BIOACCUMULATION OF METALS IN FRESHWATER CRABS (*POTAMONAUTES PERLATUS*) OF THE LOURENS RIVER, WESTERN CAPE, SOUTH AFRICA

by

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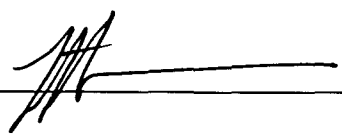
DECLARATION

I, Tim van Stormbroek, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.



Signed

2 April 2008

Date

ABSTRACT

Urban rivers are the most utilised and yet degraded rivers worldwide. The urban rivers of the Western Cape are no different. The Lourens River flows through the agricultural and urban areas of Somerset West in the Western Cape and as a result is subjected to a variety of pollution sources. In the upper reaches this river flows through two large farms where metal containing pesticides are used. Further downstream it passes through an urban area where a variety of pollution sources could contribute to the contamination of the river. The extent to which the Lourens River, and the ecosystem it supports, is affected by metal pollutants is not known. The aim of this study was to determine the concentrations of metals in the Lourens River as well as the contribution of agricultural and urban activities to metal contamination of the river. Sediment and crab (*Potamonautes perlatus*) samples were collected over a period of one year from seven sites over the length of the river. Sediment samples were also collected from a sedimentation pond on the bank of the river where orchard run-off water is re-mediated. Preliminary analysis of samples was done for ten metals (Al, Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb and Zn). Results from these analyses determined the selection of six metals (Al, Cr, Cu, Fe, Mn and Zn) for further investigation in this study. The concentrations of metals detected in collected samples varied significantly throughout the sampling period. This can be attributed to various factors such as rainfall patterns, the fact that pesticide application varies throughout the year and other urban activities. Al, Cr, Fe and Zn were found in significantly higher concentrations in the urban areas. These higher levels of contamination, relative to the upper parts of the river, can probably be attributed to various urban activities contributing to the contamination of run-off into the river. The sedimentation pond results revealed high concentrations of Al and Fe, while Cu, Cr, Mn, and Zn were found in lower concentrations. All six metals however followed the same pattern where the first four sampling occasions showed higher concentrations than the last three occasions. It can be concluded

that agricultural and urban activities do contribute significantly to the metal contamination of the Lourens River.

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- To my precious family who have been so loving and patient with me. I wouldn't change you for anything in this world.

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DEDICATION

I would like to dedicate this work of discovery to my God and Saviour, who designed it all, created it all and sustains it all. I am in awe of His great work in this universe and yet also His attention to the details of life – my life.

Isaiah 40:11&12

"He tends his flock like a shepherd: He gathers the lambs in his arms and carries them close to his heart; he gently leads those that have young. Who has measured the waters in the hollow of his hand, or with the breadth of his hand marked off the heavens? Who has held the dust of the earth in a basket, or weighed the mountains on the scales and the hills in a balance?"

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CHAPTER 1

INTRODUCTION

FRESHWATER SYSTEMS IN SOUTH AFRICA

Fresh water is a natural resource vital to the survival of man but is too often taken for granted. This complacency is especially evident in South Africa. Cartographically, South Africa has many rivers but a large percentage of these rivers are short in length or flow only during the wet season or both (Dallas and Day, 1993). Consequently, water is a limited resource in South Africa and therefore needs careful management if we are to rely on it in the years to come. Rivers are an important source of fresh water and are also ecosystems that help maintain our environment's natural balance. Our water sources need to be monitored, assessed and reported on to achieve sustainable development (River Health Programme, 2003). Urban rivers are the most utilized and yet degraded rivers worldwide. Cape Town's rivers are no different. As agriculture and urban development grow, so does the impact on our rivers. The need for more fresh water and the growing volumes of storm and waste water are negatively influencing our river water quality. The future consumption of water worldwide will increase due to a rapid rise in population, increasing standards of living, growing demands of irrigation water as well as increasing industrialisation. In South Africa alone the domestic and industrial water consumption is expected to reach 16 814 million m^3 per annum by the year 2025 based on the high scenario published in the First Edition of the South African National Water Resource Strategy in 2004.

THE POLLUTION PROBLEM

Pollution of water can be understood as the human introduction of substances or energy, directly or indirectly, into aquatic environments that result in such deleterious effects as harm to living organisms, hazards to human health, hindrance to aquatic activities such as fishing, impairment of water quality for consumption and reduction of amenities (Reish and Oshida, 1986). According

to Dallas and Day (1993) agriculture and forestry can potentially affect the integrity of receiving water bodies by contributing nutrients and dissolved and suspended solids. Processes such as land preparation (burning and land clearing), ploughing, livestock rotation, fertilising, application of biocide, afforestation with exotic species and logging may all affect the water quality of receiving bodies. Acidification of surface waters, with its direct and indirect consequences for aquatic ecosystems, is one of the largest global environmental problems of recent times (Galloway, 1989; Rhode, 1989). Acidification depends mainly on SO_x- and NO_x- emissions from industrial production and combustion processes. Surface waters receive acid inputs through precipitation and surface runoff from the terrestrial environment (Gerhardt, 1995). The range of potential toxic substances is very extensive and includes inorganic and organic poisons, heavy metals, pesticides and PCB's. Metals, pesticides and PCB's have the greatest potential for bioaccumulation (Hellawell, 1988). Two factors contribute to the deleterious effects of metals as environmental pollutants: metals cannot be destroyed through biological degradation as is the case with most organic pollutants; and metals tend to accumulate in the environment – especially in the sediment of rivers and lakes – by association with organic and inorganic matter through processes of adsorption, complex formation and chemical combination (Förstner and Prosi, 1979). According to Fatoki (2003) the accumulation of metals in an aquatic environment has direct consequences to man and the ecosystem. In most natural waters metal concentrations are very low, and thus any increase exposes aquatic organisms to levels not previously experienced. Contamination of water bodies with metals is therefore of significance and should be carefully monitored and controlled. Sources of metals include geological weathering, atmospheric pollution, industrial effluents, agricultural runoff and acid mine drainage (Dallas and Day, 1993).

IMPORTANCE OF SEDIMENT QUALITY FOR RIVER HEALTH

According to Power and Chapman (1992) contaminants found in minute quantities in water columns can accumulate in sediments at much higher levels. Sediment can also serve as a sink for contaminants and a source of contamination to the water and aquatic organisms. Water column

contaminants are variable and dynamic while sediment accumulates contaminant concentrations over time. Sediments are also an important part of the living environment of many aquatic organisms; providing habitat, feeding, spawning and rearing areas. Accumulated contaminants in sediments affect benthic organisms and other sediment associated organisms, as well as those that feed on them. Therefore, sediments are the ultimate reservoirs for the numerous potential chemical and biological contaminants that may be contained in effluents originating from urban, agricultural and industrial lands, and recreational activities. Contaminated sediments in rivers and streams, lakes, coastal harbours and estuaries have the potential to pose ecological and human health risks (Sabine *et al.,* 2004). According to Lyytikäinen *et al.* (2001) it is therefore very important to monitor aquatic sediments, since they may accumulate compounds in concentrations harmful to benthic organisms even though the concentrations in the water phase are fairly low. Fortunately many living organisms possess detoxifying mechanisms that involve a variety of mechanisms rendering the contaminant harmless to the host. Brown (1990) has reported that *Aspergilus niger* (fungus), *Pseudomonas aeruginosa* (bacterium), *Cladosporium resinae* (algae) and genus *Chamydomonas* (algae) act as detoxifying agents for Al, Cr, Cu and Mn respectively.

METALS

Ten major elements: Oxygen (O), Silicon (Si), Aluminium (Al), Iron(Fe), Calcium (Ca), Sodium (Na), Potassium (K), Magnesium (Mg), Titanium (Ti) and Phosphorus (P), constitute over 99% of the total content of the earth's crust. The remainder of the elements in the periodic table are called trace elements and their individual concentrations do not normally exceed 1000 mg kg^{-1}. In fact, most have average concentrations of less than 100 mg kg^{-1} (Mitchell, 1964). According to Birch *et al.* (1996) metals tend to be persistent in the environment, especially in sediments. Rainbow (1985 b) reported that it is the chemical characteristics of metals which are responsible for the fact that all metals ultimately become toxic at some elevated concentration. The ability of organisms to excrete, sequester or otherwise detoxify themselves can be inhibited by abnormally high concentrations of especially non-essential metals (Thorp *et al.,* 1979). Bioaccumulation of metals depends on numerous biotic

and abiotic factors such as environmental temperature, pH and dissolved ions (Gambrell, 1994). Metals can also accumulate in the food chain, through a process referred to as biomagnification (Dallinger and Rainbow, 1993). Agricultural fertilizers are an important source of metals in soils, which are eroded and carried into catchment basins where they are adsorbed by particulate material like sediment. Assessment of metal concentrations in sediment is therefore an important mechanism of pollution identification in aquatic systems (Alloway, 1995). According to Musibono (1998) the toxic effects of metals fall into three groups, namely:

- blocking the essential biologically functional groups of biomolecules (e.g. enzymes and proteins);
- displacing the essential metal ion in biomolecules; and
- modifying the active conformation of biomolecules.

AQUATIC INVERTEBRATES IN ECOTOXICOLOGICAL STUDIES

The study of the nature and action of contaminants on ecosystems, or "ecotoxicology", is of worldwide importance. Pollutants in the aquatic environment may cause death, reduction in growth and reproduction, changes in behaviour, teratogenesis and genetic disturbances in aquatic organisms. Macroinvertebrates living in river sediment are in constant contact with contaminants. Here they bioaccumulate these pollutants through gill cell osmosis and ingestion. To help us understand the effects of these pollutants, many studies have been undertaken in laboratories around the world using selected pollutants and test organisms. Their acute and chronic effects can be evaluated in this manner. Although the application of laboratory obtained results to actual ecosystems is not ideal, these results do help in understanding the probable impact of certain concentrations of selected toxic substances on the natural environment (Musibono, 1998). The effect of a toxic substance on an organism depends on the concentration and the duration of exposure. For many toxic substances, over a wide range of concentrations, these two factors act reciprocally: a low concentration over a long period has the same effect as a high concentration for a short period. The potential for accumulation of toxic substances within the tissues of organisms increases

the significance of these pollutants which may be present in water even though ambient concentrations are very low (Hellawell, 1988). According to Chapman (1989) it has been shown in numerous studies in which water quality criteria have not been exceeded that adverse effects are possible in aquatic organisms that reside and forage in or near sediment.

POLLUTION STUDIES IN THE LOURENS RIVER CATCHMENT AREA

The Lourens River is the only Protected Natural Environment (PNE) river in South Africa and has been the subject of numerous studies in the past (Tharme *et al.*, 1996; Schulz *et al.*, 2001; Dabrowski, 2001; Dabrowski *et al.*, 2002; Moore *et al.*, 2002; River Health Programme, 2003). In 1996 Tharme *et al.* of Southern Waters Ecological Research and Consulting undertook a commissioned study entitled "An assessment of the present ecological condition of the Lourens River, Western Cape, with particular reference to proposals for stormwater management". Schulz *et al.* (2001) also investigated current-use insecticide, phosphate and suspended solids in the Lourens River during the first rainfall event of the wet season. In their study they found that pesticide contamination resulting from agricultural runoff depends on the time period between application and rainfall. In the same year Dabrowski (2001) completed a risk assessment using GIS and microcosms in the prediction and ecotoxicological effects of runoff-induced pesticide contamination. Shulz (2001) also investigated the importance of a single rainfall event in sediment and pesticide input into the Lourens River from orchards. Dabrowski *et al.* (2002) also published results dealing with runoff-related pesticide input into the river. In 2002 Moore *et al.* reported mechanisms in the mitigation of chlorpyrifos runoff using constructed wetlands before discharge into the Lourens River. The Lourens River was also included in the River Health Program in 2003.

AIMS OF THE STUDY

This study aims to demonstrate the potential negative impacts of anthropogenic activities, with specific reference to agricultural activities, on the health status of the Lourens River, Western Cape. This will be achieved by:

- determining the concentrations of selected metals in the sediment of the Lourens River, Western Cape over a period of one year.
- determining the concentrations of selected metals in the crabs (*Potamonautes perlatus*) of the Lourens River, Western Cape over a period of one year.
- determining possible seasonal variations in the concentrations of selected metals in the sediment and crabs of the Lourens River, Western Cape.
- determining the effectiveness of the sedimentation pond on the Vergelegen Farm in retaining selected metals.
- determining potential sources of metal pollution along the length of the Lourens River, Western Cape.

CHAPTER 2

STUDY AREA

GENERAL DESCRIPTION

The study was undertaken on the Lourens River in Somerset West in the Western Cape. This river rises in the Hottentots Holland Mountains at an altitude of more than 1110m and has no major tributaries or in-stream dams. The upper reaches of the catchment area (Figure 2.1) are located within the Helderberg and Hottentots Holland Nature Reserves. Outside the nature reserves and before the river enters Somerset West, it passes two farms, Lourensford and Vergelegen (Figure 2.2). The river flows in a south-westerly direction for approximately 20km, before passing through Somerset West and entering False Bay at Strand beach where it forms a small estuary (Figure 2.3). As depicted in Table 2.1, land use in the Lourens River catchment area is dominated by natural and agricultural vegetation (River Health Programme, 2003).

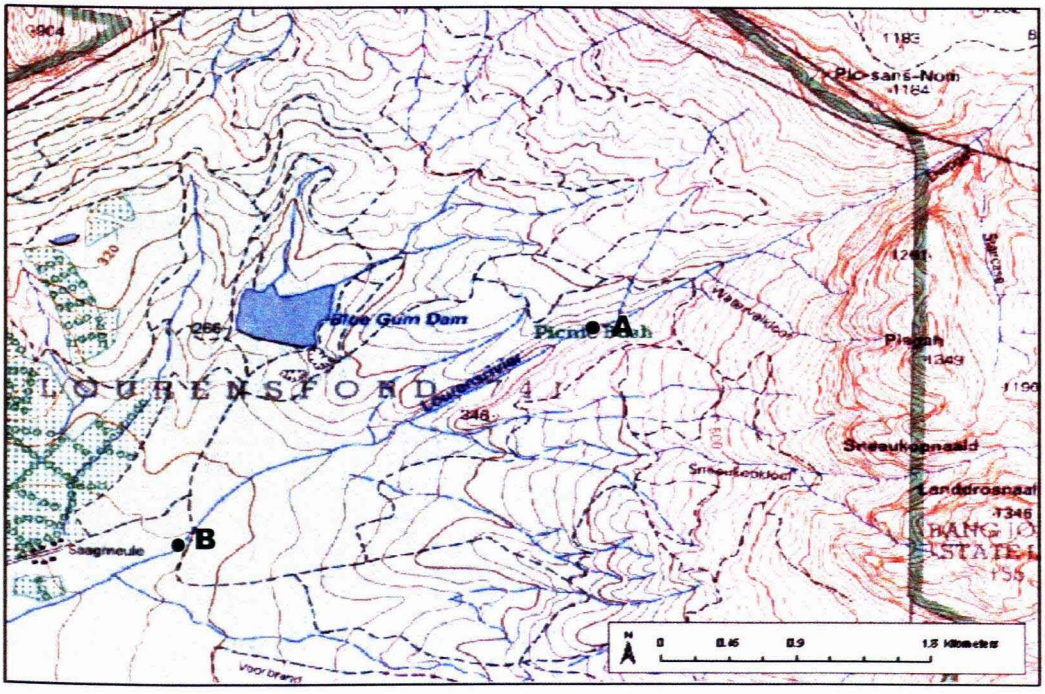

Figure 2.1 Map illustrating the upper reaches of the Lourens River and sampling positions A & B.

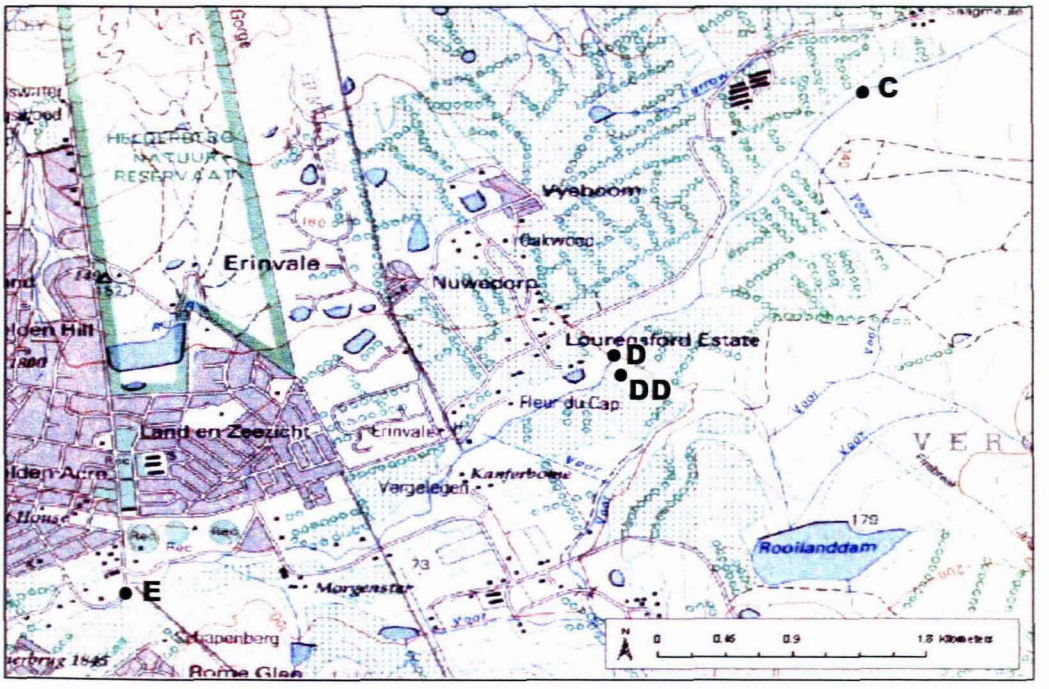

Figure 2.2 Map illustrating the middle reaches of the Lourens River and sampling positions C, D, DD (sedimentation pond) & E.

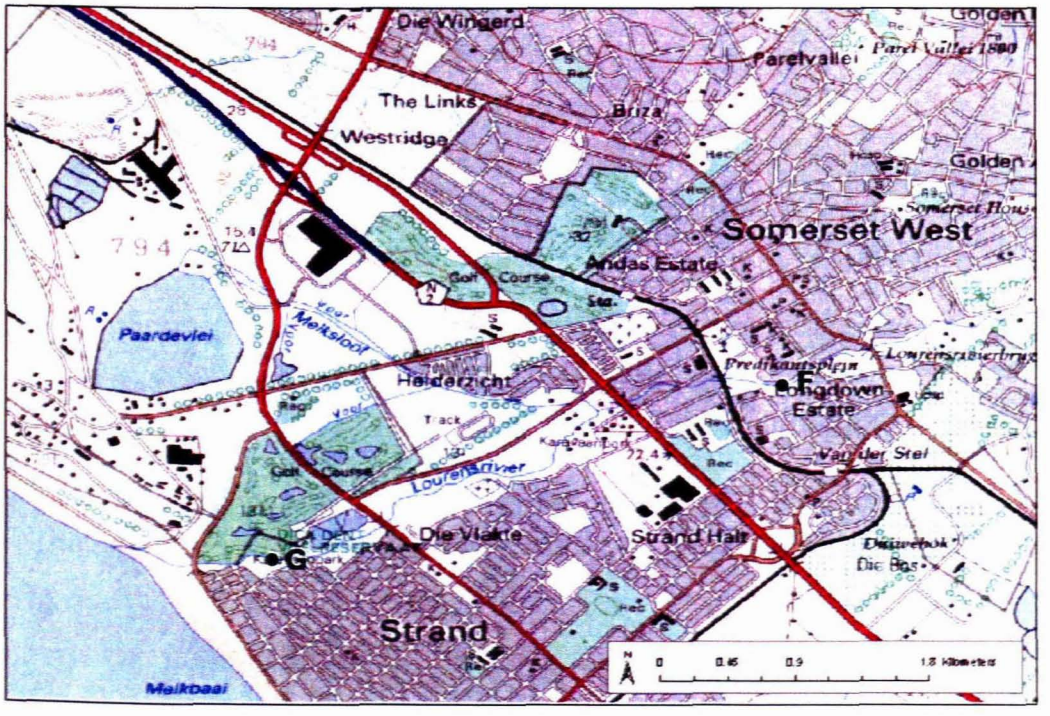

Figure 2.3 Map illustrating the lower reaches of the Lourens River and sampling positions F & G.

Table 2.1: Main land-use within the catchment area (River Health Programme, 2003).

Land use	Percentage utilisation (%)
Forestry	33
Natural Areas	28
Vineyards and Orchards	20
Urban Areas	18
Other	1

VEGETATION

The natural fynbos vegetation of the upper reaches of the catchment area (Mountain Fynbos), outside the reserves, has largely been replaced by forestry and agriculture. The indigenous vegetation commonly found in the Lourens River catchment area includes riparian trees, shrubs and herbaceous flora. Orchards and some vineyards dominate agriculture in the area. Much of the natural vegetation in the lowland area, the natural coastal renosterveld, has been replaced by urban development. Healthy riparian vegetation is essential to the health status of rivers in many ways. It stabilises riverbanks, attenuates flooding, maintains water quality, recycles nutrients and provides habitat for fauna (River Health Programme, 2003).

GEOLOGY

The geomorphology of any area influences many aspects of the landscape. River gradient is one such aspect and influences water turbulence and thus the amount of dissolved gasses in the water and the degree of erosion which in turn influences the turbidity of the water. Geological formations also vary in chemical composition and thus exert different chemical influences on river systems. Water flowing over or seeping through these rocks and soils accumulate ions in different quantities and proportions. Rock types within the catchment area of the Lourens River include quartzitic sandstone (Table Mountain Group), shale (Malmesbury Group), Cape granite and sandy sediments (River Health Programme, 2003).

the Lourens River. During some visits to the river, the presence of the Cape clawless otter (*Aonyx capensis*) and Water mongoose (*Atilax paludinosus*) was noted in the form of crab remains and scats. The Lourens River corridor also provides a favourable habitat for many water-dependent birds like giant kingfishers, ducks, herons and waders (River Health Programme, 2003).

SEDIMENT CHARACTERISATION

The character of sediment changes along the course of the Lourens River. Where the gradient, and therefore water velocity is higher, the sediment tends to be coarser. The highest percentage of gravel was found at Site A while the highest percentage of mud was found at Site G (excluding the sedimentation pond – DD).

Table 2.3: Texture Analysis of sediment samples taken from each sampling site.

Sample Site	Gravel %	Sand %	Mud %	Texture	Mean Size (phi)
A	69.36	30.59	0.04	Sandy Gravel	0.83
B	39.98	59.95	0.06	Sandy Gravel	0.41
C	21.36	78.49	0.13	Gravely Sand	0.58
D	65.25	33.93	0.81	Sandy Gravel	1.16
DD	6.54	86.48	6.97	Slightly Muddy gravely Sand	1.03
E	19.86	79.94	0.18	Gravely Sand	0.4
F	0	99.78	0.21	Sand	0.81
G	0	94.21	5.79	Slightly Muddy Sand	1.39

SAMPLING SITES

In order to determine total metal concentrations along the length of the Lourens River, seven sampling sites were selected for this study. The selection of sites was based on the different activities bordering the river, accessibility of the site, and distribution of sites from the upper reaches to the river mouth. One additional sampling site, the sedimentation pond on the Vergelegen farm, was selected. This site was chosen to determine the concentrations of metals accumulated in the sediment at the inlet to the pond. Water flow characteristics vary greatly along

the length of the river due to changing gradients and therefore influences the texture of sediment accumulation. Seasonal rainfall patterns also greatly influence the water velocity and flow patterns.

Site A: S 34° 02' 43" E 18° 55' 96"

After the selection of this site as a control/reference site, a new vineyard development was started in December of 2002, approximately 40m from the river in a north-westerly direction. Consistent with the other agricultural development along the river, a "buffer-zone" of existing vegetation, both endemic and exotic, was kept in place along the upper reaches of the river. This area was also used to dump rocks and tree stumps removed during the development of the vineyard. The width of these buffer zones varies along the course of the river and is somewhat influenced by the topography of the area. This site is densely vegetated and the trees form a canopy over the river, which provides almost constant shade. Along the lower reaches of this new vineyard development, almost 400 m of natural and exotic vegetation was removed along the banks of the Lourens River. This was cleared in early 2003 but emergency measures were taken by placing rocks in this zone to protect it from potential erosion in the event of flooding. The Lourensford Farm management were required to rectify this damage in consultation with specialists. This zone of the river is moderately steep with a geomorphological character dominated by cobble or mixed bedrock-cobble. Small pools and fast cascading water is characteristic of this area. The water at this site is clear and free of visible pollution despite signs of occasional human presence in the area. This site was considered a reference site since it is unaffected by point source pollution (Persaud *et al.*, 1992 as cited in Saulnier and Gagnon, 2006). Non-point source pollution through dust, possible spray drift and precipitation cannot be ignored though.

