The Occurrence and Accumulation of Selected Heavy Metals in Fresh Water Ecosystem Affected by Mine and Industrial Polluted Effluent

H J Schoonbee, et al

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THE OCCURRENCE AND ACCUMULATION OF SELECTED HEAVY METALS IN FRESH WATER ECOSYSTEMS AFFECTED BY MINE AND INDUSTRIAL POLLUTED EFFLUENT

Final Report to the
Water Research Commission

by

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The Steering Committee for this project consisted of the following persons:

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EXECUTIVE SUMMARY

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Background and motivation

The establishment of gold and coal mines in South Africa during the past century led to the creation of several industrial zones surrounding some of the major cities, which directly or indirectly supported the mining industry. Many of these were metal-processing factories. Processed and unprocessed effluents and seepage waters from both the mines and industries were discharged into streams and were found to contain variable amounts of heavy metals (Wittman and Förstner, 1976 a-d, 1977 a,b; Kempster *et al.*, 1980; Van der Merwe *et al.*, 1990). The pollution from the mining areas in particular led to severe acidification of a number of tributaries of the Vaal River (Harrison, 1958, 1961; Schoonbee and Van der Merwe, 1989). Water pumped from some mines varied in pH, affecting the solubility and the consequent deposition in stream and lake sediments as well as the uptake of certain metals by the flora and fauna of the affected water bodies. Estimates of the volume of water pumped from mines reaching the Elsburgspruit alone amounted to as much as 10 938 m^3/d (Wells, 1989), directly and/or indirectly through seepage and effluents from existing mine dumps.

The effect of mine effluent water containing metals on stream ecosystems has received considerable attention elsewhere in the world (Eyres and Pugh-Thomas, 1978; Salomons and Mook, 1980; Norris, 1986). From these investigations it is clear that certain metals may have a pronounced effect on the composition and presence of certain aquatic biota in lakes and rivers and that changes in the water pH and associated physical and chemical variables which affect this parameter may have a direct bearing on the toxicity of some metallic ions. Research by Abo-Rady (1980) and Mortimer (1985), to name but two researchers, showed that rooted aquatic macrophytes can be used as bio-indicators to evaluate the extent of metal pollution in aquatic ecosystems. It is also known that floating aquatic weeds can be employed in the removal of potentially toxic metals from polluted waters (Muramoto and Oki, 1983; Abbasi and Nipanei, 1985; Jain *et al.*, 1989). Some aquatic invertebrates were also found to be extremely sensitive to metal pollution (Bryan and Ward, 1965; Burrows and Whitton, 1983; Yasuno *et al.*, 1985). Aquatic vertebrates such as amphibia and fish have also been studied to determine the various routes of metal uptake via their food (Baudin, 1989), gills (Matthiesen and Brafield, 1977), through the drinking of water (Eddy, 1981) and simply through absorption via the skin. The release of toxic metals into the aquatic environment was shown to be detrimental to aquatic birds (Koeman *et al.*, 1972; Bull *et al.*, 1983).

In view of the increasing threat of metal-containing effluents to the water quality conditions of wetland areas on the Witwatersrand in particular and because of the planned and unplanned residential encroachment on these potential recreational areas and wildlife sanctuaries, the need was expressed by researchers at the Rand Afrikaans University to evaluate stream conditions and, where possible, to establish further research programmes on the problem of metal pollution of the aquatic environment on the Witwatersrand. The present series of investigations comprised the first phase of the envisaged project.

Objectives and Scope

The investigations conducted during 1988-1994 had the following objectives:

- **Selection of representative localities in lakes and rivers affected by metal-containing effluents and seepage water from mines and metal-processing industries.**
- **To determine the occurrence and concentration of selected metals in mine- and industry-polluted waters and sediments in catchments of the Natalspruit-Elsburgspruit, Blesbokspruit and in the headwaters of the Klip River in the Roodepoort municipal area.**
- **To establish metal concentrations in semi-aquatic and aquatic weeds in these mine and industrially polluted waters with observations on the ability of some plants to accumulate certain metals in their roots and/or shoots.**
- **To evaluate some benthic macro-invertebrate organisms as possible indicators of metal pollution in streams with special reference to the freshwater crab, *Potamonautes warreni*.**
- **To study the occurrence of metals in target organs and tissues of fish from mine- and industry-polluted aquatic habitats.** Nine indigenous and exotic fish species were considered for investigation from one or more of six different sampling localities on the East and West Rand. The report deals with two of the fish species concerned, namely the southern mouthbrooder *Pseudocrenilabrus philander* and the perch *Perca fluviatilis*.
- **To evaluate the clawed frog or platanna *Xenopis laevis* as a potential indicator of metal pollution in aquatic ecosystems.**
- **To compare concentrations in organs and tissues of some aquatic and semi-aquatic birds.** Attention was specifically given to the redknobbed coot, *Fulica cristata*, the sacred ibis *Threskiornis aethiopicus* and the reed cormorant, *Phalacrocorax africanus*.

The localities where the investigation took place were mainly in wetland regions on the East and West Rand, Gauteng, where the water of the polluted streams traverse expansive wetlands consisting of floating, emergent and submerged aquatic vegetation and where these plants play an important role in the recovery from pollution of the affected waters.

All the above objectives have largely been met.

Results and conclusions

Physical and chemical conditions of the water and sediments of the different localities

Research showed that the Germiston Lake had recovered substantially from mine and industrial pollution following the first steps taken to rehabilitate this lake in the early 1970's. Since then, there was a consistent decline in mineral loads of the lake water. However, metal concentrations in the lake sediments remained high and metals released into the water column appear to be gradual under the prevailing alkaline conditions. Transfer of metals through the biological component of the lake ecosystem takes place without any signs of metal toxicity to organisms studied. The lake water is discharged into the Elsburgspruit where serious metal pollution of its water by both the mines and industries took place. Seepage of water from industrial, ash and mine dumps further complicated the situation. Although considerable recovery from organic and inorganic pollution occurs in the Elsburgspruit and, despite the ability of the wetlands to assist in the recovery of the stream, considerable metal loads still pass through the dense mats of submerged and emergent weeds into the Natalspruit. A similar situation was found to exist in the main Blesbokspruit ecosystem where abnormal concentrations of Fe, Zn, Ni and at places Cu were found to exist. Water in both the Cowles and Nigel dams contained appreciable loads of most of the metals investigated, with Fe and Mn in particular, occurring in relatively high concentrations in the water and sediments of both dams. The Florida Lake showed the best recovery from past mine pollution in its catchment area.

Metal uptake by some emergent and floating aquatic weeds

The emergent aquatic weeds *Typha capensis*, *Arundo donax* and the water fern *Azolla filliculoides* were evaluated for their ability to retain metals in their organs and tissues and estimates were made on their ability to remove metals from polluted aquatic environments under local environmental conditions.

Metal uptake by the freshwater crab *Potamonautes warreni*

The concentrations in which the metals occurred in the Natalspruit and in the crab *P. warreni* followed similar trends. Indications are that the accumulation of the metals Pb, Cr and Fe, in particular, in the body of the crab may be regulated successfully in the metal-polluted waters of the Natalspruit and probably also in other similarly mine-polluted waters where it occurs.

Metal uptake by fish

Investigations showed that the southern mouthbrooder *Pseudocronilabrus philander* appears to be reasonably tolerant to various concentrations of the metals Fe, Mn, Zn, Cu, Ni and Pb in the Spaarwater dam and that a possible mechanism exists whereby concentrations of these metals are bioregulated by this fish species. Bioregulation improves with the age and size of the fish.

Concentrations of Fe, Ni and Zn were the highest of all the metals analysed in the organs and tissues of the perch *Perca fluviatilis* and closely reflected the ratio of the metals in the lake sediments. Comparatively high concentrations of Pb in most organs were mainly related to surface runoff waters from the streets which enter the lake via various stormwater drains.

Metal uptake by the platanna *Xenopus laevis*

The concentration of metals by the frogs and tadpoles of *X. laevis* showed that certain organs and tissues were able to accumulate certain metals in much higher concentrations than others. This can be linked to sites of metal uptake, storage and excretion in the frogs and also the possibility that metal bioregulation may take place. Whole body metal analysis more closely reflects the metal loads present in the water and sediments. Metal analysis of the river frog *Rana angolensis* larvae yielded similar results as those for *X. laevis*.

Metal uptake by the redknobbed coot *Fulica cristata*, the reed cormorant *Phalacrocorax africanus* and sacred ibis *Threskiornis aethiopicus*

The concentrations of cadmium, copper, nickel and lead were determined in the liver, kidney, bone and blood of all three species which occurred in the Natalspruit wetlands. The investigation suggests that the dietary preferences of these birds and the metal contents of their diets as such, may be the two most important factors which determined the metal concentrations in the bodies of these birds.

Concentration ratios

The concentration ratios for the metals investigated suffer the same weaknesses and limitations as pointed out by Bain *et al.* (1994) for the radionuclide radium in mine polluted aquatic ecosystems. Both the CR_w (water) and CR_s (sediments) of the metals were calculated to evaluate the possible efficiency of particular aquatic flora and fauna to accumulate the metals under different environmental conditions. In the present study more importance is attached to the CR_s values as they represent the history of metal pollution at a given site whereas the CR_w values evaluate the concentrations of metals in streams at a specific period and which may differ considerably within hours depending on the periodic release of effluents from mines and industries during the day.

Water quality criteria for metals

A comparison of the maximum limits suggested for the protection of aquatic life in rivers and lakes (Kempster *et al.*,1980) with results obtained during the present survey showed that all the different ecosystems investigated were endangered by excessive loads of the seven metals under consideration, in both the water and sediments. This tendency is usually not reflected by the prevailing general physical and chemical conditions at the various sites.

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1. Aquatic and semi-aquatic flora

CLASSIFICATION	POPULAR NAME	PLANT TYPE ACCORDING TO VENTER (1991)
ALGAE *Nitella* sp. *Spirogyra* sp.	Coarse algae Spirogyra	SA SA
BRYOPHYTA Bryum	Wort	MP
PTERIDOPHYTA MARSILEACEAE *Marsilea macrocarpa* Pres 1.	Water clover	RFA
AZOLLACEAE *Azolla filiculoides Lam.*	Water fern	FA
ANGIOSPERMAE-MONO-COTOLYDONEAE TYPHACEAE *Typha capensis* (Rohrb.) N.E. Br.	Bulrush	EA
POSTAMOGETONACEAE *Potamogeton pectinatus L.*	Fennel-leaved pondweed	SA
ALISMATACEAE *Alisma plantago-aquatica L.*	Water plantain	EA/MP
POACEAE *Avena* sp.	Wild oats	EA
Arundo donax L.	Spanish reed	EA/MP
Phragmitis australis (Cav.) Steud.	Common reed	EA
Agrostis Lachnantha Nees var. *lachnantha*	Bent grass	MP
Polypogon monspeliensis (L.) Desf.	Brackish grass	MP
Bromus *B. inermus Leyss*	Prairie grass	MP
B. catharticus Vahl	Broncho grass	MP
CYPERACEAE *Cyperus longus* L. var. *longus*	Water grass	EA/MP
Schoenoplectus *S.corymbosus* (Roth. ex Roem. & Schult.) J. Raynal	Mat sedge	EA/MP
S. lacustrus (L.) Palla	Prickly water sedge	EA/MP

List of aquatic and semi-aquatic plants and animals which occur in the flood plains of the metal polluted streams of the Elsburgspruit, Natalspruit and Blesbokspruit catchment areas (Continued).

1. Aquatic and semi-aquatic flora

CLASSIFICATION	POPULAR NAME	PLANT TYPE ACCORDING TO VENTER (1991)
Isolepis costata (Boeck.) A. Rich. var. Macra (Boeck.) B.L. Burtt	Common sedge	EA/MP
JUNCACEAE *Juncus lomatophyllus* Spreng.	Common rush	EA/MP
ANGIOSPERMAE-DICOTYLEDONEAE		
SALICACEAE *Populus canescens* (Ait.)J.E. Sm.	Grey poplar	EA/MP
POLYGONACEAE *Rumex conglomeratus* Murb.	Dock	MP
Polygonum lapathifolium L. subsp. *maculatum* (S.F. Gray) T. Dyer & Trim	Cock's comb	MP
CHENOPODIACEAE *Chenopodium album* L.	Pig bush	MP
BRASSICACEAE *Rapistrum rugosum* (L.) All.	Rapistrum	MP
ONAGRACEAE *Oenothera rosea* L'Herit. ex Ait	Evening primrose	MP
SCROPHULARIACEAE *Veronica anagallis-aquatica* L.	Veronica	EA/MP
PLANTAGINACEAE *Plantago lanceolata* L.	Lamb's tongue	MP
ASTERACEAE *Cotula coronopifolia*	Goose grass	EA/MP
Senecio sp.	Water groundsel.	MP
Sonchus oleraceus L.	Groundsel.	EA/MP
LEMNACEAE *Lemna gibba* L.	Duckweed	FA
HYDROCHARITACEAE *Lagarosiphon major* (Ridley) Moss ex Wager	Bobbejaantou	SA
L. muscoides Harv.		SA
NYMPHAEACEAE *Nymphaea* cf. *spectabilis* Gilg.	Water lily	RFA

EA: Emergent aquatic. FA: Floating aquatic. SA: Submerged aquatic. MP: Mudplant. RFA: Rooted floating aquatic (After Venter, 1991).

