1979). Heavy metal concentrations in the muscle tissue of 12 species of teleost from Cockburn Sound, Western Australia. **Australian Journal of Freshwater Research** 30:607-616.

Phillips, D.J.H. & Rainbow, P.S. (1993). **Biomonitoring of trace aquatic contaminants.** Chapman & Hall, London. 371pp.

Rainbow, P.S (1995). Biomonitoring of heavy metal availability in the marine environment. **Marine pollution bulletin** 31(4-12):183-192.

Rainbow, P.S. & White, S.L. (1989). Comparative strategies of heavy metal accumulation by crustaceans: Zinc, copper and cadmium in a decapod, an amphipod and a barnacle. **Hydrobiologia** 174(3):245-262.

Regnier, P. & Wollast, R. (1993). Distribution of metals in suspended matter of the Scheldt estuary. **Marine Chemistry**. 43:3-19.

Reish, D.J. (1988). The Use of Toxicity Testing in Marine Environmental Research. In: **Marine Organisms as Indicators.** D.F. Soule & G.S Kleppel (Eds), Springer-Verlag. New York. 342pp.

Romeo, M. (1987). Trace metals in fish roe from the Mauritania coast. **Marine Pollution Bulletin** 28:50-53.

Rosenberg, D.M. & Resh, V. (1993). **Freshwater Biomonitoring and Benthic Macroinvertebrates.** Chapman & Hall, New York. 488pp.

Roux, D.J, van Vliet, H.R. & van Veelen, M. (1993). Towards integrated water quality monitoring: Assessment of ecosystem health. **Water S.A.** 19(4):275-280.

Saltes, J.G. & Baily, G.C. (1984). Use of fish gill and liver tissue to monitor Zn pollution **Bulletin of Environmental Contamination and Toxicology** 32:233-237.

Schlacher, T. & Wooldridge, T.H. (1996). Ecological responses to reductions in freshwater supply and quality in South Africa's estuaries: lessons for management and conservation. **Journal of Coastal Conservation** 2:115-130.

Schmidt-Nielsen, K (1990). **Animal Physiology, Adaptations and Environments.** Cambridge University Press, London. 338pp.

Seymore, T., Du Preez, H.H. &Van Vuren J.H.J. (1995). Manganese, lead and strontium bioaccumulation in the tissues of the yellowfish, *Barbus marequensis* from the lower Olifants River, Eastern Transvaal. **Water SA** 21(2):159-172.

Singh, N.K., Varma M.C. & Munshi D.J.S. (1990). Accumulation of copper, zinc iron and cadmium in certain freshwater fishes of River Subernarekha. **Journal of Freshwater Biology**. 2(3):189-193.

So, L.M., Cheung, R.Y.H. & Chan, K.M. (1999). Metal concentrations in tissues of Rabbitfish (*Siganus oramin*) collected form Tolo Harbour and Victoria Harbour in Hong Kong. **Marine Pollution Bulletin** 39(1-12):234-238.

Sly, P.G. & Hart, B.T. (1989). **Sediment/Water interactions.** Kluwer Academic Publishers, London. 701pp.

Sorensen, E.M.B. (1991) **Metal Poisoning in Fish.** CRC Press Inc Boca Raton, Florida. 374pp.

Spry, D.J. & Wood, C.M. (1985). Ion flux rates, acid base status and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. **Canadian Journal of Fishery Science** 42:1332-1341.

Spry, D.J. & Wood, M. (1989). A kinetic measurement for the measurement of zinc influx *in vivo* in the rainbow trout, and the effects of water borne calcium on the flux rates. **Journal of Experimental Biology** 142:425-446.

Sultana, R. & Rio, D.P. (1998). Bioaccumulation Pattens of Zn Copper Lead and Cadium in grey mullet *Mugil cephalus* (L) from Harbour Waters of Visakhapatnam, India. **Bulletin of Environmental Contamination and Toxicology** 60:949-955.

Sunda, W.G., Tester, P.A. & Huntsman S.A. (1990). Toxicity of trace metals to *Acarcia tonsa* in the Elizabeth River and southern Chesapeake Bay**. Estaurine, Coastal and Shelf Science** 30:207-221.

Syderman, W.J. & Jarman, W.M. (1998). Trace metals in seabirds stellar sea lion and forage fish and zooplankton from Central Carlifornia. **Marine Pollution Bulletin** 36(10):828-832.

Tack F.M.G., Vossius, H.A.H. & Verloo, M.G. (1996). A comparison between sediment metal fractions obtained from sequential extraction and estimates from single extraction. **International Journal of Environmental Analytical Chemistry** 63:61-66.

Taylor, D., Maddock, B.G. & Mance, G. (1985). The acute toxicity of nine grey list metals (arsenic boron, chromium, copper, lead , nickel, tin, and vanadium) to two marine fish

species *Limanda limanda* and grey mullet, *Chelon labrosus*. **Aquatic Toxicology** 7:135-144.

Tewari, H., Gill, T.S. & Pant, J. (1987). Impact of chronic lead poisoning on the haematological and biochemical profiles of a fish, *Barbus conchonium* (Ham) **Bulletin of Environmental Contamination and Toxicology** 38:748-756.

Van Der Hurk, P, Eertman, R.H.M., & Stronkhorst, J. (1997). Toxicity of harbour canal sediment before dredging and after offshore disposal. **Marine Pollution Bulletin** 34:244-249.

Van Loon, J.C. (1980). **Analytical Atomic Absorption Spectroscopy: Selected Methods.** Academic Press, New York. 337pp.

Vermeulen, L. & Wepener, V. (1999). Spatial and temporal variations of metals in Richards Bay Harbour, South Africa. **Marine Pollution Bulletin** 39(1-12):304-307.

Villegas-Navarro, A. & Villereal-Trevino, C.M. (1989). Uptake of zinc, copper and lead in Texas Cichlid, *Cichhasoma cyanoguttum*. **Differential Ecotoxicology and Environmental Safety** 29:187-199.

Virkanen, J. (1998). Effects of urbanization on metal deposition in the bay of Toolonlahti, Southern Finland. **Marine Pollution Bulletin** 36(9):729-738.

Wallace, J.H. & Van der Elst, R. (1975). **The Estuarine Fishes of the East Coast of Africa V5: Ecology, Estuarine Dependence and Status.** Oceanographic Research Institute Report 42. 63pp.

Watling RJ & Watling H, R (1982a). Metal Surveys in South African Estuaries II. Knysna River. **Water SA** 8(1):36-44.

Watling, R.J. & Watling, H.R. (1982b). Metal Surveys in South African Estuaries IV. Keurbooms and Bietou Rivers (Plattenberg Bay) **Water SA** 8 (1) 14-19.

Watling, R.J. & Watling, H.R (1982c). Metal Surveys in South African Estuaries V Kroome and Gamtoos (St Francis Bay) **Water SA** 8(1):87-91.

Watling, R.J. & Watling, H.R. (1982d). Metal Surveys in South African Estuaries I. Swartkop River **Water SA** 8(1):26-35.

Watling, R.J. & Watling, H.R. (1982e). Metal Surveys in South African Estuaries VI. Sundays River **Water SA** 8(1):92-95.

Watling, R.J. & Watling, H.R. (1983a). Trace metal surveys in Mossel Bay, St Francis Bay and Algoa Bay, South Africa **Water SA** 9:57-65.

Watling, R.J. & Watling, H.R. (1983b). Metal Surveys in South African Estuaries VII. Bushman, Kariega, Kowie and Great Fish Rivers. **Water SA** 9:66-70.

Watling, R.J., Talbot, MMJ-F., Branch, E.B. & Marsland S.A. (1985). Metal Survey in South African Estuaries IX, Buffalo River, **Water S.A.** 11(2):61-66.

Weerts, S.P. & Cyrus, D.P. (1999). A review of the ichthyofauna of the Mhlathuze Estuary. **South African Journal of Aquatic Sciences** 24(1/2):100-110.

Wepener, V. (1997). Metal Ecotoxicology of the Olifants River in the Kruger National Park and the Effect hereof on Fish Haematology. **Unpublished Ph.D Thesis**, Rand Afrikaans University, Johannesburg, South Africa.

Wepener, V. & Cyrus, D.P. (1997). The effects of Inter-basin Transfer on the Hydrochemistry, Benthic Invertebrates and Ichthyofauna of the Mhlathuze Estuary and Lake Nsezi: Second Interim Report. CRUZ Investigational Report 43.37pp.

Wepener, V. & Vermeulen, L.A. (1999). Comments on the water quality of the Mhlathuze Estuary in relation to determining the ecological integrity class. **South African Journal of Aquatic Sciences** 24(1/2):87-98.

Whitton, B.A. (1975). **River Ecology.** Blackwell Scientific Publishers, Oxford. 725pp.

Whitfield, A.K. & M.N. Bruton. (1989). Some implications of reduced fresh water inflow into eastern Cape estuaries, a preliminary assessment**. South African Journal of Science** 85: 691-694.