Site B: S 34° 02' 67" E 18° 55' 57"

The second site was situated down-stream from the Red Bridge. The Red Bridge is a small vehicular bridge providing access to the agricultural land on the south-

easterly side of the Lourens River. This site is now the point of entry for run-off from the new vineyard as discussed above. A small sedimentation pond has been constructed at the lowest point of this vineyard with its overflow point immediately upstream of the bridge. This site is also moderately steep with a mixed bedrock-cobble substrate. Small pools form below the weir, which is located under the Red Bridge.

Site C: S 34° 03' 50" E 18° 54' 46"

This site was situated down stream from the sawmill and the old piggery (both located on the Lourensford farm). On the north-westerly side the buffer zone is heavily vegetated with a band of eucalyptus and some black wattle, beyond which are vineyards. The south-easterly side of the river is dominated by open, fallow land with some trees. This area has a slightly lower gradient than site A and B but is still fast flowing with only a few pools forming in the summer months. The riverbed consists mostly of cobblestone and bedrock with some alluvial sand and gravel.

Site D: S 34° 04' 17" E 18° 53' 88"

This site displays similar characteristics to Site C, although the river has started to form some deeper pools in places, especially along the cut-off banks. The sediment in these pools is finer and contains more organic matter than the sediment in other areas at this site. The north-westerly side of the river is densely vegetated with eucalyptus, wattle and shrubbery. The south-easterly bank is grassed with a sparse population of trees. The distance between the river and the orchards on the south-easterly side varies from 30 – 50 m. The riverbed is dominated by bedrock and cobble stone with some small sand bars forming where the water velocity allows.

Site DD: S 34° 04' 10" E 18° 53' 95"

This sampling site was situated in a sedimentation pond on the Vergelegen farm. This pond was constructed in 1991 along one of the Lourens River tributaries, for

the sole purpose of allowing sedimentation of contaminants from orchard run-off before the water enters the Lourens River. The pond is densely vegetated with Bulrush (*Typha capensis),* Sea rush *(Juncus kraussii) and* Papyrus *(Cyperus dives)* (Schulz and Peall, 2000). Sediment samples and physico-chemical data was collected at a small rock weir near the water inlet point. The sediment found here is fine and contains much organic matter. Due to the dense reed growth in this pond, no crab samples were collected here. The outlet/overflow point for this pond into the Lourens River, is located upstream of sampling site D.

Site E: S 34° 04' 69" E 18° 52' 14"

This sampling site was situated at the point where existing urban development starts, although during the sampling period, agricultural land was being developed for housing purposes upstream of this site. The south-eastern side of the river in this area is dominated by agricultural land, while the north-western side is used for recreation. Both of these areas are separated from the river by a corridor of indigenous and exotic trees, grass and shrubbery.

The riverbed is dominated by cobblestone with large sandy slip-off banks and deeper pools in the cut-off banks.

Site F: S 34° 05' 38" E 18° 50' 91"

This sampling site was situated upstream of the railway bridge next to the Longdown Estate. A green corridor, used as a walking trail and for other recreational activities, is situated on either side of the river in this area. The riverbed is dominated by cobble stone but in some areas alluvial soil and silt is present. The cut-off banks are heavily vegetated by grass and shrubbery with some deeper pools. In some areas the cut-off banks have been reinforced using rock cages.

Site G: S 34° 06' 01" E 18° 49' 28"

Site G was situated alongside the Strand Golf Course. A green belt, used for recreation, flanks the south-eastern riverbank. The water velocity has slowed

considerably in this stretch of the river although there are some rocky areas, which cause the water to cascade. The riverbanks are well vegetated with grass, reeds, aquatic plants and shrubbery. The riverbed is dominated by some small cobble stones and sediment. This site is situated less than 500 m from the river mouth, which forms a small, inter-tidal estuary.

RIVER PROFILE

The figure (2.4) below shows the gradient of the Lourens River from sampling site A in the upper reaches of the river to site G near the river mouth. Distances between sites vary from 1 to 2 km.

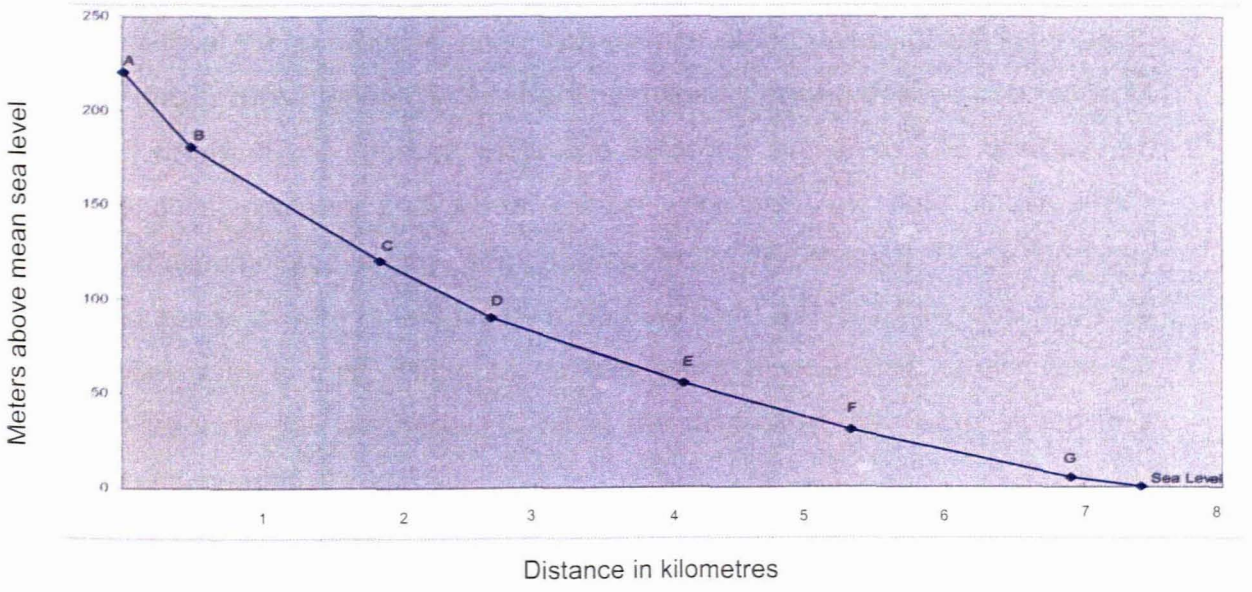

Figure 2.4: The profile of the Lourens River, Western Cape.

CHAPTER 3

MATERIALS AND METHODS

FIELD SAMPLING

Field sampling was undertaken every two months, from 29 October 2003 till 28 October 2004. Sediment samples and five crabs (whenever possible) were collected at each site over a two-day period.

Sediment

At each site, sediment was collected using a plastic (polypropylene) scoop attached to a 1,2 m wooden pole. This device aided collection of samples in deeper pools. Small amounts of surface sediment were randomly collected from different areas of the riverbed within the sampling site area. The downstream side of rocks, boulders and slip-off banks, were often the only places where sediment accumulated in large enough amounts to be collected. These randomly selected samples were mixed together and stored in a PVC bottle (one bottle for each site) with a tight fitting lid, labelled and placed in a cooler box for transportation to the laboratory. Once in the laboratory they were stored in a freezer until needed.

Freshwater crabs: *Potamonautes perlatus*

According to Mason (1996) macroinvertebrates are the organisms most commonly used to assess the biological quality of running fresh waters. Freshwater crabs (*Potamonautes perlatus*) are the largest naturally occurring macroinvertebrates in Southern Africa rivers (Hill and O'Keefe 1992). They are nocturnal and occur in rivers in the south-western region of the Western Cape (Barnard 1950). According to Hill and O'Keefe (1992), *P. perlatus* play an important role in processing organic material. Through feeding, they reduce the surface area of leaf litter in river sediment by 99.95%. Crabs are known to be

generalist feeders. Results from foregut analysis of samples (*P. perlatus*) collected in the Buffalo River, show significant amounts of detritus in the form of unrecognisable organic debris from decomposing plants. Moss and algae, vascular plant material, small amounts of bark and wood, aquatic invertebrates, and some invertebrate tissue was found. *P. perlatus* occur along the whole length of the Lourens River (Tharme *et al.*, 1996).

At each site, every effort was made to collect a full sample size of five crabs. However, during the first sampling occasion, no crabs were caught at site E. This site was subsequently moved upstream by approximately 100m to an area where crabs were more plentiful. The sampling size at site G on the second sampling occasion totalled four, as was site D during the sixth occasion. Only two crabs were caught during the sixth sampling occasion at site G. During the last sampling occasion, only three crabs were collected at site C. Whenever possible, full sample sizes of five crabs, of a similar size (21mm-48mm carapace width) were collected. Of the crabs collected, 40% were female and 60% male. Sheep heart was used as bait, which was placed inside a fine mesh nylon bag and attached to the end of a fishing rod by means of fishing line. A small stone was placed in the bag, along with the heart, to allow it to sink. The rods were placed in suitably deep sites along the riverbank and left till they had attracted crabs. A scoop net was used to "catch" the crabs once the fishing rod was lifted. The crabs were placed in plastic containers with some river water, lidded, labelled and stored in a cooler box for transportation to the laboratory. Once in the laboratory they were killed by freezing and stored frozen until needed.

LABORATORY TECHNIQUES

Sediment

Frozen sediment was allowed to defrost over night. Once defrosted, five sub-samples were taken from each sediment sample by means of a plastic spoon and placed in plastic petri dishes and labelled. These petri dishes were placed in a desiccation oven at 60 °C for a minimum of 48 hrs. Once dry, each sample

was removed and sieved to remove larger stones and organic matter. The sieved samples are then placed in plastic vials. Each sub sample was weighed (*Precisa XB220A balance*, accurate to 0,0001 grams) to between 0,4 grams and 0,5 grams and placed into labelled glass test tubes. To each sample, 10 ml of 55% nitric acid was added and the test tubes were placed in the heating blocks (*Grant UBD4)* for digestion. The samples were heated at 60 °C for 1 hr and then at 120 °C for a minimum of 3 hrs or until digested. The samples were then removed and filtered (Whatman no 6 Qualitative filter paper) into 20 ml volumetric flasks to remove remaining sludge. Each sample was made up to 20ml using distilled water and then filtered again using Whatman Cellulose nitrate membrane filters (0, 45µm). Completed samples were stored in labelled 30 ml plastic vials under refrigeration, until ICP Analysis was done (Varian Liberty II radial ICP-AES).

Crabs

Frozen crabs were allowed to defrost over night. Once defrosted, crabs were placed on plastic petri dishes and labelled. These petri dishes were placed in a desiccation oven at 60 °C for a minimum of 72 hrs. Once dry, each whole crab was weighed, measured and sexed before being crushed using a pestle and mortar. The crushed crabs were stored individually in 10 ml plastic vials from which a 0,4 to 0,5 gram (*Precisa XB220A balance*, accurate to 0,0001g) homogenous sample was taken from each. Each weighed sample was placed into glass test tubes, to which 1ml of 55% nitric acid was added and allowed to stand until the initial vigorous reactions had taken place. The remaining 9 ml of 55% nitric acid was added and the test tubes placed in the heating blocks (*Grant UBD4)* for digestion. The samples were heated at 60°C for 1 hr and then at 120°C for a minimum of 3 hrs or until digested. The samples were then removed and filtered (Whatman no 6 Qualitative filter paper) into 20 ml volumetric flasks to remove sediment residue. Each sample was made up to 20 ml, using distilled water and then filtered again using Whatman Cellulose nitrate membrane filters (0,45 µm). Completed samples were then stored in labelled 30 ml plastic pill vials under refrigeration until ICP analysis was done.

METAL SELECTION

Ten different metals were initially selected (Al, Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb and Zn) for preliminary analysis of sediment and crab samples. The results from these preliminary tests are provided in appendix 1 and 2. Of these ten metals, six (Al, Cu, Cr, Fe, Mn and Zn) were chosen, based on the concentrations obtained from the preliminary results.

PHYSICO – CHEMICAL CONDITIONS

Physico-chemical factors may affect the acute toxic effect of pollutants (Mason, 1996). According to Mason (1996) temperature is an important environmental factor because it influences the metabolic activity, and therefore the behaviour of organisms which may affect their exposure to a pollutant. Temperature may also alter the physical and chemical state of a pollutant influencing its bioavailability. According to Felts and Heath (1984) and Khangarot and Ray (1989), toxicity of metals generally increases with temperature. Reducing canopy vegetation along the river course increases light and therefore temperature (Dallas and Day, 1993) and subsequent changes in biotic communities. The maintenance of "buffer" strips in the riparian zone adjacent to streams or rivers will alleviate these effects to a certain extent.

Acid soils occur in nearly one-half of all non-irrigated, arable lands in the world (Fageria *et al.*, 1988). The pH of natural water is determined by geological and atmospheric influences. Since pH is a log scale, a change of one unit means a ten-fold change in $[H^+]$ (Dallas and Day, 1993). According to Mason (1996) the pH level in water is especially important in influencing the toxic effects of pollutants. Campbell and Stokes (1985) have described two contrasting responses of an organism to metal toxicity with a decrease in pH:

a) If there is little change in speciation and metal binding is weak at the biological surface, a decrease in pH will decrease toxicity due to competition for binding sites from hydrogen ions.

b) Where there is a marked effect on speciation and strong binding of the metal at the biological surface, the dominant effect of a decrease in pH will be to increase metal availability.

Although much present-day acidification of soil is as a result of fertilizer use, acid precipitation and other anthropogenic sources, it should be pointed out that acidification can also be a natural process (Sutcliffe, 1983). Although the processes involved are complex, in essence, acidification is likely to occur whenever soil lacks base cations, particularly Ca^{2+} and Mg^{2+}. The process is exacerbated when low-nutrient soils, particularly polyphenolics, decompose to form humic and other weak acids. A typical example is the fynbos region of the south-western Cape (Dallas and Day, 1993).

During this study, the temperature and pH were measured at each site, using field probes and the data recorded.

Temperature:

Water temperature was measured at each site during each sampling occasion using a digital thermometer (Table 3.1).

Table 3.1: Water temperature (°C) at each sampling location (A-G) during the sampling period (Oct 2003 – Oct 2004). #: Denotes no data.

	29 Oct 2003	15 Jan 2004	27 Feb 2004	30 Apr 2004	16 Jun 2004	20 Aug 2004	28 Oct 2004
A	15	16.9	19	15.8	13.4	13	14.7
B	18	18.5	24.4	17.6	14.1	13.4	18.4
C	19.5	20.4	26	17.6	14.8	13.8	19.2
D	22	18.6	22.7	15.7	15.4	13.1	21
DD	#	20.6	20.1	16.1	16.4	14.7	19.3
E	21	19.2	23.4	15.4	14.4	13.6	18.5
F	20	18.6	21.9	15.6	14.2	12.8	19.2
G	19	24.1	22.7	15.8	13.9	12.4	19.6

pH:

The pH was determined using a *Hanna HI 9025 pH Meter* at each sampling site during the sampling period (Table 3.2).

Table 3.2: pH measured at each sampling site (A-G) during the sampling period (Oct 2003 – Oct 2004). #: Denotes no data.

	29 Oct 2003	15 Jan 2004	27 Feb 2004	30 Apr 2004	16 Jun 2004	20 Aug 2004	28 Oct 2004
A	5.5	5.5	5.7	5.5	5	5.5	5.5
B	5.5	5.5	6	5.2	5.2	5.5	5.5
C	5.5	5.5	6	5.2	5.3	5.4	5.6
D	5.5	6	6	5.8	5.5	5.7	6
DD	#	5.5	5.7	5.6	5.4	5.5	5.8
E	6	6	6	5.5	5.5	5.8	5.8
F	6	5.5	6	6	5.8	5.9	5.9
G	5.5	7	7	6	5.8	5.8	5.9

STATISTICAL ANALYSIS

Data was analysed by means of the Sigmastat 3.1 statistical package. Analysis of variance was used to compare metal concentrations for each occasion over the length of the river, as well as, per site over time. All pairwise multiple comparison procedures were done using the "Student-Newman-Keuls Method".

CHAPTER 4

ALUMINIUM

INTRODUCTION

Aluminium (Al) is one of the most plentiful metallic elements in the earth's crust and the third most common of all the elements, after oxygen and silicon. Most rocks, soils and minerals contain aluminium compounds. It is also naturally present in water and air (Agriculture and Agri-Food Canada, 2003). Al exhibits a pH-dependent solubility, which drops drastically from pH 5 to pH 7 (Rankama *et al.*, 1950). It is mostly insoluble at neutral pH with typical dissolved concentrations seldom exceeding 50 $\mu g\ l^{-1}$ (Baird, 1995).

The most dramatic effects of acid precipitation on watershed ecosystems is the increase in Al mobilisation. This may result from long-term atmospheric inputs of H_2SO_4 and HNO_3 (Cronan and Schofield., 1979). This has a direct impact on soil pH and when the pH of the soil drops below about 4.2, Al leaching from soil and rocks becomes particularly significant. Such acidification has occurred in some regions in central Europe, including Poland, the former Czechoslovakia, and eastern Germany, and the resulting solubilisation of aluminium may have contributed to the forest dieback observed there in the 1980's (Baird, 1995). A study has shown that a number of large rivers in the UK contain levels of Al, mostly in colloidal and particulate form, up to 450$\mu g\ l^{-1}$ at circumneutral pH 7-8 (Dixon and Gardner, 1998). At alkaline pH values, Al is normally present as soluble or insoluble, biologically unavailable hydroxide complexes. At intermediate pH values, Al is sparingly soluble and probably occurs as hydroxo- and polyhydroxo-complexes. Under acidic conditions (pH<5.0), it occurs as the soluble, available toxic hexahydrate (aquo) species (Al^{3+}). In waters naturally acidic because of the presence of acidic compounds, Al is also present but is not always bioavailable because it could be absorbed onto organic molecules (Musibono, 1998). The concentration of aluminium ions in natural waters is normally quite low, typically about 0.027 $mg\ l^{-1}$. This low value is the consequence of the fact that in the pH range of 6-9, which is usual for natural waters, the solubility of

the aluminium contained in rocks and soils to which the water is exposed is very small. The fact that aluminium is not very soluble in water is controlled by the insolubility of $Al(OH)_3$. For every decrease of the pH by one unit the concentration of aluminium ion increases by a factor of 10^3, and so it reaches 0.027 mg l^{-1} at pH of 5 and 27 mg l^{-1} at pH of 4. Thus, aluminium is much more soluble in highly acidified rivers and lakes than in those where the pH values do not fall below 6 or 7 (Baird, 1995).

According to Dallas and Day (1993) Al is one of the most toxic of the major elements, and is probably not an essential nutrient in any organism. It is generally accepted that low molecular weight aluminium species, of largely inorganic character, are primarily responsible for the metal's aquatic toxicity (Dixon and Gardner, 1998). Herrmann (1987) has shown that freshwater invertebrates disappear in acidic waters as a response to low pH and aluminium.

In a laboratory study, Elangovan *et al.* (1997) has shown that the pond snail, *Lymnaea stagnalis,* accumulates Al significantly, when exposed to concentrations similar to those reported by Dixon and Gardner (1998) (6.9 - 458 µg l^{-1}). Observations by Elangovan *et al.* (1997) suggest that availability of Al at neutral pH to aquatic invertebrates may be markedly influenced by feeding habits, mucous secretion and respiration. Even particulate Al seems to be bioavailable to key members of the freshwater biota, such as grazers and filter feeders. Although speciation of Al is dependent on pH changes, Elangovan *et al.* (1996) has shown that aluminium is clearly available to the snail, *Lymnaea stagnalis* even at neutral pH, the most likely route of entry being the gut.

In many species, gills are thought to be a further important route of uptake of Al (Pynnonen, 1990). Rosseland *et al.* (1990) has shown that, in the aquatic environment, Al acts as a toxic agent on gill breathing organisms such as fish and some invertebrates. This is caused by a loss of plasma- and haemolymph ions leading to osmoregulatory failure. Buckler *et al.* (1995) has shown that fish exposed to 0.033 mg kg^{-1} had concentrations of 0.003 mg g^{-1} Al in their

whole body tissue. Those exposed to 0.264 mg kg^{-1} Al had concentrations of 0.096 mg g^{-1} in their tissues. Although evidence for food chain accumulation of Al is still weak (Herrmann *et al.*, 1987), bioaccumulation of Al in some invertebrates could facilitate entry of the metal into the food chain (Elangovan *et al.*, 1996).

MATERIALS AND METHODS

Refer to Chapter 3

RESULTS: Sediment

The concentrations of total Al measured in sediment samples, collected over 12 months from seven sites is depicted in Figure 4.1.

(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Al concentrations measured in sediment, at a particular site over the 12 month sampling period

Pairwise multiple comparison procedure of sediment Al concentrations at the different sites over time, revealed the following results. At site A, comparisons of occasions 2 vs 4, 5 & 7; 4 vs 5 and 1 vs 6 were found not to be statistically significant ($P>0.05$). Pairwise multiple comparisons made between sediment Al concentrations on all sampling occasions at site B displayed no statistical difference ($P>0.05$) for any of the combinations. Statistical differences ($P<0.05$) were only found when occasion 5 (1615 mg kg^{-1}) was compared to all other occasions at site C, while all other comparisons resulted in no statistical differences. At site D, when sampling occasion 6 was compared with occasions 1, 2, 3, 4, 5 and 7 results showed statistical differences ($P<0.05$) for each combination, while every other comparison at this site did not. Statistical differences were found when occasions 1 and 7 were compared to all other occasions at site E. No statistical differences ($P>0.05$) were found for 2 vs 4 & 6; 4 vs 6; and 1 vs 5 at site F. At site G all pairwise multiple comparisons were statistically significant except for comparisons 6 vs 7 and 1 vs 6 & 7. The lowest Al concentration measured was 1270.7 ±109.5mg kg^{-1} at site A during occasion 1. The highest mean Al

concentration measured was 17599,6 ±577.8mg kg^{-1} at site G during the 3rd sampling occasion.

Comparisons of total Al concentrations measured in sediment per sampling occasion over the length of the river

Comparisons of Al concentrations measured at site A – G on sampling occasion 1, revealed the following results. Site A vs B, D vs G and E vs F did not show statistical differences ($P>0.05$). There were no statistically significant differences ($P>0.05$) between the site comparisons of C vs A & B and A vs B for occasion 2. No statistical significance ($P>0.05$) was found for pairwise multiple comparisons made between C vs D & E, E vs A & D, and D vs A for sampling occasion 3. Pairwise multiple comparisons for sampling occasion 4 revealed statistical differences ($P<0.05$) for all comparisons except G vs F. Occasion 5 pairwise multiple comparisons revealed no statistical significance ($P>0.05$) for all site comparisons. Comparisons for occasion 6 revealed only B vs A to be statistically insignificant ($P>0.05$) while for occasion 7, results revealed statistical significance ($P<0.05$) for B and E vs C, D, F, G and A vs G.

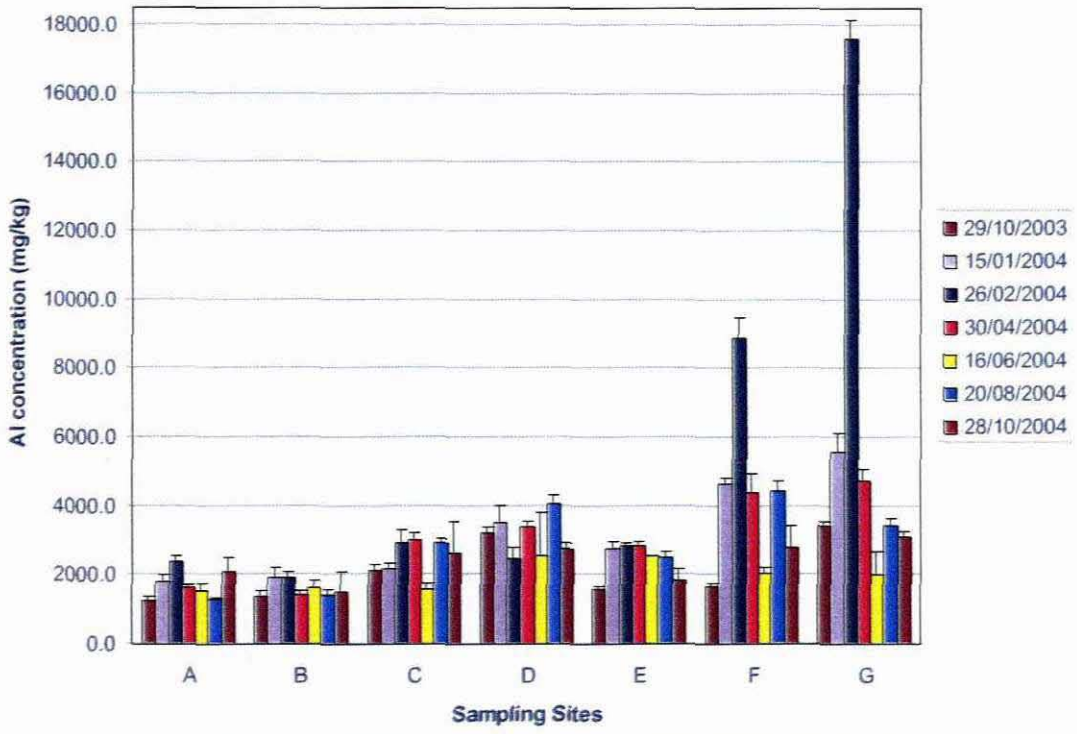

Figure 4.1 Mean concentrations of total aluminium (mg kg^{-1}) (±SD) in sediment samples collected from seven sites (A – G) of the Lourens River over a 12 month period. ND = no data.