List of aquatic and semi-aquatic plants and animals which occur in the flood plains of the metal polluted streams of the Elsburgspruit, Natalspruit and Blesbokspruit catchment areas (Continued).

2. Aquatic and semi-aquatic fauna

CLASSIFICATION	POPULAR NAME
CRUSTACEA (DECAPODA)	
Potamonautidae	
Potamonautes warreni (Calman)	Freshwater crab
OSTEICHTHYS	
Cichlidae	
Pseudocrenilabrus philander (Weber)	Southern mouthbrooder
Percidae	
Perca fluviatilis L.	European perch
AMPHIBIA	
Pipidae	
Xenopus laevis (Daudin)	Common platanna
Ranidae	
Rana angolensis Bocage	Common river frog
AVES	
Phalacrocoracidae	
Phalacrocorax africanus (Gmelin)	Reed cormorant
Plataleidae	
Threskiornis aethiopicus (Latham)	Sacred ibis
Rallidae	
Fulica cristata Gmelin	Redknobbed coot

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1. INTRODUCTION

Goldmining on the Witwatersrand and elsewhere in the Transvaal and the deposition of processed and unprocessed waste material above ground, have led to the continuous, persistent leaching, over decades, of dissolved minerals and metals from these sites into streams, lakes and rivers. These mining activities were accompanied by the establishment of secondary industries and in particular metal-processing plants which further added to the problem of a variety of effluents being discharged into these water bodies. Such effluents and seepage waters contributed substantially towards the acidification (Harrison, 1958), mineralization (Wittmann and Förstner 1976a, 1976b), eutrophication (Schoonbee, 1991) and metal contamination (Van der Merwe *et al.*, 1990; de Wet *et al.*, 1990) of aquatic habitats in this region. Changes in physical and chemical conditions such as pH and dissolved oxygen further affected the speciation and biological availability of certain metals in the water and substrates of the affected waters (Förstner and Prosi, 1979; Campbell and Stokes, 1985; Campbell and Tessier, 1985), enabling organisms such as aquatic plants (Whitton *et al.*, 1981; Van der Merwe, *et al.*, 1990; De Wet *et al.*, 1990) and benthic macro-invertebrate organisms (Nehring 1976; Eyres and Pugh-Thomas, 1978; Steenkamp *et al.*, 1993) to bioaccumulate certain metals directly or indirectly via certain aquatic food chains. Transfer of these metals then occurs via several possible pathways to fish (Heath, 1987; de Wet *et al.*, 1990), amphibia (Fleischer, 1993) and aquatic birds (Van Eeden and Schoonbee 1992, 1993). Changes in the physical and chemical conditions of the aquatic environment may also affect the degree of toxicity of such metals to these organisms (Whitley, 1968; Stumm and Morgan, 1979).

During the period 1986 - 1993 a number of investigations were conducted into the presence and concentrations of selected metals in wetland ecosystems of the Elsburgspruit-Natalspruit, the Klip River and Blesbokspruit catchment areas. Specific attention was given to the presence of Zn, Mn, Cu, Ni, Fe, Pb, Cr and to a lesser extent Cd as these metals were largely associated with effluents and seepage waters from mines and industries in this region. In addition to the metal concentrations in the abiotic environment, analyses were also done on selected aquatic and semi-aquatic plants, macro-invertebrate organisms, a number of fish species, amphibia (*Xenopus laevis*) and aquatic and semi-aquatic birds. Since a number of research papers have already been published or are in the process of publication, this report mainly summarises the major results obtained during this research project.

The main objectives of and recommendations on the present report on metals in mine-, sewage- and industry-polluted aquatic environments are dealt with in the following sections:

1. **Establishment of the occurrence and concentration of selected metals in mine- and industry-polluted waters and sediments in catchments of the Natalspruit-Elsburgspruit, Blesbokspruit and in the headwaters of the Klip River in the Roodepoort municipal area.**

2. **Determination of metal concentrations in semi-aquatic and aquatic weeds in these mine- and industry-polluted waters with observations on the ability of some plants to accumulate certain metals in their roots and/or shoots.**

3. **Evaluation of some benthic macro-invertebrate organisms as possible indicators of metal pollution in streams with special reference to the freshwater crab, *Potamonautes warreni.***

4. **Investigations into the occurrence of metals in target organs and tissues of fish from mine- and industry-polluted aquatic habitats.**

5. **Determination of metal concentrations in the adult and larval stages of the African clawed toad *Xenopus laevis.***

6. **Comparison of metal concentrations in organs and tissues of some aquatic and semi-aquatic birds.** Attention was specifically given to the redknobbed coot, *Fulica cristata*, the sacred ibis *Threskiornis aethiopicus* and the reed cormorant, *Phalacrocorax africanus*.

7. **Discussion of the major findings of the survey with recommendations on further work which needs to be done in the study area.**

These objectives have largely been achieved during the present series of investigations. A total of six M.Sc. dissertations and two Ph.D. theses emanated from this study. Twelve articles on various aspects of the study appeared in national and international scientific journals. Some of the results were presented at international and national scientific conferences.

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2. DESCRIPTION OF SAMPLING AREAS

A total of seven sampling areas, covering different stretches in metal-polluted rivers and localities in two lakes on the Witwatersrand, were investigated for physical and chemical conditions with special emphasis on the concentrations of selected metals of the water and sediments, some algae and semi-aquatic and aquatic weeds, benthic macroinvertebrates, fish, amphibia (mainly *Xenopus laevis*) and aquatic and semi-aquatic birds. A brief description of each site at the time of the respective surveys follows.

2.1 The Germiston Lake

The Germiston, or Victoria Lake, near Johannesburg (Figure 2.1) has been severely polluted for decades by mine and industrial effluents and seepage waters (Schoonbee, *et al.*, 1985; Bezuidenhout *et al.*, 1990).

The elimination of the major sources of pollution from the lake since 1968, over a period of approximately 15 years, led to a major recovery of the lake from pollution so that a number of fish species, including *Clarias gariepinus* (Burchell), *Cyprinus carpio*, L., *Tilapia sparrmanii* Smith and *Micropterus salmoides* Lacépède were successfully introduced into the lake during 1972-76. The recovery in the water quality of the lake coincided with a general increase in pH from 3,5 - 5,5 to 7,0 - 8,5 at present (Bezuidenhout *et al.*, 1990).

This led to the development of excessive growths of submerged aquatic weeds in the lake, in particular by the fennel-leaved pondweed *Potamogeton pectinatus* L. The Chinese grass carp *Ctenopharyngodon idella* (Val.) was employed successfully to combat the excessive growths of the water weeds in the lake (Schoonbee, 1991).

Investigations showed that metals deposited in the lake sediments and dissolved in the overlying water column found their way into the aquatic flora and fauna of the lake ecosystem including the fish (Bezuidenhout *et al.*, 1990) and aquatic birds (Adendorff, 1993; van Eeden, 1994). A total of nine sampling sites were selected for the collection of the water, sediments and aquatic organisms. Specific attention was given to metal accumulation by the algae *Spirogyra* sp. and *Nitella* sp. as well as emergent aquatic macrophytes *Phragmitis australis*, *Typha capensis* and the emergent marginal vegetation *Alisma plantago-aquatica* and *Polygonum monspeliensis*. Organs and tissues from fish in the lake analysed for metals included *Clarias gariepinus*. Aquatic birds investigated for metal contamination included the red-knobbed coot, *Fulica cristata* (van Eeden, 1994).

2.2 The Elsburgspruit at Germiston

A total of fifteen different sampling localities on the Elsburgspruit and on one of its tributaries (Figure 2.2) were selected largely on the basis of prevailing pH conditions in the streams, but also for the reasons that follow. These localities were used to evaluate the effects of the acidic mine waters on the metal contents of the water, the sediments and the two aquatic plants *Arundo donax* and *Typha capensis*. Investigations by Van der Merwe (1990) and Schoonbee and Van der

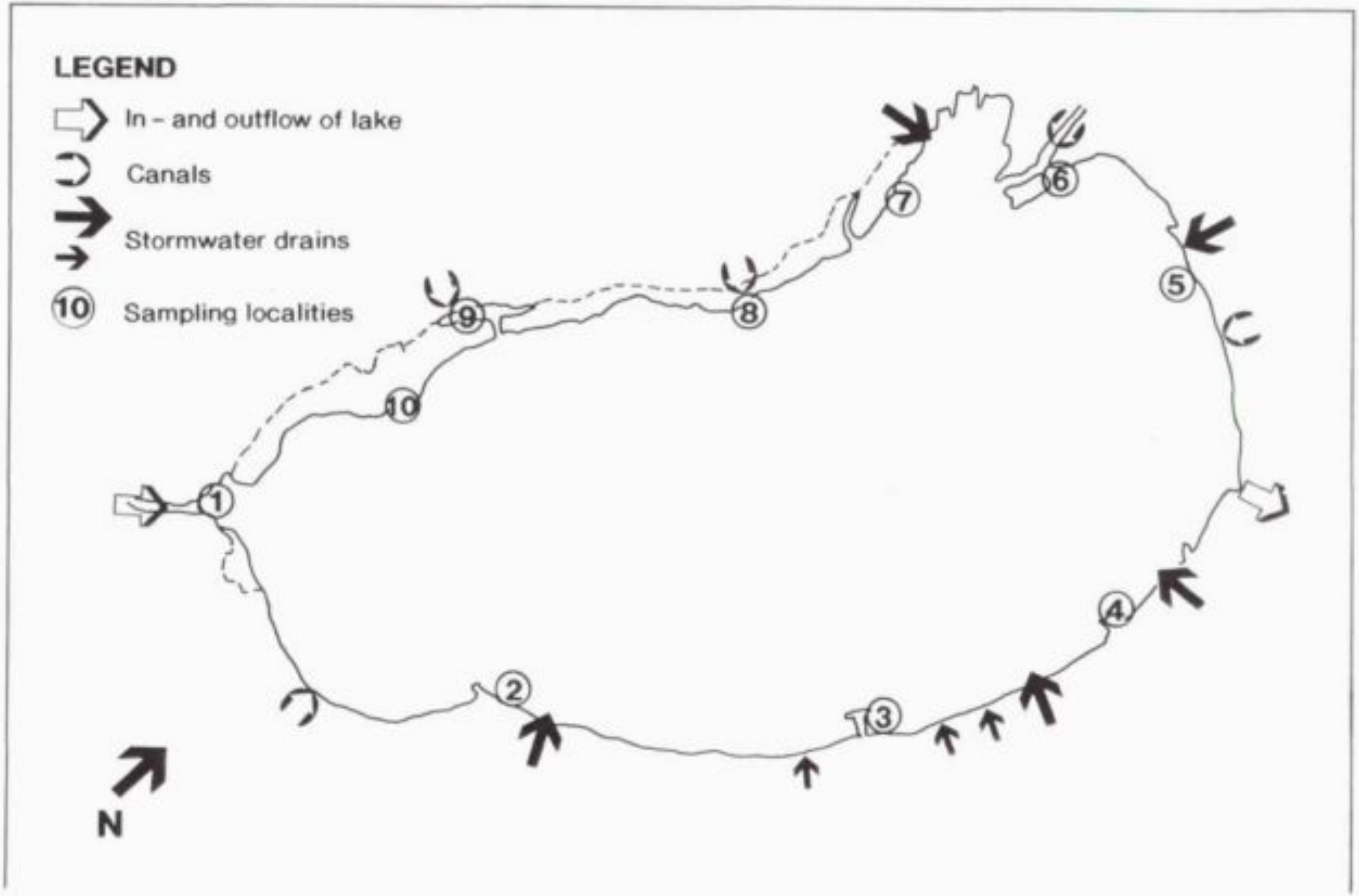

Figure 2.1: Map of Germiston lake showing the sampling localities used during the survey as well as the number of stormwater drains which enter the lake around its perimeter.