Whitfield, A.K. (1992). A characterisation of southern African estuarine systems. **South African Journal of Aquatic Sciences** 18(1/2):89-103.

Williams, P.R., Attrill, M.,J. & Nimmo, M. (1998). Heavy metal concentrations and bioaccumulation within Fal Estuary , A Reappraisal. **Marine Pollution Bulletin** 36(8):463-467.

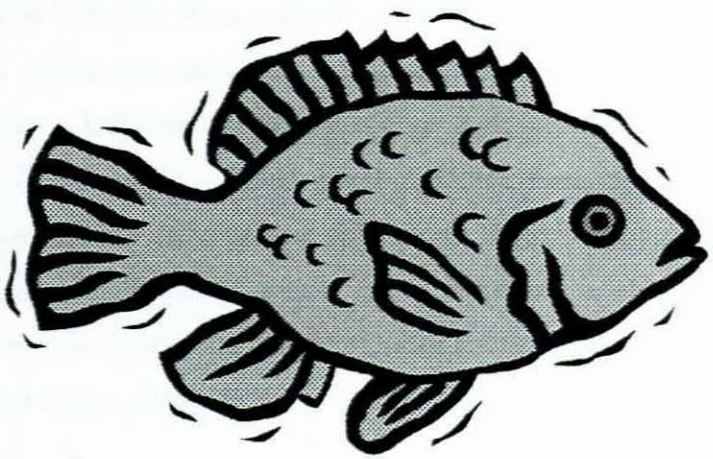
Williams, R.R. & Willward, G.E. (1998). Dynamics of particulate trace metal in the tidal reaches of the Ouse and Trent U.K. **Marine Pollution Bulletin** 37 (3-7):306-315.

Wolfe, D.A., Thayer, G.W. & Adams, S.M. (1976). Manganese, Iron, Copper and Zinc in Eelgrass, (*Zostera marina)* Community. In **Radioecology and Energy Resource.** C.E. Cushing (Ed.), Proceeds of the National Symposium on Radioecology, 4th, Oregon State University Corvallis, Dowden Hutchinson and Ross Stroudsburg.

Wollast, R. (1982). Behaviour of organic carbon, nitrogen and phosphorus in the Scheldt estuary. **Thalassia Jugoslavia** 18:11-34

Zar, J.H. (1984). **Biostatistical Analysis.** Prentice-Hall Inc. Englewood Cliffs, New Jersey. 718pp.

Chapter 5



CHAPTER 5

The effects of Cu and Pb on the haematology and acid-base balance of *L. dumerelii*

5.1 Introduction

Trace metals are of biological importance because of their role as micronutrients and toxins (Mason, 1991). These metals could act either through stimulatory or inhibitory effects. The increased anthropogenic impacts on aquatic systems have intensified investigations into interactions between these trace metals and aquatic organisms (Mance, 1987; Roux *et al.*, 1993).

Detailed investigations on the individual effects of heavy metals have been widely reported (Farmer, Ashfield & Samant, 1979; Van der Putte & Part, 1982; Taylor, *et al.*,1985). As natural waters receive effluents from different industries the impact on ecosystems is due to mixtures of metals rather than individual component metals. When metals are discharged into water, they show antagonistic, synergistic or additive effects on aquatic organisms (Mukhopahyay & Kona, 1985). Trace metal mixtures have been recorded to decrease reproduction success of fish (McFairlane & Frazin, 1978). Wong, Luk & Choi (1977) concluded that a mixture of metals within safe concentrations were very toxic to algae. Copper concentration that is toxic to plakton *Cyclops viridis* decreased in toxicity when Fe and Zn were added (Mukhopahyay & Kona, 1985).

Sublethal responses at biochemical level induce a sequence of functional alterations at higher level of organisation in an individual (Heath, 1987). This can take different forms of physiological stress. Laboratory studies must therefore also address diagnostic anomalities at low exposure levels (Cairns, 1981; Heath, 1987). The close association of the blood of the fish and the external medium enable haematology and acid base balance to be reliable tools in determining the pollution effects of waterborne pollutants to fish (Van Vuren *et al.*, 1994).

Gills can be considered as the interface of the organisms and its ambient environment (Sultana & Rio, 1998). Any change in the environment will be transferred through the gills and will consequently be reflected in the blood of the fish (Wepener, van Vuren & Du Preez,

1992a, b). Heavy metals are known to cause lifting of gill lamellae and disrupt the normal functioning ionic regulation and respiration (Cyriac, Anton & Nambisan, 1989). This may increase the amount of intracellular CO_2 that can result in disturbance in the pH regulation of the organism (Heisler, 1984, 1986). Gill damage places new demands on respiration and can result in anaerobic respiration thus increasing lactic acid production (Nikinmaa & Jensen, 1986).

Haematological procedures have been used for a long time by fishery biologists as a screening method to assess the state of health of fish stocks in fish hatcheries and fish biology research (Jensen, Nikinmaa & Weber, 1983; Nikinmaa & Jensen, 1986; Nikinmaa, 1992). Thus theory has been applied to investigate the conditions and to detect and diagnose diseases and parasite attacks on fish (Blaxhall, 1972). Besides diseases, many other factors, both environmental and endogenous can affect haematological characteristics. For example season, nutritional state, age and size, sex, sexual maturation and spawns, migrations, hormonal status and various stresses can all influence haematological parameters in fish (Wedemeyer & Mcleay, 1981).

Changes in haematological parameters caused by metals in fish have been widely examined (Wepener, 1991; Wepener *et al.*, 1992a, b; van Vuren *et al.*, 1994). Copper, (Nussey, van Vuren & Du Preez, 1995) and lead (Somero *et al.*, 1977) have both been found to alter the haematological parameters in fish. Copper toxicity in aquatic fresh water environments have been widely documented. Most studies on marine vertebrates are on acute toxicity (e.g. Mance, 1979; Taylor *et al.*, 1985). Copper was found to reduce the activity of branchial Na^+-K^+ ATPase in *Ictalurus punctatus* (Eddy, 1981). Van Vuren *et al.* (1994) found an increase in glucose and lactate and a decrease in mean cell volume following exposure of *Clarius gariepinus* to sublethal copper concentrations. Copper toxicity has been reported to decrease with the increased salinity (Mance, 1987, Janus, Cantona, van Gestel & Heijna-Merkus, 1989). The authors attributed this reduction in toxicity to the complexing action of the Cu ions in water. This action results in an antagonistic reaction. High concentrations of Pb have been shown to inhibit ALA-D activities (Villereal-Trevino *et al.*, 1986), and may inhibit erythropoiesis (Johansson-Sjöbeck & Larsson, 1979).

While it is important to quantify the amount of metals accumulated in water, sediments and biota (see Chapter 4), it is equally important to determine their effects on the metabolic

processes of aquatic organism. This involves exposing the biota to different metals and metal mixtures at environmentally relevant concentrations. The resultant effects can then be related to tissue concentrations, for the comprehensive assessment of metals as pollutants in the aquatic ecosystem. This chapter, therefore, addresses the sublethal effects of Cu, Pb and their mixture on the haematology and acid-base balance of *L. dumerelii*.

5.2 Materials and methods.

5.2.1 Sampling protocol

Groovy scaled mullet (*L. dumerelli*), ranging from 110-160 mm (mean 131.75) in standard length and 23.5 g to 68.7 g (mean 40.63) in mass were obtained from the Richards Bay Harbour using seine nets. Fish were transported to the hatchery of the Department of Zoology, University of Zululand in a transport tank containing aerated seawater. An antibiotic (13.2% Anchorpharm Furaltadone) was added to the water to reduce bacterial infection during transport. At the hatchery of the Department of Zoology, fish were placed into 1000l holding tanks. Water salinity in holding tanks was kept at 15‰ during the initial stages of acclimation. Water was recirculated through a biological filter. Fish were allowed to acclimate to laboratory conditions for three months. During acclimation fish were fed with protein rich (48%) commercially available fish pellets.

5.2.2 Description of bioassay

Following a three months acclimation period healthy fish were transferred to 10 x 40l tanks in the experimental flow through system in environmental control rooms (Fig. 5.1). Fish were allowed to acclimate for a further week prior to metal exposure. Water used for this experiment was obtained from the Richards Bay Harbour. Water was diluted to a salinity of 29‰ using dechlorinated water. The exposure salinity represents an average salinity at the Mhlathuze Estuary during the summer season. The water temperature of the experimental system was kept at 28° C. The mean water quality values recorded during the exposure of fish to Cu, Pb and combinations of Cu and Pb concentrations are presented in Table 5.1.

Salinity was measured using a temperature compensated salinometer, pH was measured using an ORION pH meter and dissolved oxygen was measured using a WTW OXI 92 Oximeter. Total ammonium (NH_4^+) was measured with commercially available reagent kit using a Merck S112 Spectrophotometer.