RESULTS: Crabs

The concentrations of total Al measured in crabs, collected over 12 months from seven sites is depicted in Figure 4.2.

(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Al concentrations measured in crabs collected at a particular site over the 12 month sampling period

All pairwise multiple comparisons for site A and B revealed the same results with statistical differences ($P<0.05$) found for comparisons between occasion 1 and all other occasions at both sites. At site C, the results were similar. Only 1 vs 2 - 7 and 3 vs 5 were found to be statistically different ($P<0.05$). Statistical significance ($P<0.05$) was found for comparisons 1 vs 2 - 7 and 3 vs 2, 4, 5, 6 & 7 at site D. No statistical differences ($P<0.05$) were found for any comparisons made for site E. It should be noted that no crab samples were collected on occasion 1 for this site due to lack of availability. Pairwise multiple comparisons for site F revealed statistical significance ($P<0.05$) for

comparisons 1 vs 2 - 7 only. Site G only revealed statistical significance ($P<0.05$) for comparison 2 vs 5. It should be noted that comparisons for site G which may appear statistically significant on the graph below (Figure 4.2), appear so due to the difference in sampling size, and therefore the mean Al concentration. During occasion 2 and 6 only four and two crab specimens were collected, respectively.

Comparisons of total Al concentrations measured in crabs per sampling occasion over the length of the river

Pairwise multiple comparisons for sampling occasion 1, revealed no statistical significance ($P>0.05$) for C vs D, G, & F; D vs C & F and C vs F. Occasion 2 had a sample size of four crabs and only revealed statistical differences ($P<0.05$) for comparisons A vs G and B vs G. Statistical differences ($P<0.05$) were found when sites F and G were each compared with sites A, C and D during occasion 3. Comparisons for occasion 4 revealed statistical significance ($P<0.05$) when site A was compared to site D, F and G. Occasion 5 revealed statistical significance ($P<0.05$) for all pairwise multiple comparisons made between site A and all other sites. Site B, for this occasion, compared to site E and F was also statistically significant ($P<0.05$). Pairwise multiple comparisons for occasion 6, when site A is compared to C, D, E, F and G and Site B with D, F and G, revealed statistical significance ($P<0.05$). The sample size for site D and G was four and two respectively. Occasion 7 revealed statistical significance ($P<0.05$) when site G was compared to all other sites. Comparisons A vs B, E and F were also found to be statistically significant ($P<0.05$) for this occasion. The sample size for site C on this occasion was three.

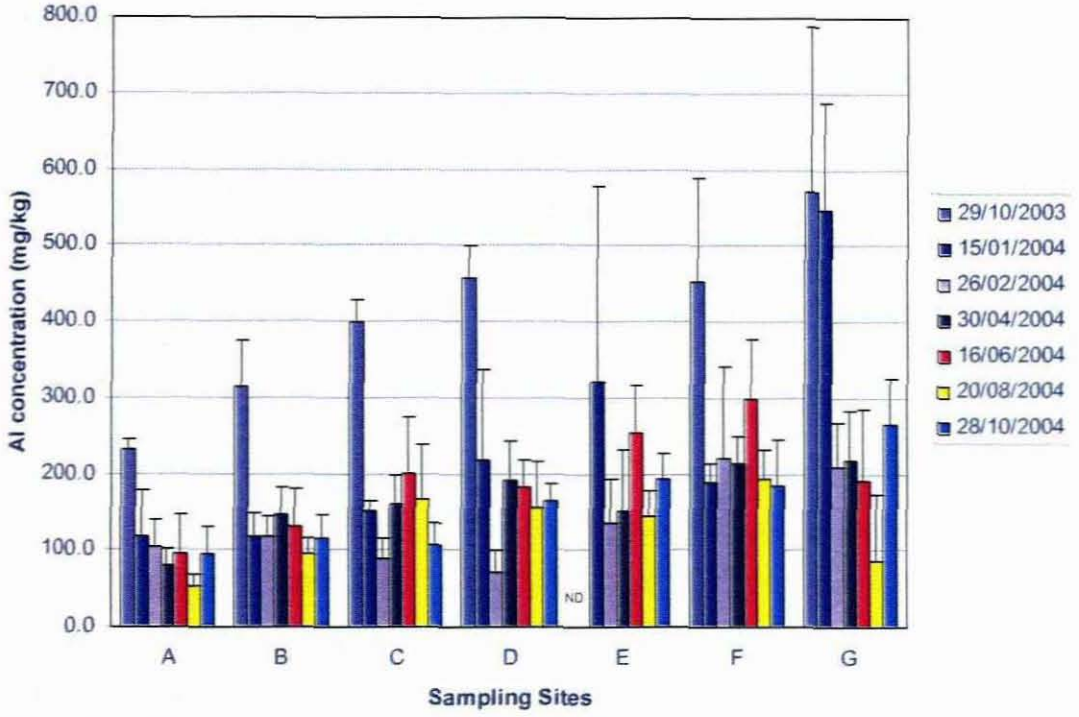

Figure 4.2 Mean concentrations of total aluminium (mg kg^{-1}) (±SD) in crab samples collected from seven sites (A – G) of the Lourens River over a 12 month period. ND = no data.

DISCUSSION

Al concentrations measured in the sediment from all sites monitored over 12 months generally showed concentrations of less than 4070 mg kg^{-1}. A few exceptions were however found at sites F and G, where the Al concentrations were significantly higher. During the third sampling occasion (27/02/04) the mean Al concentrations of 8887,2 ±608.9 mg kg^{-1} and 17599,6 ±577.8 mg kg^{-1} were measured in the sediment samples collected from site F and G respectively. These comparatively high concentrations cannot be attributed to pH fluctuations since the pH values measured (6 and 7 respectively) during this sampling occasion were equal to or higher than those measured during the same sampling occasion at the sites higher up in the river. These previous sites (A to E) did not display the same peak in total Al concentrations for the same occasion. These high concentrations can also not be attributed to agricultural runoff since sites A - E, located within the agricultural area, would then have displayed a similar range of Al concentrations. According to

Alloway (1995), agricultural and horticultural materials and practices are not a known source of Al contamination even in technologically advanced countries. Rainfall figures recorded during the month of February show only 6.2 mm of precipitation. It seems such little rainfall during this sampling occasion would not affect urban storm water runoff to the point where such high concentrations are reached. Rainfall figures from five other months during which sampling was done showed results which ranged from 45.6 mm to 108.4 mm. Mean Al concentrations recorded from sediment samples collected during these months did not show significantly higher values than those recorded during the dryer months (Jan and Feb 2004). With the exception of site E, it appears that mean Al concentrations increased gradually as the river passes through urban areas. This means that increased anthropogenic influence in urban areas, could possibly be a contributing factor in the steady rise in Al concentrations detected at these sites in the Lourens River. Sites F and G were the only two sites located in, and just after the town of Somerset West. It is therefore also possible that the high Al concentrations measured during occasion three at these sites are as a result of some urban effluent input into the river.

As discussed in the introduction to this chapter, acidification of soil allows Al leaching to take place which ultimately increases the concentrations of Al in natural waters. As indicated in chapter three, acidification of soil can occur naturally as well as through human interference. The removal of large tracts of vegetation, as has occurred on the Lourensford and Vergelegen farms, also removes base cations (i.e. Ca^{2+} and Mg^{2+}) that have been taken up by the vegetation (Cresses and Edwards, 1988). Lydersen *et al.* (2002) have shown in a laboratory study that the addition of base cations, such as Ca^{2+} or Na^{+}, to Al-rich water reduced Atlantic salmon mortality significantly, compared to Al-only exposures. The River Health Programme (2003) reported that 33% of the Lourens River catchment area is planted under forestry. The logging of trees and removal of natural vegetation to make way for further agricultural development is likely to increase the acidification of these soils (Dallas and Day, 1993). As mentioned in chapter three, the Lourens River is located in the fynbos biome and therefore the impact of this vegetation on natural

acidification of the soil must also be taken into account. Although humic and other weak acids produced by the decomposition of this vegetation type can increase the acidity of soils, Tipping *et al.* (1991) has shown that humic acids reduce the bioavailability of Al by binding this metal. A study conducted by Desouky *et al.* (2002) has shown that 48 hrs after the addition of Al (500 µg L^{-1}), 83% was lost from the water column through the reducing action of oligomeric silica (20 mg L^{-1}) and humic acid (10 mg L^{-1}). Bioavailability of Al may further be decreased through its binding capacity with alginate in the presence of calcium and also with organic matter (Musibono, 1998). These aspects could therefore all be contributing factors in explaining the comparatively low Al concentrations detected in crab (*P. perlatus*) samples. The predominant source of Al in the sediment of the Lourens River is therefore probably naturally occurring Al leaching from parent rock as a result of an increase in the acidity of soils in the area and not anthropogenic (Cronan *et al.*, 1979).

Total Al concentrations in soil or sediment do not predict the potential risk to organisms from that particular concentration. Certain organisms can modify the environment of soil particles such that the partitioning of the metals is altered. For example, factors such as the pH of the gut of an invertebrate may play an important role in controlling the availability of metals. It is the ability of the metal to partition from the solid phase to a soluble phase, to a reaction with an important receptor site that leads to risk to the organism (Allen, 2002). According to Musibono (1998) and Herrmann (1987) the bioavailability and toxicity of Al in the sediment is largely dependent on the pH of the water. It must however also be mentioned that in naturally acidic waters Al is present but is not always bioavailable, because it is absorbed onto organic molecules. At intermediate pH values, as encountered in this study, Al is known to be sparingly soluble. This is a possible reason for the lower concentrations detected in the crabs compared to those detected in the sediment. Changing the pH of water changes the concentration of both H^+ and OH^- ions. This affects the ionic and osmotic balance of aquatic organisms. Relatively small changes in pH are not normally lethal, although sublethal effects such as slow growth and reduced fecundity may occur due to increased physiological

stress placed on the organism by increased energy requirements (Dallas and Day, 1993). Ramamoorthy (1988) has reported that Al is most toxic to fish at a pH of between 4.5 and 5.1. Although the pH values measured during the 12 months of sampling never entered the above mentioned range, the lowest values did reach 5.2 on three occasions. Two of these occurred during the forth sampling occasion at site B and the other during the fifth at site C. Research conducted by Brodeur *et al.* (1999) has shown that river water (Fossbeck River) with a pH of 5.2 and an Al concentration of 94 μg L^{-1} resulted in the death of most fish (*Salmo salar*) before the end of the 48 hr exposure period. A large elevation in heart rate was observed together with a decrease in plasma chloride concentrations and an increase in hematocrit, plasma glucose and plasma cortisol levels.

Crabs collected during sampling occasion one (29/10/2003), showed significantly higher Al concentrations than those collected during the remaining occasions. Results from the same month (28/10/2004) one year later did not show a similar increase. Only very small differences in water temperature and pH have been noted between these two occasions and are therefore unlikely grounds for such large differences in Al concentrations. It could be that an Al contaminated food source became more readily available during that time, therefore increasing the concentration in the crabs. Another possibility is that the pH could have changed for a period of time before this sampling occasion resulting in an increased bioavailability of Al and therefore an increased concentration in the crabs when sampled. In general the mean total concentration of Al found in the crabs is only between 5 and 10% of those concentrations found in the sediment. According to Hill and O'Keeffe (1992), crabs are known to be generalist feeders where a significant part of their diet consists of detritus. It is possible that Al is actively taken up in this manner. Although proofs of food chain accumulation of Al are still weak (Herrmann, 1987), Nyholm (1981) has suggested that bioaccumulation of Al in invertebrate prey organisms, as a possible explanation for impaired hatching success observed in birds. Al contaminated invertebrates and plants might thus be a link for aluminium to enter into terrestrial food chains (Rosseland, 1990). As mentioned in chapter two, the Lourens River provides habitat for

the Cape clawless otter (*Aonyx capensis*), water mongoose (*Atilax paludinosus*) giant kingfisher (*Megaceryle maxima*) and other water dependent birds which feed on crabs (Purves *et al.*, 1994).

However, the significantly lower concentrations found in *P. perlatus* may indicate their ability to manage Al as a result of internal detoxification mechanisms. According to Simkiss and Taylor (1989), many terrestrial invertebrates are able to manage toxic metal concentrations and essential trace elements predominantly through cellular processes. These include the removal of whole degenerated cells, exocytosis or extrusion of metal containing vesicles into the digestive tract. This can be understood through the elucidation of the concentration factor. Dallinger *et al.* (1993) explains the phenomenon of the concentration factor in the following way. "It refers to the ratio between the assimilated constant and the eliminated constant and therefore indicates by how much a metal is concentrated in an animal in relation to environmental concentrations", in this case, sediment concentrations. Although Dallinger *et al.* (1993) has applied this concept to terrestrial invertebrates, it appears that the application of the concentration factor for the classification of macroconcentrators, microconcentrators and deconcentrators could be applied to the regulation of Al by crabs in the Lourens River. The comparatively lower Al concentrations found in the crabs suggests that they could be classified as deconcentrators.

The interactions of various metals must also be considered when analysing such data as synergistic interactions may have adverse effects on aquatic organisms. Musibono (1998) has shown that mortality of freshwater amphipods (*Paramelita nigroculus*), when exposed to solutions of Al and Copper (Cu), is higher than for those exposed to mixtures of Al, Cu and Manganese (Mn) in acidic waters (pH = 5 ± 0.8) in both acute and chronic tests. Al and Mn are antagonistic as are Al, Cu and Mn. According to results for the experiment on survival, the toxicity of different mixtures to *P. nigroculus* is, from the most to the least toxic, as follows: Cu + Mn > Al + Cu > Al + Mn > Al + Cu + Mn. Therefore the toxicity of Al can be influenced either synergistically or antagonistically when exposure is mixed with Cu and

Mn as was found in this study even though mean concentrations of each metal differed significantly (see Chapters 5 and 8).

In studies conducted by Odendaal and Reinecke (1998) results have shown that the terrestrial isopod *Porcellio laevis* exhibits the ability to distinguish between and avoid food sources contaminated with high levels of cadmium sulphate. This is considered a contributing factor in allowing this animal to tolerate high environmental concentrations (LC_{50} of 26 700 mg kg^{-1}) of this contaminant. In another study, Weibenburg *et al.* (2003) showed avoidance of copper contaminated leaf litter by *Porcellio scaber*. A similar behavioural response has been obtained by Fountain *et al.* (2001). In the Fountain *et al.* (2001) study, springtails (*Folsomia candida*) were observed avoiding food contaminated with Cd, Cu, Pb and Zn. In the aquatic environment, De Lange *et al.* (2005) have shown that two freshwater invertebrate species, *Gammarus pulex* and *Asellus aquaticus*, avoid polycyclic aromatic hydrocarbon (PAH) spiked sediment. Although some of these studies were conducted on terrestrial invertebrates, it is possible that *P. perlatus* could also have the ability to distinguish between and avoid contaminated food sources.

These four aspects: bioavailability, detoxification mechanisms, metal interactions and avoidance are all possible factors influencing the uptake and management of Al by *P. perlatus* from its environment.

CHAPTER 5

COPPER

INTRODUCTION

The geological composition of the study area in the present investigation is sandstone and shale. Typical Cu concentrations found in sandstone and shale are 5 – 20 mg kg^{-1} and 30 – 150 mg kg^{-1} respectively (Adrinao, 1986). Like many other metals copper (Cu) is an essential element in the human body (Friberg *et al.*, 1986) and plants (Alloway *et al.*, 1995) and is also one of the world's most widely used commercial metals (Musibono, 1998). In nature it is widely distributed in its free state, as well as in sulphides, arsenides, chlorides and carbonates. Cu is also absorbed and assimilated by plants in varying concentrations. The availability of Cu to plants refers to the readiness with which the available ion $[Cu(H_20)_6]^{2+}$ is absorbed by plants in acidic soils and $Cu(OH)_2$ in neutral and alkaline soils. This availability is related to the chemical potential of the respective Cu species in the soil solution, which is dependent on pH. The abundance of essential micronutrients in plants generally rank in the order Fe>Mn>B>Zn>Cu>Mo>Cl. Typically Cu concentrations in plants range from 5 – 20 mg kg^{-1}. Cu can also be specifically absorbed or "fixed" in soils, making it one of the trace elements which moves the least (Alloway *et al.*, 1995). In the aquatic environment, Cu can occur in particulate, colloidal and soluble form and its speciation is influenced by physico-chemical, hydrodynamic and biological factors (Moore and Ramamoorthy, 1984).

Mining of Cu has taken place for many years. The all-time mine production of Cu is estimated in excess of 307 million metric tons. More than 50% of this production is consumed by the electrical industry providing power transmission, electronics and electrical equipment (U.S. Minerals Yearbooks, 1911-1979 as sited in Moore *et al.*, 1984). According to Nriagu (1979) 75% of atmospheric emissions of Cu are from anthropogenic sources. Production of nonferrous metals is the largest single anthropogenic source followed by

wood combustion and iron/steel production. Alloway *et al.* (1995) reported that fertilisers, sewage sludge, fungicides and bactericides are all known anthropogenic sources of Cu. Another is manure from poultry and swine fed on a Cu-enriched diet to increase feed efficiency and greater growth rates. Moore and Ramamoorthy (1984) reported that the most important natural discharge of Cu into the atmosphere is wind-blown dust.

Copper is a micronutrient, forming an essential part of cytochrome oxidase and various other enzymes involved in redox reactions in the human body (Friberg *et al.*, 1986). In crabs however, it is an essential component of hemocyanin, the hemolymph pigment responsible for oxygen transport (Engel, 1987; Engel and Brouer, 1987; Tulasi and Ramanarao, 1988). Most studies conducted on the distribution of metals in crustacean tissues show that the midgut gland is the most important organ in the storage of Cu (Bryan, 1976; Baggato and Alikhan, 1987). Since hemocyanin has a remarkably high affinity for Cu ions, this metal is absorbed by the hemolymph and then moved to and stored in the midgut gland. This helps to maintain constant Cu levels in the hemolymph and other internal organs. In a study done by Steenkamp *et al.* (1994) it has been shown that *Potamonautes warreni* is able to bioaccumulate comparatively high levels of Cu.

For some organisms however, Cu is known to be toxic, even in low doses. For some algae it is lethal at only 0.5 mg kg^{-1}. Under certain conditions, Cu is highly toxic to most marine invertebrates. Copper's $LC_{50's}$ are generally known to be less than 0.5 mg l^{-1} though they can range from 0.006 to >225.0 mg l^{-1} (Moore and Ramamoorthy, 1984). The United States Environmental Protection Agency considers Cu as "potentially hazardous" (Duffus, 1980). Its toxicity can however be reduced in the presence of Zn, molybdenum and sulphate (Dallas and Day, 1993). In aquatic ecosystems calcium, magnesium or high levels of alkalinity or TDS (total dissolved solids) are known to reduce the toxicity of copper (Dallas and Day, 1993).

MATERIALS AND METHODS

Refer to Chapter 3

RESULTS: Sediment

The concentrations of total Cu measured in sediment samples, collected over 12 months from seven sites is depicted in Figure 5.1.
(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Cu concentrations measured in sediment, at a particular site over the 12 month sampling period

Pairwise multiple comparison procedures for site A revealed that statistical differences ($p<0.05$) were found for occasion 2 vs 1 and 3 and occasion 1 vs 3. Occasion 4 vs 5, 6 & 7; 5 vs 6 & 7 and 5 vs 7 were also found not to be statistically significant ($P>0.05$). Results for site B show that only when occasion 1 is compared to occasion 4, 5 and 7 and occasion 5 is compared to occasion 2, 3, 4, 6 and 7, do we find statistical differences ($p<0.05$). Cu concentrations for occasions 1 and 5 were found to be 5.8 ±1 and 0 mg kg^{-1} respectively. Comparisons with occasion 5 revealed these results due to its zero value. Sampling occasions 2 and 5 revealed zero concentrations of copper at site C. No statistical differences ($P>0.05$) were found for 4 vs 6 and 7, 6 vs 7 and 5 vs 2 at this site. Results for site D revealed 6 comparisons of no statistical significance ($P>0.05$) namely: 1 vs 3 & 6, 3 vs 6, 4 vs 5 & 6 and 5 vs 7. At site E, statistical significance ($P<0.05$) was found only when occasion 1 and 5 were compared to 2, 3, 4, 6 and 7 respectively. No statistical differences ($P<0.05$) were found for any pairwise multiple comparisons for site F. All comparisons made for site G were found to be statistically significant ($P<0.05$) with the exception of occasion 2 vs 1, 4, 5 & 6; 4 vs 1, 5 & 6; 6 vs 1 & 5 and 1 vs 5.

Comparisons of total Cu concentrations measured in sediment, per sampling occasion over the length of the river

Sampling occasion 1, compared over all sites revealed the following results. Statistical differences ($P<0.05$) were only found for comparisons made between site E and F with A, B, C, D and G. No Cu was detected at sites C

and D, therefore all comparisons with these sites revealed statistical differences ($P<0.05$). Site A and B showed significantly higher mean Cu concentrations during occasion 2 than those concentrations measured at C, D, E, F and G. Occasion 3 had the highest Cu concentration (20.5 ±1.3mg kg^{-1}) at site G. Cu concentrations at this site were found to be statistically significantly higher ($P<0.05$) when compared to all other sites. The same result was found when site E was compared to A, B, C, D and F. Cu concentrations measured at site G were statistically significantly higher than those from all other sites ($P<0.05$). All comparisons made for occasion 5 revealed no statistical differences ($P=0.056$). The highest Cu concentration for occasion 6, was once again found at site G (8.6 ±8.6 mg kg^{-1}) but no statistical differences ($P>0.05$) were found for any comparisons. The same result was found for comparisons made for occasion 7. Concentrations measured at each site varied little from each other and therefore resulted in no statistical differences ($P=0.136$).

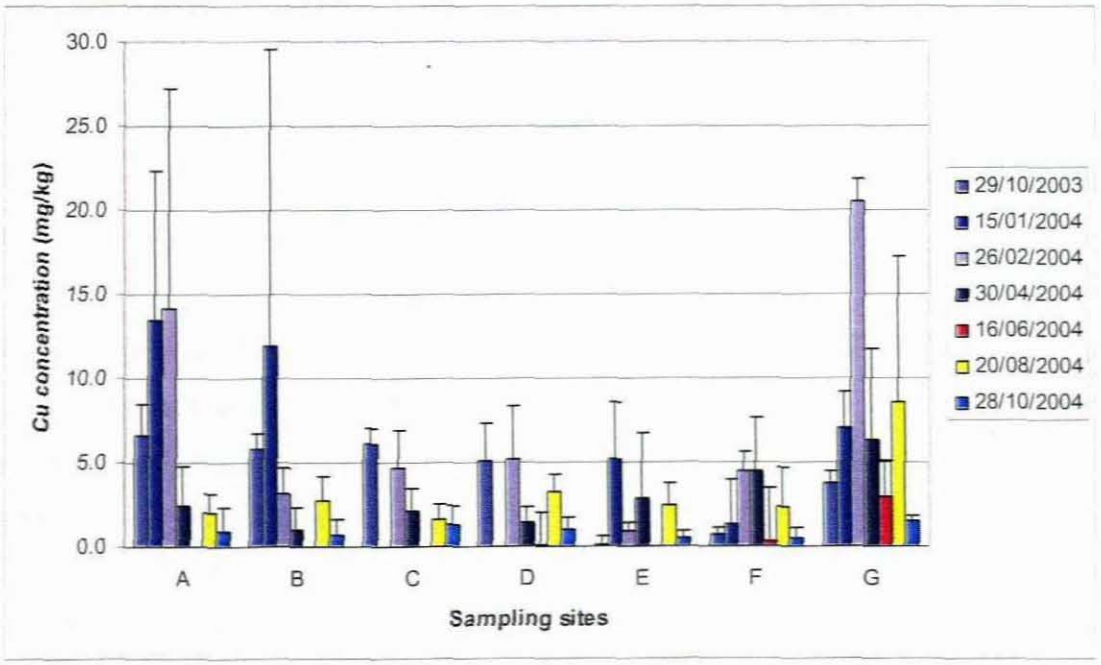

Figure 5.1 *Mean concentrations of total copper (mg kg^{-1}) (±SD) in sediment samples* collected from seven sites (A – G) of the Lourens River over a 12 month period.

RESULTS: Crabs

The concentrations of total Cu measured in crabs, collected over 12 months from seven sites is depicted in Figure 5.2.