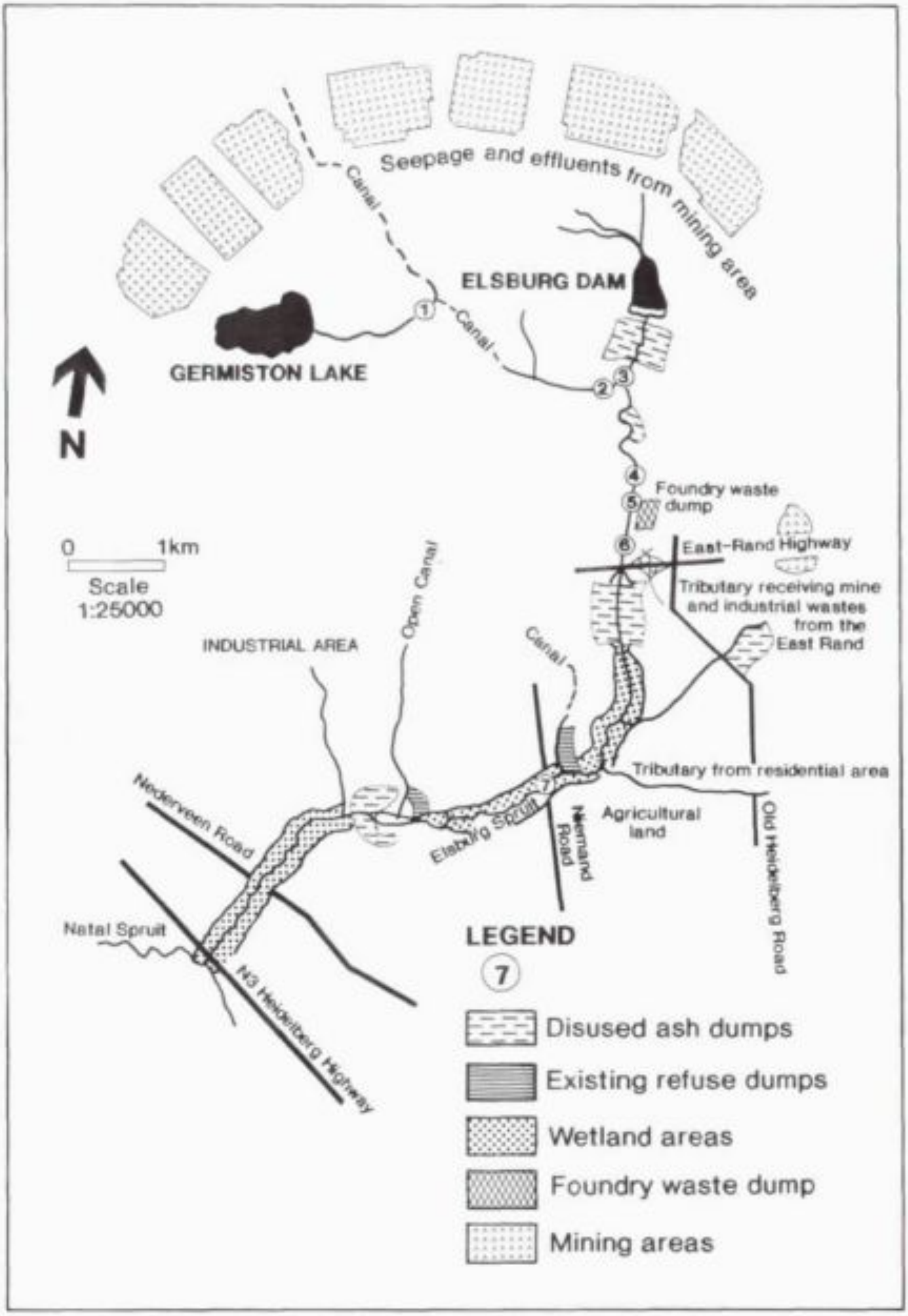

Figure 2.2: Map of the Elsburgspruit showing the seven sampling localities as well as the different potential sources of metal pollution of the stream.

Merwe, (1989) had shown that the water of the Elsburgspruit contained abnormally high concentrations of various metals and that the pH of the water was very low at the headwaters of this stream (Locality 1), fluctuating between 3,6 and 6,6. Locality 2 was situated approximately 2,5 km downstream from Locality 1, where the pH of the water showed some improvement, varying mainly between 4,4 and 5,9. Locality 3, situated on a tributary which also receives acidic mine effluent waters, was also shown to receive fluxes of relatively high concentrations of the metals concerned (Schoonbee and Van der Merwe, 1989). Sampling Locality 4 was situated on the main stream again in a predominantly wetland area (Fig. 2.2), after the stream had flowed through densely-vegetated *T. capensis* and *A. donax* communities for more than 6 km. At this point the pH of the water already showed some recovery by fluctuating between 6,1 and 8,8.

The absorption and accumulation of selected metals under these different pH conditions were investigated for *A. donax* and *T. capensis* (Van der Merwe *et al.*, 1990). A qualitative assessment of the ability of other semi-aquatic plants to accumulate metals was also made (Van der Merwe, 1990).

2.3 The Natalspruit below the confluence of the Elsburgspruit

Investigations by Van Eeden (1990) van Van Eeden and Schoonbee (1991, 1992, 1993) showed this part of the Natalspruit to be contaminated by effluents from upstream mines and industries in the head waters of the Natalspruit and Elsburgspruit as well as partly treated and treated sewage effluents. This part of the Natalspruit (Fig. 2.3) is covered by an extensive wetland region consisting mainly of the emergent aquatic weeds *T. capensis* and *A. donax*. Five localities were selected for the various surveys into the metal contamination of the water, sediments and a number of aquatic organisms including the two plants mentioned, *A. donax* and *T. capensis*, a macro-invertebrate *Potamonautes warreni*, and the clawed frog *X. laevis*. Birds investigated included the reed cormorant *Phalacrocorax africanus*, the red-knobbed coot *Fulica cristata* and the sacred ibis *Threskiornis aethiopicus* (Lathum).

2.4 The Blesbokspruit river system on the East Rand

This river basin is situated on the East Rand and traverses the municipal areas of Benoni, Springs, Nigel and Heidelberg (Fig. 2.4A). It has been subjected to effluents and seepage waters from mines, industries and agricultural land for decades (Viljoen, 1974; De Wet, 1990). Several distinct aquatic ecosystems exist including the Alexander, Cowls, Nigel and Spaarwater dams and the Springs Marievale and Daggafontein bird sanctuaries.

Relatively high concentrations of lead, nickel, zinc, chromium, iron, manganese and copper were found at localities in this wetland region (De Wet, 1990).

A number of separate investigations were made into the fate of metals in aquatic ecosystems in the Blesbokspruit catchment area (Figs. 2.4A and B). In addition to metals in the water and sediments, those in organisms studied included the occurrence of the seven metals Fe, Cu, Ni, Pb, Zn, Mn and Cr in the moss *Bryum* sp. as well as in the aquatic weeds *T. capensis*, *P. australis*, *P. monspeliensis*, *A. plantago-aquatica*, *Lemna minor* and *Azolla filiculoides*. Specific attention was given to the bioaccumulation

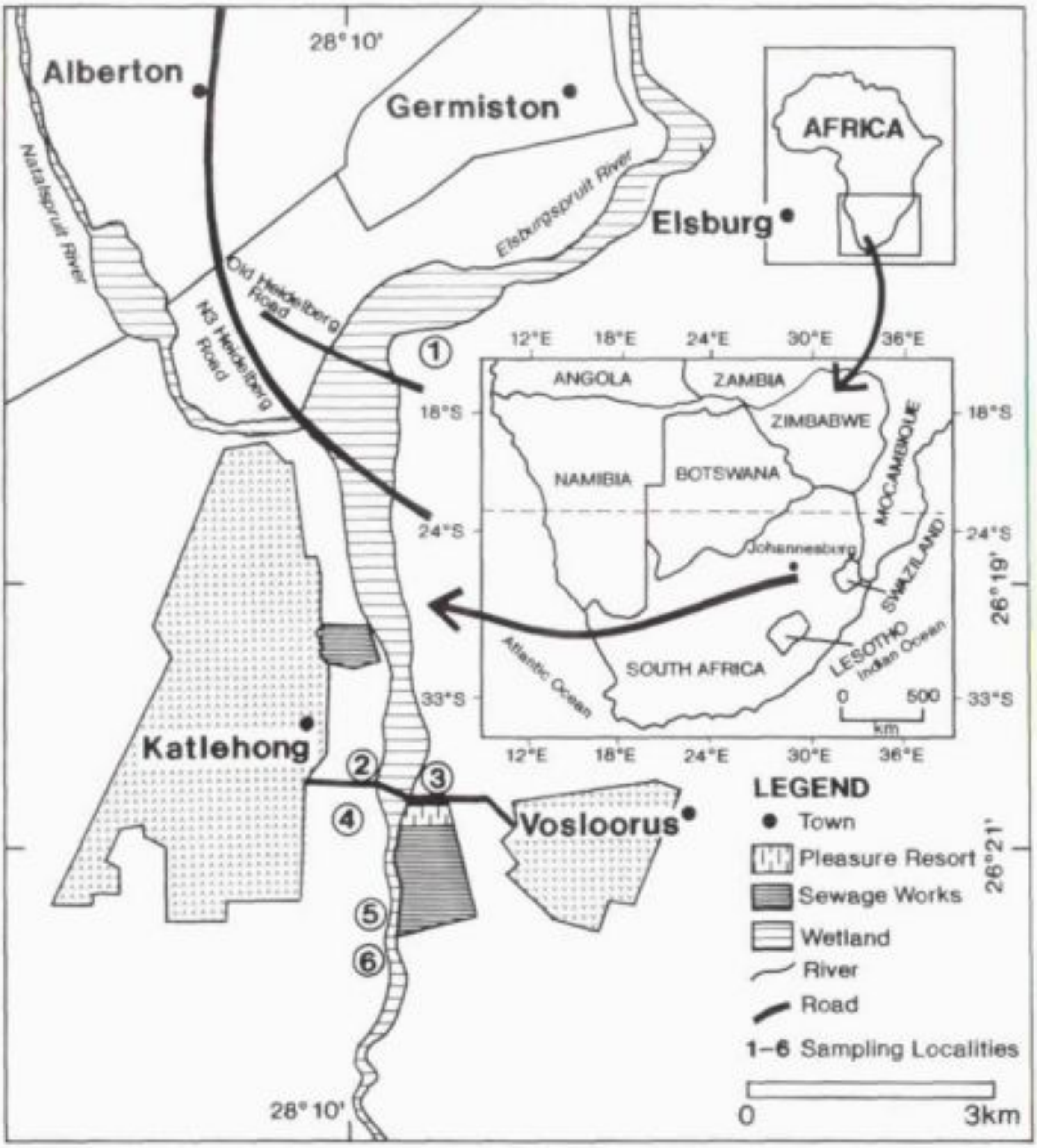

Figure 2.3: Sampling localities in the Natalspruit wetland.