Fish were subjected to 14h light and 10h dark rhythms. These are day:night regimes as found in the Mhlathuze Estuary. The exposure concentrations of Cu and Pb in this experiment represent the maximum concentrations measured in water of Mhlathuze Estuary (Cu - 200 µg/l and Pb - 670 µg/l). The chemicals used were purchased from Merck and were of analytical grade. Stock solutions of 1000mg/l copper and lead were made in distilled water from copper (I) chloride (CuCl) and lead chloride ($PbCl_2$). The volume of CuCl or $PbCl_2$ stock solution necessary to obtain the required concentration was added to the storage tank.

Each experimental tank contained two fish. The flow rate of the tanks was maintained at 12 ml/min that allowed for total water replacement within 24 hours. Control fish were maintained simultaneously but without the addition of the metal to water. Fish were not fed for the duration of the exposure. Exposure period for each experiment (Cu, Pb, and Cu and Pb mixture) including the control was 96 hours. One long-term exposure was conducted, over 28 days, where fish were exposed to the mixture of Cu and Pb.

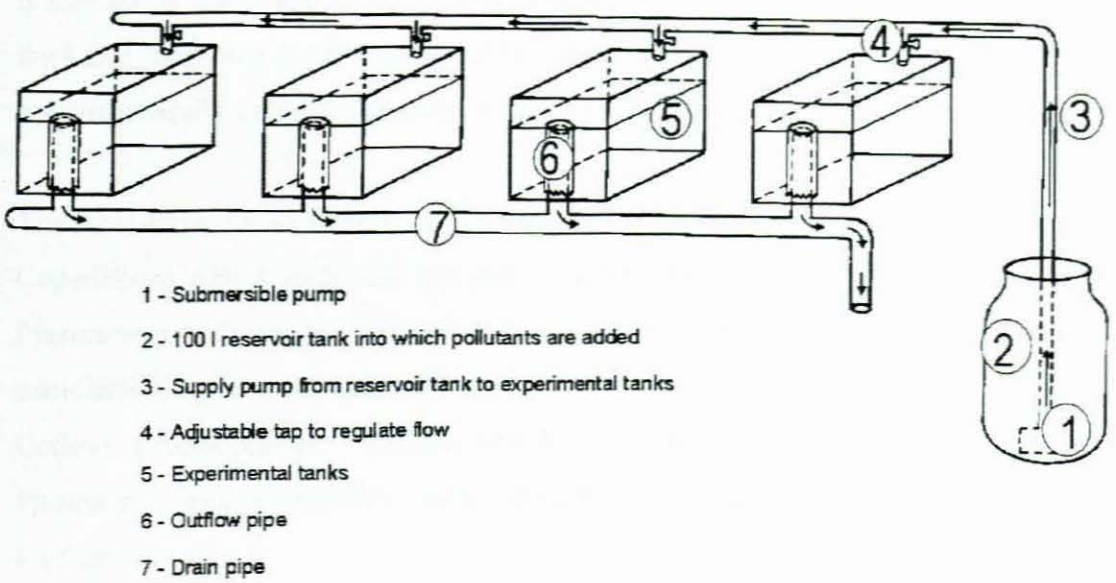


Fig. 5.1 Diagrammatic representation of the flow-through system used during the study.

5.2.3 Physiological variables

The control and experimental fish were removed individually at the end of each exposure period by means of the hand net. Standard length and mass of the fish were measured. Blood samples were immediately collected from the caudal aorta using a 1 mm heparinised plastic syringe. No anaesthesia was used as it can have an adverse effect on the blood thus affecting results. The eyes of the fish were covered to minimise struggling (Van Vuren *et al.*, 1994).

The haematological parameters measured were: white blood cell count (WBC), mean cell volume (MCV) red blood cell count (RBC), haematocrit (Hct), haemoglobin concentration (Hb), blood glucose concentration, blood lactate concentration, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Acid-base and ionic regulation parameters measured were sodium (Na), potassium (K), chloride (Cl), pH, carbon dioxide partial pressure, (PCO_2), and bicarbonate (HCO_3^-).

Blood cell counts and MCV were measured with the Sysmex CC120 Microcell Counter. Haemoglobin concentrations were determined with the cyan-met haemoglobin method and haematocrit was measured with the microcentrifuge method as described by Korzhuev (1964). Mean cell haemoglobin and MCHC were calculated according to the formulae described by Dacie and Lewis (1963). Plasma lactate was determined colourmetrically using the LDH, GPT and NAD method of Boehringer Mannheim. Blood glucose was determined colourmetrically using the glucose/ GOD-PAP method.

The acid-base parameters (pH, PCO_2 and HCO_3^-) were measured using a Radiometer Copenhagen ABL3 acid-base analyser. Blood was centrifuged for 10minutes at 5000 rpm. Plasma was extracted from the centrifuged solution from which plasma chloride, Na, K and osmolarlity were measured. Plasma Cl concentrations were measured using a Buchner Cotlove Chloridometer. Sodium and K were measured using Radiometer FLM3 Flame Photometer and osmorlality was measured using a Wescor 5100B Vapour pressure Osmometer.

5.2.4 *Statistical analyses*

Statistical determination of differences between control values and different exposures was carried out using the unpaired Student t-test. Data was presented as mean ± standard error of the mean. Statistical analysis between groups was performed using ANOVA and Tukey's multiple comparison test. The significance level was taken as $P< 0.05$.

5.3 Results

The mean water quality values recorded during the exposure of fish to Cu, Pb and combinations of Cu and Pb concentrations are presented in Table 5.1.

Table 5.1 Mean water quality values as recorded during the different bioassay experimental studies.

Exposure	Salinity (‰)	pH	Ammonia (mg/l)	Dissolved O_2 (mg/l)	Temperature (°C)
Holding tank	15.5±0.267	7.68±0.012	0.028	6.64±0.082	21
Control	29	7.27±0.023	0.041	6.7±0.089	28
Cu 96 h	29	6.86±0.039	0.019	6.9±0.145	28
Pb 96 h	29	7.20±0.028	0.017	7.2±0.101	28
CuPb 96 h	29	7.12±0.038	0.025	6.8±0.198	28
CuPb 28 d	29	7.55±0.051	0.079	7.4±0.210	28

5.3.1 *Haematological parameters*

Slight increases in RBC (Fig. 5.2A) were recorded following short-term exposure to Pb and long term exposure to Cu and Pb mixture. A significant increase ($P<0.05$) in RBC was recorded following short-term exposure to the metal mixture. An insignificant decrease in RBC was recorded following exposure to Cu. Slight increases in Hb (Fig 5.2B) were recorded following short-term exposure to Pb and short-term exposure to Cu and Pb mixture.

A significant increase in Hb was recorded following long-term exposure to the metal mixture. An insignificant decrease in Hb was recorded following exposure to Cu.

Slight decreases in Hct (Fig. 5.2C) were recorded following short-term exposure to Cu and short term exposure to Pb. Insignificant increases in Hct were recorded following short term and long term exposures to Cu and Pb mixture. Slight increases in MCV (Fig. 5.2D) were recorded following exposure to short term Pb, short term Cu and Pb and long-term exposure to metal mixtures. An insignificant decrease in MCV was recorded following exposure to Cu. Slight increases in MCH (Fig 5.2E) were recorded following short-term exposure to Cu, and short- and long-term exposure to Cu and Pb mixture. Slight increases in MCHC (Fig 5.2F) were recorded following short-term exposures to Pb and Cu and Pb mixture as well as long-term exposure to Cu and Pb mixture. No change was recorded in MCHC following exposure to Cu. Slight increases in WBC (Fig. 5.2G) were recorded following short-term exposure to Pb and short- and long-term exposures to Cu and Pb mixtures. An insignificant decrease in WBC was recorded following exposure to Cu. Slight increases in glucose (Fig 5.2H) were recorded following all short-term exposures and a significant increase ($P<0.05$) was recorded following long-term exposure to metal mixture.

5.3.2 Acid-base balance and osmoregulatory parameters

Slight increases in lactate (Fig. 5.3A) were recorded following short-term exposure to Pb and short- and long-term exposure to Cu and Pb mixtures. An insignificant decrease in lactate was recorded following exposure to Cu. Slight decreases in Na (Fig. 5.3B) were recorded following short-term exposure to Pb and an insignificant increase was recorded following a long-term exposure to metal mixtures. A slight increase in K (Fig. 5.3C) was recorded following short-term exposure to Pb and long-term exposure to Cu and Pb mixture. Slight increases in Cl (Fig. 5.3D) were recorded following exposure to Cu and short-term exposure to Pb, whereas the exposures to the metal mixture did not result in any changes in Cl levels. A slight increase in osmolarity (Fig. 5.3E) was recorded following short-term exposure to Pb. Insignificant decreases were recorded following short-term exposure to Cu and short-term exposure to the metal mixture.