(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Cu concentrations measured in crabs collected at a particular site over the 12 month sampling period

A full sample size of five crabs was collected for each occasion at site A. Pairwise multiple comparisons of Cu concentrations found at this site in each sample group revealed no statistical differences (P=0.061). With the exception of occasion 2, all concentrations measured at site B were found to be analogous. Due to the higher concentration found for occasion 2 (59.4 ±24.4 $mgkg^{-1}$), all comparisons made between this occasion and others revealed statistical differences (P<0.05). All other comparisons did not (P>0.05). At site C sampling occasion 3 displayed the highest Cu concentration (54.4 ±4.6 mg kg^{-1}). Pairwise multiple comparisons made at this site only revealed statistical differences (P<0.05) for the comparison between occasion 3 and 6. No comparisons made between concentrations found at site D were statistically significant (P=0.131). Site E revealed the same results (P=0.074). The only comparison found to be statistically different (P<0.05) within this site was 1 vs 5. The low mean concentration illustrated in the graph below for occasion 6 at site G, appears so, as a result of the small sample size. Only two crabs were collected on this occasion. No statistical differences (P>0.05) were found for any comparisons made within this site.

Comparisons of total Cu concentrations measured in crabs, per sampling occasion over the length of the river

During sampling occasion 1, site B was found to be statistically different (P<0.05) when compared to A, C, D, F and G. No crabs were collected during this occasion at site E. This sampling occasion also showed the highest mean Cu concentration (65.2 ±20.3 mg kg^{-1}) at site F. No statistical differences (P>0.05) were found for all pairwise multiple comparisons made for occasion 2, 3, 4 and 5. During sampling occasion 6, a sample size of four crabs was collected from site D and two crabs from site G. Statistical differences were found for comparisons made between site A and all other sites and F vs B, C, D, E and G. Sampling occasion 7 revealed statistical differences (P<0.05) for comparisons between site C and G when each was compared to all other sites.

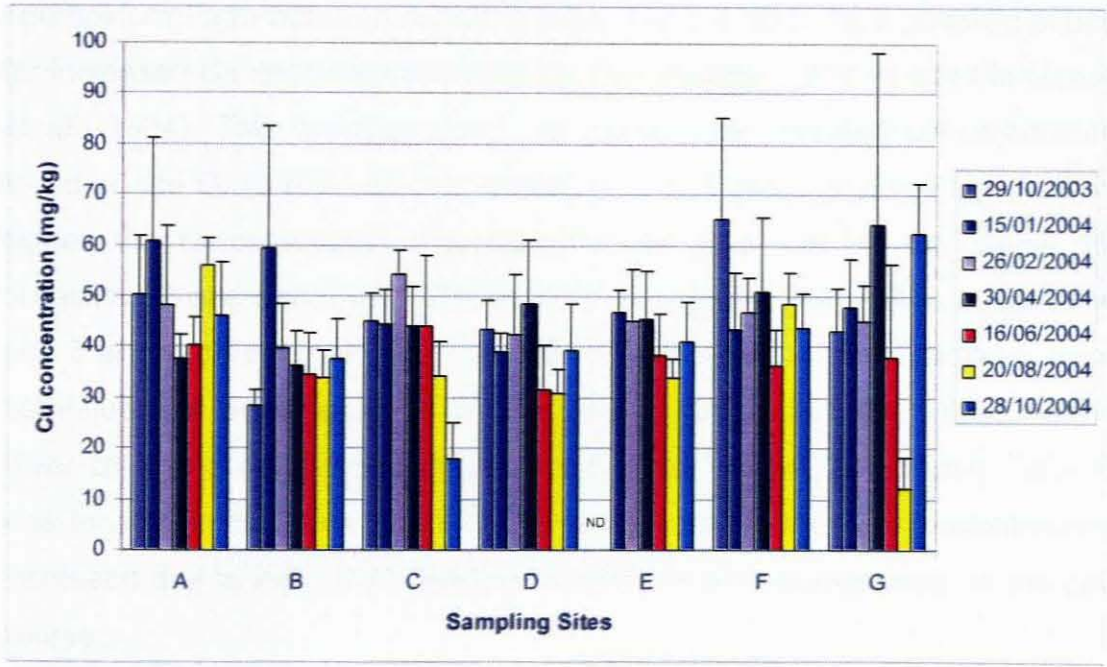

Figure 5.2 Mean concentration of total copper in crab samples collected from seven sites (A – G) of the Lourens River over a 12 month period.

DISCUSSION

Mean Cu concentrations found in the sediment during this study were not as high as those reported by Steenkamp *et al.* (1994). In their study concentrations from three polluted sites were found to be 102.9 ±111.4 mg kg^{-1} in the Natalspruit River; 45.8 ±32.3 mg kg^{-1} in the Bronkhorstspruit River and 12.9 ±2.1 mg kg^{-1} in the Nooitgedacht Dam. In the present study on the Lourens River mean Cu concentrations in the sediment ranged from 0 to 20.5 ±1.3 mg kg^{-1}. Mean concentrations varied significantly within this range without displaying any clear patterns of distribution. The highest concentrations were found at sites A, B and G. A piggery was in operation for many years (1980 - 2001) on the Lourensford Farm, positioned approximately 500m from the river between sampling sites B and C. Since small channels allow water runoff from this area to enter the river, it was expected that sediment Cu concentrations would be elevated at these sites. This was however not the case. The construction of a small sedimentation pond (approximately eight years ago) at the end of one of the main channels could have prevented the piggery from having any significant influence on sediment Cu concentrations at these sites. The creation of excessive dust

through land clearing and planting of vineyards (Dec 2002 – Nov 2004) on the Lourensford Farm between sampling sites A and B could be a possible cause for increased Cu concentration at these sites (Nriagu, 1979 as sited in Moore *et al.*, 1984). This however does not explain the elevated concentrations found at site G. At this site only occasion 2, 3, 4 and 6 showed significantly higher Cu concentrations. Precipitation can also not be the cause as occasions 2 and 3 showed 13.8 and 6.2 mm respectively, while occasion 4 and 6 showed 75.8 108.4 mm rainfall respectively. Cu concentrations were not influenced by an increase or decrease in rainfall. However, the Lourens River creates a natural boundary for part of the Strand Golf course. Site G was located in this area and it is therefore possible that Cu concentrations increased due to input from fertilizer, fungicides or manures used on the golf course.

Corresponding with results reported by Snyman *et al.* (2002), all crabs were found to have significantly higher mean Cu concentrations than the sediment. In the present study mean Cu concentrations ranged from 12.4 ±5.8 to 65.2 ±20.3 mg kg^{-1}. In the study of Van Eeden and Schoonbee (1991) Cu concentrations were detected as high as 99.3 mg kg^{-1} dry weight in whole crabs. Although significantly higher mean Cu concentrations were detected in the sediment during some occasions at sites A, B and G, no comparable increases can be seen in the concentrations detected in crabs collected during those occasions. According to Bryan (1976) the most important mechanism by which tissue Cu concentrations increase, may be via the intake of Cu-rich food. Although food is the major source of Cu in decapod crustaceans, Cu may also be obtained from the surrounding water column. The midgut gland and gills seem to be the worst affected by Cu contamination. Rainbow (1985) and Rainbow and White (1989) have shown that decapod crustaceans have the ability to control body concentrations of essential metals, one of these being Cu. Although at higher ambient Cu concentrations this regulation mechanism breaks down, which results in an increase in mean Cu levels in crabs (Bryan, 1976; White and Rainbow, 1984).

In the study conducted by Steenkamp *et al.* (1994) seasonal variations were found in the bioaccumulation of Cu in *P. warreni* in one of the three sites investigated, namely the Natalspruit River. Concentrations of Cu were found to be lowest during the colder months of the year (May, July and September) and increased during the hotter months. It is known that water temperature affects the metabolism of invertebrates and therefore would also affect the organism's uptake of contaminants. In the present study however, no clear pattern of temperature-related Cu increase in *P. perlatus* was observed. These results correspond with the results obtained from the remaining two sites (Bronkhorstspruit River and Nooitgedacht dam) in the Steenkamp *et al.* (1994) study. Snyman *et al.* (2002) showed similar results, where Cu concentrations found in the digestive gland of *P. perlatus* showed no seasonal differences. It must be remembered, that bioaccumulation of metals in aquatic biota is not determined by total mean environmental concentrations but rather by the speciation of that metal. Changes in speciation and therefore toxicity of Cu may be caused by changes in the physico-chemical conditions of the water body (Dallinger and Rainbow, 1993). Steenkamp *et al.* (1994) have shown that the crab *P. warreni* is able to regulate Cu concentrations in its various tissues and is therefore not a suitable indicator of the presence of Cu in aquatic environments. Snyman *et al.* (2001) have also shown that *P. perlatus* cannot be used to predict environmental concentrations since it is also capable of regulating Cu.

In their study, Steenkamp *et al.* (1994) have shown that gender plays no significant role in total body bioaccumulation of Cu in decapod crustaceans. This is confirmed by Alikhan *et al.* (1990) and Bardeggia and Alikhan (1991). Although the sexes of all crabs collected during this study were noted, no correlations of sex to metal concentrations were studied.

Musibono (1998) has shown that the combination of Cu and Mn are toxic to *Paramelita nigroculus*. Only 40% survived after 96 hours of exposure to mixed concentrations of these elements. The combination of Al and Cu is less toxic; 54% survived after 96 hours of exposure. When Al, Cu and Mn are combined the results showed that they are not acutely toxic as 87% of *P. nigroculus*

survived the same exposure time. As described in the previous chapter, total Al concentrations in the sediment of the Lourens River were high. Mean manganese concentrations (refer to chapter 8) ranged from 10.2 ±2.6 to 176.7 ±98.8 mg kg^{-1} with the majority of results being less than 50 mg kg^{-1}. These mixed concentrations could have reduced the toxicity of Cu in the present study.

CHAPTER 6

CHROMIUM

INTRODUCTION

The earliest known use of Cr was in the production of paint pigments in the 1800's in France, Germany and England. Today, Cr is used in the stainless steel, chrome-plating, paint pigment, tanning, textile colouring and other industries. It is widely distributed in the earth's crust with an average concentration of 125 mg kg^{-1} but is rare in natural waters. Chromite is the only commercially important ore for the production of Cr. South Africa has become one of the leading producers of Chromite with a production of 3.2 million metric tons in 1979 (US Minerals Year Book: 1920 – 1979, as sited in Moore and Ramamoorthy, 1984). In his synoptic review of the hazards of Chromium (Cr) to fish, wildlife and invertebrates, Eisler (1986) states that most authorities agree on seven points:

1: In the vicinity of electroplating industries, publicly-owned municipal treatment plants, tanneries, oil drilling operations and cooling towers, Cr levels in soil, air, water and biota are elevated.

2: Hexavalent chromium (Cr^{6+}) is known to be the most biologically active of all Cr species.

3: Cr is an essential trace element in humans and some laboratory animals.

4: Cr is mutagenic, tetratogenic and carcinogenic at high environmental concentrations.

5: No biomagnification of Cr has been observed in food chains and concentrations are usually highest in the lowest trophic levels.

6: Toxic and sublethal properties of Cr are modified by a variety of biological and abiotic factors.

7: Organism sensitivity to Cr varies, even among closely related species.

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6: Toxic and sublethal properties of Cr are modified by a variety of biological and abiotic factors.

7: Organism sensitivity to Cr varies, even among closely related species.

Cr can be carried in the air when associated with particulate matter (>5 µm in diameter) and originates from windblown dust and natural soil-forming processes (Lide, 1998). Cr occurs in several oxidation states ($^{-2}$ to $^{+6}$), of which Cr^{6+} (hexavalent Cr) is known to be the most toxic. Hexavalent chromium exists only as oxy species such as CrO_3 and is strongly oxidising (Moore and Ramamoorthy, 1984). According to Mattuck *et al.* (1995) Cr speciation, and therefore toxicity, depends primarily on pH and redox conditions. Results from their studies have shown that Cr binds strongly to sediment particles and requires a decrease in pH to allow it to leach from sediments. It is not yet understood why, but some algae accumulate and tolerate Cr concentrations of up to 4000 times (Dallas and Day, 1993). Cr is naturally found in small quantities in the RNA of a few organisms. Although Cr is known to be one of the least toxic of the trace elements and is also an essential part of fat and carbohydrate metabolism in mammals (Dallas and Day, 1993), its toxic effects can still be seen across a wide range of taxa. Mammalian bodies can tolerate 100 to 200 times their total natural body content of Cr without adverse effects (Moore and Ramamoorthy, 1984). At higher concentrations though, it can become tetratogenic, mutagenic, genotoxic and carcinogenic in mammals (Dallas and Day, 1993). The stomach acidity of mammals reduces Cr^{6+} to the much less toxic Cr^{3+} form where gastrointestinal absorption is less than 1% (Moore and Ramamoorthy, 1984).

According to Moore and Ramamoorthy (1984) acute toxicity of Cr to freshwater invertebrates is highly variable. Cr is however readily transferred through food to invertebrates and is therefore probably a more significant source of Cr than water. There is however no indication of macroconcentration of Cr in any invertebrate species. Pfeiffer *et al.* (1982) have shown that fish and emergent grass concentrate Cr from water and/or sediment and then return the metal to their environment as detritus. Hexavalent Cr has been shown to cause a temporary decrease in hemolymph glucose levels in the freshwater prawn, *Macrobrachium lamarrei*, at 1.84 mg kg^{-1} over 96 hrs of exposure (Murti *et al.*,

1983). Fromm and Stokes (1962) have shown that various freshwater fish can regulate Cr over a range of environmental concentrations. Bioaccumulation in freshwater invertebrates from polluted waters generally ranges up to 25 mg kg^{-1} dry weight compared to ≤5 mg kg^{-1} for unpolluted waters. Unlike some metals, Cr does not normally concentrate significantly in any one specific organ or tissue.

MATERIALS AND METHODS

Refer to Chapter 3

RESULTS: Sediment

The concentration of total Cr measured in sediment samples, collected over 12 months from seven sites is depicted in Figure 6.1.
(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Cr concentrations measured in sediment, at a particular site over the 12 month sampling period

Pairwise multiple comparison procedure of sediment Cr concentrations at the different sites over time revealed the following results. At site A, occasion 2 (4.4 ±1.1 mg kg^{-1}) and 3 (5.8 ±3.6 mg kg^{-1}), showed statistical differences ($P<0.05$) from all other occasions in pairwise comparisons. No other statistical differences were found for any other comparisons within this site. Occasions 2 and 3 also showed statistical differences ($P<0.05$) when compared to all other occasions within site B. Statistical differences ($P<0.05$) were also found for comparisons made between occasion 7 and occasions 1, 4 and 6 for this site. All comparisons made between occasion 2 and 3 with all other occasions at site C were found to differ statistically. However, mean Cr concentrations for occasion 2 and 3 was found not to be statistically different ($P>0.05$) when compared to each other at site C. At site D, all comparisons made between occasion 2 and 3 with all other occasions were found to be statistically different ($P<0.05$), except when occasion 2 and 3 were compared to each other. The same results were found for comparisons made for site E, F and G. Sampling occasions 2 and 3 showed

consistently higher mean Cr concentrations than all other occasions with a significant peak during occasion 3 at site G (40.1 ±1.8 mg kg^{-1}).

Comparisons of total Cr concentrations measured in sediment, per sampling occasion over the length of the river

Comparisons made between Cr concentrations found at each site during sampling occasion 1 showed statistical differences for all, except A vs B; C vs E and F; D vs C, E and F and F vs E. The Cr concentration (2.7 ±1 mg kg^{-1}) found during occasion 2 at site B, resulted in statistical differences ($P<0.05$) when compared to all other sites during this occasion. Results from comparisons within occasion 3 showed no statistical differences ($P>0.05$) for A vs D and E; C vs A, D and E, and D vs E. As stated above, site G during this occasion showed a significantly higher Cr concentration (40.1 ±1.8 mg kg^{-1}). Multiple pairwise comparisons made for occasion four, showed statistical differences ($P<0.05$) for all comparisons, except C vs A and E; D vs A, E and C and E vs A. No statistical differences were found for any comparisons made for occasion five. Results from occasion six showed no statistical differences ($P>0.05$) for comparisons made between sites C, D, E and F. Site G revealed a mean Cr concentration of 5.5 ±0.7 mg kg^{-1}, and was the only site found to be statistically different ($P<0.05$) from all other sites during sampling occasion 7.

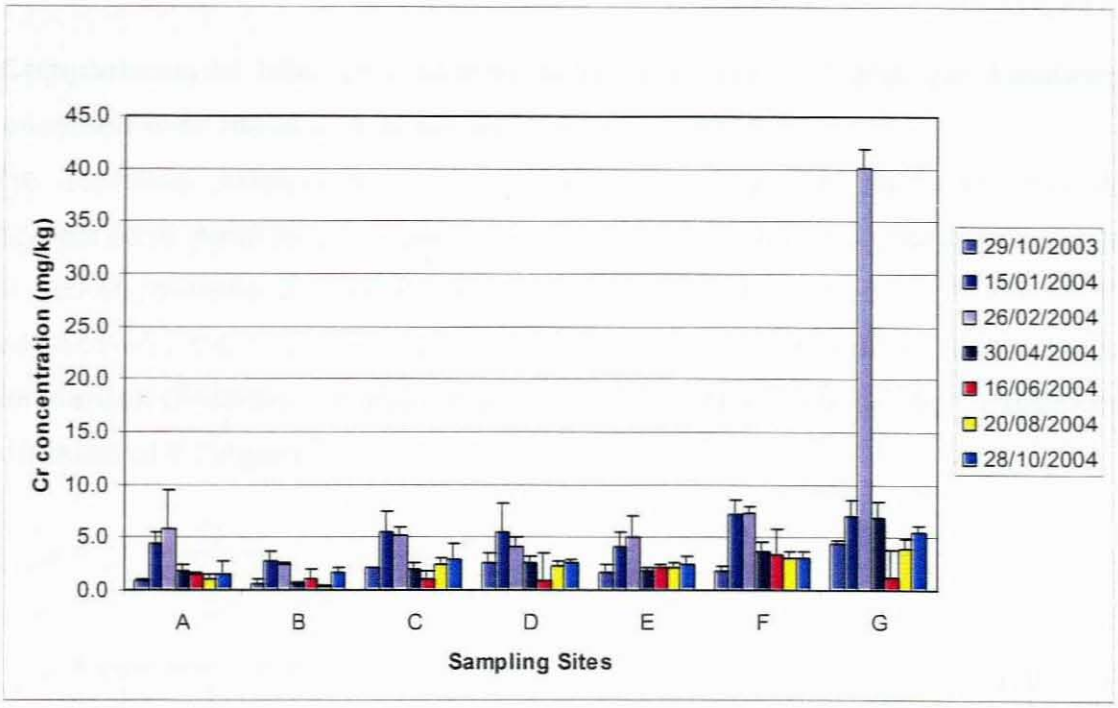

Figure 6.1 Mean concentrations of total chromium (mg kg^{-1}) (±SD) in sediment samples collected from seven sites (A – G) of the Lourens River over a 12 month period.

RESULTS: Crabs

The concentrations of total Cr measured in crabs, collected over 12 months from seven sites is depicted in Figure 6.2.

(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Cr concentrations measured in crabs collected at a particular site over the 12 month sampling period

Cr was only detected in crab samples during occasions 3, 5 and 7. During occasion 5, Cr concentrations ranged from 1,5 – 2,5 mg kg^{-1}. Site F revealed the highest concentration and site G the lowest. Occasions 3 and 7 were the only other occasions where Cr was detected. Sites A, B, C, D, E and F all showed statistical differences ($P<0.05$) when occasion 5 is compared to all other occasions within individual sites. At site C, occasion 3 was found to be statistically different ($P<0.05$) from all other occasions. At site G occasions 5 and 7 were statistically different from occasions 1,2,4 & 6 respectively.

Comparisons of total Cr concentrations measured in crabs, per sampling occasion over the length of the river

No statistical differences (P>0.05) were found for any pairwise multiple comparisons made for occasions 1, 2, 3, 4, 5 and 6. Although occasion 3, at site C and G, revealed Cr concentrations of 0.3 ±0.4 mg kg^{-1} and 0.4 ±1.7 mg kg^{-1} respectively, the high standard deviation for each resulted in no statistical differences (P<0.05). Occasion 7 at site G revealed 0.5 mg kg^{-1} with a standard deviation of 0.2 mg kg^{-1}.

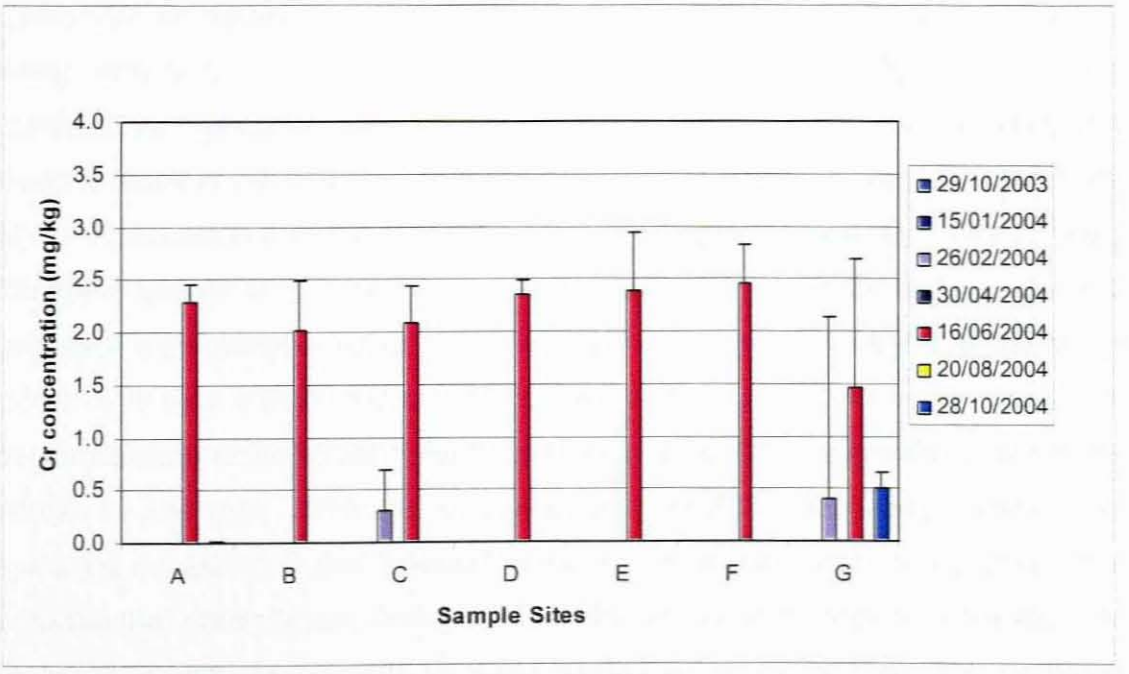

Figure 6.2 Mean concentrations of total chromium (mg kg^{-1}) (±SD) in crab samples collected from seven sites (A – G) of the Lourens River over a 12 month period.

DISCUSSION

Sediment Cr concentrations can vary greatly. In a study conducted by Pfeiffer *et al.* (1982), concentrations were recorded as high as 17 536 mg kg^{-1} in the Irajá River, Brazil - more than a thousand times higher than previously published data for freshwater systems. Carton *et al.* (2000) recorded sediment Cr concentrations ranging from 50 – 630 mg kg^{-1} in the River Aire in Yorkshire, U.K.