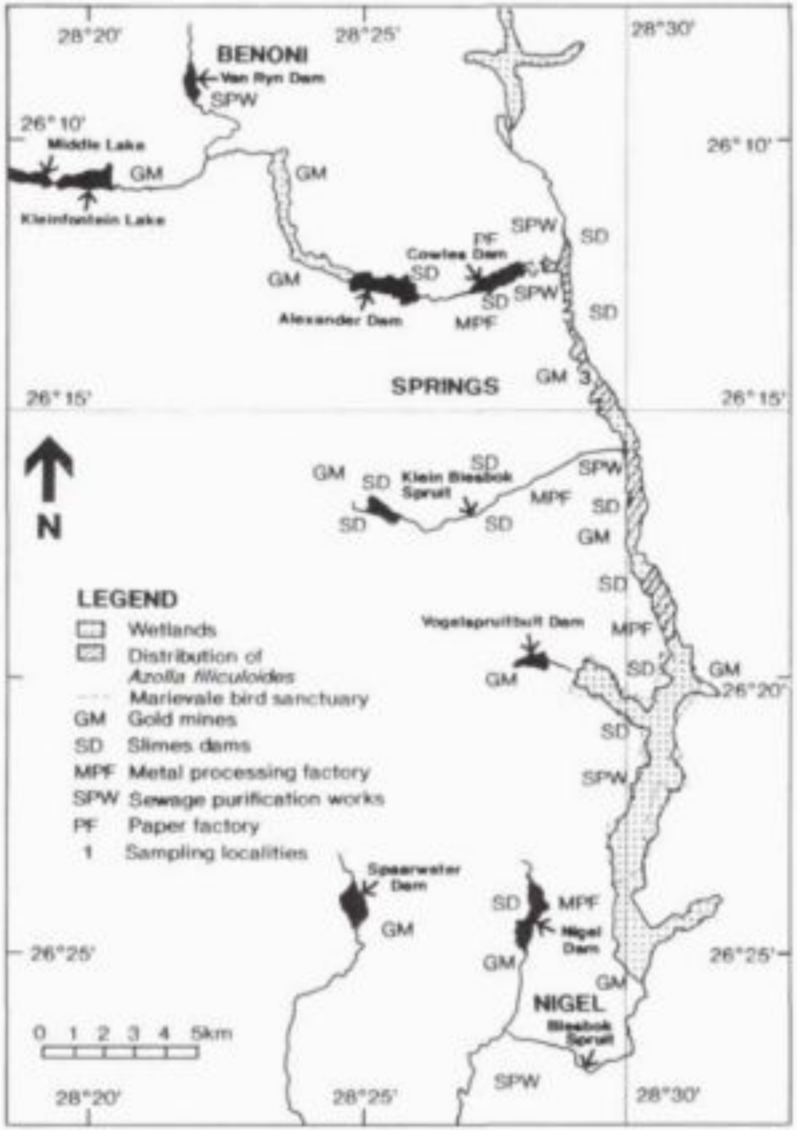

Figure 2.4A: The Blesbokspruit wetland in the vicinity of Springs and Nigel, Gauteng, with an indication of the four sampling localities as well as the various sources of pollution.

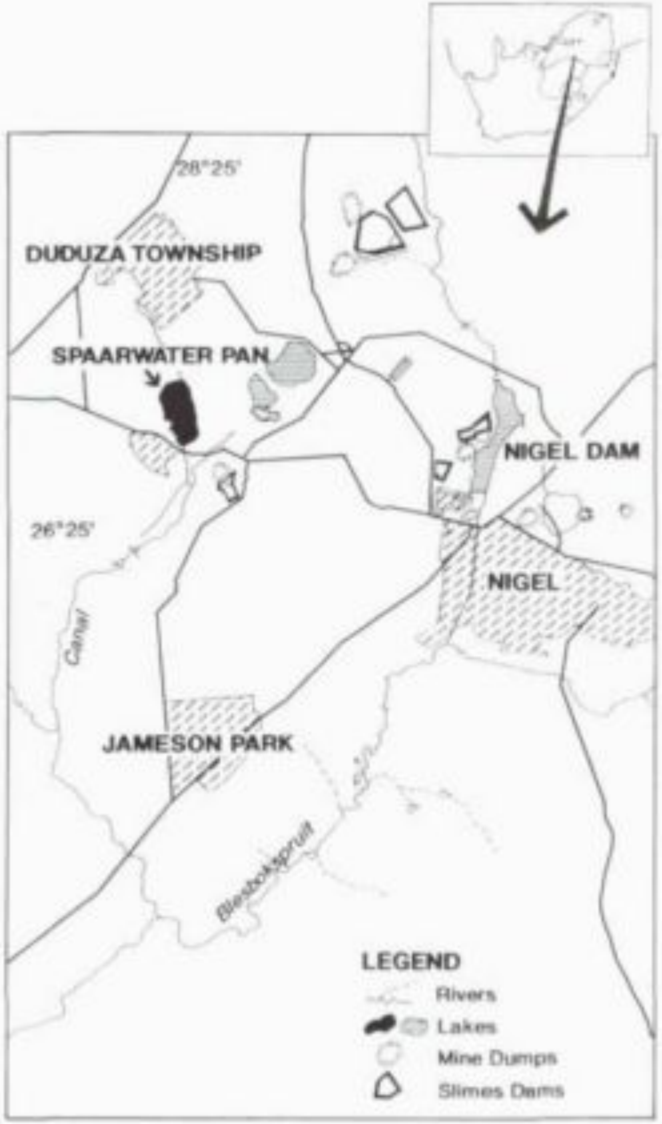

Figure 2.4B: Map showing the location of the Spaarwater Pan near Nigel.

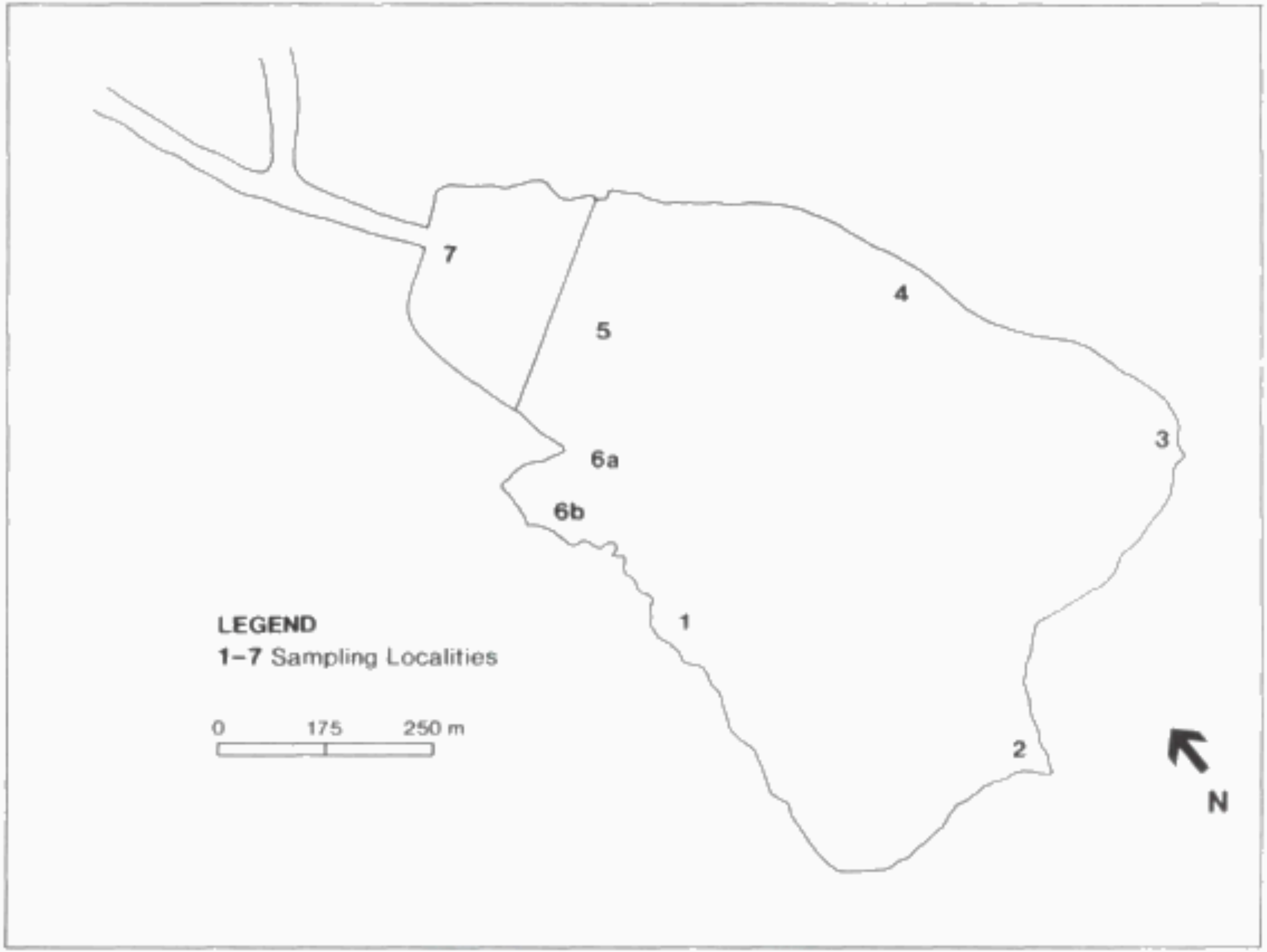

Figure 2.5: Sampling localities on the Florida Lake used for investigation into the metal contamination of the lake ecosystem.

of these metals in organs and tissues of *A. filiculoides* (De Wet *et al.*, 1990), *T. capensis*, *P. australis*, *P. monspeliensis* and *A. plantago-aquatica*.

Metal accumulation by the dominant benthic macro-invertebrate fauna was investigated in the Cowles and Nigel dams. Fish species studied included *Pseudocrenilabrus philander* with special emphasis on the ability of various sizes of the latter fish to accumulate the different metals in the metal-polluted Spaarwater dam (De Wet *et al.*, 1994) (Fig. 2.4B).

The red-knobbed coot, *F. cristata* from this area was investigated for comparative studies on the bio-accumulation of selected metals by this bird at some mine-polluted localities on the East and West Rand.

2.5 The Florida Lake

The Florida Lake (Fig. 2.5) is located in the Klip River municipal area of Roodepoort. The existing lake was created when the river on the farm Vogelstruisfontein was impounded during 1887 and 1888 for irrigation purposes and also to provide the then existing Bantjies gold mine with water. It is a relatively small lake with a surface area of approximately 26,5 ha, when full. Schuurman (1932) described the Florida Lake as a water body with a variety of bird and fish life, which at that time was already used for recreational purposes. Swans were introduced from England in 1936, the offsprings of which are still kept in an enclosure in the head water region of the lake where a dense growth of *P. australis* occur.

Currently the lake is not severely polluted by effluents originating from industries, mines or agricultural activities (Venter, 1991; Venter & Schoonbee, 1991). Although the Bantjies mine had been inactive for many decades, it still contributes to sediment-bound metal pollutants in the lake water. At present, the only possible major sources of metal-containing water discharged into the lake include drainage waters and street runoff from surrounding urban areas.

In addition to selected metals studied in the lake water and sediments, investigations were made into their bio-accumulation by *Spirogyra* sp., *P. pectinatus*, *P. australis*, *Lagarosiphon* spp. and *Nymphaea*. Organs and tissues of fish species investigated for their metal content included the perch *Perca fluviatilus*. Metal accumulation in the organs and tissues of the coot, *F. cristata* was also included in the study.

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3. MATERIALS AND METHODS

3.1 Collection of samples.

All equipment used for the collection, storage and processing of water, sediment and biological samples was thoroughly cleaned and acid washed to remove all traces of metals according to procedures described by Giesy and Wiener (1977).

Water and bottom sediment samples collected from the different sites were either kept cooled or frozen until analysis would take place in the laboratory. Bottom sediment samples consisted of core samples of the upper 5-10 cm layer of the substrate.

Aquatic plants and algae used for metal analysis were all kept frozen or dried until processed. Collections of the benthic macroinvertebrate fauna, including the freshwater crab, *Potamonautes warreni*, were properly rinsed in the field from sand and mud and preserved with the organic material in 10% formalin for later analysis.

Amphibia which primarily consisted of adults and juveniles of the clawed frog *Xenopus laevis* were collected using handnets or baited traps, respectively. Live specimens were cooled to -14° until dead and then processed in the laboratory. Fish were collected using either electrofishing techniques (Moore, 1968) or seine and gill nets. Fish were individually weighed and their total length taken in the field. They were then transferred to the laboratory for further analysis. Traps or 0,22 shotguns were employed to collect representation material of bird species under investigation. These were kept cooled in separate metal-free plastic bags for processing in the laboratory.

3.2 Laboratory processing and analysis of material.

Selected physical and chemical analyses of the water and sediments were conducted according to standard international procedures (APHA, 1989). Metal analysis including Fe, Mn, Zn, Ni, Cr and Pb, which commonly occur in sewage and industrial effluents and mine drainage waters discharged into the streams and lakes under investigation, were made on the water and sediments as well as all the processed biological samples collected during the various surveys. In the case of the water samples, 20ml quantities well-mixed water were used. Approximately 1g quantities of previously dried and homogenized samples of the sediments and all processed biological samples were used. Selected organs and tissues or whole specimens of the various plants and animals as described in more detail in each of the relevant chapters were used for metal analysis. Acid digestion of samples was used according to procedures described by Agemian and Chu (1976) and van Loon (1980). The concentrations of the metals, expressed as μg/l (water) or μg/g (sediment and biological samples), were analysed using flame atomic absorption spectrophotometers (AAS) according to standard procedures (APHA, 1989; Varian, 1989).