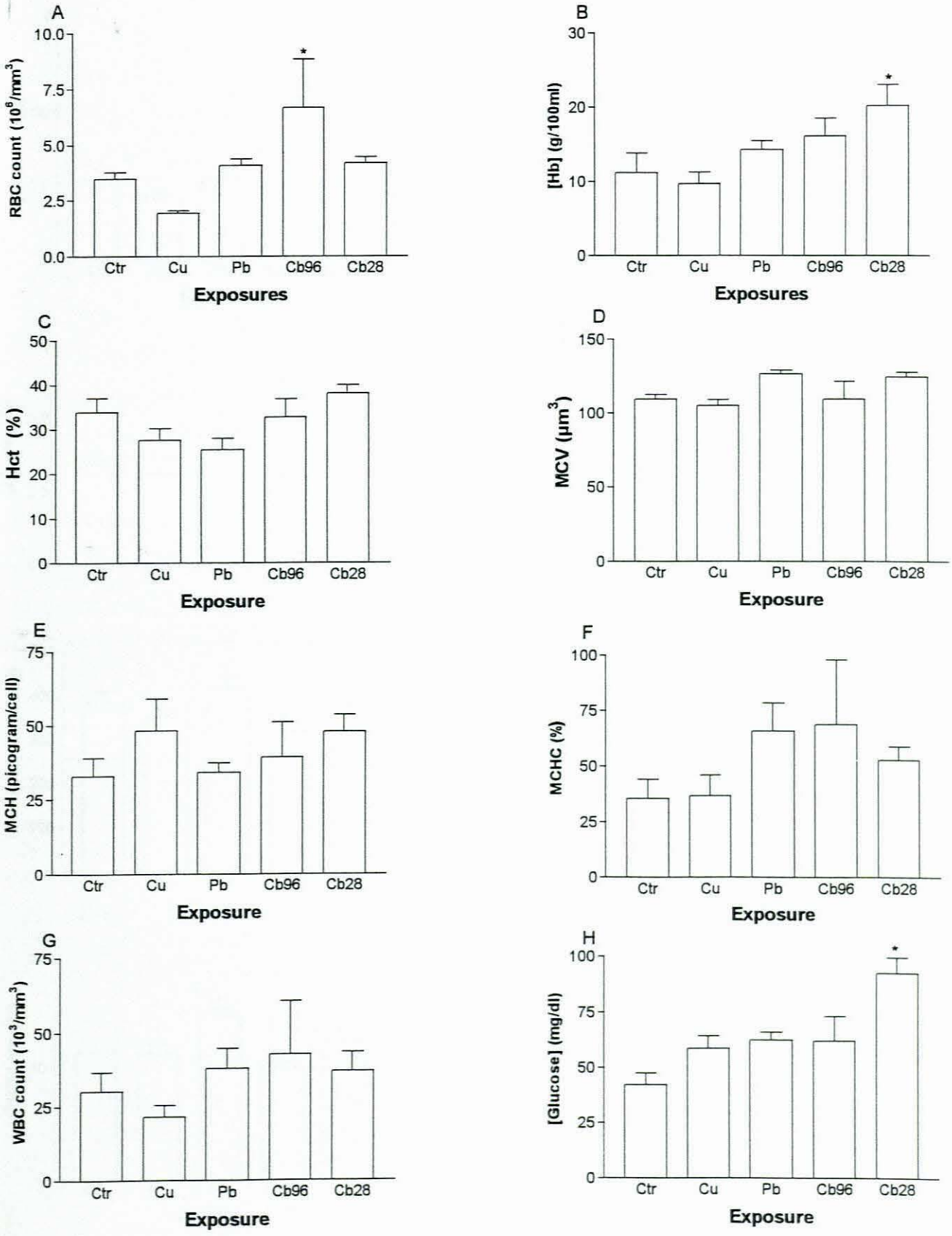


Fig. 5.2 Effects of exposure to Cu and Pb as single solutions and as mixtures on RBC (A), Haemoglobin (B), Haematocrit (C), MCV (D), MCH (E), MCHC (F), WBC (G) and Glucose (H). Exposure groups are represented as follows: Ctr = control, Cu = Copper, Pb = Lead, Cb96 = Cu and Pb 96 hours Cb28 = Cu and Pb long-term (28 days). Results reported as mean ± standard error and asterisks (*) indicate significant differences between controls and metal exposures ($p<0.05$).

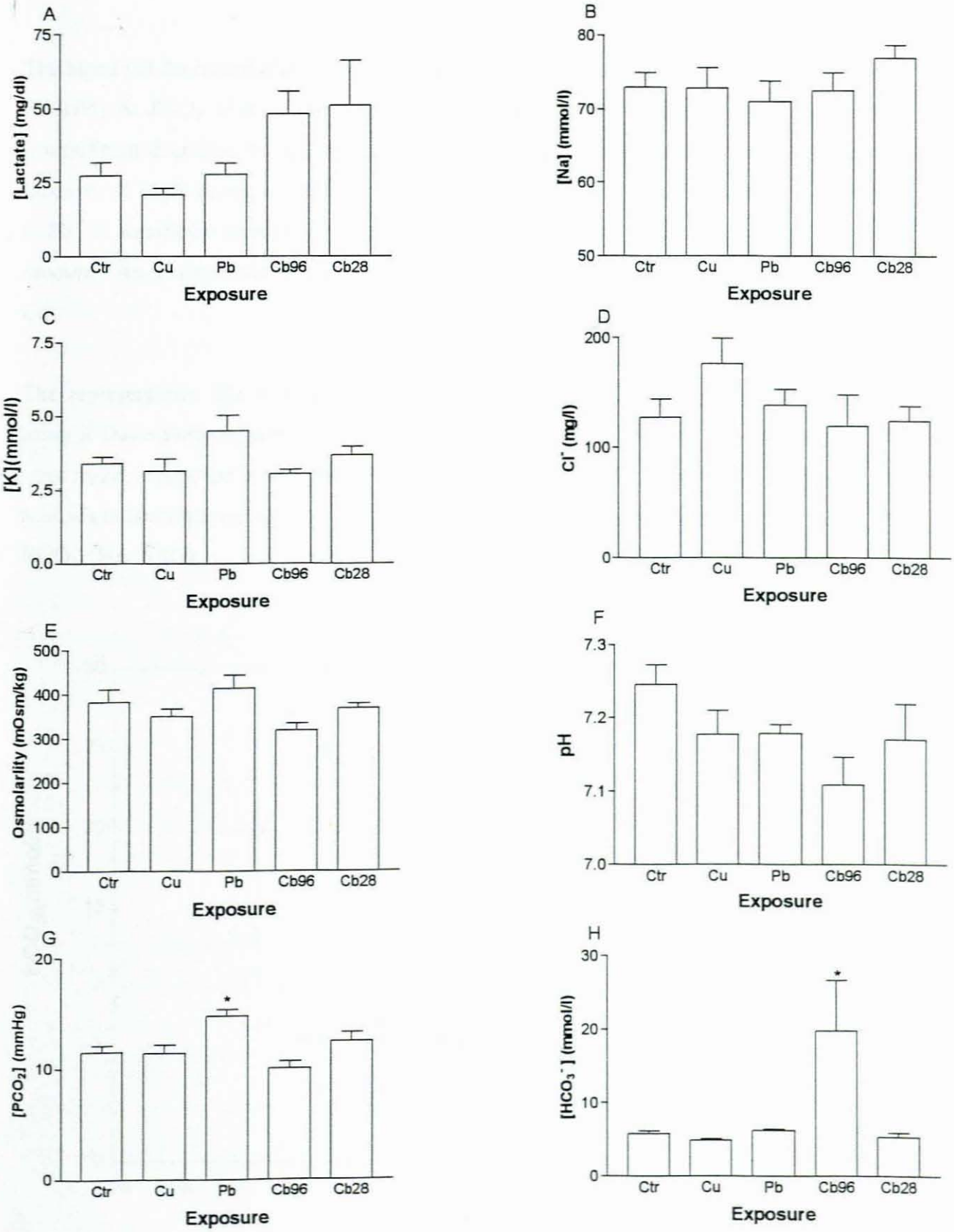


Fig. 5.3 Effects of exposure to Cu and Pb as single solutions and as mixtures on, lactate (A), Na (B), K (C), Cl (D), Osmorlality (E), pH (F), PCO_2 (G) and HCO_3 (H). Exposure groups are represented as follows: Ctr = control, Cu = Copper, Pb = Lead, Cb96 = Cu and Pb 96 hours Cb28 = Cu and Pb long-term (28 days). Results reported as mean ± standard error and asterisks (*) indicate significant differences between controls and metal exposures ($p<0.05$).