Comparisons of total Cr concentrations measured in crabs, per sampling occasion over the length of the river

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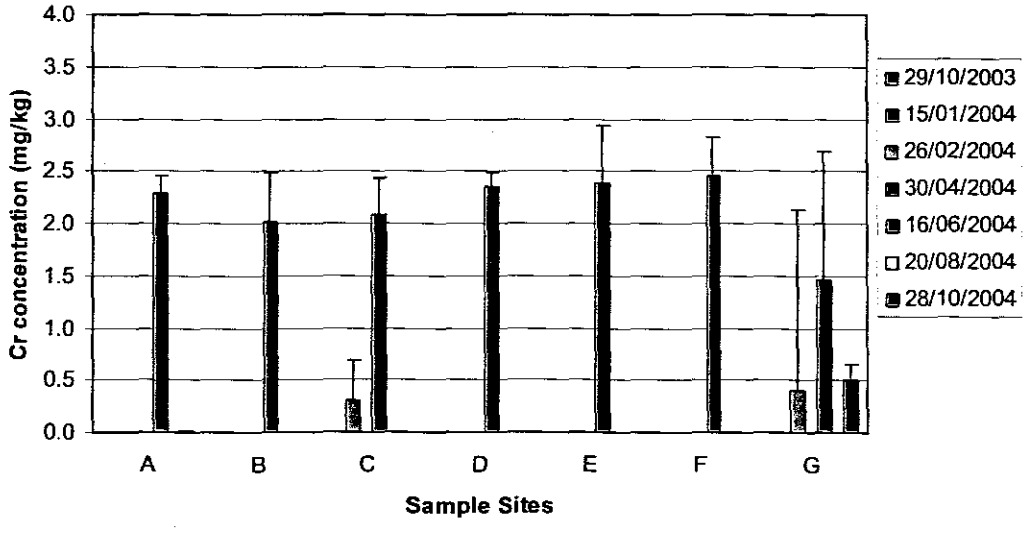

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In the present study, almost all sediment Cr concentrations remained below 5 mg kg^{-1}. Only occasions 2 and 3 showed Cr concentrations higher than 5 mg kg^{-1} at various sites as did occasion 4 and 7 at site G. With the exception of occasion 3 at site G, the Lourens River can be considered an unpolluted site in terms of sediment Cr concentrations (Fromm and Stokes, 1962). Sampling occasion 3 at site G showed a remarkable increase in Cr to reach a mean concentration of 40.1 ±1.8 mg kg^{-1}. These elevated concentrations cannot be attributed to runoff from precipitation since only 13.8 and 6.2 mm of rain fell during occasions 2 and 3 respectively. According to Young *et al.* (1973), municipal waters are known to release considerable amounts of Cr into the environment. The Southern Californian municipal treatment plants discharge 600 – 700 metric tons of waste water with a Cr concentration of 40 – 800 µg L^{-1} annually. The high Cr concentration reached at site G (occasion 3) in the present study could possibly be as a result of waste water discharge just prior to sampling. With the exception of site B, occasion 2 and 3 showed consistently higher concentrations of Cr along the entire course of the river. According to Carton *et al.* (2000), Cr in suspended sediment may increase up to 100% during and immediately after a single storm event. This may provide the conditions needed for Cr contaminated sediment to be transported down stream resulting in decreased Cr concentrations along the course of the river. With the exception of May 2004 (sampling occasion 5), sampling occasions 2 and 3 were the months with the lowest precipitation. It is possible that precipitation, during the months when concentrations were found to be lower, is partly responsible for these decreased concentrations. The pH levels measured during occasions 2 and 3 did not differ much from those measured during other sampling occasions with the exception of site G where the pH was found to be 7 at both these sites (Table 3.2). According to Mattuck *et al.* (1995), Cr found in these conditions would be tightly bound to sediment particles with little speciation occurring. A decrease in Cr concentration at such a site could occur through transport of suspended sediment through water turbulence.

Cr is one of the ingredients used in the preservation of wood (chromated copper arsenate) and therefore the sawmill and wood treatment plant on the Lourensford farm was expected to have an impact on the Cr concentrations found in sediment samples. This was however not the case. No marked increase in Cr concentrations were found in the sediment in the vicinity of the sawmill (sites C and D). In a similar study, Lyytikäinen *et al.* (2000) found that transportation of wood preservatives from a sawmill area to its surroundings was fairly low.

During this study there were also no known Cr containing pesticides, fungicides or herbicides in use on either the Lourensford or Vergelegen farms.

In a field experiment conducted by Berge and Brevik (1996) it was shown that crabs (*Cancer pagurus*) accumulated Cr in claw tissue, the hepatopancreas and additional carapace tissue when exposed to sediment concentrations of 670 mg kg^{-1}. Although the Cr concentrations measured in the crabs were small, (< 0.25 mg kg^{-1} for each) in each case an increase in bioaccumulation of more than 100% was evident with increased exposure time (after 1 month). Burger *et al.* (2002) found Cr levels of 0.095 ±0.09 mg kg^{-1} in the apoderme of horseshoe crabs (*Limulus polyphemus*). The apoderme accumulated more Cr than both the eggs and leg muscle. Studies done by Neff *et al.* (1978) have suggested that benthic invertebrates have only a limited ability to accumulate Cr from sediments or clays. According to Moore and Ramamoorthy (1984) Cr is readily taken up by invertebrates through food and is therefore probably a more significant source of Cr than water. The presence of Cr in the sediment of the Lourens River therefore probably makes this element available to *P. perlatus* during feeding. With the exception of occasion 5, the low or undetectable concentrations of Cr found in the crab samples possibly indicates the ability of *P. perlatus* to regulate Cr concentrations in their body. Significantly higher Cr concentrations in crabs were detected during sampling occasion 5 at all sites. This increase in the crabs cannot be attributed to the sediment concentration during this occasion. At site C, D and G, the sediment Cr concentrations during occasion 5 were in fact lower

than all other occasions. The mean water temperatures measured during the month of June 2004 (occasion 5), although slightly cooler, are not very different from those temperatures measured in the previous and later sampling periods (Table 3.1). According to Karbe et al. (1977) seasonal variations in bioaccumulation are normally evident because Cr uptake is often temperature dependent. However, results from the present study do not show a link between temperature and bioaccumulation. The increased concentrations detected during occasion 5 could possibly be as a result of some single event, anthropogenic source in the upper reaches of the Lourens River. Wind blown dust could also be a possible influencing factor as Cr can be carried by airborne particulate matter (Lide, 1998). Any number of these factors could have increased the bioavailability of Cr during occasion 5 even though the sediment concentration did not change much.

According to Moore and Ramamoorthy (1984), toxic interactions with other metals have not been fully documented. However, Spehar and Fiandt (1986) have shown that mixtures of As, Cd, Cr, Cu, Hg and Pb, at concentrations based on United States water quality criteria, induced chronic effects in two species of fish and one invertebrate (the daphnid *Ceriodaphnia dubia*). Biesinger *et al.* (1986) too has shown, in chronic toxicity tests, that binary mixtures of Cd, Cu and Cr exerted toxic action on *Daphnia magna* while no significant effects were seen when these metals were tested singly. Although the concentrations of Cr detected in this study are low, possible synergistic effects with other metals must be considered. However, considering its lower toxicity (relative to some other metals), low sediment concentrations and the apparent bio regulation in crabs, it is unlikely that Cr poses significant health risks to aquatic biota in the Lourens River.

CHAPTER 7

IRON

INTRODUCTION

According to Friberg *et al.* (1986), Iron (Fe) is one of the most abundant metals and constitutes 5% of the earth's crust. Bear (1964) claimed that the Fe content, expressed as percentage Fe_2O_3, makes up 1 - 6% of soils which is comparable to 7% of the earth's crust. Fe occurs in a variety of inorganic compounds such as oxides, carbonates, disulphides, sulphates and chlorides and also in some carbonyls. Fe has been mined for many years and in various countries. It is used primarily in the production of steel, which has become one of the cornerstones of the industrialised world. In 1982 the total world iron ore production was 780 million metric tons with the major world producers being Russia, Brazil, Australia and China (Klinger, (1982) as cited in Friberg *et al.* (1986)). Fe has various other applications, with Fe oxides being used in paint and plastic pigments while certain Fe salts are frequently used for the treatment and prevention of Fe deficiency in humans (Friberg *et al.*, 1986). Bowen (1966) has reported that Fe concentrations in soil can vary from 7000 mg kg^{-1} to extreme values of 550 000 mg kg^{-1}. Although guideline values for Fe in freshwater were published by Kempster *et al.* (1980) and Kempster and Smith (1985), the Canadian Water Quality Guidelines (1992) set a lower value of 0.3 mg lt^{-1} for both hard and soft water. In 1996 the Department of Water Affairs and Forestry set a new guideline for Fe concentrations in South African waters, which states that Fe concentrations should not vary by more than 10% of the background concentration for that area.

Fe is an essential micronutrient in all organisms where it forms a part of haeme-containing respiratory pigments (e.g. haemoglobin), catalases, cytochromes and peroxidases. The bioavailability of aqueous iron is affected by water pH, which

changes its speciation. It appears that while the ferric ion (Fe^{3+}) is biologically unavailable, the ferrous (Fe^{2+}) ion is bioavailable to organisms (Albergoni and Piccinni, 1983). According to the Canadian Water Quality Guidelines (1992), the ferric ion is generally the predominant form in aerated, surface waters although it can be reduced to the ferrous state under reducing conditions. Although at high concentrations its toxic properties include inhibiting various enzymes, it is not easily absorbed through the gastro-intestinal tract of invertebrates (Dallas and Day, 1993). However, Heath (1987) reports that altered physiological functions result when Fe reaches sufficiently high concentrations in body cells. Gerhardt (1995) has reported that the toxic effects of Fe at low pH in the aquatic environment, have been underestimated in the literature. Because Fe compounds are easily oxidised, high concentrations of Fe in aquatic environments can cause oxygen depletion (Dallas and Day, 1993). In a study conducted by Myllynen *et al.* (1997), results suggest that the hatching success of roe lamprey, a commercially important species, is negatively influenced by an increase in total Fe concentrations in Finnish rivers. Pynnönen (1995) has shown that a sediment Fe concentration of just 200 mg kg^{-1} decreased the burrowing activity of the marine isopod, *Saduria entomon*. The same Fe concentration was avoided by *S. entomon* when contaminated and uncontaminated sediments were present in a laboratory experiment. Roldan and Shivers (1987) have shown that high Fe levels have caused pathological changes in the ultrastructure of certain cells in the hepatopancreas in crayfish. They have also demonstrated pathological responses in the antennal gland of the same species. Hellawell (1986) has reported that other aquatic organisms, such as fish, could be indirectly affected through the killing off of benthic prey organisms. It was also shown that higher levels of Fe in water can be detrimental to aquatic organisms by forming a precipitate on their gills and thereby reducing respiration surface area in these animals.

MATERIALS AND METHODS

Refer to Chapter 3

RESULTS: Sediment

The concentrations of total Fe measured in sediment samples, collected over 12 months from seven sites is depicted in Figure 7.1.

(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Fe concentrations measured in sediment, at a particular site over the 12 month sampling period.

Pairwise multiple comparisons made for groups within site A revealed no statistical differences ($P<0.05$). At site B, only occasion 7 (3384 mg kg^{-1}) revealed statistical significance ($P<0.05$) when compared to all other occasions. Occasion five at site C was significantly different ($P<0.05$) from all other sampling occasions within the same site. Occasion three vs one and two was also statistically significant ($P<0.05$). Although the mean Fe concentration found at site D during sampling occasion 5 was considerably lower than other occasions, its standard deviation was high (1333.3 mg kg^{-1}). No statistical differences were found within site D ($P=0.070$). At site E, sampling occasion 3 vs 1 – 7 and 4 vs 1 – 7 were found to be statistically significant ($P<0.05$). Statistical differences ($P<0.05$) were found for all comparisons made with occasion one and three at site F. The highest Fe concentration measured in the sediment was found at site G during occasion three (11242.1 mg kg^{-1}). All comparisons with this occasion were found to be statistically significant ($P<0.05$).

Comparisons of total Fe concentrations measured in sediment per sampling occasion over the length of the river.

During sampling occasion one, comparisons of Fe concentrations measured in the sediment showed statistical differences ($P<0.05$) in all groups except C vs F and E and E vs F. The highest Fe concentration during occasion one was recorded at site G (3644.8 mg kg^{-1}) while the lowest was recorded at site B

(1103.1 mg kg^{-1}). Occasion two showed statistical significance ($P<0.05$) for all comparisons except D vs A and E; A vs C and E and C vs B. All comparisons made for occasion three showed statistical differences ($P<0.05$) except E vs C and D; C vs A and D and D vs A. As stated above occasion three showed the highest Fe concentration at site G (11242.1 mg kg^{-1}). No differences ($P<0.05$) were found when site C was compared to sites A and E for sampling occasion four. All comparisons made for occasion five showed no statistical differences ($P>0.05$). Pairwise multiple comparisons made for occasion six showed statistical differences ($P<0.05$) in all groups except D vs C and G. No statistical differences $P>0.05$) were found for any comparisons made between sites during sampling occasion seven.

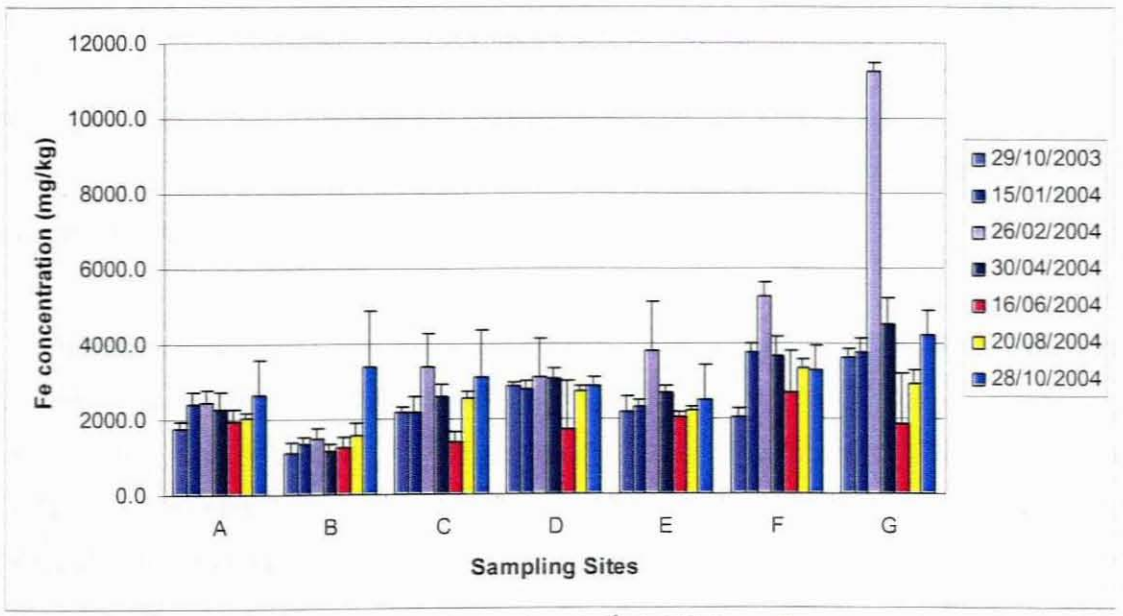

Figure 7.1 Mean concentrations of total iron (mg kg^{-1}) (±SD) in sediment samples collected from seven sites (A – G) of the Lourens River over a 12 month period.

RESULTS: Crabs

The concentrations of total Fe measured in crabs, collected over 12 months from seven sites is depicted in Figure 7.2.

(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Fe concentrations measured in crabs collected at a particular site over the 12 month sampling period.

The mean Fe concentration (643.9 mg kg^{-1}) measured during occasion seven at site A, proved to be the highest. The number of crabs collected during occasion seven was however, only three. All multiple pairwise comparisons made with this occasion were statistically significant ($P<0.05$), while all others were not. No significant ($P=0.981$) differences were found for any comparisons made within site B. Although the mean concentrations of occasions one and three at site C were relatively high, the elevated standard deviation for each, resulted in few statistically significant comparisons. Occasion three was found to be statistically significant ($P<0.05$) when compared to one and seven. No significant differences ($P>0.05$) were found for any comparisons made for site D. No crabs were available for collection during occasion one at site E. Statistical differences ($P\leq0.01$) were found when occasion three, four and six were compared to two five and seven. All comparisons within site F showed a P-value of 0.142 and therefore none were found to be statistically significant. Site G showed no statistical differences either ($P=0.041$).

Comparisons of total Fe concentrations measured in crabs per sampling occasion over the length of the river.

During sampling occasion one, site D proved to be the highest (462, 4 mg kg^{-1}). Only comparisons B vs C and D were found to be statistically significant ($P<0.05$). Similar results were found for comparisons within sampling occasion two. Site B was found to be significantly different to site E and G ($P=0.004$). During occasion three, site F was significantly different ($P<0.05$) from all other sites and G was significantly different to C. During sampling occasion four, sites A, B and C showed similar, relatively low mean concentrations (99, 1 – 144, 6 mg kg^{-1}) compared to sites D, E, F and G (174, 3 – 475, 0 mg kg^{-1}). All comparisons between these two groups was found to be statistically significant ($P<0.001$). A similar result was found for comparisons made for occasion five. Sites A, B and C were found to be statistically different from sites D, E, F and G ($P<0.05$). Site D,

F and G during occasion six, appear to be significantly different from the remaining sites however no statistical differences were found for any comparisons made for this occasion. Due to the high standard deviations, only D vs B was found to be statistically different during occasion seven (P=0.016).

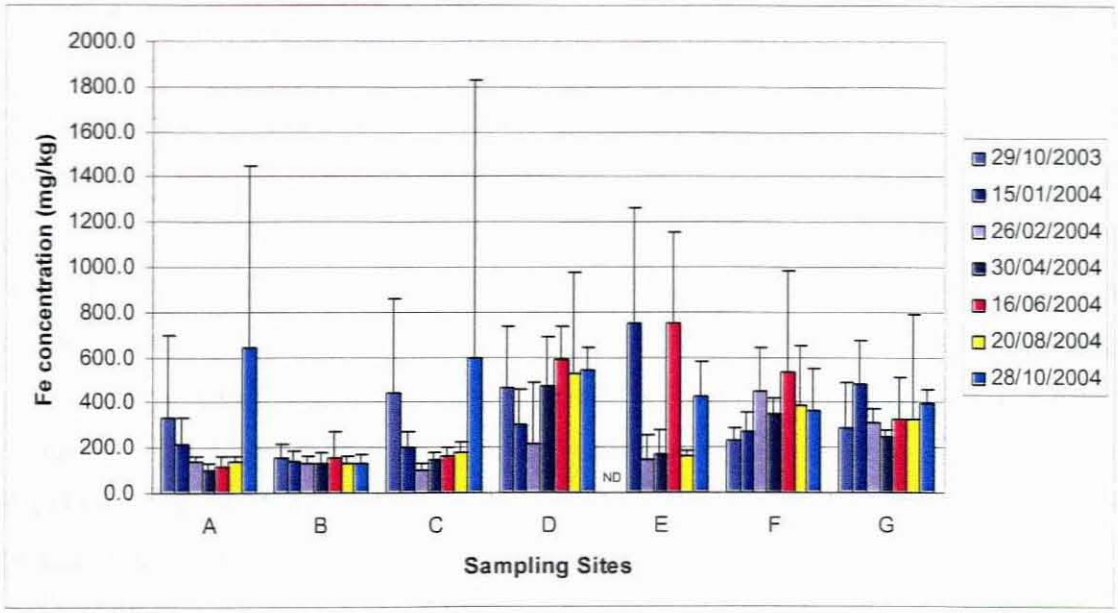

Figure 7.2 Mean concentrations of total iron (mg kg^{-1}) (±SD) in crab samples collected from seven sites of the Lourens River (A – G) over a 12 month period. ND = no data.

DISCUSSION

The sediment Fe concentrations detected in the present study ranged from 1103.1 ±273 mg kg^{-1} to 11242.1 ±249.0 mg kg^{-1}. These values were much lower than those concentrations reported by Steenkamp (1992), where concentrations ranged from 21 650 ±21 752.5 to 45 531.1 ±33 205.5 mg kg^{-1} in the Bronkhorstspruit and Natalspruit Rivers. These high Fe concentrations were largely attributed to mining activities in the headwaters of these rivers. In the present study, with the exception of site B, a small increment in Fe concentration can be seen along the length of the river.

With the exception of occasion one and seven, most comparisons showed statistical differences when sites A, B and C, were compared to D, E, F and G. These lower sites therefore showed generally higher values than the first three

sites during the same sampling occasions. What is the difference between site C and D that makes a significant difference to the Fe concentration within the crabs?

Increased Fe concentrations in the sediment did not seem to influence the bioaccumulation in the crabs on every occasion. This can be seen at site G, occasion 3 where the sediment Fe concentration was 11242.1 ±249 mg kg^{-1} (highest at site G) and the Fe concentration detected in the crabs was only 313.7 ±54.6 mg kg^{-1} (3^{rd} lowest at site G). This is confirmed by the study conducted by Steenkamp (1993) where an increase in sediment Fe concentrations in the Natalspruit River were not always reflected by an increase in crab (*P. warreni*) tissue Fe concentrations. *P. warreni* is however able to bioaccumulate high levels of Fe. Concentrations ranged from 158.7 to 1068.8 mg kg^{-1} in the study conducted by Van Eeden and Schoonbee (1991). In a study conducted by Sanders *et al.* (1997), it was concluded that although temporal variations in Fe concentration in *P. warreni* exist, the recommendation was still made to incorporate these decapods into biomonitoring protocols. This is because in general, higher Fe concentrations were detected in *P. warreni* collected from sites where environmental levels were higher than those collected from areas where lower environmental concentrations exist. In the present study, *P. perlatus* has shown a similar propensity, when compared to *P. warreni*, to bioaccumulate Fe with concentrations ranging from 99.1 ±33.4 to 751.8 ±515.6 mg kg^{-1}.

Since Fe binds strongly to sediment particles, variation in total crab Fe concentrations could be as a result of differences in the amount of iron-rich sediment present in the gut of sampled crabs. This would be influenced by feeding behaviour prior to collection. It is possible that variation in gut sediment quantity would affect the total Fe concentrations detected in the crabs. A similar rationale was used by Ong Che and Cheung (1998). They found a wide disparity in Fe concentrations between individual samples of both *Metapenaeus ensis* (commercially important shrimp) and *Rriocheir sinensis* (Chinese mitten crab)

collected from shrimp and fish ponds where Fe sediment concentrations were known. In their study, sediment Fe concentrations varied from 3168.1 – 15478.7 mg kg^{-1}, while shrimp and crab Fe concentrations ranged from 91.8 – 2304.0 mg kg^{-1}. No clear correlation between pond sediment Fe concentrations and organism Fe concentrations could be drawn.
In the study conducted by Steenkamp (1993) a significant rise in bioaccumulation of Fe in *P. warreni* occurred for some periods, although no distinct seasonal variation was observed. Neither was a specific gender-related trend observed.

In a study conducted by Forsyth *et al.* (2006) on the effects of runoff, sediment loss and water quality from gravel forest roads in a *Pinus* plantation, it was concluded that Fe concentrations in road water runoff were higher than the Fe concentrations detected in the general plantation area. This may have a general influence on the input of Fe into the Lourens River during rain events. The proximity of gravel roads to the river however seems not to influence sediment Fe concentrations in the Lourens River. This can be demonstrated at the "Red Bridge" at site B, where the gravel road crosses the Lourens River, yet it was this site which almost always revealed the lowest Fe concentrations when compared to other sites.

CHAPTER 8

MANGANESE

INTRODUCTION

Manganese (Mn) is a whitish-grey metal, harder than Fe but more brittle. It is found next to Fe in the periodic table and is similar to it in its chemical behaviour. It can exist in compounds in the oxidation states of I, II, III, IV, VI and VII. Manganese oxidises superficially in air and rusts in moist air. It is a ubiquitous metal in the earth's crust and is the 12th most abundant element. Its most common mineral form is pyrolusite (MnO_2) (Steenkamp, 1992). South Africa, Brazil, Gabon, Australia, India, China and the former USSR were/are the leading producers of Mn. In 1980 the total worldwide production of Mn ores was 26, 7 million tons (Adriano, 1986). It is used mainly in metallurgical processes where it is an essential ingredient in steel. It is used in metal alloys for the electrical industry, ship propellers, and its compounds are used in matches, ceramics, batteries, electrical coils, welding rods, glass, dyes, paints, drying industries and fertilizers (Friberg *et al.*, 1986). Bowen (1979) reported that average natural concentrations of Mn in sandstone is 460 mg kg^{-1}, while shale (Friberg *et al.*, 1986) and igneous rock (NAS, 1973) contain Mn concentrations that range from 390 – 1620 mg kg^{-1}. According to Berrow and Reaves (1984) a mean Mn concentration of 450 mg kg^{-1} can be expected for world soils with an absolute maximum being 4000 mg kg^{-1}. In natural waters, Mn occurs as dissolved, colloidal and complex forms. Stable Mn and organic matter complexes may form and have been known to cause problems in water treatment processes (Galvin, 1996). The Department of Water Affairs and Forestry (1996) set a guideline value of 180 µg litre^{-1} for Mn in aquatic ecosystems. However, Mn precipitates/adsorbs well to suspended matter in natural waters thus causing higher concentrations of particulate Mn. This usually results in high ratios of dissolved to particulate Mn (Salomons and Förstner, 1984).

Mn is an important nutrient to plants, as it activates many enzyme reactions involved in the metabolism of organic acids, phosphorus and nitrogen. It also plays a role in photosynthesis. Plants may also experience Mn deficiency causing numerous diseases, especially in crop plants. Although Mn plays a number of important roles in plants, it can also be toxic. Mn toxicity frequently occurs with Al toxicity. According to Foy and Campbell (1984) Al and Mn toxicities are the most important growth-limiting factors in many acidic soils. In soils that are well drained, Mn toxicity normally occurs when the pH is below 5.5 (NAS, 1973) while concentrations of Mn in plant tissues exceeding 500 mg kg^{-1} (dry weight) can be associated with Mn toxicity (Friberg *et al.*, 1986). Second to Al, Mn is considered to be the most important toxic metal in acidic soils. According to Friberg *et al.* (1986) several investigators have found that pH has the greatest effect on the bioavailability of Mn, followed by organic matter and moisture. Lucas and Knezek (1972) reported that great increases in Mn availability can be expected when soil pH drops below 5.5.