3.3 Concentration Ratio (CR)

A concentration ratio (CR) calculated for the various metals (expressed as %) was determined using the formula:

$$\mathrm{CR} = \frac{\text{Concentration of the metals in the organism tissues } (\mu\text{g / g dry mass})}{\text{Concentration of the metal in the sediments } (\mu\text{g / g dry mass})}$$

This formula corresponds to the Goldschmidt enrichment principle (Goldschmidt, 1937) as used by Hutchinson (1943) and Cannon (1960). Sediment rather than water metal concentrations were used because they were taken as more representative of the long-term history of metal contamination of the aquatic environment at a particular site.

3.4 Statistical analysis of data.

Where possible, statistical evaluation of the data was made using Lotus 123 and Harvard Graphics software packages.

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4. PHYSICAL AND CHEMICAL CONDITIONS OF THE WATER AND SEDIMENTS AT THE VARIOUS SAMPLING LOCALITIES

4.1 Conditions in Germiston Lake

Germiston or Victoria Lake covers a total surface area of 58 ha when full. It had been severely polluted for decades by effluents and seepage waters from mines and industries. As a result, it was devoid of fish life for more than 40 years prior to 1970. The elimination of the major sources of pollution during 1968-1975 has led to a rapid recovery of water quality in the lake (Schoonbee *et al.*, 1985; Table 4.1). Following recovery, the lake was invaded by submerged aquatic weeds, notably the fennel-leaved pondweed *Potamogeton pectinatus* and *Lagarosiphon major* and *L. muscoides* (Vermaak *et al.*, 1981). The successful biological control of these weeds is described in more detail by Schoonbee *et al.* (1985) and Schoonbee (1991). The elimination of the various sources of pollution in the lake coincided with an increase in pH and the consistent decline in mineral loads in the water which reflected the extent of recovery of the lake ecosystem from pollution by the mines in particular (Table 4.1). Mine dumps responsible for the seepage and effluents discharged into the lake were removed in recent years during the process of extraction of gold and uranium. This further facilitated the rapid recovery from pollution of the lake water. Today, only a small number of metal-processing industries remain which occasionally still contribute to intermittent pollution of the lake.

Recent investigations into the ecology of Germiston Lake pointed towards the occurrence of abnormal concentrations of certain metals in the lake water and sediments and in the flora and fauna inhabiting the lake (De wet, 1990; Bezuidenhout *et al.*, 1990; Adendorff, 1993; Van Eeden, 1994). Results on the physical and chemical conditions which prevailed during 1989 - 1990 are summarized in Tables 4.1 - 4.3.

The dramatic improvement in the water quality condition of Germiston Lake following rehabilitation is clearly demonstrated by changes in the physical and chemical conditions since 1969 (Table 4.1). The pH of the water, which at some sampling localities was as low as 3,5, recovered to become completely alkaline by 1978, with values which stabilized between 7,8 and 8,8. The progressive reduction in dissolved solids in the lake water is reflected over the years by a consistent decline in values of parameters such as conductivity, hardness, chlorides and sulphates.

Plant nutrients such as phosphate and nitrate occurred at levels sufficiently high to promote algae and water weed blooms (Schoonbee, 1991). A summary of results on the seasonal physical and chemical investigations during 1989 - 1990 is recorded in Table 4.2. These findings confirm the extent of rehabilitation of the lake from the various sources of pollution. Values for pH of the lake water during this period remained alkaline during all seasons with values exceeding 8 during three of the four seasons, except spring. Conductivity, reflecting the amount of dissolved salts in the water, remained below 600 μS/cm. Dissolved oxygen concentrations were generally high, with the exception of a few isolated values at one locality during spring, at a point where significant quantities of organic material were washed into the lake during heavy rains when concentrations as low as 2,3 mg/l were recorded. Values for total hardness and sulphate, although relatively low compared to previous years (Table 4.1) still reflected the influence of deposited sediments in the lake from mines. Analysis of N and P in 1989 still reflected moderate loads of these nutrients in the lake. Of the seven mine- and industry-associated metals analysed for, iron concentrations were clearly the highest with mean values fluctuating between 307 (summer) and 803 mg/l (winter).

Zinc concentrations varied between 69,3 μg/l (summer) and 496,4 μg/l (Winter). Metal-processing industries were found to contribute most to the loads of this metal in the lake water and sediments. Values for manganese fluctuated greatly during the various seasons, from a relatively low mean concentration of 35,6 μg/l in summer to a maximum of 410,2 μg/l during spring (Table 4.2). Concentrations of this parameter were clearly affected by seepage waters from the mining area. The order of magnitude of concentrations of dissolved copper, chromium and nickel in the lake water remained reasonably constant, showing some fluctuations during the wet and dry seasons of the year. Results suggest that mine as well as industrial effluents contributed towards the metal loads which occurred in the lake water and sediments. Surface run-off waters from the streets of Germiston, which enter the lake via a number of storm water drains, clearly added to the loads of lead recorded in the lake.

Except for sites on the southeast perimeter of the lake, most other areas are characterized by a muddy soft bottom substrate rich in organic material deposited over the years in layers of the lake sediment. Decaying material of aquatic weeds, in particular that of *P. pectinatus* and *Lagarosiphon*, contributed much to this situation. The percentage composition of organic material in the lake sediments averaged 31% with a maximum of 79% at one site (Table 4.3). Partly because of this, the concentrations of the various metals analysed for in the sediments were markedly higher than in the water column (compare Tables 4.2 and 4.3). Iron concentrations and, to a lesser extent, those of manganese, were particularly high in the lake sediments at most localities. Zinc was another metal which occurred in comparatively higher concentrations in the sediments than was the case for lead, chromium, nickel and copper, which all occurred in similar concentrations in the sediments. Of these, lead concentrations fluctuated most, probably influenced by variable loads washed into the lake from the streets during rains.

4.2 Conditions in the Elsburgspruit

As mentioned, the Elsburgspruit, which forms part of the Natalspruit catchment area (Figure 2.2), receives a variety of metal-containing effluent and seepage water from various industrial sources and from the mining region in the upper catchment of this stream. Because of intensive gold-mining activities over many decades in its head water region, the streams of the Elsburgspruit are at places severely polluted by effluents from disused and, until recently, actively operating gold mines (Van der Merwe, 1988, 1990). In addition, a number of metal-processing industries bordering the Elsburgspruit further contributed to the metal loads recorded here during investigations of this stream during 1988 - 1989 (Van der Merwe *et al.*, 1990). Treated water pumped from the mines varied in pH and contained significant concentrations of a number of metals. Specific attention was given to Cr, Cu, Fe, Mn, Pb, Zn and Ni in the water and sediments. The effects of acidification of the streams on aspects of the fauna and flora investigated will be reported on later.

The physical and chemical conditions of the Elsburgspruit at seven localities investigated over ten successive seasons from autumn 1987 to the winter of 1989 are summarized in Table 4.4. Water temperatures ranged between 5 and 21°C. The pH of the streams in the upper catchment of the Elsburgspruit (localities 1-3, Table 4.4) which were also in closest proximity to the mining areas was lowest, varying between 3,3 and 5,8. Mining activity affected parameters such as conductivity, alkalinity and hardness. Values recorded for the various metals were, however, mostly affected by contributions from the mines and from seepage water from metal waste, refuse and ash dumps situated on or near the banks of the different streams (Figure 4). Concentrations of most of these metals were usually found to be the highest at localities 1 - 4 with some decline in values at the downstream sites 5 - 7, where the Elsburgspruit flows through expansive wetland areas covered mainly by *Arundo donax* reedbeds and by dense growths of the cattail *Typha capensis*. Chromium and copper concentrations in the water at these localities were usually the lowest for all seven metals investigated with lead, zinc and nickel occurring in more variable quantities at stations 1 - 4, being largely influenced by both effluents from the mines and metal-processing industries. An analysis of the organic material components from the bottom sediments at localities 1 - 6 (Figure 2.2, Table 4.5) showed that the sediments contained comparatively higher concentrations of the seven metals analysed, than in the corresponding overlying water column.

Almost without exception, the more alkaline certain sections of the Elsburgspruit were, the higher were the concentrations of the metals in the organic material of the stream sediments (Table 4.5). Iron concentrations were clearly the highest of all metals, with a mean concentration recorded for all six localities investigated, exceeding 3 900 µg/g. This was followed by manganese with a mean concentration fluctuating between 350 µg/g (locality 2) and 6 428 µg/g (locality 6). The concentrations of Zn were likewise extremely high, with an overall mean of 1 872 µg/g. Nickel was the only other metal which averaged more than 1 000 µg/g in the sediment organic material for all six localities investigated. Mean concentrations for copper in the sediments corresponded more closely with those for lead. Chromium had, on average, the lowest overall concentration of all seven metals, namely 156 µg/g.

4.3 Conditions in the Natalspruit wetlands

Physical and chemical conditions in the Natalspruit wetlands (Figure 2.3) were mainly affected by a combination of certain environmental variables. In addition to the upstream sources of metal and inorganic pollution by industries and mines in the Elsburgspruit and in the headwater region of the Natalspruit, the water of the lower Natalspruit region below the confluence of these two streams is further contaminated by partly treated and treated sewage effluents discharged into the stream immediately upstream of the sampling area of localities 2A and 2B. With the water of the Natalspruit flowing through dense mats of *Arundo donax*, the prevailing water temperature at the time of sampling was low, varying between 11 and 18°C. The pH of the river water at this time of the survey remained alkaline, fluctuating mainly between 7,4 (locality 2B) and 8,3 (locality 4) (Table 4.6). However, the effects of mine effluent and seepage waters on the water quality of the stream in this study area is clearly demonstrated by conductivity values recorded which ranged between a mean of 853 µS/cm (locality 4) and 1 728 µS/cm (locality 1). Mean concentrations of dissolved oxygen were seldom lower than 4,0 mg/l with the highest values occurring at localities 4 and 5 (Table 4.6). Concentrations of total hardness and sulphates further reflected the influence of the water from the mines on the water quality conditions of this stream. However, comparatively high values for ammonia, nitrate and, to a lesser extent phosphate, pointed towards the eutrophication of the Natalspruit water, largely caused by the sewage effluents discharged into the river, upstream of localities 2A and 2B (Figure 2.3). Of the metals in the stream water, nickel (54 - 161 µg/l), zinc (21 - 288 µg/l), iron (63 - 268 µg/l) and manganese (45 - 167 µg/l) all occurred in appreciable quantities in the water column of the stream with mean concentrations for chrome (31 - 39 µg/l) and copper (18 - 27 µg/l) being the lowest. The concentration of lead did not exceed 60 µg/l in the stream water itself at any of the six sampling localities investigated (Table 4.6). An analysis of metal concentrations in the various components of the stream sediments showed appreciable quantities of iron to occur in all fractions (Table 4.7) with the highest concentration of this metal present in the organic and silt components, respectively. Manganese occurred in the second highest concentrations in the sediments after iron, again with its highest concentrations in the silt and organic material fractions. This tendency was also reflected by the distribution of zinc and copper. It was only chromium, which on average was fairly evenly distributed amongst the silt, organic and inorganic fractions of the stream bottom sediments (Table 4.7).

4.4 Conditions in the Blesbokspruit catchment area

Investigations during 1989 and 1992 into the water quality conditions in the Blesbokspruit wetlands, and the effects of metal concentrations on aspects of the biology of the aquatic environment there, were primarily confined to part of the Blesbokspruit upstream and downstream of the Marievale bird sanctuary as well as the Cowles, Nigel and Spaarwater dams (Figs. 2.4A and B).

The Blesbokspruit, which was investigated at four localities during the winter and summer of 1989 (Table 4.8) was mainly affected by seepage and effluent waters from surrounding mines, metal-processing plants, a paper and pulp factory and sewage purification installations. The expansive wetland region through which the river flows, is covered by dense growths of *Phragmites* and *Typha capensis* which play an important role in the recovery of water quality conditions of the stream. The Cowles dam in the Blesbokspruit is directly affected by organic rich effluents discharged from a paper factory into the dam. Despite the presence of organic rich black deposits of sediment in the dam, its fish life appeared to be healthy and no serious fish kills were observed during the survey. The Nigel and Spaarwater dams, which both occur on tributaries in the lower catchment area of the Blesbokspruit, downstream of the Marievale bird sanctuary, are both affected by effluents from nearby gold mines. In addition, the Nigel dam receives effluent water from a metal-processing plant.