The blood pH decreased (Fig. 5.3F) during all metal exposure groups. A significant increase ($P<0.05$) in PCO_2 (Fig. 5.3G) was recorded following short-term exposure to Pb and insignificant decreases in PCO_2 were recorded following short-term exposure to Cu and Pb mixture. A slight increase in HCO_3^- (Fig. 5.3H) was recorded following short-term exposure to Pb. A significant increase ($P<0.05$) was recorded following short-term exposure to metal mixture. An insignificant decrease in HCO_3^- was recorded following short-term exposure to Cu.

The representative acid-base changes during the different exposure groups are illustrated using a Davenport diagram (Fig. 5.4). The only significant changes in acid-base balance were found during the mixed Cu and Pb exposure that resulted in a metabolic acidosis. The acidosis is accompanied by concomitant significant increases in blood lactate (Fig. 5.3A) and HCO_3^- (Fig. 5.3G).

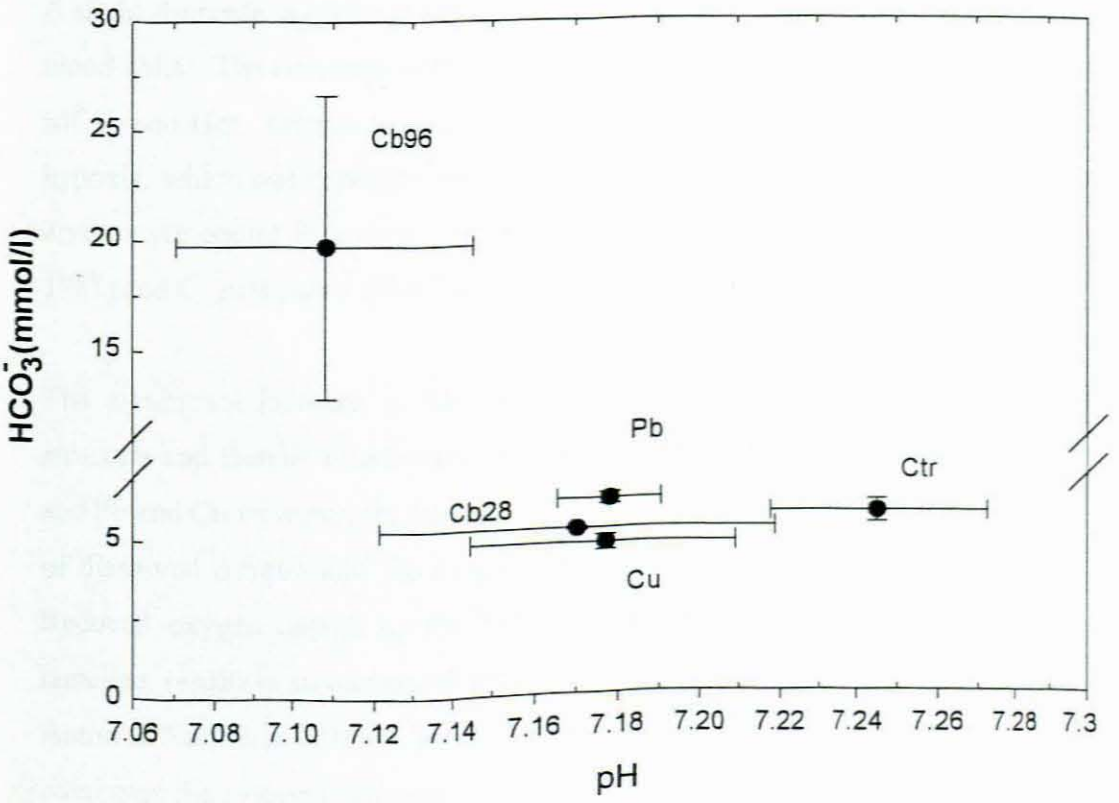


Fig. 5.4 Davenport diagram showing the effects of metals on the acid-base balance *of L. dumerelii*.

5.4 Discussion

Fish gills are in direct contact with the environment and the change occurring in the environment will invariable be reflected in the physiology and in particular the haematological parameters (Blaxhall, 1972). This means that the haematology is affected by the water quality in which a given population of fish inhabits.

It is known that haematology is also influenced by different environmental factors such as temperature, physiological condition of the organism (Van Vuren, 1977). In order to ensure that haematological values measured during the metal exposures could not have been caused by the change in environmental parameters, temperature and photoperiod were kept constant, and fish of the same size were used.

5.4.1 Haematological changes

A slight decrease in erythrocytes during Cu exposure indicates an inhibited production of red blood cells. The decrease, although not significant, was accompanied by a decrease in Hb, MCV, and Hct. Immature cells with less haemoglobin were being produced in response to hypoxia, which was probably the result of metal induced gill damage. Similar reductions in erythrocyte counts following exposure to Cu were recorded in *Clarias lazera* (El Domiaty, 1987) and *C. gariepinus* (Van Vuren *et al.*, 1994).

The significant increase in RBC (erythrocytosis) can be attributed to alterations in gill structure and thereby interference in oxygen uptake. During exposure of *L. dumereli* to Pb, and Pb and Cu mixtures, the fish developed an oxygen deficiency. The decreased availability of dissolved oxygen and the oxygen debt resulted in internal hypoxia (Tort *et al.*, 1987). Reduced oxygen uptake by the gills, which may have caused by the lifting of the gill lamellae, results in an increase in the release of RBC's from the haemopoeitic tissues (Cyriac, Anton & Nambisan, 1989). The increase in RBC's indicates the strategy *L. dumereli* uses to overcome the oxygen deficiency.

Mean cellular haemoglobin and MCHC provide an indication of RBC status. They reflect the supply of cost constituents for the haemoglobin synthesis (Larsson, Haux & Sjöbeck,

1985). Increase in RBC's with no change in Hb and Hct and a resultant increase in cell size (MCV), MCH and MCHC are indicative of haemodilution (Van Vuren *et al.*, 1994). Either cellular swelling or mortality of small immature cells accomplishes the increased RBC size. Increases in the RBC accompanied by an increase in Hb and a slight increase in MCV also indicate cellular swelling through haemodilution to compensate for increased oxygen requirements. Haemodilution is regarded as a mechanism by which an irritating factor in the circulatory system is reduced. The swelling of the red blood cells through this process is the result of the stimulation of the β-adregenic receptors by catecholamines (Smit *et al.*, 1979) and is considered a general stress response (Cameron, 1978).

With the increase of RBC and MCV in observed during the short-term exposure to the Cu and Pb it would be expected that the Hb would also increase. However they remained constant. That would suggest that ALA-D, which synthesises haemoglobin was inhibited. The enzyme ALA-D catalyses the formation of porphobilinogen from delta aminolevulinic acid thus taking part in haemoglobin synthesis. Lead poisoning inhibits ALA-D activity and less haemoglobin is formed (Somero *et al.*, 1977; Sorensen, 1991). Therefore increased RBC release takes place in response to Hb inhibition (Johansson-Sjobeck & Larsson, 1979). Lead poisoning also causes direct erythrocyte injury and the cells become fragile and easily distorted (Sordyl, 1990; Sorensen, 1991). According to Somero *et al.* (1977) fish respond to lead induced disturbance by erythrocyte formation and stimulation of erythropoiesis in various haematopoietic tissues.

Haematocrit readings are valuable in determining the effects of stressor on the health of fish (Wedermeyer & Yasutake, 1977). It is also used to determine the oxygen carrying capacity of blood. A high Hct value would imply polycythemia induced by stress and haemoconcentration caused by gill damage and impaired osmoregulation (Wedemeyer & McLeay, 1981). However during this study the Hct values remained largely unaffected by changes in cell counts. This is demonstrated by the significant increase in RBC (Fig 5.2A) and increase in WBC (Fig 5.2G) recorded during the short-term metal mixture exposure, which does not result in a concomitant increase in Hct (Fig 5.2C).

White blood cells play a major role in the defence mechanism of fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Each type of leukocyte has unique

specific functions. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue, lymphocytes produce antibodies (Ellis *et al*, 1978; Wedemeyer & McLeay, 1981) and thrombocytes have an important function in blood clotting (Wepener, 1991). Dawson (1985) reported an increase in WBC (trombocytes monocytes and eosinophyls) following long-term exposure of fish to Pb. The increases in WBC observed during this study could be attributed to a stimulation of the immune system in response to tissue damage caused by Pb and the Pb and Cu mixtures. Histological damage to tissue is most likely to occur on the gills, liver and muscle tissues during metals exposure (Van Vuren *et al.*, 1994).