Mn is also an essential element in vertebrates in glycosal transrefase enzymes which are important in proteoglycan synthesis. Deficiency of Mn in vertebrates can lead to skeletal deformities, heart disease and impaired growth (Galvin, 1996). High concentrations are however toxic and lead to a variety of disturbances in metabolic pathways and inhibition of dopamine formation in the central nervous system (Dallas and Day, 1993; Seymore *et al.*, 1995; DWAF 1996). Hernroth *et al.* (2004) showed that Mn exposure of the Norway lobster, *Nephrops norvegicus*, suppressed fundamental immune mechanisms. According to their findings, these results identify potential harm for other organisms exposed to higher concentrations of bioavailable Mn. According to Snyman (1996) very little research has been done on the concentrations of Mn in freshwater decapod crustaceans. France (1987) studied the crayfish *Orconectes virilis* while the bioaccumulation of Mn in the crab *P. warreni* was also studied by Van Eeden and Schoonbee (1991) and Steenkamp *et al.* (1994). More recently,

Sanders *et al.* (1998), studied *P. warreni* as a bioaccumulative indicator of Fe and Mn pollution. Due to the fact that ultimate levels of Fe and Mn bioaccumulation in *P. warreni* vary according to the site, it was concluded that this organism be incorporated into biomonitoring protocols.

MATERIALS AND METHODS

Refer to Chapter 3

RESULTS: Sediment

The concentrations of total Mn measured in sediment samples, collected over 12 months from seven sites is depicted in Figure 8.1.
(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Mn concentrations measured in sediment, at a particular site over the 12 month sampling period

The highest Mn concentration (176.7 ±98.8 mg kg^{-1}) in the sediment was found during sampling occasion four at site A. Statistical differences ($P<0.05$) were only found for comparisons with this occasion. At site B, occasion one proved to be the lowest mean Mn concentration (10.2 mg kg^{-1}) with a standard deviation of just 2.6 mg kg^{-1}. All multiple pairwise comparisons with this occasion showed statistical differences ($P<0.05$). Occasion one, two, five and six showed the lowest mean Mn concentrations at site C. Occasions three, four and seven showed statistical differences ($P<0.05$) when compared to these occasions. Occasions one and two were also statistically different ($P<0.05$) from occasions five and six, as was occasion five from occasion six. No statistical differences were found for any comparisons within site D. All occasions compared to occasions three and four showed statistical differences ($P<0.05$) except when occasion three and four were compared to each other. Occasion one at Site F showed the lowest concentration (19.4 ±2.6 mg kg^{-1}) for that site. All comparisons with this occasion showed statistical significance ($P=0.001$). All Mn concentrations detected for site G showed low standard deviations

(1 - 2.9 mg kg^{-1}). All multiple pairwise comparisons within this site showed statistical differences except when occasions five, six and seven are compared to each other.

Comparisons of total Mn concentrations measured in sediment per sampling occasion over the length of the river

Occasion one at site A proved to be the highest (60.5 ±45.8 mg kg^{-1}) for all sites during this occasion. All comparisons with occasion one showed statistical differences ($P<0.05$) except for D vs A and C; A vs C; E vs F and G and F vs G. All multiple pairwise comparisons for occasion two showed statistical differences except D vs A and F; A vs F and G vs E. No statistical differences were found for any comparisons made for occasion three. Occasion four at site A was found to have the highest Mn concentration (176.7 ±98.8 mg kg^{-1}). Only comparisons made with this concentration resulted in statistical differences ($P<0.001$). All multiple pairwise comparisons made for occasion five showed statistical differences except for sites A vs D; E vs B, C and G; G vs B and C and B vs C. Comparisons between sites D vs F; C vs B, D and E; G vs B and E and B vs E were the only comparisons found not to be statistically different from one another. Statistical differences ($P<0.05$) were found when site A and C were compared to B, D, E, F and G for occasion seven. All other comparisons were not.

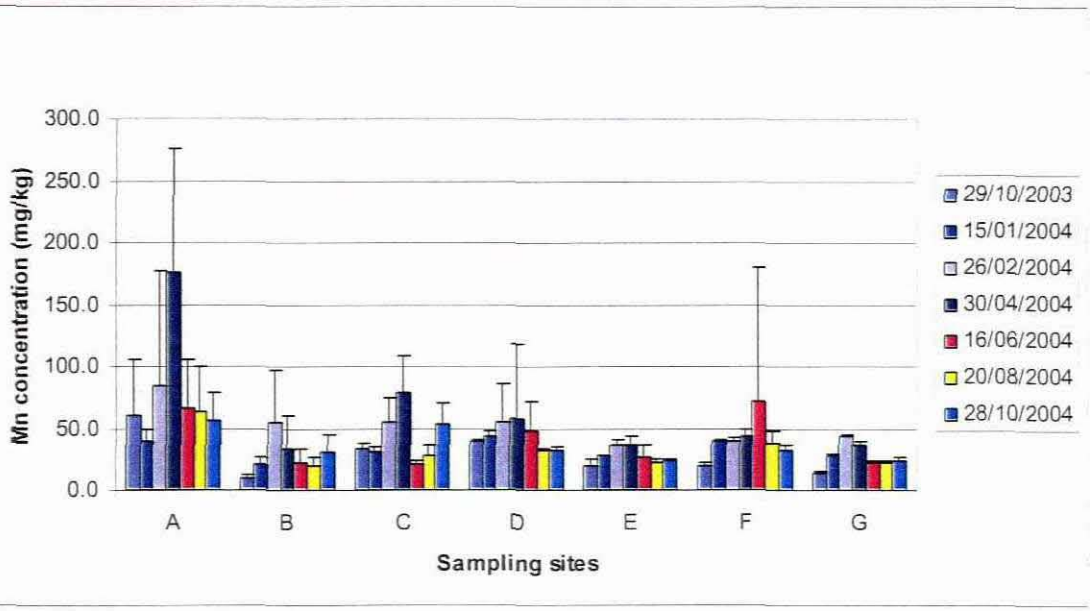

Figure 8.1 Mean concentrations of total manganese (mg kg^{-1}) (±SD) in sediment samples *collected from seven sites (A – G) of the Lourens River over a 12 month period.*

RESULTS: Crabs

The concentrations of total Mn measured in crabs, collected over 12 months from seven sites is depicted in Figure 8.2.

(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Mn concentrations measured in crabs collected at a particular site over the 12 month sampling period

The lowest mean Mn concentration (48.1 ±14.1 mg kg^{-1}) measured over the entire twelve month period can be found during sampling occasion one at site A. No statistical differences were found for any multiple pairwise comparisons within site A ($P=0.148$). The same result was found for site B and C ($P>0.05$). At site D, only one comparison, occasion 6 vs 7, revealed statistical significance ($P<0.05$). No statistical differences were found for any comparisons made within site E, F or G. Relative standard deviation for these three sites ranged from 16.6 – 50.4 mg kg^{-1}. The highest concentration measure over all sites during the twelve month period was 152.2 ±46.2 mg kg^{-1} at site F during occasion four.

Occasions 2, 4, 5, 6, and 7 all showed the highest mean Mn concentrations at site F.

Comparisons of total Mn concentrations measured in crabs per sampling occasion over the length of the river

The highest mean Mn concentration (102.5 ±10 mg kg^{-1}) measured in crabs during occasion one was found at site D. All multiple pairwise comparisons made between C, D, F and G vs A and B during occasion one, showed statistical significance (P=0.005). No statistical differences (P>0.05) were found for any comparisons made within sampling occasion two. During occasion three G vs A, B, C, D, and E and F vs A were found to be statistically different (P<0.001). Sites F vs A, B, C and D and G vs B showed statistical differences (P<0.05) when compared with each other during sampling occasion four. No differences were found during sampling occasion five. Multiple pairwise comparisons between sites C, D, F and G vs A and B showed statistical differences (P<0.05) during occasion six. Sampling occasion seven revealed statistical differences (P<0.001) for comparisons made between site G and sites A and B.

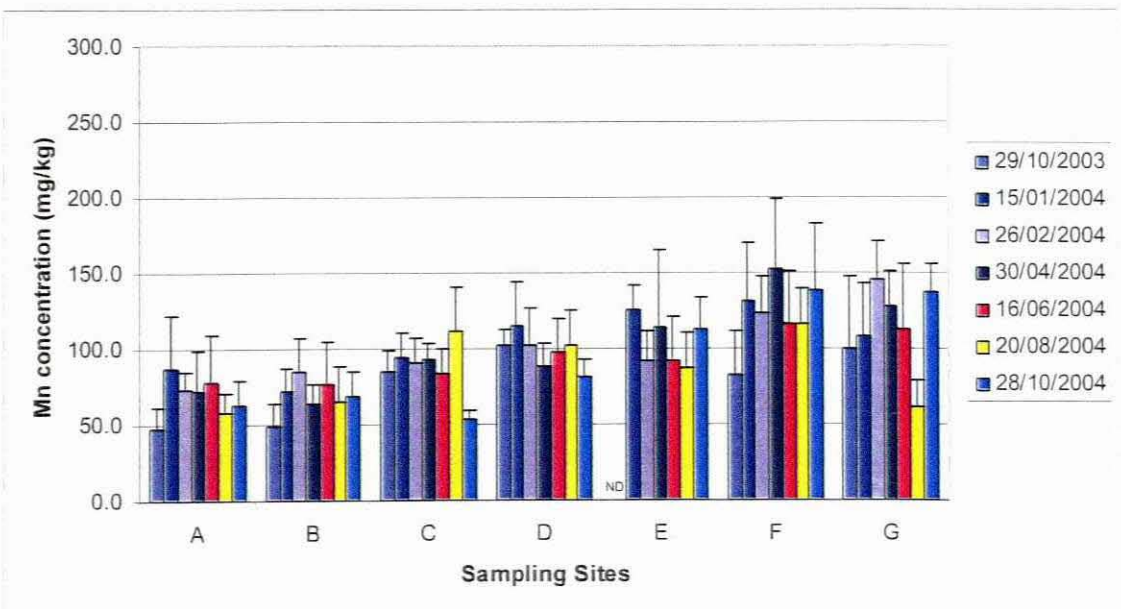

Figure 8.2 Mean concentrations of total manganese (mg kg^{-1}) (±SD) in crab samples collected from seven sites of the Lourens River (A – G) over a 12 month period. ND = no data.

DISCUSSION

Mn concentrations detected in the sediment samples ranged from 10.2 ±2.6 mg kg^{-1} to 176.7 ±98.8 mg kg^{-1}. The upper limit of this range is however misleading, since the majority of mean sediment Mn concentrations were found to be less than 50 mg kg^{-1}. This is considerably lower than Mn concentrations detected in other studies. In the study conducted by Sanders *et al.* (1998) Mn sediment concentrations ranged from 1190 to 7426 mg kg^{-1}. These significantly higher concentrations could however be due to the fact that the Sanders *et al.* (1998) study was conducted on more sedentary water bodies (Potchefstroom Dam and Germiston Lake) and a different geomorphological area with different background Mn concentrations. In the present study (at all sampling sites), Mn concentrations found in occasions three (February) and four (April) were consistently higher than other occasions forming a noticeable peak at each site. According to Adriano (1986) Mn is available in organic and inorganic fertilizers. The most commonly used Mn-containing fertilizer is $MnSO_4$ $4H_2O$ (24% Mn) and is highly water soluble in both acidic and alkaline environments. Application rates of fertilizers differ depending on soil conditions and crop type. Foliar application rates for Mn-containing fertilizer can range from 2 to 4.5 kg/ha. Fertilizers used on the Lourensford and Vergelegen Farms could therefore contribute towards the Mn concentrations in the Lourens River.

The Vergelegen farm uses the pesticide, Dithan, (with active ingredient – Mancozeb) on pears and plums, from early August till late November, at an application rate of 2.02 to 3.38 kg/ha. Manganese sulphate is also sprayed on the plumbs during October and November at an application rate of 1.5 kg/ha. On the Lourensford farm, manganese sulphate is sprayed during November and December on pears, plums and apples at an application rate ranging from 1.92 to 6 kg/ha. However, the Mn concentrations detected in the sediment did not display a corresponding pattern with the spraying programmes on either farm. In fact, sediment Mn concentrations generally increased in the months when no Mn-

containing applications were sprayed. According to Shultz *et al.* (2001) runoff-related pesticide input into the Lourens River is very much dependent on the time between the last pesticide application and the first rainfall. Rainfall data during these peaks showed little for the month of February (6.2 mm) but during April and June 75.8 and 75.4 mm fell respectively. It is therefore possible that increased precipitation and consequently runoff into the Lourens River during these periods increased the Mn concentrations detected in the sediment.

It is well known that the pH of natural waters plays an important role in the bioavailability and toxicity of Mn to aquatic organisms. The dissolved fraction of Mn may therefore increase through changes in pH, as well as redox potential, dissolved oxygen and organic matter (Canadian Water Quality Guidelines, 1992). A decrease in pH results in an increase in the bioaccumulation and toxicity of Mn (France, 1987 and Rouleau *et al.*, 1996). During February and April, there were eight samples at various sites where the pH was found to be ≥6. All remaining samples during February and April were found to have a pH value of <6. In contrast to other occasions, pH measured during February and April seemed to be slightly higher than that measured during other occasions. More pH values during February and April were found to be 6 and higher, relative to the pH values found during other occasions. These sediment Mn concentrations are therefore not likely to be clearly linked to pH changes in the Lourens River.

In invertebrates, Musibono (1998) has shown in a laboratory study, that the addition of Mn, Al and Cu mixtures reduces the risk of death to *Paramelita nicoculus,* when compared to the addition of Al and Cu alone. Mixtures of Al and Cu are therefore more toxic than mixtures of Al, Cu and Mn. He concluded that Mn reduces the toxicity of Al and Cu. According to Richard and Bourg (1991) and Driehaus *et al.* (1995) the most important effect of Mn is its action in mixtures because dissolved Mn (Mn^{2+}) may increase or decrease the toxicity of other metals. Based on their ionic and covalent indices, the toxicity of these three elements can be arranged from most to the least toxic, as follows: Cu > Al > Mn.

Al and Mn alone were found to be antagonistic (Musibono, 1998). The antagonistic relationship between Mn and Fe in higher plants has also been well documented (Sideris and Young, 1949; Ohki, 1975; Vlamis and Williams, 1964). Wallace (1962) has reported that high Fe concentrations in the soil or addition of Fe to the growing medium can decrease the toxicity of Mn. The high Fe concentrations detected in the Lourens River (1103.1 ±273 mg kg^{-1} to 11242.1 ±249.0 mg kg^{-1}) could therefore further play a role in reducing the toxicity of Mn in this river. This however, does not affect the availability or the uptake of Mn by organisms.

Bioaccumulation of Mn in decapods can vary greatly. In their study on *P. warreni*, Van Eeden and Schoonbee (1991) detected Mn concentrations ranging from 568.0 to 1675.0 mg kg^{-1}. In the present study, Mn concentrations detected in the crabs, although much lower than some other studies, were normally higher than those concentrations detected in the sediment. Crab Mn concentrations ranged from 48.1 ±14.1 mg kg^{-1} to 152.2 ±46.2 mg kg^{-1}. This indicates a tendency for *P. perlatus* to bioaccumulate Mn. It is also clear from Figure 8.2 that crabs collected from lower sites in the river bioaccumulated higher Mn concentrations than those higher up in the river. This cannot be related to sediment Mn concentrations as the highest sediment concentrations were detected at site A, while in the lower reaches of the river, sediment concentrations were lower. *P. perlatus* therefore does not always seem to bioaccumulate Mn proportionally to environmental concentrations. The bioavailability of Mn must also be considered as a possible explanation of this scenario.

The characteristics of sediment could influence the uptake of Mn by crabs since the smaller the sediment particles, the more easily they can be ingested along with food. Bryan and Ward (1965) found that food was an important source of Mn for the lobster, *Homarus vulgaris*. Sanders *et al.* (1998) showed in their study that the concentration of Mn in sediment was almost always found to be higher in the finer fractions of sediment rather than the courser fractions. According to Kindler

and Savim (1990) higher metal concentrations in the finer fractions of sediment are usually associated with pollution, while elevated levels in the coarser fractions are normally due to the geology of the area. In the present study, higher Mn concentrations were detected higher up the river where the average particle size was larger. The lower reaches of the Lourens River displays finer sediments than those collected at higher sampling sites. The sediment collected from site G, at the golf course, was classified as "slightly muddy sand" and contained almost 6% mud which was considerably higher than the other sites. No gravel was found at site G while higher up in the river, gravel concentrations were much higher. Even though sediment Mn concentrations did not increase along the length of the river, this gradual decrease in particle size along the length of the river could therefore influence the uptake of Mn by crabs during feeding. Bioaccumulation through the gills must also be considered as a possible significant route of uptake. In a laboratory study, Baden *et al.* (1995) discovered that Mn uptake was the greatest in the gills and haemolymph of the decapod, *Nephrops norvegicus*. Corrêa *et al.* (2005) found that the gills in the crab *Ucides cordatus* displayed higher Mn concentrations than the hepatopancreas and the muscle.

Seasonality is also known to influence the bioaccumulation of metals in aquatic invertebrates due to changes in water temperature, light, activity and food availability, quantity and quality (Marsden and Rainbow, 2004). Snyman (1996) found a clear relationship between seasonality and whole-body Mn concentrations in *P. perlatus,* where a summer time peak was found in whole-body Mn concentrations. In the present study however, no clear pattern indicating any kind of seasonal influence could be seen. It is possible that normal seasonal influences were distorted by the predominant tree canopy cover over most of the river. Good canopy cover is known to reduce the impact of environmental temperature changes on the temperature of the river water (See Chapter 3: Table 3.1) and therefore impacts the invertebrates' environment (Noel *et al.*, 1986).

The studies conducted by Van Eeden and Schoonbee, (1991); Steenkamp *et al.* (1994); DWAF, (1995) and Sanders *et al.* (1998) imply that *P. warreni* is unable to successfully regulate Mn. However, according to Sanders *et al.* (1998) regulation of Mn in *P. warreni* is difficult to identify since it can start at any stage when pollution levels become too high and regulation of Mn itself can be triggered by increased environmental concentrations. Extremely high levels of Mn can possibly destroy normal regulation mechanisms.

CHAPTER 9

ZINC

INTRODUCTION

Zinc (Zn) occurs relatively frequently in nature (Galvin, 1996) and is mined in over 30 countries with world production topping 100 million tons between 1961 and 1980. Canada was/is the largest world producer of Zn, contributing 25% of all Zn consumed in the Western world. It occurs in nature as both sulphide and carbonate ores. The metallurgy of Zn has been known for over 1000 years even though Zn, originally described as "false silver", has been known for over 2000 years (Moore and Ramamoorthy, 1984). The uses of Zn are varied but include galvanising iron and steel, diecasting (Zn based alloys), brass, dry battery production, photo engraving, lithography printing plates, roofing sheets and guttering. It is also used as an ingredient in paint, a catalyst in the vulcanisation of rubber, agricultural, medicinal and cosmetic products as well as in printing, textile dying and the purification of fats (Moore and Ramamoorthy, 1984).

Natural sources of Zn include eroded soil particles, vegetation and to a far lesser extent sea salt sprays. Nriagu (1979) reported that anthropogenic emissions of Zn exceeded natural emissions by 700%. He also reported that the production and use of non-ferrous metals accounts for 43% of anthropogenic Zn released into the atmosphere. Incineration of waste and other combustion processes, pesticides, rubber and plastics, sewage, electroplating, metal finishing and mining effluents are also important contributing sources (Förstner and Prosi, 1979).

According to HSAB (Hard-Soft-Acid-Base), Zn is classified as a border element. This is because as carbonic anhydrase, Zn acts as a soft acid metal by binding to the halide ions $I^- > Br^- > Cl^- > F^-$. In an aqueous solution however, $Zn^{(+2)}_{aq}$ binds

$F^- > Cl^- > Br^- > I^-$ therefore behaving as a hard acid metal. At a pH of ≥8 Zn forms the relatively stable $Zn(OH)_2$ while it begins hydrolysing at pH 7 – 7.5. At pH 6.7, Zn is present as divalent Zn, which is available for sorption onto suspended mineral colloids and complexation with organic matter (Moore and Ramamoorthy, 1984).

Zn is an important trace element in mammals where it plays a role in carbohydrate, lipid, protein and nucleic acid metabolism (Friberg *et al.* 1986). According to Moore and Ramamoorthy (1984), more than twenty Zn metalloenzymes have been identified in mammals. These include alkaline phosphatase, alcohol dehydrogenase and carbonic anhydrase. Although an essential trace element, exposure to higher concentrations of Zn can lead to poor appetite, delayed healing of wounds, non-viable newborns, erosion of cartilage, retarded growth, poor calcium and phosphorus retention and changes in tissue minerals (Mills, 1989; Richardson and Gangolli, 1994).

Zn is an unusual metal in that its toxicity to humans is relatively low but has a much higher toxicity to fish (Alabaster and Lloyd, 1980 as sited in Fatoki and Awofolu, 2003). Much research has been done on the impact of Zn on marine decapods. Brouwer and Engel (1982) suggested that hemocyanin acts as a major vehicle for the transport of Zn in blue crabs. Engel and Brouwer (1987) have reported that Zn binds almost entirely to the copper protein, hemocyanin, which is a respiratory pigment. Two metallothioneins (metal binding proteins) found in the midgut gland of decapods, have a very high affinity for metals such as Zn and therefore play an important role in the regulation of Zn in crabs (Rainbow and Scott, 1979). Giesy *et al.* (1980) has shown that the crayfish *Procambarus acutus* eliminates Zn derived from water almost twice as fast as Zn derived from food sources. A number of studies conducted on fresh water ecosystems have suggested that decapod crustaceans tend to regulate Zn well (Steenkamp, 1992). However, this regulation of Zn does not apply to all crustacean taxa (Bryan, 1976; Rainbow, 1985; Rainbow and White, 1989). In a

laboratory study conducted by Rainbow and White (1989) the decapod *Palaemon elegans* was found to be able to regulate body Zn concentrations at a constant level over a wide range of dissolved Zn concentrations. It was able to maintain this regulation until exposure to high Zn concentrations (1000 and 3162 $\mu g\ l^{-1}$) was reached, where regulation processes then broke down and net accumulation occurred. Steenkamp (1992) reported that Zn is clearly regulated by the freshwater crab *P. warreni*. Most of the crabs in her study were able to excrete a higher proportion of Zn under contaminated conditions and thus were able to regulate relatively constant whole body concentrations. She concluded that there is no certainty that the Zn concentrations detected in body tissues of *P. warreni* are an accurate reflection of the degree of Zn contamination in its aquatic environment. According to Du Preez *et al.*, (1993) *P. warreni* are able to regulate the concentration of Zn in their various tissues to a better extent than copper.

MATERIALS AND METHODS

Refer to Chapter 3

RESULTS: Sediment

The concentrations of total Zn measured in sediment samples, collected over 12 months from seven sites is depicted in Figure 9.1.

(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Zn concentrations measured in sediment, at a particular site over the 12 month sampling period

Mean concentrations of Zn measured in sediment remained below 20 mg kg^{-1}, with the exception of occasion four of site F and three and four of site G. Occasions 3 and 4 of site A showed statistical significance when compared to all other occasions. The highest mean Zn concentration (6.4 mg kg^{-1}) found at site B was during occasion four. Occasion four proved to also have the highest concentrations at sites C, D, E and F. Occasions two, five and six were found to

be statically different ($P<0.05$) from occasions one, three, four and seven at site B. At site C occasions three, four and seven showed statistical significance ($P<0.05$) when compared to occasions one, two, five and six. Site D was the first site to show detectable mean Zn concentrations for each sampling occasion however, only occasion four showed statistical differences ($P=0.027$) when compared to all other occasions. All multiple pairwise comparisons for site E showed statistical differences ($P<0.05$) except for four vs one and two, two vs one and seven vs six. At site F occasion four once again showed statistical significance ($P<0.001$) when compared to occasions one, two, five, six and seven. Occasion three showed the same results. Occasion three at site G showed a mean Zn concentration of 100.1 mg kg^{-1}, with a standard deviation of just 5.1 mg kg^{-1}. All multiple pairwise comparisons made between occasions three and four with all other occasions showed statistical differences ($P<0.05$).

Comparisons of total Zn concentrations measured in sediment per sampling occasion over the length of the river

The highest mean Zn concentration (17.7 ±1.1 mg kg^{-1}) measured during occasion one was recorded at site G. All multiple pairwise comparisons were found to be statistically significant ($P<0.05$) except comparisons between sites A, B, C and D. During occasion two, G vs E and F; E vs F; A vs B and C and C vs B were found not to be statistically different ($P>0.05$). Multiple pairwise comparison made between site A and F during occasion three showed no statistical significance ($P>0.05$) while all other comparisons did. With the exception of site A, occasion four showed an incremental increase in mean Zn concentration at each site along the course of the river. During occasion five only sites F vs B, C and E proved to be statistically different from one another. Occasion six showed a similar incremental increase in concentration along the course of the river to occasion four. Only D vs A, C and E; E vs A and C and C vs A were found not to be statistically different ($P>0.05$) from each other. Occasion seven, once again showed a general incremental increase in concentration along the course of the river. Zn concentrations for this occasion ranged from 4.3 ±2.4 to

18.8 ±2.4 mg kg^{-1}. All multiple pairwise comparisons for occasion seven made between site G and all other sites proved to be statistically different (P<0.001), while all other comparisons were not.