Results on the water chemistry of the water and sediments from these water bodies, based on investigations carried out during 1989 and 1992, are summarized in Tables 4.8 - 4.11. The water of the Blesbokspruit sampled during 1989 (Table 4.8) showed that the stream was alkaline during the period of investigation with the pH of the water ranging between 7,1 and 8,3. However, values for conductivity, sulphate and hardness still reflected the influence of mine effluent water on the water quality of the Blesbokspruit. The contribution of treated sewage effluents to the nutrient loads of the water of the stream is evidenced by some high values obtained for both ammonia and nitrate. Concentrations of phosphate were much lower but still occurred at levels which facilitated algal and plant growths in the wetland. Amongst the metals in the water, manganese occurred in extremely high concentrations with mean values for winter and summer fluctuating between 2 125 μg/l and 3 050 μg/l. In contrast, concentrations of iron were much lower with a highest individual value of 980 μg/l recorded during summer (Table 4.8). Nickel (451,7 μg/l) and zinc (454,2 μg/l) were the other two metals which occurred in appreciable concentrations in the river water during summer. Mean concentrations for copper, lead and chromium occurred at much lower levels with maximum values not exceeding 150 μg/l for any of these metals (Table 4.8). The water of the Spaarwater dam (Table 4.8) was also alkaline during the investigation in 1992. Relatively high values for conductivity (850 - 1 300 μS/cm) as well as sulphates were some indications of the influence of mine water on the water quality of this dam. Values for ammonia, nitrite, nitrate and phosphate were low compared to conditions elsewhere in the Blesbokspruit catchment. The Cowles and Nigel dams, although separated from each other (Figure 2.4A), displayed similar physical and chemical water quality conditions. Both dams were alkaline in nature but contained high mineral contents with maximum conductivities exceeding 1 000 μS/cm during all four seasons of investigation (Table 4.9). In addition, high values for total hardness and sulphates were among the other parameters which reflected the influence of the mines on the water quality conditions in both dams. The concentrations of the nutrients ammonia and nitrate, and to a lesser extent phosphate, showed the effects of sewage effluents in particular on these waters. Decaying organic material from the paper factory further complicated these values in the Cowles dam.

A comparison of results on the metal concentrations in the water of Spaarwater, Cowles and Nigel dams (Tables 4.8 and 4.10) showed iron and manganese to occur in the highest concentration at all three sites. Where values for all the other metals were relatively low in the Spaarwater dam, concentrations for nickel, lead and zinc were reasonably high in both the Cowles and Nigel dams. Copper and chromium occurred in relatively low concentrations in the water of both these water bodies. A seasonal analysis of all these metals in the sediments of the Nigel and Cowles dams (Table 4.11) reflected a similar picture obtained for all the other water bodies already discussed, namely that the sediment metal concentrations were almost without exception much higher than in the overlying water columns. This applied particularly to iron concentrations in the bottom sediments of both dams where extremely high concentrations of iron were recorded during all four seasons (Table 4.11). Nickel and copper concentrations were also particularly high in Cowles dam compared to values obtained for these metals in the Nigel dam. Despite the use of chrome in processes of the paper factory, sediment values for this metal in both dams were very similar.

4.5 Conditions in the Florida Lake, Roodepoort

The presence of measurable quantities of certain metals in the water and bottom sediments in the Florida Lake can mainly be related to pollution by gold mines which occurred some 60 years ago when the impounded lake was used for the recirculation and storage by the Bantjies Mine Company. Leaching of metals deposited in the underlying layers of sediments into the water column still occurs today. The presence of metals in the lake and in particular the high concentrations of iron in the water and sediments are perhaps the few reminders of the effects of mining activities on the water quality conditions of this water body. Investigations into the physical and chemical conditions and the occurrence of certain metals in the abiotic and biotic environment of the lake (Venter, 1991) coincided with steps to rehabilitate the lake from excessive growths of the submerged water weeds *Potamogeton pectinatus* and *Lagarosiphon* species in the lake (Venter and Schoonbee, 1991). The collection of water and sediment samples as well as aquatic plants and fish for analysis took place during a bimonthly sampling program at eight localities (Figure 2.5) over a period of one year. These findings are summarized in Tables 4.12 and 4.13. The mean water temperature ranged between a maximum of 23,7°C in summer (February 1990) to a winter low of 10,9°C (June 1990). Recorded pH values were fairly high on occasions, fluctuating between 7,0 (October 1990) and 9,9 (February 1991).

As all measurements were made between 9:00 and 12:00, values for dissolved oxygen were usually high, ranging between 6,1 mg/l in summer and 8,3 mg/l in winter. The dissolved salts as reflected by dionic conductivity were reasonably constant above 100 μS/cm.

Values obtained for alkalinity and total hardness reflected moderately alkaline conditions. Concentrations of ammonia were fairly low, ranging between 0,037 mg/l (February 1990) and 0,657 mg/l (June 1990) with an unexpectedly high mean concentration of 1,128 mg/l in December 1990. This coincided with peak values obtained for conductivity (157,5 μS/cm) suggesting the possible influx of polluted waters into the lake, which coincided with rains.

Results recorded for nitrate were generally high and, together with the occasionally high values for orthophosphates, might explain the excessive growths of submerged weeds and algae in the lake at the time of the survey. Peak values for sulphates were recorded during the summer when the highest values for parameters such as conductivity, ammonia and nitrites were obtained. High turbidity readings of 13,1 FTU-units were recorded during the spring rainy season.

The quantities of iron in both the water and sediments were considerably higher than any of the other metals. Of the other metals measured in the water column, the mean concentration for lead (230,3 μg/l) was the highest, followed by nickel (76,6 μg/l), zinc (73,7 μg/l) and manganese (67,2 μg/l). Concentrations for copper (22,9 μg/l) were the lowest of the six metals analysed. In the sediments, the tendency was similar with the mean concentrations for Mn, Ni and Zn being of the same order, ranging between 108,4 μg/g (Ni) and 134,7 μg/g (Mn). A mean lead concentration of 95,0 μg/l was also recorded in the lake sediments.

4.6 Metal water quality criteria

Kempster *et al.* (1980) compiled criteria on the desirable limits of certain water quality constituents from a number of sources, for 15 major uses of water in the world. From this, the suggested limits proposed for metal concentrations to protect aquatic life in rivers and dams were taken to compare with the findings on metal concentrations recorded at the various localities during the present study.

4.6.1 Metal concentrations in the Germiston Lake

The mean and/or individual metal concentrations in both the water column and lake sediments (Tables 4.2 and 4.3) for all seven metals, were found to exceed the proposed maximum limits laid down for the protection of aquatic life during one or more of the seasons of investigation. Spring and to a lesser extent, winter and autumn yielded for both mean and maximum metal concentrations values which exceeded the suggested maximum limits laid down in Kempster *et al.* (1980). In the lake sediments, Fe, Zn, Cr and Ni all exceeded the proposed maximum concentrations for these metals. Cu (3 localities), Pb (5 localities) and Mn (3 localities) also tended to exceed the proposed maximum limits in certain areas of the lake. These findings suggest that despite the remarkable recovery of the lake from mine and industrial pollution since 1968 in terms of the general physical an chemical conditions, abnormally high concentrations of these seven (and possibly other) metals, still exist in the lake water and sediments which threaten the stability and maintenance of its aquatic fauna and flora. This situation particularly applies to the metal concentrations in the lake sediments from where a constant leaching takes place into the water column.

4.6.2 Metal concentrations in the Elsburgspruit and Natalspruit wetlands

The concentrations of Zn, Ni and Fe in the water of the Elsburgspruit (Table 4.4) were clearly the three metals of which the concentrations exceeded the maximum limits laid down for the protection of aquatic life in rivers and dams. Cr (2 localities) and Cu (2 localities) were the only metals out of the seven where the proposed maximum limits were not exceeded in the water of this stream at all the localities sampled. In the case of the stream sediments (Table 4.5), four of the metals namely Fe, Zn, Ni and Pb exceeded the maximum concentrations at all six localities investigated during the survey. Mn and Cr (4 localities) also produced concentrations in excess of the proposed maximum limits for these metals. A comparison of the results on the metal concentrations of the water and sediments in the Natalspruit (Tables 4.6 and 4.7) further suggests that the concentrations of the various metals in the sediments usually exceeded those in the overlying water column of the stream. All seven metals in the sediments exceeded the maximum limits laid down for the protection of aquatic life. However, it was only Ni and Zn and to a lesser extent Cr where the concentrations exceeded the maximum limits in the water of the Natalspruit at three or more of the localities investigated (Table 4.6)

4.6.3 Metal concentrations in the Blesbokspruit river system

Metal analysis were made of the water of the Blesbokspruit (Table 4.8) and the Spaarwater dam as well as the water and sediments of the Cowles and Nigel dams (Tables 4.10 and 4.11). Metals which exceeded the maximum limits were Mn (Blesbokspruit, both winter and summer), Fe (Spaarwater dam, summer) as well as

Ni, Pb, Zn and Cr (summer). Metal concentrations in the water of the Cowles and Nigel dams were characterized by excessively high concentrations of Ni, Pb and Zn during all four seasons of 1989 (Table 4.10). Mn (all four seasons) and Cr (winter and spring) in the Nigel dam also exceeded the maximum limits laid down for the protection of aquatic life in rivers and dams. With the exception of Mn, the concentrations of all the other metals in the bottom sediments of the Cowles and Nigel dams, all exceeded the proposed maximum limits during all four seasons of the investigation (Table 4.11).

4.6.4 Metal concentrations in the Florida Lake

The mean concentrations for Fe, Ni, Zn and Pb in the Florida Lake water and in the bottom sediments (Table 4.13) all exceeded the maximum limits laid down for the protection of aquatic life in rivers and dams.

To summarize : Metal concentrations in both the water and sediments of all the wetlands investigated during the present survey were found to exceed the maximum concentrations proposed in the literature for the maintenance and protection of aquatic life in rivers and dams. Concentrations were generally higher in the sediments from where a direct or indirect release of metals may take place into the overlying water, to be taken up by the biotic components of the wetland ecosystems.

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Table 4.1. Improvement of physical and chemical conditions in the water of Germiston Lake over a period of twenty years between 1969 and 1989

PHYSICAL AND CHEMICAL ANALYSIS	**1969-1970**		**1978**		**1983**		**1989**	
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
pH	-	3,5-6,9	-	7,8-9,2	-	7,8-8,8	-	7,3-8,6
Conductivity (μS/cm)	2009	1644-2580	982	922-1061	793	700-880	572	500-780
Dissolved Oxygen (mg/l)	6,9	6,5-7,6	9,8	6,1-12,4	-	-	6,4	2,3-9,5
BOD (mg/l)	1,7	1,1-2,4	2,4	1,1-4,4	-	-	-	-
Total Alkalinity (mg/l as $CaCO_3$)	20	11-31	73	65-82	-	-	5,8	25-107
Total Hardness (mg/l as $CaCO_3$)	1074	890-1290	429	403-460	329	300-347	222	170-269
Mg-Hardness (mg/l as $CaCO_3$)	395	310-515	165	144-190	-	-	-	-
Ca-Hardness (mg/l as $CaCO_3$)	678	580-785	264	247-785	-	-	-	-
Chlorides (mg/l Cl)	122	100-153	87	80-98	72	62-85	-	-
Sulphate (mg/l SO_4)	978	886-1094	297	280-320	272	240-320	153	143-168
Orthophosphate (mg/l PO_4)	0,05	0-0,30	0,09	0,05-0,20	0,11	0,003-0,300	0,40	0,15-0,92
Nitrate - N (mg/l NO_3)	2,76	2,2-3,6	1,59	1,45-1,75	0,90	1,50-1,00	0,89	0,22-1,98

Table 4.2. Summary of physical and chemical conditions of the water of Germiston Lake during 1989-1991. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