Increases in blood glucose reveal a disruption in carbohydrate metabolism, which is probably a hormonal response (Nikinmaa, 1982). This occurs by induction of glucocorticoids. Tewari, Gill & Pant (1987) found an increase (42%) of glucose in blood and depletion of glycogen and that was attributed to secondary stress response mediated through hormones of the pituitary (adrenalin). The increase in blood glucose observed during all exposures was probably due to an elevated cortisol release from the interrenal cells. Schreck & Lorz (1978) showed a rapid and persistent elevation of cortisol in salmon exposed to Cu at sublethal concentrations. This hormone seemingly mobilises liver glycogen and glucose synthesis from proteins. Hyperglycaemia is a common response to stress in fish caused by factors such as handling or hypoxia (Heath, 1991). Heath (1991) showed an increase in glucose concentrations in bluegill, *Lepomis macrochirus* when exposed to water-borne Cu. Hyperglycaemic conditions observed during the long-term exposure could be related to inhibition the functioning of hepatic hormones such as insulin (Tewari *et al.*, 1987; Van Vuren *et al.*, 1994). Haux, Larsson, Lithner & Sjöbeck (1986) recorded significant increase in blood glucose concentration when whitefish, *Coregonus* spp. was exposed to lead contaminated water. Significant increase in blood glucose concentrations were also reported by Tewari *et al.* (1987) following exposure of *Barbus conchonius* to Pb poisoning.

The increase in lactate after exposure to the metal mixture implies an increase in anaerobic glycolysis. This occurs in the muscles as a result of insufficient oxygen supply from the ambient water to the blood due to gill damage (Nikinmaa & Jensen, 1986). As the mechanism for removal of lactate in fish is a fairly slow process (Jensen, Nikinmaa & Weber, 1983) exposure of fish for an extended period like the 28 days exposure in this study, resulted in a significant increased in lactate concentration in plasma.

5.4.2 *Acid-base balance and osmoregulation*

Estuarine organism may be exposed to solutions that are either hypertonic or hypotonic. The organisms exposed to hypotonic media responds to the increased osmotic entry by increased urine production. This leads to potential loss of salts such as Na^+ and Cl^- and to balance up the loss ions are taken up actively across the gill against the concentration gradient (Jones 1975; Eddy 1981). An organism exposed to near seawater has a tendency to be dehydrated. Seawater is taken up through the mouth and water together with salts is absorbed in the intestine. Active pumping of ions occurs across the gills. This branchial fluxes are associated with chloride cells which contains an enzymes Na^+-K^+ ATPase responsible for pumping Na^+ out of the cells and K^+ in the opposite direction (Eddy 1981). This whole process by which the total electrolyte content, and the water volume are held constant is termed osmoregulation (Heath, 1987).

Pollutants may cause disturbances in the osmoregulatory mechanism of fish. Measuring the blood plasma sodium, potassium, chloride and osmolarity (Heath 1987), generally elucidates changes in osmoregulation due to some stressor. Metal exposure of fish for a short duration has been found not to severely alter osmorality of fish (Heath, 1987). Fish exposed to metals for a long duration of up to 6 months have been found to reduce the activity of Na^+-K^+ ATPase, which leads to permeability of the gills and passive movement of ion such as K^+ into the cells and Na^+ out of the cells (Lewis & Lewis 1981). Reabsorption of Na^+ via renal tubules is reduced due to the decrease in Na^+-K^+ ATPase.

There were no significant alterations in osmoregulation in all short-term and long term exposures. This could be attributed to relatively short length of experiments. According to Eddy (1981), changes in osmoregulatory mechanism may not occur immediately in response to stress but may take days to develop and similarly normality may only be achieved a number of days after removal of stress. Copper elicits a slight increase in blood osmolarity after 29 days (Heath, 1987). Slight increases in K^+ and slight decreases in Na^+ were found in fish exposed to Pb in brackish water for 30 days (Heath, 1987). Copper administered to flounder only resulted in a slight increase in osmoregulation after 42 days.

There were no drastic changes in the plasma K^+ during Pb short-term exposure. An increase, although insignificant during Pb exposure may have been a response by fish to balance the osmotic difference resulting from the loss of Na^+ ions. This is the mechanism employed by fish for osmotic balance. After short-term exposure to Cu slight increase changes in Cl^- were recorded. Copper poisoning in fish cause damage to gills, which would impair the functioning of chloride cells (Nussey *et al.*, 1995). The increase in Cl^- would be a result in the inability of cell membranes to eliminate Cl^- that had been taken up in the intestine or the renal tubules. Increased permeability of gill lamellae as a result of metal pollution could result in passive uptake of Cl^- from seawater into the blood. Copper also induces the proliferation of chloride cells. (Heath, 1987). The increase may be caused by an increased number of cells taking up Cl^-.

Heavy metals are one class of pollutants, which have a disruptive influence on the structural organisation of the gill tissue. Because the gills are intimately associated with the ionic regulation it is predictable that heavy metals will influence some aspect of osmotic and ionic regulation in fish (Eddy, 1981). Lewis & Lewis (1971) found a significant decrease in osmotic pressure of channel catfish, *Ictalurus punctatus* when exposed to Cu with osmotic pressures reduced by 83% in seawater fish.

The role of gills in both acid and ionic regulation is intimately linked via the $Na/H^+(NH_4^+)$ and Cl^-/HCO_3^- exchange mechanisms (Spry & Wood, 1985). During normal steady state conditions in fish, there is a continual production of surplus H^+ and OH^- ions. These ions are eliminated from the body fluids by the excretory organs of the fish at the same rate as they are produced such that the pH of the body compartment is kept constant within narrow limits (Rankin & Jensen 1992). Fish blood contains proteins and bicarbonate buffers, which are responsible for controlling transient changes in extracellular pH (Heisler, 1986; Rankin & Jensen 1992). In order to compensate for acid-base disturbances, changes in the transfer of acid-base relevant ions between fish and the environment are induced (Heisler, 1986).

During stress however gills are damaged and this affects the exchange of ions across the gill surface. Protein and bicarbonate buffers can also be disturbed (Heath, 1987). This results in changes in pH values of the blood, which is referred to as acidosis or alkalosis (Heisler, 1984; Heisler, 1986). Acidosis induced by stress can be respiratory induced through elevation of

blood CO_2 tension or metabolic through elevation of lactic acid concentration (Nikinmaa & Jensen, 1986).

It is evident from the Davenport diagram (Fig. 5.4) that only the short-term exposure to the metal mixture resulted in significant acid-base disturbances. The decreases noted in blood pH during all the other exposures are only related to increased levels of blood lactate with no concomitant changes in bicarbonate or ion levels. These transient disturbances in pH are probably already compensated for, and have returned to near normal conditions, as shown by the insignificant decrease in pH.

In the case of the metal mixture exposure the changes in blood pH initiated a compensatory reaction involving increase in bicarbonate buffer stores (Cameron, 1978). The production of lactic acid acidifies the blood (Nikkinmaa & Jensen, 1986) and causes a marked extracellular acidosis and as a compensatory mechanism, HCO_3^- levels are increased to neutralise the acidic conditions. However, the over-production of HCO_3^- is probably the result of β-adrenergic stimulation of red blood cells, which affect the bicarbonate by slowing the conversion of bicarbonate to CO_2 (Nikkinmaa, 1992), as well as increased influx of bicarbonate ions across the gills. The β-adrenergic stimulation of the red blood cells is evident from the cellular swelling (increased MCHC) recorded during this exposure. A constant supply of oxygen is maintained by increases in red blood cells (Holeton *et al.*, 1983).

5.5 Conclusion

From the results of this study it is clear that short-term exposure to the combination of Cu and Pb resulted in the most stress to the test organisms. Exposure to the individual metals did not alter the haematology or acid-base balance and osmoregulation significantly. It is clear that a number of compensatory mechanisms contributed towards maintaining homeostasis. The results also indicated that the physiological responses were related to secondary reactions to reduced oxygen uptake due to disturbances at the gill-water interface. In order to delineate the toxicokinetic response it is recommended that tissue concentrations of metals be measured in conjunction with physiological parameters. This would provide an indication of

whether the metals were taken up, which tissues were affected and whether the metals were regulated.

5.6 References

Blaxhall, P.C. (1972). The haematological assessment of the health of freshwater fish: A review of selected literature. **Journal of Fishery Biology** 4:593-604.

Cairns J. Jr. (1981). Biological monitoring Part IV: Future needs. **Water Research** 15:941-952.

Cameron, J.N. (1978). Regulation of blood pH in teleost fish. **Respiration Physiology** 33:129-144.

Cyriac, P.J., Antony, A. & Nambissan, P.N.K. (1989). Haemoglobin and hematocrit values in the fish *Oreochromis mossambicus* (Peters) after short-term exposure to copper and mercury. **Bulletin of Environmental Contamination and Toxicology** 43:221 320.

Dacie, J.V. & Lewis, S.M. (1963). **Practical Haematology.** J & A Churchill, London. 435pp.

Dawson, A.B. (1985). The haemotopoetic response in catfish, *Amicrus nebulosus* to chronic lead poisoning. **Biology Bulletin** 68:335-343.

Eddy, F.B. (1981). Effects of Stress on Osmotic and Ionic Regulation in Fish. In **Stress and Fish.** A.D. Pickering (Ed.), Academic Press, London. 367pp.