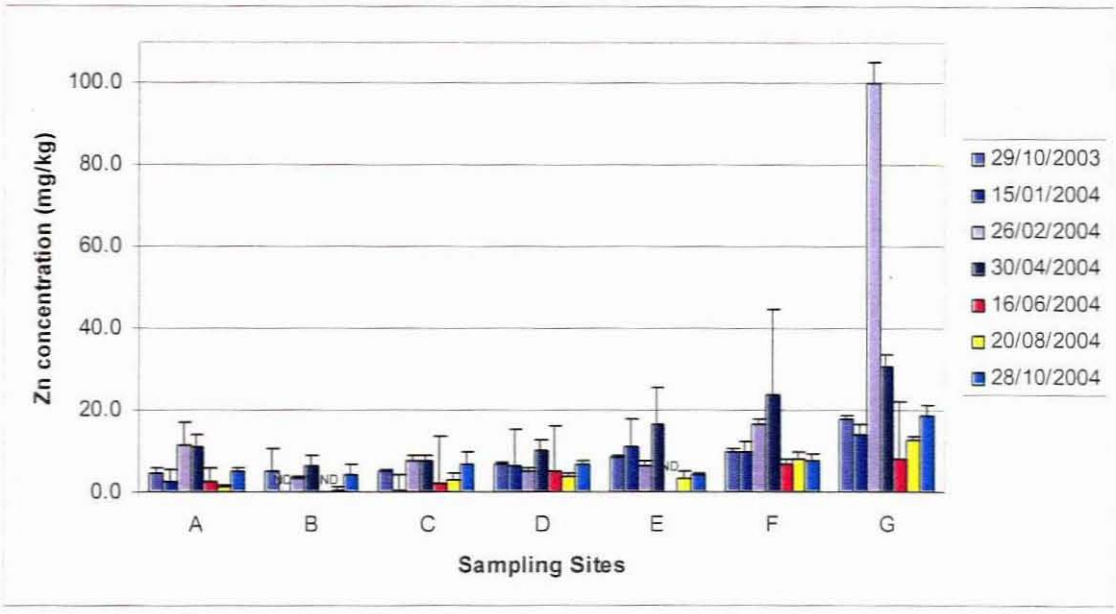

Figure 9.1 Mean concentrations of total zinc (mg kg^{-1}) (±SD) in sediment samples collected from seven sites (A – G) of the Lourens River over a 12 month period. ND = no data/not detected

RESULTS: Crabs

The concentrations of total Mn measured in crabs, collected over 12 months from seven sites is depicted in Figure 9.2.

(A – G refers to sites and 1 – 7 refers to sampling occasions)

Comparisons of total Zn concentrations measured in crabs collected at a particular site over the 12 month sampling period

Mean Zn concentrations detected in crabs collected during the full sampling period generally showed a three fold increase when compared to those concentrations detected in the sediment. At site A, only occasions two, three and four vs one were found to be statistically different (P=0.004) from one another. At site B the lowest concentration detected (50.0 mg kg^{-1}) was during occasion one. Occasions two to five at the same site showed statistical differences (P<0.05)

when compared to this occasion. Although the highest and lowest mean concentrations (85.1 and 33.8 mg kg^{-1}) at site C, indicated on figure 9.2, for occasion one and seven respectively, appear significantly different from other occasions, this is not so. The high standard deviation (31.4 mg kg^{-1}) for occasion one and the small sample size (3 crabs) for occasion seven, resulted in no statistical differences (P=0.1) for any comparisons. No statistically significant (P>0.05) differences were found for any comparisons made between concentrations found at sites D, E and G. Site F only showed statistical differences (P=0.010) for multiple pairwise comparisons made between occasion F with all other occasions.

Comparisons of total Zn concentrations measured in crabs per sampling occasion over the length of the river

No crabs were available for collection at site E during sampling occasion one. All comparisons between sites C and F, during this occasion, with all other sites proved to be statistically different (P<0.05). All other comparisons were not. The highest mean Zn concentration (124.0 mg kg^{-1}) detected was during occasion one at site F. During occasion two, only site A vs all other sites resulted in statistical significance (P<0.001). Results for comparisons made between sites during occasions three, four, five and six showed no statistical differences (P>0.05). During occasion seven, multiple pairwise comparisons showed statistical significance for G vs A, B, C, D and F and E vs C and B. The lowest mean Zn concentration (33.8 mg kg^{-1}) was detected at site C during occasion seven. This was less than half the value of the highest concentration measured (73.7 mg kg^{-1}) at site G during the same occasion.

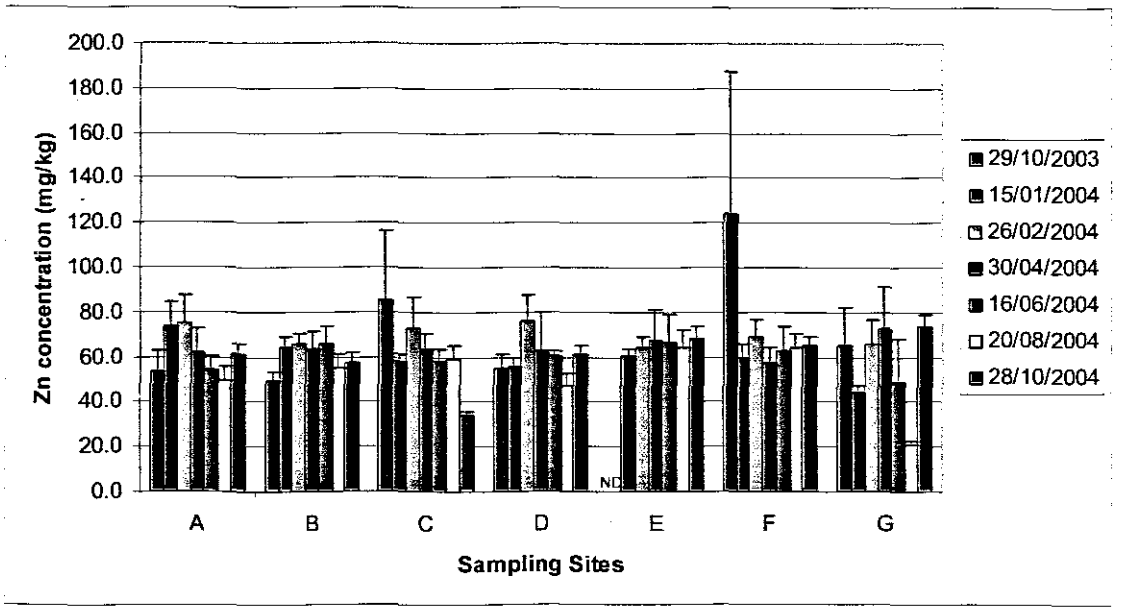

Figure 9.2 Mean concentrations of total zinc (mg kg^{-1}) (±SD) in crab samples collected from seven sites of the Lourens River (A – G) over a 12 month period. ND = no data.

DISCUSSION

The concentrations of Zn detected in the sediment of the Lourens River ranged from ND (not detected) to 100.1 ±5.1 mg kg^{-1}. This upper limit is however, very misleading since the majority of the results were found to be < 20 mg kg^{-1} and 35 of all results (49) were ≤ 10 mg kg^{-1}. Nevertheless, a small but identifiable increment in mean Zn sediment concentrations can be seen along the length of the river (See Fig. 9.1). Mean sediment Zn concentrations have been known to vary from 81 to 3800 mg kg^{-1} dry weight and are clearly influenced by natural background concentrations and anthropogenic activity in the area (Maxfield *et al.*, 1974; Pagenkopf and Cameron, 1979; Harding and Witton, 1978; Moore, 1979; Mathis and Cummings, 1973). Four of these five studies sited above, were conducted at sites where mining was an important anthropogenic source and displayed the highest mean concentrations. The other study however, Mathis and Cummings (1973) was conducted in an area where the polluting sources were described as industrial and municipal. According to Moore and Ramamoorthy (1984) total Zn concentrations in river sediments in the vicinity of mines often exceeds 1000 mg kg^{-1} however, in urban areas the concentrations are normally

much lower. Snyman (1996) reported sediment Zn concentrations ranging from 8.5 to 102.5 mg kg^{-1} (wet mass) in the Eerste River.

The small increases in Zn sediment concentrations along the length of the Lourens River may be partially due to the natural downstream transportation of Zn in particulate form or bound to organic matter. Montgomery and Santiago (1978) reported a near 50% increase in particulate Zn from the head waters of the Guanajibo River to the coastal area. Hart and Davies (1981) reported that the majority (75%) of the filterable fraction of Zn in the Yarrah River was transported downstream. However, the influence of runoff from urban and industrial activities in the vicinity of the Lourens River as it passes through the town of Somerset West is probably a more significant contributing factor.

Although $ZnCl_2$ is often used as a wood preservative (Moore and Ramamoorthy, 1984), no identifiable influence of this could be seen in sediment Zn concentrations. The distance between the saw mill on the Lourensford farm and the Lourens River is probably the mitigating factor here. The use of pesticides, herbicides and fungicides on both farms could also contribute through spray drift and runoff, to the total Zn concentrations found in the sediment. Zinc oxide, "Zinc Max" and "Mancozeb" containing applications are widely used on both farms on vines, pears, and plumbs. Spraying times, dilution rates and application rates per hectare differs considerably. These Zn containing agricultural applications must also be considered as possible contributors to Zn contamination of the Lourens River.

During occasion three at site G (along side the Strand Golf Course) the highest mean Zn concentration was detected: 100.1 ±5.1 mg kg^{-1}. This peak Zn concentration was found to be statistically different ($P< 0.05$) when compared to all other concentrations. According to Moore and Ramamoorthy (1984) large quantities of Zn are transported and deposited by rain. These aerial inputs of Zn can account for more than 50% of the total Zn deposited in an area

(Peyton *et al.* 1976). Elgmork *et al.* (1973) and Landy and Peel, (1981) reported that Zn concentrations in Antarctica averaged 0.06 µg L^{-1}. Although the potential aerial deposition of Zn into the Lourens River through precipitation must be considered as a possible source of Zn, it cannot reasonably be considered an influencing factor in the peak Zn concentrations detected at site G during occasion three (26 February 2004). Such a localised peak in any metal concentration cannot be as a result of a widespread source such as precipitation. Moore and Ramamoorthy (1984) have reported that flooding in industrial areas may produce total Zn residues of ≥3000 µg L^{-1} in receiving waters. This however, is unlikely to be the main contributing factor since total precipitation during the month of February was only 6.2 mm. It is possible that Zn contaminated effluent from urban or industrial point sources entered the river at this locality to cause this significant peak in Zn sediment concentrations.

Total Zn concentrations detected in crabs in the present study show results which are generally at least 2 to 3 times higher than the concentrations detected in the sediment. The lowest and the highest total Zn concentrations stand alone at 21.3 ±1.4 mg kg^{-1} and 124.0 ±63.3 mg kg^{-1}. Almost all the remaining results fall within a similar range from 50 to 75 mg kg^{-1}. Sanders *et al.* (1999) reported Zn concentrations in *P. warreni* that ranged from 52.0 to 413.4 mg kg^{-1}. Van Eeden and Schoonbee (1991) reported total body Zn concentrations ranging from 73.3 ±0.03 to 138.3 ±0.01 mg kg^{-1}. As mentioned in Chapter 3, fresh water crabs live and feed on, and in the sediments of the river. According to Bryan (1967, 1979) in most cases the most important source of Zn in decapod crustaceans is food and particulates. Due to the fact that Zn is an essential element in *P. perlatus* and that they are in constant contact with Zn contaminated sediment (albeit very low concentrations), these total body crab Zn concentrations are not surprising. According to Bryan (1979) the excretion of Zn in decapod crustaceans is a controlled process. It can be increased or decreased depending on the uptake of Zn from its environment and the immediate needs of the individual. As

mentioned earlier though, this regulation can break down when environmental concentrations become too high. This results in net accumulation of the metal.

The interaction of different metals and their effect on the bioaccumulation of metals in invertebrates must always be considered as a possible influencing factor. In the terrestrial environment, Odendaal and Reinecke (2004) have shown in a laboratory study on the isopod, *Porcellio laevis,* that cadmium (Cd) and zinc exhibit the ability to influence the bioaccumulation of each other in mixed metal exposures. Nickel and Zinc are said to be synergistic, in that they are more than five times more toxic in combination than when either is alone (Dallas and Day, 1993). However, in the preliminary sampling for this study cadmium and nickel concentrations were shown to be non existent or negligible (See Annexure 1).

An increase in water temperature normally results in an increase in the overall activity and therefore, metabolism of fresh water crabs. This would naturally increase an invertebrate's exposure to metals and in this case Zn. This however, is a generalisation as an increase in water temperature does not always result in an increase in Zn uptake by biota. This is because an increase in the rate of detoxification can be initiated by the increase in metal concentrations and the effect of temperature change can therefore be eliminated or reduced (Cairns *et al.*, 1975). Snyman (1996) actually observed peaks in whole body Zn concentrations in *P. perlatus* during the colder winter months. This possibly indicates the effects that decreased activity has on the rate at which Zn is eliminated from the body. In the present study, no clear or consistent seasonal variations in Zn concentrations in *P. perlatus* were evident.

P. perlatus seems to be able to regulate Zn well in the Lourens River and the increment growth in environmental Zn concentrations in the sediment of the lower parts of the river were not reflected in the Zn concentrations detected in the

crabs. This implies that *P. perlatus* in this freshwater ecosystem cannot be considered a reliable bio indicator of potential environmental Zn pollution.

CHAPTER 10

THE SEDIMENTATION POND

INTRODUCTION

According to Power and Chapman (1992) sediment can be divided up into four main components. The largest, accounting for over 50% by volume, is interstitial water. The next large component is rock and shell fragments, which are the result of natural erosion. Organic matter occupies a lower volume but is important since it plays a role in the bioavailability of many pollutants. The final component is that which results from anthropogenic sources and includes any contaminated materials and eroded topsoil. Sediments act as final reservoirs for many chemical and biological contaminants. They integrate numerous contaminants from multiple sources within a watershed system, which makes it difficult to pinpoint the sources of selected contaminants (Apitz *et al.*, 2005). Chapman (1989) reported that many studies in which water quality criteria were not exceeded, adverse effects are possible in benthic organisms dependent on sediment for their survival. According to Wenning and Ingersoll (2002) it is a well known fact that contaminated sediment has detrimental effects on benthic organisms. These effects are sometimes difficult to identify and therefore require a variety of investigation and risk-assessment tools which include chemical testing, analyses of macroinvertebrate communities, modelling of hydrodynamics and sediment transport, toxicity testing and analysis of habitat.

Like many other substances, metals are often adsorbed onto the surfaces of particulates – especially organic particulates – suspended in water rather than being simply dissolved in the water as free ions or as complexes with soluble bio-molecules such as fulvic acids. Particles eventually settle to the bottom of lakes, dams or rivers and become buried when other sediments accumulate on top of them. This "burial" represents an important sink for many water pollutants and is

a mechanism by which the water is cleansed. Before they are covered by later sediments however, freshly deposited matter at the bottom of a body of water can re-contaminate the water above it, by desorption of the chemicals since adsorption and desorption establish equilibrium. Furthermore, the adsorbed pollutants can enter the food chain if the particles are consumed by benthic organisms (Baird, 1995). Yousef *et al.* (1991) reported that pond sediments are commonly rich in metals such as lead, zinc, copper, nickel, cadmium and chromium. Of these metals, copper, lead and zinc have been reported as contributing up to 90% of all metals. The organic component of sediment also plays a role in the accumulation of metals. According to Coquery and Welbourn (1995), the presence of high concentrations of organic matter in sediments is shown to decrease the availability of metals to the plant species, *Eriocaulon septangulare*. Matagi *et al.* (1998) reported that the rate at which heavy metals are deposited into the environment exceeds their removal by natural processes. This necessitates the need for remediation of runoff before it enters receiving water bodies.

Natural wetlands and artificially built sedimentation/detention ponds have become an economic means of runoff remediation. The value of these wetlands and sedimentation ponds has long been established. Both natural and artificial wetlands are being favoured over conventional treatment processes because they require low investment, are cost effective and need no external energy input (Tam and Wong, 1994). The potential functions of wetland areas include wildlife and fish habitat, recreational activities, denitrification, nutrient processing, water quality enhancement, etc. The use of wetlands has been proposed as a potential best management practice to mitigate the effects of pesticide associated agricultural runoff (Moore *et al.*, 2002). There are four main processes that take place in wetlands to remove metals. These include physical, chemical, biological and biochemical processes. These processes incorporate sedimentation, adsorption, co-precipitation, cation exchange, complexation, microbial activity and plant uptake. There are also four main compartments in which these

processes take place: the water, biota, substratum and suspended solids (Matagi *et al.* 1998). Metals move between these four compartments as they are acted on by any of the processes mentioned above. The aquatic flora play an important role in these contaminant-removal mechanisms in wetlands. Aquatic macrophytes function in oxygen production, the recycling of nutrients, water quality control, the stabilization of sediment and providing shelter for aquatic organisms (Mohan and Hosetti, 1999). Zurayk *et al.* (2001) reported that rooted plants appear to accumulate heavy metals from sediment and water. Demirezen and Aksoy (2005) found that although all the plants investigated in their study accumulated Fe and Mn, *Phragmites australis* seemed to reflect environmental conditions best. Dunbabin and Bowmer (1992) have commented that to illustrate the removal processes of metals from wetlands is difficult. This is because all these processes are dependent on one another, making the mechanism very complex. Nevertheless the rate at which reactions occur is dependent on sediment pH, redox status, the nature of the contaminant, plant genotype, the composition of sediment, and the amount and type of clay, minerals, hydrous oxides and organic matter.

In 1991 a vegetated 0.44 ha sedimentation pond was constructed on one of the small Lourens River tributaries on the Vergelegen Farm. This was done to minimize the input of sediment into the Lourens River from agricultural runoff (Schulz and Peall, 2001). It was constructed approximately 15 m before this small tributary discharges into the Lourens River and concrete pipes form the inlet and outlet of the pond. The approximate length of the pond is 134 m and it is 36 m at its widest. When constructed, the average depth was 1.5 m but that has been significantly reduced through sedimentation. Schultz and Peall (2001) reported depths of between 0.3 and 1 m. During sampling in the present study however, the depth at the inlet of the pond had diminished further to between 0.1 and 0.4 m. It was also noted during the present study that the entire surface area of the pond has been covered by *T. capensis*, *J. kraussii* and *C. dives*. The catchment area of this pond includes approximately 18 ha of forest, 10 ha of

pastures and 15 ha of orchards (Schulz and Peall, 2001). Ten years after the construction of this pond a study was conducted by Schulz and Peall (2001) to assess its effectiveness in the control of sediment, pesticides and nutrients originating from agricultural runoff. In their study they found the pond to be effective in removing all organophosphorus pesticides resulting in an 89% reduction in the toxicity of the outlet water (using an in situ bioassay: *Chironomus*). During dry weather conditions, 15, 54 and 70% of total suspended solids, orthophosphate and nitrate, respectively, were retained. During wet conditions the retention of these substances increased to 78, 75 and 84%, respectively. Water-diluted azinophos-methyl retention was found to be between 77 and 93%, while the pesticides, chlorpyrifos and endosulphan, were undetectable in the outlet water.

According to our knowledge, no studies have been conducted on this sedimentation pond to determine the total concentrations of selected metals accumulated in the pond itself.

MATERIALS AND METHODS

One sampling site was selected in the sedimentation pond to determine the total concentrations of selected metals entering the pond. This was situated just inside the inlet to the pond. A sampling site was also selected in the Lourens River a short distance downstream from the outlet (Site D). Sediment sampling was conducted on the same occasions as the samples collected from the Lourens River itself. The sampling methodology used was consistent with that used on the river (See Chapter 3).

RESULTS: Sediment

The concentrations of total Cu, Cr and Zn measured in sediment samples, collected over 12 months from the sedimentation pond are depicted in Figure 10.1.

Comparisons of total individual metal concentrations measured in the sediment, of the sedimentation pond over the 12 month sampling period

Copper

The highest Cu sediment concentration detected was during occasion four (11.2 ±2.8 mg kg^{-1}) and the lowest was detected during occasion seven (1.7 ±0.6 mg kg^{-1}). Occasion four was found to be statistically different ($P<0.05$) from all other occasions. Occasion three was found to be statistically different ($P<0.05$) from occasions five, six and seven while occasion seven was statistically different from occasions one and two. Occasion two showed a standard deviation of 14.4 mg kg^{-1}. All other comparisons showed P-values > 0.05.

Chromium

Total Cr sediment concentrations in the sediment pond ranged from 1.7 ±0.8 to 22.7 ±1.2 mg kg^{-1}. Only four comparisons showed no statistical significance ($P>0.05$): when occasion five, six and seven are compared with each other, and when occasion one was compared to occasion three. All other comparisons were statistically different ($P<0.05$).

Zinc

Zn sediment concentrations showed higher values than Cu and Cr. Concentrations ranged from 1.2 ±1.3 to 42.7 ±2.9 mg kg^{-1}. The highest concentration was once again detected during occasion four. All comparisons were found to be statistically significant ($P<0.05$).

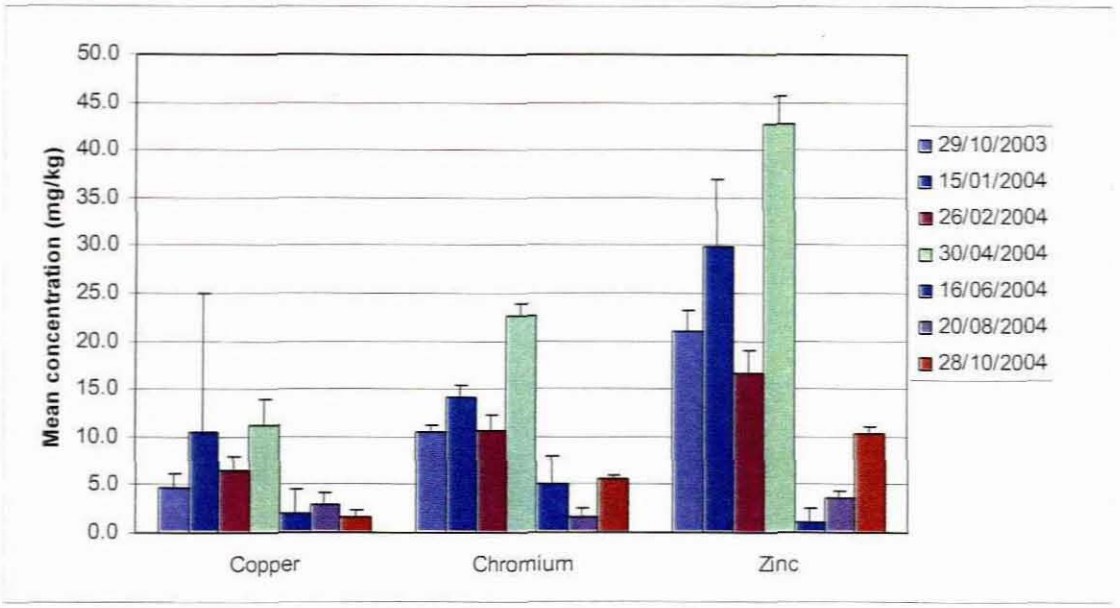

Figure 10.1 Mean concentrations of total copper, chromium and zinc (mg kg^{-1}) (±SD) in sediment samples collected from the sedimentation pond on the Vergelegen Farm, Western Cape, over a 12 month period.

Aluminium

The concentration of Al detected in the pond sediment ranged from 3366.3 ±283.3 to 45071.3 ±1894.5 mg kg^{-1}. Al once again showed a similar graphical pattern to Cu, Cr and Zn with occasion four displaying the highest concentration and occasions five, six and seven consistently lower. All multiple pairwise comparisons for Al showed statistical differences ($P<0.05$), except for occasion one vs three and five vs seven.

Iron

The highest concentration of Fe detected in the pond sediment reached 49 938.0 ±2054.2 mg kg^{-1} while the lowest was 3044.2 ±289.7 mg kg^{-1}. All multiple pairwise comparisons were found to statistically different ($P<0.05$) except occasion five vs occasion six ($P>0.05$).