PHYSICAL AND CHEMICAL ANALYSIS	SUMMER			AUTUMN			WINTER			SPRING		
	$\bar{x}$	Range n = 9	SD	$\bar{x}$	Range n = 9	SD	$\bar{x}$	Range n = 9	SD	$\bar{x}$	Range n = 9	SD
Temperature (°C)	22,8	22,0-24,0	0,7	16,8	16,0-17,5	0,5	12,1	11,5-13,0	0,5	17,2	16,5-18,0	0,4
pH	7,5	7,3-7,8	0,1	7,8	7,6-8,1	0,2	8,0	7,9-8,2	0,1	8,2	7,5-8,6	0,3
Conductivity (μS/cm)	594,4	500-650	36,9	563,3	530-600	18,9	512,2	590-540	21,5	621,1	590-780	57,0
Dissolved Oxygen (mg/l)	6,7	4,4-9,5	1,5	7,1	6,4-8,4	0,6	7,5	5,8-8,4	0,8	4,4	2,3-5,2	0,9
Alkalinity (mg/1 $CaCO_3$)	31,7	25-36	2,7	50,1	45-55	3,2	62,2	60-70	3,2	86,1	69-107	12,7
Total hardness ((mg/1 $CaCO_3$)	195,8	170-258	25,9	206,8	194,235	16,4	222,4	211-248	10,3	261,0	254-269	4,2
Ammonia (mg/1 NH_3)	0,48	0,22-1,71	0,44	0,81	0,68-1,41	0,22	1,26	1,12-1,40	0,10	0,94	0,77-1,46	0,25
Nitrate (mg/1 NO_3)	13,4	10,6-17,6	2,7	9,8	8,3-14,8	2,2	8,0	4,0-16,7	3,4	4,7	2,2-19,8	5,4
Nitrite (mg/1 NO_2)	0,40	0,25-0,66	0,13	0,30	0,20-0,48	0,09	0,19	0,12-0,31	0,05	0,26	0,15-0,55	0,11
Orthophosphate (mg/1 PO_4)	0,44	0,28-0,60	0,12	0,44	0,33-0,52	0,06	0,43	0,29-0,49	0,06	0,29	0,15-0,92	0,23
Sulphate (mg/1 SO_4)	168,0	154-188	9,2	156,6	150-170	6,0	145,0	140-155	4,7	143,3	130-150	5,8
Turbidity (FTU units)	3,1	1-10	2,9	5,0	3-6	0,9	7,8	4-10	1,7	6,4	4-12	2,4
Iron (μg/1 Fe)	306,7	160-670	156,1	583,3	280-810	165,2	803,3	590-**1050**	143,4	623,7	**424-1012**	187,4
Zinc (μg/1 Zn)	69,3	**41-94**	16,3	**200,0**	50-**730**	204,5	**496,4**	**274-818**	172,5	**311,0**	33-**2043**	620,9
Copper (μg/1 Cu)	83,3	20-**260**	84,3	50,0	30-130	46,2	53,3	10-170	53,1	62,4	13-**372**	110,7
Lead (μg/1 Pb)	36,7	20-70	14,9	43,3	30-60	8,2	27,8	20-50	9,2	68,0	**41-164**	35,9
Chromium (μg/1 Cr)	46,7	10-90	25,8	50,0	20-**120**	29,8	61,1	10-**160**	41,2	53,2	**24-104**	25,2
Nickel (μg/1 Ni)	**68,9**	20-**170**	50,2	**54,4**	30-**60**	28,7	43,3	10-**170**	49,0	**60,7**	**38-112**	21,7
Manganese (μg/1 Mn)	35,6	10-120	31,-0	212,2	100-**1730**	536,8	205,6	100-**1520**	466,9	410,2	40-**3618**	1134,1

Table 4.3. Metal analysis of the bottom sediments at nine sampling localities in Germiston Lake with an indication of the organic contents of the sediments at each site. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

SAMPLING LOCALITIES	METAL CONCENTRATIONS IN µg/g							
	Iron	Zinc	Copper	Lead	Chromium	Nickel	Manganese	Sediment organic content (%)
1	**14575**	**1423**	**418**	**387**	**124**	**97**	409	24
2	**52063**	**1023**	102	**926**	**334**	**263**	279	20
3	**30648**	**2189**	**522**	**550**	**200**	**824**	**1348**	79
4	**14620**	**595**	99	85	**189**	**122**	478	16
5	**14698**	**892**	155	80	**106**	**257**	384	35
6	**12041**	**597**	106	8	**141**	**146**	440	8
7	**16008**	**808**	132	**109**	**203**	**174**	792	15
8	**13502**	**695**	99	-	**122**	**133**	**1010**	23
9	**17520**	**1715**	**314**	**144**	**192**	**426**	**2307**	56
$\bar{x}$	**20630**	**1104**	**216**	254	**179**	**271**	828	31
S.D.	12256	524,4	151,8	293,1	65,4	217,6	618,2	22,9
C.V.	59	48	70	115	36	80	75	75

$\bar{x}$: Mean values
S.D. : Standard deviation
C.V. : Coefficient of variability
With the exception of S.D., all values were rounded off to the nearest figure after calculations.

Table 4.4 Physical and chemical conditions of the water of the Elsburgspruit, Germiston, investigated over a period of ten successive seasons at seven localities during autumn 1987 - Winter 1989. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

PHYSICAL AND CHEMICAL PARAMETERS	Mean and Range	LOCALITY 1 n = 36	LOCALITY 2 n = 36	LOCALITY 3 n = 36	LOCALITY 4 n = 36	LOCALITY 5 n = 36	LOCALITY 6 n = 36	LOCALITY 7 n = 36
Temperature °C	min-max	6-20	5-20	4-20	5-20	5-21	5-21	5-21
pH	min-max	5,8-7,4	3,3-6,6	3,6-7,4	4,4-7,1	6,7-8,1	7,0-8,3	5,6-8,8
Dissolved Oxygen	$\bar{x}$	7,39	7,25	7,56	7,28	5,39	6,90	7,38
(as mg/l)	min-max	6,20-7,50	6,80-7,80	7,10-8,20	6,80-7,60	3,40-7,10	4,80-7,80	6,90-8,00
Conductivity	$\bar{x}$	1020	2880	2530	1820	490	580	1480
(as µS/cm)	min-max	730-1300	2300-3250	1630-3100	1430-2300	410-560	520-670	550-2380
Total Alkalinity	$\bar{x}$	91	3,9	13,4	4,7	118,9	126,3	110,0
(as mg/l $CaCO_3$)	min-max	61-105	1-12	2-32	1-12	106-143	127-153	20-192
Total Hardness	$\bar{x}$	366	1969	1550	1078	187	255	737
(as mg/l $CaC0_3$)	min-max	248-432	1503-2280	953-2066	743-1376	157-205	215-288	237-1246
Chromium	$\bar{x}$	<0,02	<0,02	<0,08	<0,08	**0,10**	0,05	**0,32**
(Cr as mg/l)	min-max	-	-	-	-	0,05-**0,36**	0,02-0,07	0,02-**0,90**
Copper	$\bar{x}$	-	0,13	<0,07	**0,39**	0,03	0,05	**0,32**
(Cu as mg/l)	min-max	-	0,07-0,17	0,06-0,07	0,16-0,62	0,07-0,13	0,02-0,07	0,02-**0,90**
Iron	$\bar{x}$	**1,97**	**6,54**	1,00	**4,07**	0,67	0,97	1,17
(Fe as mg/l)	min-max	**1,11-2,57**	**1,60-21,03**	0,57-**1,90**	0,39-**12,64**	0,37-**1,55**	0,69-**1,53**	0,69-**1,90**
Manganese	$\bar{x}$	1,03	6,11	4,85	6,34	0,16	0,78	2,82
(Mn as mg/l)	min-max	0,50-1,50	4,40-12,00	2,77-6,60	3,40-8,73	0,10-0,59	0,38-1,06	0,76-8,70
Lead	$\bar{x}$	0,12	0,15	0,18	0,12	0,23	0,20	<0,19
(Pb as mg/l)	min-max	0,10-0,13	0,13-0,20	0,12-0,33	0,11-0,14	0,12-0,51	0,11-0,32	0,10-0,51
Zinc	$\bar{x}$	**0,28**	**1,12**	**0,71**	**1,04**	**0,27**	**0,21**	**0,52**
(Zn as mg/l)	min-max	**0,15-0,47**	**0,76-1,65**	**0,36-1,00**	**0,34-3,86**	0,06-**0,88**	0,06-**0,50**	**0,33-0,91**
Nickel	$\bar{x}$	**0,12**	**2,02**	**1,37**	**2,62**	**<0,14**	**<0,14**	**0,81**
(Ni as mg/l)	min-max	**0,06-0,18**	**1,26-3,20**	**1,02-1,53**	**1,13-3,75**	-	-	**0,12-1,90**

Table 4.5. Metal concentrations (μg/g) in the organic component of bottom sediment material from six sampling localities on the Elsburgspruit during winter 1988 and the summer and winter of 1989. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

LOCALITIES	MEAN AND RANGE	METAL CONCENTRATIONS IN μg/g						
		Fe	Mn	Zn	Ni	Pb	Cu	Cr
1	$\bar{x}$	**2599**	**1232**	**1148**	**231**	**261**	158	**447**
	min-max	**2112-3651**	432-**2722**	**885-1348**	**41-585**	**187-364**	128-190	**146-983**
2	$\bar{x}$	**3976**	350	**2359**	**430**	**306**	**312**	**141**
	min-max	**1507-5925**	141-473	**375-5925**	17-**949**	**146-600**	103-**535**	44 - **244**
3	$\bar{x}$	**3135**	348	**656**	**76**	77	**272**	55
	min-max	**1803-5450**	110-639	**471-953**	**55-103**	20-**152**	**45-474**	22-94
4	$\bar{x}$	**3711**	**2765**	**2251**	**1124**	**287**	**299**	40
	min-max	**3370-4312**	**1049-4060**	**965-3370**	**860-1431**	**100-640**	**247-371**	2-80
5	$\bar{x}$	**5080**	**3469**	**2371**	**1177**	**568**	**346**	**152**
	min-max	**1228-11340**	**2184-4940**	**1134-4238**	**922-1380**	**150-874**	**250-466**	**122-174**
6	$\bar{x}$	**5069**	**6428**	**2464**	**2698**	**175**	**221**	**103**
	min-max	**2912-8649**	**851-8935**	**1310-3465**	**1045-4529**	**125-233**	165-**278**	21-**203**
1-6	$\bar{x}$	**3928**	**2432**	**1875**	**1056**	**279**	**268**	**156**
	min-max	**1228-11340**	110-**8935**	**375-5925**	17-**4529**	20-**874**	**45-535**	20-**983**

Table 4.6. Summary of water quality condition at six localities on the Natalspruit wetlands during the four seasons of 1989. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

PHYSICAL AND CHEMICAL ANALYSIS	SAMPLING LOCALITIES					
	1	2A	2B	3	4	5
	$\bar{x}$ ± SD n = 4	$\bar{x}$ ± SD n = 4	$\bar{x}$ ± SD n = 4	$\bar{x}$ ± SD n = 4	$\bar{x}$ ± SD n = 4	$\bar{x}$ ± SD n = 4
pH	7,8 ± 0,3*	7,9 ± 0,2*	7,4 ± 0,4*	8,0 ± 0,1*	8,3 ± 0,4*	8,2 ± 0,3*
Conductivity (μS/cm)	1728 ± 182	1398 ± 466	1130 ± 223	1215 ± 252	853 ± 74	1198 ± 103
Temperature (°C)	11 ± 1	13 ± 3	14 ± 2	14 ± 2	15 ± 2	16 ± 4
Dissolved Oxygen (mg/l)	6,6 ± 0,2	6,9 ± 0,3	5,5 ± 1,8	6,6 ± 0,4	7,4 ± 1,0	8,6 ± 0,3
Alkalinity (mg/l $CaCO_3$)	101 ± 31	227 ± 97	93 ± 32	98 ± 29	95 ± 24	95 ± 26
Total hardness ((mg/l $CaCO_3$)	1052 ± 180	723 ± 6	599 ± 135	488 ± 156	273 ± 181	350 ± 62
Ammonia (mg/l NH_3)	0,49 ± 0,14	0,37 ± 0,44	4,25 ± 0,11	2,45 ± 1,6	3.53 ± 1,1	1,98 ± 0,2
Nitrate (mg/l NO_3)	0,12 ± 0,08	0,14 ± 0,08	0,84 ± 0,36	0,59 ± 0,2	0,9 ± 0,4	0,88 ± 0,2
Nitrite (mg/l NO_2)	6,9 ± 3,8	5,12 ± 3,33	9,5 ± 2,5	8,9 ± 2,7	34,0 ± 6,6	20,5 ± 4,9
Orthophosphate (mg/l PO_4)	0,87 ± 0,17	0,12 ± 0,13	1,54 ± 0,91	1,04 ± 0,12	1,44 ± 0,74	1,4 ± 0,5
Sulphate (mg/l SO_4)	350 ± 42	468 ± 124	310 ± 39	366 ± 41	195 ± 58	292 ± 34
Lead (μg/l Pb)	<60 ± 20	<60 ± 20	<60 ± 20	<60 ± 20	<60 ± 20	<60 ± 20
Nickel (μg/l Ni)	**161** ± 12	54 ± 5	**128** ± 25	**107** ± 23	**112** ± 21	**126** ± 14
Zinc (μg/l Zn)	**132** ± 80	21 ± 3	**289** ± 236	**65** ± 9	**78** ± 18	**175** ± 171
Chromium (μg/l Cr)	36 ± 3	36 ± 4	31 ± 3	39 ± 4	37 ± 4	38 ± 6
Iron (μg/l Fe)	90 ± 12	63 ± 39	**242** ± 89	94 ± 48	**108** ± 36	**268** ± 228
Manganese (μg/l Mn)	92 ± 34	45 ± 8	167 ± 183	159 ± 162	62 ± 30	153 ± 71
Copper (μg/l Cu)	27 ± 3	18 ± 2	22 ± 5	21 ± 5	22 ± 5	20 ± 4