EL-Domiaty, N.A. (1987). Stress response of juvenile *Clarius lazera* elicited by copper. **Comparative Biochemistry and Physiology** 88C:259-262.

Ellis, E.A., Roberts, R.J. & Tyler, P. (1978). **Fish Pathology**. Bailliere-Tindall, London. 318pp.

Farmer, G.J., Ashfields, D. & Samant, H.S. (1979). Effects of zinc on juvenile atlantic salmon *Salmar salar* :Acute toxicity, food intake, growth and bioaccumulation. **Environmental Pollution** 19:103-117.

Heath, A.G. (1987). **Water Pollution and Fish Physiology.** CRC Press Inc. Boca Raton, Florida. 245pp.

Heath, A.G. (1991). Effects of water-borne copper on the physiological responses of bluegill, *Lepomis macrochirus* to acute hypoxic stress and subsequent recovery. **Comparative Biochemistry and Physiology** 3:559-564.

Haux, C., Larsson, A., Lithner, G. & Sjöbeck, M (1986). A field study of physiological effects on fish in lead contaminated lake. **Environmental Toxicology and Chemistry** 5:283-288.

Heisler, N. (1984). Acid-Base Regulation in Fishes. In: **Fish Physiology Vol X.** W.S. Hoar & D.J. Randall (Eds.), Academic Press, New York. 456pp.

Heisler, N. (1986). Mechanisms and Limitations of Fish Acid-Base Regulation. In: **Fish Physiology:Recent Advances.** S. Nilsson, & S Holmgren, (Eds.), Croom Helm, London. 198pp.

Heisler, N. (1993). Acid-base Regulation in Response to Changes of Environmental Characteristics and Capacity. In: **Fish Ecophysiology**. . J.C. Rankin & F. B. Jensen (Eds), Chapman and Hall, London. 421pp.

Janus, J.A., Cantona, J.H., van Gestel, C.A.M. & Heijna-Merkus, E. (1989). **Integrated Criteria Document: Copper Effects.** National Institute of Public Health and Environmental Protection Report 758474009. 91pp.

Jensen, F.B., Nikkinmaa M. & Weber, R E. (1983). Effects of exercise on acid base balance and respiratory function in blood of the teleost, *Tinca tinca*: Respiratory physiology. **Elsevier Medical Press** 51:291-301.

Johansson-Sjobeck, M-L. & Larsson, A. (1978). The effects of cadium on the haematology and the activity of aminolevulinic acid dehydratase (ALA-D) in blood and haematopoietic tissues of the flounder *Pleuronectes flesus* L. **Environmental Research** 17:191-204.

Jones, M.B. (1975). Synergistic effects of salinity, temperature and heavy metals on mortality and osmoregulation in marine estuarine Isopod (Crustacea). **Marine Biology** 30:13-20.

Korzhuev, P.A. (1964). Methods of Study of Blood in Fish. In: **Techniques for the Investigation of Fish Physiology**. E.P. Pavlovski (Ed.), Israel Programme for Scientific Translation, Jerusalem. pp 2-10.

Larsson, A., Haux, C., Sjöbeck, M. & Lithner, G. (1984). Physiological effects of an additional stressor on fish exposed to a simulated heavy-metal-containing effluent from sulfide ore smeltry. **Ecotoxicology and Environmental Safety** 8:118-128.

Lewis, S.D. & Lewis W.M. (1971). Effects of zinc and copper on the osmolarity of the blood serum of the channel catfish *Ictalurus punctatus* and golden shine *Noternigonus crysoleucas*. **Transactions of American Fishery Society** 100:639-643.

Mance, G. (1987). **Pollution Threat of Heavy Metals in Aquatic Environments.** Elsevier Applied Science, England. 372pp.

Mason, C.F (1991). **Biology of Freshwater Pollution.** Longman Scientific & Technical, New York. 347pp.

Mc Fairlane, G.A. & Franzin, W.G. (1980). An examination of Cd, Cu and Hg concentrations in the liver of northern pike, *Esox lucius* and white sucker, *Catostomus commersoni* from five lakes near a base metal at Flin Flon, Manitoba. **Canadian Journal of Fish Aquat.** 37:1573-1578.

Mukhopahyay, M.K. & Kona, S.K. (1985). Effects of copper zinc and iron mixtures on fish and aquatic ecosystems. **Environment and Ecology** 3(1):58-65.

Nikinmaa, M. (1982). Effects of adrenalin on cell volume and concentration gradient of proteins across the red cell membrane in rainbow trout, *Salmo gairdneri*. **Molecular Physiology** 2:287-297.

Nikkinmaa, M. (1992). How does environmental pollution affect red cell function in fish? **Aquatic Toxicology** 22:227-238.

Nikkinmaa, M. & Jensen, F.B. (1986). Blood oxygen transport and acid base status of stressed trout *Salmo gairdneri* : Pre- and post branchial values in winter fish. **Comparative Biochemistry and Physiology** 84A(2):391-396.

Nusssey, G., van Vuren, J.H.J. & Du Preez, H.H. (1995). Effects of copper on the haematology and osmoregulation of the Mozambique tilapia, *Oreochromis mossambicus*. **Comparative Biochemistry and Physiology** 3:369-380.

Rand, G.M. & Petrocelli, S.R. (1985). **Fundamentals of Aquatic Toxicology.** Hemisphere Publishers, New York. 666pp.

Rankin, J.C. & Jensen, F.B. (1992). **Fish Ecophysiology.** Chapman and Hall, London. 421pp.

Roux, D.J., van Vliet, H.R. & van Veelen, M. (1993). Towards integrated water quality monitoring: Assessment of ecosystem health. **Water S.A.** 19(4):275-280.

Smit, G.L., Hattingh, J. & Burger, A.P. (1979). Haematological assessment of the effects of the anaestethic MS 222 in natural and neutralised form in three freshwater fish species: Interspecies differences. **Journal of Fish Biology** 15:633-643.

Schreck, C.B. & Lorz, H.W. (1978). Stress response of coho salmon, *Onchorynchus kisutch*, elicited by cadium and copper and potential use of cortisol as an indicator of stress. **Journal of Fish Biology** 35:1124-1131.

Sordyl, H. (1990). Influence of exposure time and H^+ concentrations of the water on the effects of sublethal Pb^{2+} loads on blood parameters of the rainbow trout *(Salmo gairdneri)* **Zoological Journal of Physiology** 94:31-41.

Somero, G.N., Chow, T.J., Yancey, P.H. & Snyder, C.B. (1977). Lead accumulation rates in tissues of the estuarine teleost fish, *Gillichthys mirabilis*: Salinity and temperature effects. **Archives of Environmental Contamination and Toxicology** 6:337-348.

Sorensen, E.M.B. (1991). **Metal Poisoning in Fish.** CRC Press Boca Raton, Florida. 374pp.

Spry, D.J. & Wood, C.M. (1985). Ion flux rates, acid base status and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. **Canadian Journal of Fishery Science** 42:1332-1341.

Sultana, R. & Rio, D.P. (1998). Bioaccumulation patterns of zinc, copper, lead and cadium in grey mullet *Mugil cephalus* (L) from harbour waters of Visakhapatnam, India. **Bulletin of Environmental Contamination and Toxicology** 60:949-955.

Tart, L., Torres, P. & Flos, R. (1987). Effects on dogfish haematology and liver composition after acute copper exposure. **Comparative Biochemistry and Physiology** 87C:349-353.

Tewari, H., Gill, T.S. & Pant, J. (1987). Impact of chronic lead poisoning on the haematological and biochemical profiles of a fish, *Barbus conchonius* (Ham). **Bulletin of Environmental Toxicology and Chemistry** 38:748-752.

Van der Putte, I. & Part, P. (1982). Oxygen and chromium transfer in perfused gills of rainbow trout (*Salmo giardneri*) exposed to hexavalent chromium at two different pH levels. **Aquatic Toxicology** 2:31-45.

van Vuren, J.H. (1977). The effects of toxicants on the heamatology of *Labeo umbratus* (Teleost). **Comparative Biochemistry and Physiology** 83C(1):155-159.

van Vuren, J.H.J., van der Merwe, M. & Du Preez, H.H. (1994). The effects of copper on the blood chemistry of *Clarius gariepinus* (Clariidae). **Ecotoxicology and Environmental Safety** 29:187-199.

Villereal –Trevino, C.M., Obregon-Morales, C.M., Lozano-Morales, M.E. & Villegas-Navarro, J.F. (1986). Bioaccumulation of lead iron and zinc by fish in a transect of the Santa Catarina River Cadereyta Jiminez, Neuro Leon , Mexico. **Bulletin Environmental Contamination Toxicology** 37:395-401.

Wedemeyer, G.A. & Mcleay, D.J. (1981). Methods of Determining the Tolerance of Fishes to Environmental Stressors. In **Stress and Fish.** A.D. Pickering (Ed.), Academic Press, New York. pp247-275.