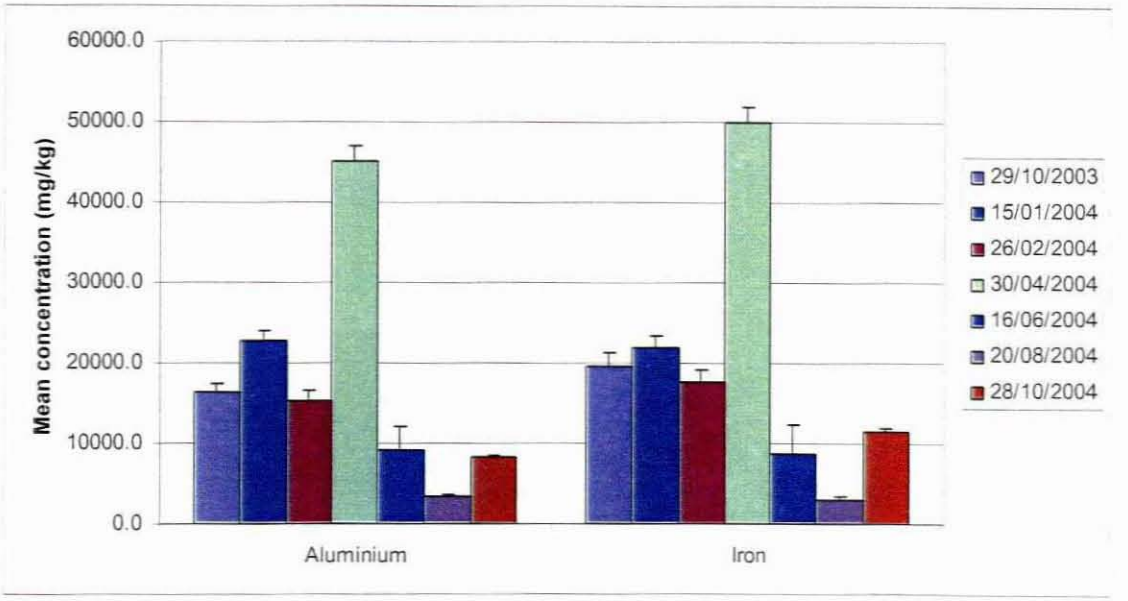

Figure 10.2 Mean concentrations of total aluminium and iron (mg kg^{-1}) (±SD) in sediment samples collected from the sedimentation pond on the Vergelegen Farm, Western Cape, over a 12 month period.

Manganese

Mn sediment concentrations ranged from 21.2 ±2.4 to 489.9 ±24.8 mg kg^{-1}. Graphically, Mn followed the same pattern as the previous metals. Multiple pairwise comparisons between occasion two and three showed no statistical difference ($P>0.05$), while all other comparisons did ($P<0.05$).

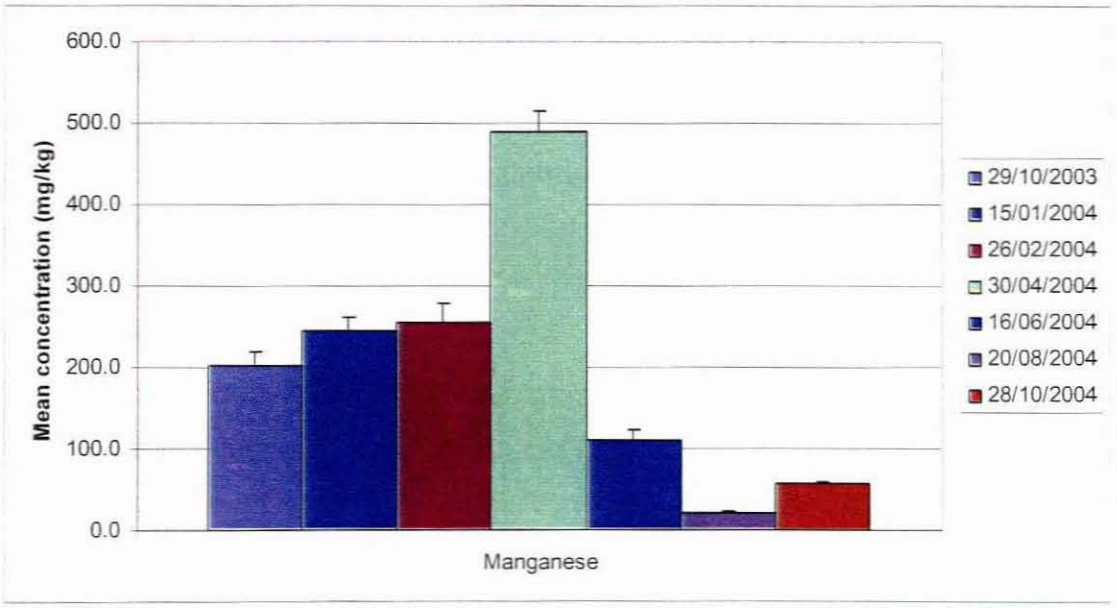

Figure 10.3 Mean concentrations of total manganese (mg kg^{-1}) (±SD) in sediment samples collected from the sedimentation pond on the Vergelegen Farm, Western Cape, over a 12 month period.

Comparisons of total individual metal concentrations measured in the sediment, of the sedimentation pond and of site D in the river, over the 12 month sampling period

Copper

Multiple pairwise comparisons between Cu concentrations detected in the sedimentation pond and the river showed varied results. Occasions one, three, six and seven showed no statistical differences ($P>0.05$). Cu concentrations detected in the sedimentation pond on occasions two, four and five were found to be statistically ($P<0.05$) different from those found in the river.

Chromium

The Cr sediment concentration detected in the river during occasion six was 2.3 ±0.4 mg kg^{-1} and the pond sediment concentration was 1.7 ±0.8 mg kg^{-1}. This was the only occasion where no statistical difference ($P>0.05$) was found. All other comparisons showed statistical differences ($P<0.05$).

Zinc

The Zn sediment concentration detected in the river during occasion six was 3.9 ±1.0 mg kg^{-1} and the pond sediment concentration was 3.7 ±0.6 mg kg^{-1}. Once again this was the only occasion where no statistical difference ($P>0.05$) was found. All other comparisons showed statistical differences ($P<0.05$).

Aluminium

Total Al concentrations in the river at site D ranged from 2467.8 ±354.3 to 4069.8 ±234.6 mg kg^{-1} while in the pond they ranged from 3366.3 ±283.3 to 45 071.3 ±1894.5 mg kg^{-1}. All multiple pairwise comparisons were found to be statistically different ($P<0.05$) from each other.

Iron

The total Fe concentration found in the river sediment during occasion six was the only occasion where no statistical difference ($P>0.05$) was found when compared to the total Fe concentration detected in the pond. All other comparisons showed statistical differences ($P<0.05$).

Manganese

Mn concentrations in the river at site D ranged from 32.0 ±2.9 to 57.7 ±60.4 mg kg^{-1} while pond sediment concentrations were much higher ranging from 21.2 ±2.4 to 489.9 ±24.8 mg kg^{-1}. All multiple pairwise comparisons were found to be statistically different ($P<0.05$).

DISCUSSION

A similar pattern in total metal concentrations for each sampling occasion can clearly be seen in all the graphs above (Fig 10.1 to 10.3). The samples collected during the month of April (occasion four) showed consistently higher concentrations for all six metals (Al, Cr, Cu, Fe, Mn and Zn). Sampling occasion 5, 6 and 7 (16 June, 20 August and 28 October 2004) showed consistently lower concentrations for all metals. In fact, every metal displayed a remarkable drop in concentration after occasion 4. Schulz *et al.* (2001) found that pesticide contamination of surface waters resulting from agricultural runoff is dependent on the time lapsed between the last pesticide application and the first major rainfall event. They also found that in the second rainfall event, which exceeded the first by 4.8 mm/day, no increases in pesticide levels were detected. In the present study, the high metal concentrations recorded for all the metals during sampling occasion 4 (30 April 2004), are therefore probably due to the first major rainfall of the wet season, where 75.8 mm of rain fell. Although 35.6 mm fell during the previous month, these high metal concentrations are probably as a result of the increased rainfall runoff during April. In the Western Cape, the last spraying during the summer growing season, normally takes place in February. The above mentioned results reported by Schulz *et al.* (2001) from their study in the same area were also recorded in the month of April.

The range of total Al concentrations detected in sediment from the pond generally showed higher concentrations than the range detected in the Lourens River. All multiple pairwise comparisons between these two sites were found to be statistically different ($P<0.05$). The range of Cu concentrations from the pond were, on the other hand, very similar to those detected in the river with only three comparisons being statistically different ($P<0.05$). Many similarities in Cr concentrations from the pond and the river can been seen, although the upper limit of the concentrations from the pond was higher (excluding the concentration detected on occasion 3 at site G). The range of total Fe concentrations detected in the pond was generally much higher than those detected in the river. Only

sampling occasion 5, 6 and 7 of the pond fell within the range of Mn sediment concentrations found in the river. All the other values were much higher. With the exception of occasion 2 and 4, most of the pond sediment Zn concentrations were similar to those detected in the Lourens River itself.

Table 10.1 Lowest and highest metal concentrations (±SD) found in sediment samples collected from the inlet of the sedimentation pond.

Metal	Total lowest conc. (mg kg^{-1})	Total highest conc. (mg kg^{-1})
Aluminium	3366.3 ±283.3	45071.3 ±1894.5
Copper	1.7 ±0.6	11.2 ±2.8
Chromium	1.7 ±0.8	22.7 ±1.2
Iron	3044.2 ±289.7	49938.0 ±2054.2
Manganese	21.2 ±2.4	489.9 ±24.8
Zinc	1.2 ±1.3	42.7 ±2.9

An increase in total metal concentrations can also be seen during occasion 2 for all metals except Mn. In most metals this increase drops once again in the following sampling occasion. It is possible that this increase is as a result of uncharacteristic rainfall-induced runoff during the month of December, where 52.6 mm of rain fell. Most metals show an incremental growth in total sediment concentrations over the dry season, which is probably as a result of a slow but steady discharge of contaminated runoff into the pond. It must be noted that although the water flow into the pond decreases in the dry months, there is a small but constant flow. Because much of the summer season spraying stops during winter, and the pond has the ability to remove metals from the sediment (Matagi *et al.*, 1998), the decrease in total sediment metal concentrations is not surprising. It must also be remembered though, that metals adsorbed onto settled sediment particles at the inlet to the pond can also be moved further into the pond through increased turbulence during winter rainfall events, thus reducing the concentration of those metals at the inlet. In a study on the mobility

of Cr in freshwater wetlands, Mattuck *et al.* (1995) have shown that freshwater wetlands are highly effective in immobilizing Cr. The wetland reduces Cr(VI) and precipitates it as a relatively insoluble Cr(III)-hydroxide.

Schulz and Peall (2001) found that during dry and wet conditions, 15 and 78% respectively of suspended solids were retained in the sedimentation pond. Metals adsorbed onto these suspended particles would then, in all probability, be retained at the same rate. If metals were leaving the pond to enter the river above site D, a general increase in total metal concentrations would be seen at this site. This however is not the case. No pattern of an increase in total metal concentrations can be seen in the river at site D. It appears that the sedimentation pond has been effectively removing metals from runoff water on the Vergelegen Farm.

CHAPTER 11

CONCLUSION

The accumulation of Al, Cr, Cu, Fe, Mn and Zn in the sediment and crabs (*P. perlatus*) of the Lourens River is affected by numerous natural and anthropogenic factors. Sediment concentrations of all six metals varied widely with Al and Fe showing the highest average concentrations. The following conclusions for each individual metal have been reached:

- The main source of total Al found in the sediment of the river was probably due to acidic leaching of parent rock. Gradual increases in Al concentrations, found in the sediment along the course of the river, seemed to be influenced more by urban anthropogenic activities, lower down the river, than agricultural activities. Total Al concentrations found in *P. perlatus* were only 5 to 10% of the concentrations detected in the sediment. *P. perlatus* seems to be able to regulate Al concentrations fairly well.

- Sediment Cu concentrations were found to be relatively low [when compared to those reported by other studies (Steenkamp *et al.*, 1994).] Congruent with results obtained by Snyman *et al.* (2001), *P. perlatus* appears to be able to regulate Cu well even when sediment concentrations increase.

- Considering its lower toxicity (relative to some other metals), low sediment concentrations and an apparent degree of bioregulation in *P. perlatus*, it is unlikely that Cr poses significant health risks to aquatic biota in the Lourens River.

- Although Fe concentrations in sediment were found to be high, they were still much lower than those reported in other SA studies (Steenkamp, 1992). Sediment concentrations generally increased along the course of the river with some occasions showing significant peaks. However, increased Fe concentrations in the sediment, were not always reflected by the crab Fe concentrations. This is confirmed by other SA studies (Steenkamp, 1993). Variations in crab Fe concentrations could partly be the result of Fe-rich sediment present in the gut of some crab samples.

- Most of the sediment Mn concentrations were found to be lower than those reported in other SA studies (Sanders *et al.,* 1998). Although numerous Mn containing agricultural applications are used on both farms from August till December, no corresponding pattern can be seen in sediment Mn concentrations. Bioaccumulation of Mn in crabs can vary greatly (Van Eeden and Schoonbee, 1991). In the present study, it is clear that crabs in the lower reaches of the river, generally accumulated higher concentrations of Mn even though sediment concentrations are lower. The characteristics of sediment particle size could play a role in increasing the bioavailability of Mn in the lower reaches of the river.

- Crab Zn concentrations were found to be 2 to 3 times higher than the concentrations detected in the sediment. Peak Zn sediment concentrations, in the lower reaches of the river, could be due to downstream transportation of Zn containing sediment, or other point-source, anthropogenic activities in the urban areas. Crab concentrations however, did not follow the small increases seen in the sediment Zn concentrations, along the course of the river. *P. perlatus* seem to regulate Zn fairly well in the Lourens River.

The following aspects must also be considered important influencing factors concerning metal pollution in the Lourens River:

- It is likely that agricultural activities on both the Lourensford and Vergelegen farms contribute to point source metal pollution of the Lourens River. It is expected that agricultural activities increases the rate of erosion and thus sedimentation rates, resulting in acceleration of natural metal erosion releasing processes.

- Clear cutting (removal of canopy vegetation) during forestry and other land clearing work may influence the water quality of the Lourens River. Newbold *et al.* (1980) and Noel *et al.* (1986) have shown that clear cutting without the maintenance of a "buffer zone" reduces canopy shade and thus increases mean water temperatures. Increased temperatures influence the diversity and density of many micro and macroinvertebrates. Every effort should therefore be made to protect and maintain the "buffer zone" vegetation along the length of the Lourens River.

- The sedimentation pond on the Vergelegen Farm was shown to be effective in removing metals from surface water run-off. A significant and consistent drop in all six metal's concentrations was seen after sampling occasion four. This system of run-off remediation should be seen as an important environmental intervention in reducing the impact of agricultural activities on the Lourens River system.

- Urban anthropogenic sources of Al, Cr and Zn between sites F and G is possibly responsible for the peak concentrations observed during occasion three at site G. Further investigation into possible point sources of various metal contamination from the urban areas is recommended.

- Although Cu, Mn and Zn containing applications are regularly used on both the Lourensford and Vergelegen farms, no sediment or crab results showed clear point source pollution from these chemicals. However, the impact of these chemicals on metal concentrations in the Lourens River must not be underestimated.

- Mean sediment size and characterization of sediment varies at each site. These factors may also influence the concentrations of various metals found in the sediment at specific sites and therefore the bioaccumulation by *P. perlatus* through feeding.

- It appears the sawmill on the Lourensford Farm has little or no detectable influence on the concentration of metals in the sediment and crabs of the Lourens River.

- It is possible that higher metal concentrations at the lower sampling sites are a function of the slower water current velocity which increases the sedimentation rate of suspended matter and therefore the metal concentrations detected in the sediment.

- Changing bioavailability of metals (Tipping *et al.*, 1991); detoxification mechanisms (Simkiss and Taylor, 1989); various interactions with other metals (Musibono, 1998) and avoidance of metal contaminated food sources (Weibenburg *et al.*, 2003) are all possible factors influencing the uptake and management of metals by *P. perlatus*.

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APPENDIX 1

Results from preliminary metal analyses of sediment samples (mg kg^{-1} dry mass)

ELEMENT	Al	Cd	Co	Cr	Cu
Wavelength	396.152	228.802	228.616	267.716	324.754
Control	0.09	ND	ND	0.22	0.12
A1	1151.79	ND	0.36	0.88	5.30
A2	1180.55	ND	0.37	0.73	5.08
A3	1377.62	ND	0.38	1.05	9.51
A4	1254.70	ND	0.36	1.07	7.42
A5	1388.97	ND	0.36	0.86	6.03
B1	1600.71	ND	ND	0.94	4.85
B2	1382.75	ND	ND	0.93	5.69
B3	1277.38	ND	ND	0.81	7.38
B4	1465.31	ND	ND	0.02	5.78
B5	1196.52	ND	ND	0.21	5.17
C1	1929.81	ND	0.34	2.07	4.64
C2	1995.65	ND	0.34	2.10	6.80
C3	2131.18	ND	0.32	2.11	6.36
C4	2172.60	ND	0.35	2.07	7.06
C5	2365.15	ND	ND	2.12	5.68
D1	3191.04	ND	0.38	4.22	8.44
D2	3296.33	ND	1.20	2.05	5.76
D3	3521.42	ND	0.81	2.13	5.22
D4	3100.55	ND	0.72	2.37	2.38
D5	3080.44	ND	0.68	2.18	3.60
DD1	14731.21	ND	2.82	9.43	4.04
DD2	16826.79	ND	3.16	10.73	4.37
DD3	16799.93	ND	3.24	10.81	7.20
DD4	17642.78	ND	3.35	11.17	3.40
DD5	16133.64	ND	3.14	10.37	4.19
E1	1563.03	ND	0.36	1.46	0.00
E2	1550.19	ND	0.35	1.08	0.00
E3	1369.95	ND	0.29	2.56	0.54
E4	1696.04	ND	0.37	1.31	0.66
E5	1607.53	ND	0.37	1.60	0.00
F1	1702.88	ND	0.36	2.34	0.47
F2	1574.69	ND	0.36	1.20	1.10
F3	1732.96	ND	0.35	1.87	0.79
F4	1752.34	ND	0.35	1.64	1.20
F5	1636.52	ND	0.35	2.00	0.21
G1	3458.33	ND	0.34	4.59	2.43
G2	3580.85	ND	0.37	4.74	3.82
G3	3523.37	ND	0.37	4.34	4.30
G4	3255.74	ND	0.39	4.40	3.86
G5	3457.30	ND	0.33	3.82	4.31
Detection limit	0.01	0.01	0.02	0.01	0.02

ND: Not detected

Results from preliminary metal analyses of sediment samples (mg kg^{-1} dry mass)

ELEMENT	Fe	Mn	Ni	Pb	Zn
Wavelength	259.94	257.61	231.604	220.353	213.856
Control	0.79	0.02	0.12	ND	0.07
A1	1541.88	28.00	1.03	ND	2.93
A2	1706.23	29.57	0.00	ND	5.08
A3	2001.62	28.88	0.00	ND	2.14
A4	1842.61	128.90	0.61	ND	3.01
A5	1813.65	86.98	0.27	ND	2.92
B1	1192.94	13.03	0.05	ND	1.74
B2	1533.88	12.64	0.57	ND	0.44
B3	847.19	6.99	0.00	ND	0.26
B4	1015.66	8.80	0.13	ND	0.00
B5	925.90	9.51	0.06	ND	13.26
C1	2084.06	29.80	1.00	ND	4.01
C2	2104.64	30.37	0.21	ND	3.55
C3	2260.61	39.11	0.66	ND	3.77
C4	1986.47	36.74	0.76	ND	2.83
C5	2386.03	35.58	0.52	ND	2.99
D1	2988.69	40.78	1.39	ND	5.50
D2	2781.52	38.86	1.24	ND	4.84
D3	3005.41	41.54	1.21	ND	5.35
D4	2732.65	37.73	1.36	ND	4.60
D5	2825.30	38.71	1.27	ND	4.73
DD1	17204.73	173.14	3.24	8.58	15.95
DD2	20864.91	204.67	2.69	8.21	18.84
DD3	19962.56	205.78	2.87	9.72	19.14
DD4	21228.50	224.71	3.43	8.85	20.02
DD5	18955.26	197.46	3.08	9.22	22.12
E1	2146.92	15.46	0.57	ND	7.25
E2	2033.21	16.34	1.04	ND	6.62
E3	2412.61	20.69	0.87	ND	5.34
E4	1932.19	26.98	0.62	ND	6.48
E5	2022.46	16.47	0.87	ND	7.14
F1	2014.54	20.37	1.19	0.18	9.30
F2	1882.41	19.07	1.01	0.18	8.03
F3	2110.03	22.51	0.34	0.17	7.76
F4	2394.09	19.62	0.71	7.66	8.51
F5	1936.16	15.37	0.59	0.17	7.67
G1	3313.83	13.01	1.65	5.86	14.51
G2	3741.57	15.53	1.91	6.61	17.03
G3	3726.44	15.60	1.70	7.21	15.84
G4	3903.45	14.51	2.21	5.07	16.69
G5	3538.52	13.25	1.48	7.19	15.39

ND: Not detected

APPENDIX 2

Results from preliminary metal analyses of crab samples (mg kg^{-1} dry mass)

ELEMENT	Al	Cd	Co	Cr	Cu
Wavelength	**396.152**	**228.802**	**228.616**	**267.716**	**324.754**
Control	0.25	ND	ND	0.24	0.07
A1	248.17	0.20	ND	0.00	46.20
A2	237.08	0.40	0.07	0.00	68.98
A3	215.73	0.18	0.05	0.00	38.38
A4	234.45	0.19	0.06	0.00	51.93
A5	230.69	0.28	ND	0.00	45.96
B1	390.78	0.06	0.07	0.00	27.27
B2	269.93	ND	ND	0.00	26.36
B3	357.90	0.06	ND	0.00	27.05
B4	309.95	0.04	0.05	0.00	29.36
B5	241.97	0.04	0.05	0.00	33.49
C1	381.49	0.14	0.05	0.00	49.51
C2	435.35	0.06	0.43	0.00	49.66
C3	379.71	0.07	ND	0.00	43.91
C4	428.64	0.18	ND	0.00	46.38
C5	377.73	0.04	0.29	0.00	36.14
D1	466.17	0.17	0.07	0.00	46.80
D2	481.33	0.24	0.32	0.00	47.72
D3	378.79	0.16	0.27	0.00	34.38
D4	485.81	0.19	ND	0.00	47.54
D5	473.82	0.06	0.75	0.00	41.73
E1	No data				
E2					
E3					
E4					
E5					
F1	664.27	0.22	ND	0.00	84.25
F2	515.39	0.35	0.20	0.00	37.93
F3	355.90	0.11	0.22	0.00	66.98
F4	390.94	0.04	0.23	0.00	52.13
F5	344.20	0.16	0.05	0.00	84.50
G1	437.87	ND	ND	0.00	49.01
G2	471.53	ND	ND	0.00	42.75
G3	483.70	ND	ND	0.00	41.05
G4	956.30	ND	ND	0.00	30.60
G5	507.44	ND	ND	0.00	52.93
Detection limit	0.01	0.01	0.02	0.01	0.02

ND: Not detected

Results from preliminary metal analyses of crab samples (mg kg^{-1} dry mass)

ELEMENT	Fe	Mn	Ni	Pb	Zn
Wavelength	259.94	257.61	231.604	220.353	213.856
Control	0.98	0.02	0.12	ND	0.42
A1	240.26	56.95	0.08	ND	47.47
A2	168.60	30.90	0.26	0.68	67.95
A3	117.09	51.26	0.00	ND	43.46
A4	158.56	64.80	0.00	0.55	50.93
A5	979.67	36.80	0.00	ND	57.65
B1	234.34	73.79	0.00	ND	55.14
B2	69.73	48.22	0.00	ND	47.60
B3	193.86	32.37	0.00	ND	45.98
B4	159.23	48.47	0.00	ND	48.37
B5	106.83	43.27	0.24	ND	52.88
C1	265.78	91.93	0.00	ND	125.03
C2	260.11	96.87	0.22	0.70	110.70
C3	196.69	72.87	0.00	ND	71.92
C4	1190.19	63.68	0.00	ND	67.21
C5	288.92	96.17	0.00	ND	50.56
D1	317.32	107.73	0.00	ND	58.85
D2	444.95	84.77	0.19	ND	62.37
D3	229.88	107.01	0.00	0.52	46.31
D4	928.17	105.04	0.00	ND	56.91
D5	391.70	108.11	0.30	ND	52.20
E1	No data				
E2					
E3					
E4					
E5					
F1	317.94	55.08	0.07	0.71	232.47
F2	174.19	54.42	0.00	4.27	101.06
F3	202.18	92.74	0.00	0.34	123.92
F4	227.16	124.15	0.00	0.50	74.83
F5	227.87	86.28	0.00	0.49	87.51
G1	159.65	85.59	0.00	ND	62.98
G2	208.79	68.55	0.00	ND	56.85
G3	195.82	110.36	0.00	ND	59.15
G4	637.60	176.78	0.00	ND	51.26
G5	240.50	59.79	0.00	ND	95.21
Detection limit	0.02	0.005	0.03	0.1	0.02

ND: Not detected