Wedemeyer, G. & Yasutake, W.T. (1977). Clinical methods for the assessment of the effects of environmental stress on the fish health. **U.S. Fish and Wildlife Service** 89:1-18.

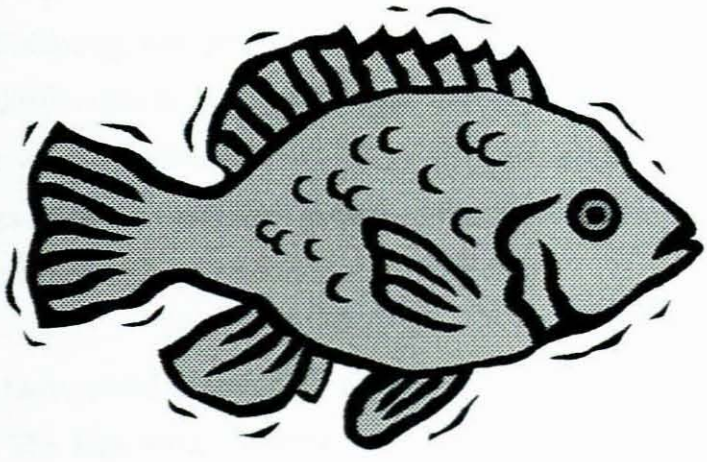
Wepener, V. (1991). The effects of heavy metals at different pH on the blood physiology and metabolic enzymes in *Tilapia sparmanii* (Cichlidae). MSc-Thesis, Rand Afrikaans University South Africa.

Wepener, V., van Vuren, J.H.J. & du Preez, H.H. (1992a). The effects of hexavalent chromium at different pH values on the haematology of *Tilapia sparmanii* (Cichlidae). **Comparative Biochemistry and Physiology** 101C(2):375-381.

Wepener, V., van Vuren, J.H.J. & du Preez, H.H. (1992b). Effects of manganese and iron at neutral and acidic pH on the haematology of the banded tilapia, *Tilapia sparmanii* (Cichlidae). **Comparative Biochemistry and Physiology** 49:613-619.

Wong, M.H., Luk, C.K. & Choi, K.Y. (1977). The effects of zinc and copper salts on *Cyprinus carpio* and *Ctenopharygodon idellus*. **Actam Anatomy** 99:450-454.

Chapter 6



CHAPTER 6

Conclusions and recommendations

6.1 Conclusions

When compared to previous studies on the Mhlathuze Estuary it is clear that there has been an overall increase in the metal concentrations in water and sediment. Metals exist in aquatic media in different forms. They may exist as dissolved ions or as suspended matter adsorbed to particulate material. The physical and chemical interaction between the dissolved metal and the particulate suspended metals is controlled by physicochemical factors, which include turbidity, salinity, dissolved oxygen and pH. These interactions are the contributing factors in the spatial differences of metal concentrations in water in the Mhlathuze Estuary.

In the Mhlathuze Estuary, metals in water were differentiated into two groups; the first comprised metals showing similarity of increasing in dissolved and decreasing particulate phases in high salinity areas and secondly metals that increase in dissolved and decrease in particulate phases when salinities are low. Those that increase in their particulate phases in high turbidity areas are those that favour the adsorption process whereas the particulate phase increases by precipitation in areas with decreased oxygen.

It was found that heavy metal concentrations in the Mhlathuze Estuary differed both spatially and temporally. The high metal concentrations measured in water during autumn/winter periods could have been caused by the low flows experienced resulting in the concentration of metals in aquatic systems due to evaporation. Floods also contribute to increasing metal concentrations in water in the estuary. This is due to metals that are transported as particulate bound complexes during flood events to estuaries or increases the soluble fraction of metals, and consequently their bioavailability to organisms, due to increased freshwater input into the estuary.

Metal concentrations in sediment did not differ significantly on a temporal scale. Of all the metals studies Al and Fe were present in sediment of the Mhlathuze Estuary in very high

concentrations. These are the metals that become available to organisms when resuspended in the water column and in the case of the Mhlathuze, this was exacerbated by the shallow nature and the high silt content of the system. The importance of the sediment as source of metal bioaccumulation was demonstrated with the significant relationships between metal concentrations in the sediment and sediment dwelling invertebrates.

Different metals are taken up by organisms through different routes and are then bioaccumulated in different tissues. In fish (mullet), the gill tissue was the main site for metal uptake. The highest metal concentrations were measured in the liver tissue. Metals such as Cu and Fe appeared to be accumulated from the sediment or with sediment particles during feeding whereas other metals, (Zn, Cr, Mn, Pb and Al) were most probably taken up from the water.

The accumulation of metals by fish in the estuary was relatively low when compared to benthic invetebrates. It therefore supports the fact that most of the metals accumulated by fish was taken up from water and very little, if any, due to biomagnification. Fish are good metal regulators and are therefore not good indicators of metal bioaccumulation. This was reflected in the low metal content in the muscle tissue and that did not differ with seasons.

Two metals, i.e. an essential (Cu) and non-essential metal (Pb) were selected as exposure toxicants for the sublethal bioassays. Environmentally relevant concentrations of Cu and Pb had an effect on the haematology and acid-base balance of the test fish species. Short-term exposure to mixture of Cu and Pb had an impact on the haematology and acid-base balance *of L. dumerelii*. Acid-base disturbances were more pronounced during short-term (96 hr) exposure than when fish was exposed to the metals for 28 days. This shows that the fish were able to regain homeostatic control after 28 days. Exposure of fish to Cu decreased the values of haematological parameters whereas the exposure to lead increased the values of haematological parameters. The combination of the two metals had an effect that increased the haematological parameters. Exposure of organisms to metals for an extended period results in organisms acclimating to contaminants and thus lowering the effects of the pollutant to the organism. Apart from the increased glucose concentrations there were no significant changes in values of both the haematological and acid-base parameters following long-term exposure of fish to the toxicants.

The presence of metals in high levels in the estuary, as determined during this study, must be treated as a matter of concern. This is particularly important as the estuary is designated as a National Marine Protected area. Pollution by metals and other pollutants would have serious implications for the ecology and management of the system.

6.2 Recommendations

The importance of the Mhlathuze Estuary can never be overemphasised. Different studies on the Mhlathuze Estuary (Adams *et al.*, 1998; Cyrus 1999; Mackay & Cyrus 1999; Weerts & Cyrus 1999; Cyrus *et al.*, 2000) found that in terms fauna and flora, the estuary rates among the best in the whole of South Africa. Since there appears to be increased metal accumulation in the estuary when compared to earlier research, further research and monitoring should be instituted to ascertain the causal effects of such bioaccumulation and the true source of heavy metals. This should be part of an appropriate management plan for the Mhlathuze Estuary and its catchment that is currently being developed by the Mhlathuze Catchment Management Authority.

The study of accumulation of metals in fauna, water and sediment gives an understanding on the level of accumulation in different media and on the rate of uptake and prediction on subsequent accumulation of those metals by the organism over time. However, it does not predict the amount of toxicity of a particular metal at that specific load on an individual organism. The alternative approach to establishing a relationship between the levels in the media, accumulation, and toxicity is to use a tripartite method (Chapman *et al.*, 1992). The method uses toxicity evaluation in addition to assessment of accumulation patterns in community and medium. This study underlined the fact that sediments and suspended matter are major sources of metal pollution in estuaries and future research should investigate sediment toxicity using the sediment triad method referred to in the previous sentence.

6.3 References

Adams, J.B., Bates, G.C. & Riddin, T. (1998). Freshwater Requirements of the Mhlathuze Estuary: Vegetation. Report submitted to the institute for Water Research on behalf of the Department of Water Affairs and Forestry, 30pp.

Chapman, P.M., Power, F.A. & Burton, G.A. (1992). Integrative Assessment in Aquatic Ecosystems. In: **Sediment Toxicity Assessment.** G.A Burton (Ed), Lewis Publishing, Boca Raton. 480pp.

Cyrus, D.P. (1999). A review of the aquatic avifauna of the Mhlathuze Estuary. **South African Journal of Aquatic Sciences** 24(1/2):131-140.

Cyrus, D.P., Wepener, V., Mackay, C.F., Weerts, S.P. & Viljoen, A. (2000). The Effects of Intrabasin Transfer on the Hydrochemistry, Benthic Invertebrates and Ichthyofauna of the Mhlathuze Estuary and Lake Nsezi. WRC Report No. 722/1/99:255pp.

Mackay, C.F. & Cyrus, D.P. (1999). A review of the macrobenthic fauna of the Mhlathuze Estuary: Setting the ecological Reserve**. South African Journal of Aquatic Sciences** 24 (1/2):111-129.

Weerts, S.P. & Cyrus, D.P. (1999). A review of the ichthyofauna of the Mhlathuze Estuary. **South African Journal of Aquatic Sciences** 24(1/2):100-110.