* Mean of -Log $[H^+]$

Table 4.7. Metal concentrations ($\bar{x}$ ± SD) (µg/g as dry mass) in the sediments as well as in its inorganic, organic and silt fractions of the soft bottom substrate based on composite samples from six localities in the Natalspruit wetlands during the 1989 investigation. Values for coefficient of variability in brackets. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

METALS	METAL CONCENTRATIONS IN THE SEDIMENT AS WELL AS IN ITS DIFFERENT FRACTIONS			
	Total sediment $\bar{x}$ ± SD n = 24	Inorganic component $\bar{x}$ ± SD n = 20	Organic component $\bar{x}$ ± SD n = 24	Silt component $\bar{x}$ ± SD n = 24
Lead	**110,8** ± 99,7 (90,0)	59,5 ± 92,5 (155,5)	**112,2** ± 99,8 (88,9)	**170,0** ± 124,2 (73,1)
Nickel	**374,8** ± 449,8 (120,0)	**120,3** ± 64,2 (53,4)	**1240,0** ± 1233,9 (99,5)	**949,1** ± 1169,2 (123,2)
Zinc	**827,0** ± 1112,5 (134,5)	**163,0** ± 102,1 (62,6)	**2244,9** ± 1393,8 (62,1)	**2062,7** ± 1991,7 (96,6)
Chrome	**137,5** ± 58,2 (42,3)	**138,1** ± 121,3 (87,8)	**126,2** ± 134,4 (106,5)	**148,5** ± 51,3 (34,5)
Iron	**33842** ± 15096 (44,6)	**19873** ± 18666 (93,9)	**26746** ± 9079 (33,9)	**32566** ± 11305 (34,7)
Manganese	**4067,4** ± 3929,8 (96,6)	**1731,8** ± 1571,0 (90,1)	**6145,8** ± 10734,9 (7362,8)	**4484,5** ± 3901,2 (87,0)
Copper	102,9 ± 78,8 (76,6)	114,8 ± 140,1 (122,0)	184,3 ± 80,9 (43,9)	189,2 ± 113,5 (60,0)

Table 4.8. Improvement of physical and chemical conditions in the water of the Blesbokspruit (summer and winter, 1989) and the Spaarwater Dam (summer, 1992). Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

PHYSICAL AND CHEMICAL ANALYSIS	BLESBOKSPRUIT				SPAARWATER DAM	
	Winter		Summer 1985		Summer 1992	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
	n = 12		n = 12		n = 4	
Temperature (°C)	12,6±0,4	12-13,0	19,2±1,1	18-21	22,5	22-23
pH*	7,6±0,3*	7,2-8,1*	7,8±0,3*	7,1-8,3*	7,9*	7,6-8,1*
Conductivity (µS/cm)	-	-	1087±126	15	1038	850-1300
Dissolved Oxygen (mg/l)	5,8±0,8	4,7-6,6	6,1±1,6	2,8-8,1	3,9	1,4-6,2
Alkalinity (mg/l as $CaCo_3$)	-	-	111±21	73-142	196	168-228
Total Hardness (mg/l as $CaCo_3$)	-	-	219±111	120-535	179	159-191
Sulphate (mg/l So_4)	294±34	240-300	257±73	135-420	175	150-200
Ammonia (mg/l NH_3)	3,9±1,4	2,8-6,2	4,1±4,7	1,0-16,8	0,3	0,2-0,4
Nitrite (mg/l NO_2)	0,12±0,05	0,60-0,17	1,2±2,6	tr - 9,3	<0,01	
Nitrate - N (mg/l NO_3)	2,1±0,4	1,8-2,6	5,9±6,1	1,6-22,0	0,8	0,6-1,2
Orthophosphate (mg/l PO_4)	0,4±0,2	0,1-0,6	0,6±0,3	0,3-1,5	0,06	0,04-0,07
Turbidity (FTU)	6,8±5,40	0,1-15,0	3,4±2,9	0-0 - 8,0	<5	-
Iron (µg/l)	23±4	20-30	550±231	300-980	**1119**	-
Manganese (µg/l)	**2125**±2930	400-**7200**	**3050**±3442	40-**7410**	88	-
Copper (µg/l	87,5±54,5	0-150	58,3±14,9	30-80	7	-
Nickel (µg/l)	-	-	**451,7**±396,5	**80-1010**	37	-
Lead (µg/l)	-	-	98,3±15,7	80-**130**	48	-
Zinc (µg/l)	-	-	**454,2**±368,7	**130-930**	34	-
Chromium (µg/l)	57,5±25,9	20-90	48,3±24,6	0-**100**	48	-

* Mean of - Log $[H^+]$

Table 4.9. Physical and chemical conditions of the Cowles and Nigel dams in the Blesbokspruit catchment area during the four seasons of 1989.

PHYSICAL AND CHEMICAL ANALYSIS		COWLES DAM			NIGEL DAM		
		$\bar{x}$	SD N = 4 per season	Range	$\bar{x}$	SD N = 5 per season	Range
Temperature (°C)	SUMMER	25,0	2,7	22,0-29,0	23,1	0,5	22,5-24,0
pH		7,7	0,4	6,9-8,0	7,8	0,1	7,7-8,1
Dissolved Oxygen (mg/l)		4,7	1,4	2,9-6,7	5,9	0,8	4,9-6,9
Conductivity (µS/cm)		1137,5	338,0	800,0-1500,0	980,0	98,0	800,0-1100,0
Total hardness (mg/l $CaCO_3$)		275,8	158,9	93,0-503,0	603,4	153,9	313,0-734,0
Alkalinity (mg/l $CaCO_3$)		68,3	43,5	17,8-124,5	22,1	1,5	21,0-25,0
Ammonia (mg/l NH_3)		10,2	9,0	1,0-19,5	3,0	1,3	1,2-4,8
Nitrate (mg/l NO_3)		4,1	1,6	2,2-6,6	7,1	2,1	3,5-9,7
Nitrite (mg/l NO_2)		0.3	0,2	0,1-0,7	0,2	0,05	0,2-0,4
Orthophosphate (mg/l PO_4)		0,3	0,1	0,1-0,6	0,3	0,07	0,2-0,4
Sulphate (mg/l SO_4)		207,5	8,3	200,0-220,0	328,0	61,4	260,0-380,0
Turbidity (FTU units)		12,1	12,7	0,1-30,0	4,0	3,0	0,1-8,0
Temperature (°C)	AUTUMN	18,6	4,6	15,5-26,5	17,1	0,7	16,0-18,0
pH		7,8	0,2	7,4-8,0	1,7	0,2	7,4-7,9
Dissolved Oxygen (mg/l)		4,5	1,6	3,1-7,1	6,3	0,8	5,3-7,4
Conductivity (µS/cm)		1125,0	301,0	650,0-1450,0	1080,0	40,0	1000,0-1100,0
Total hardness (mg/l $CaCO_3$)		249,3	113,2	136,0-437,0	633,2	74,0	547,0-765,0
Alkalinity (mg/l $CaCO_3$)		57,6	25,2	21,3-87,0	17,5	0,9	16,0-18,5
Ammonia (mg/l NH_3)		5,7	4,5	0,81-11,6	3,8	1,0	2,4-5,2
Nitrate (mg/l NO_3)		5,8	1,8	3,0-7,9	6,4	2,0	2,8-5,4
Nitrite (mg/l NO_2)		0,3	0,1	0,2-0,5	0,1	0,03	0,09-0,2
Orthophosphate (mg/l PO_4)		0,3	0,09	0,2-0,4	0,2	0,04	0,2-03
Sulphate (mg/l SO_4)		205,0	5,0	200,0-210,0	339,0	61,7	260,0-435,0
Turbidity (FTU units)		18,3	16,8	2,0-45,0	4,6	2,3	1,0-8,0

Table 4.9 Continued

PHYSICAL AND CHEMICAL ANALYSIS		COWLES DAM			NIGEL DAM		
		$\bar{x}$	SD N =4 per season	Range	$\bar{x}$	SD N = 5 per season	Range
Temperature (°C)	WINTER	14,5	4,5	10,0-22,0	12,4	0,4	12,0-13,0
pH		8,2	0,3	7,7-8,6	7,3	0,2	7,1-7,5
Dissolved Oxygen (mg/l)		5,2	1,7	3,5-8,1	6,8	0,6	7,1-7,7
Conductivity (μS/cm)		1275,0	567,3	500,0-2100,0	1290,0	37,4	1250,0-1350,0
Total hardness (mg/l CaCO$_3$)		239,5	58,0	171,0-310,0	673,2	93,2	577,0-823,0
Alkalinity (mg/l CaCO$_3$)		57,5	15,4	34,0-77,0	14,1	0,2	14,0-14,5
Ammonia (mg/l NH$_3$)		2,9	1,6	0,6-4,9	4,8	0,8	3,7-6,2
Nitrate (mg/l NO$_3$)		7,9	3,2	8,8-11,0	6,3	2,8	1,8-9,2
Nitrite (mg/l NO$_2$)		0,2	0,1	0,06-0,3	0,04	0,01	0,02-0,06
Orthophosphate (mg/l PO$_4$)		0,7	0,5	0,1-0,6	0,1	0,03	0,1-0,2
Sulphate (mg/l SO$_4$)		215,0	20,6	190,0-240,0	351,0	60,4	280,0-440,0
Turbidity (FTU units)		25,8	23,2	2,0-64,0	5,6	2,1	2,0-8,0
Temperature (°C)	SPRING	21,0	3,1	18,0-26,0	21,2	1,4	19,0-24,0
pH		7,7	0,1	7,5-7,9	7,6	0,1	7,4-7,7
Dissolved Oxygen (mg/l)		4,7	1,7	3,2-7,6	6,5	0,6	5,8-7,3
Conductivity (μS/cm)		2100,0	61,2	2050,0-2200,0	2040,0	382,6	1800,0-2800,0
Total hardness (mg/l CaCO$_3$)		147,8	29,2	112,0-193,00	274,6	74,3	166,0-376,0
Alkalinity (mg/l CaCO$_3$)		96,3	20,7	83,0-132,0	14,8	0,7	14,0-16,0
Ammonia (mg/l NH$_3$)		9,2	6,4	3,1-16,8	14,6	7,2	1,2-20,0
Nitrate (mg/l NO$_3$)		6,1	3,3	2,2-11,0	17,9	9,3	8,8-33,0
Nitrite (mg/l NO$_2$)		2,4	4,0	0,03-9,24	0,3	0,2	0,04-0,6
Orthophosphate (mg/l PO$_4$)		0,7	0,4	0,4-1,3	0,4	0,1	0,2-0,5
Sulphate (mg/l SO$_4$)		221,3	38,8	180,0-285,0	339,0	62,6	300,0-420,0
Turbidity (FTU units)		8,5	5,8	3,0-18,0	3,6	0,8	2,0-4,0

Table 4.10. Metal concentrations in the water (µg/l) at nine selected localities of the Cowles and Nigel dams during the four seasons of 1989. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

METALS		COWLES DAM		NIGEL DAM	
		x̄ ± SD (µg/l)	Range (µg/l)	x̄ ± SD (µg/l)	Range (µg/l)
Fe	S	525 ± 226	210-850	382 ± 221	140-740
Cu	U	78 ± 48	40-160	76 ± 54	30-180
Ni	M	**165** ± 94	**90-320**	**940** ± 219	**670-1180**
Pb	M	**180** ± 52	90-**220**	**174** ± 48	**140-270**
Zn	E	**213** ± 97	80-**350**	**726** ± 168	**420-900**
Mn	R	398 ± 230	10-610	**6312** ± 1717	**4060-8040**
Cr		23 ± 11	10-40	24 ± 12	10-40
Fe	A	448 ± 158	180-570	446 ± 251	168-800
Cu	U	80 ± 67	20-190	120 ± 52	70-**220**
Ni	T	**173** ± 111	**70-360**	**1026** ± 246	**730-1290**
Pb	U	**163** ± 85	50-**250**	**216** ± 68	**120-330**
Zn	M	**208** ± 131	40-**390**	**840** ± 205	**450-1040**
Mn	N	418 ± 267	0-740	**6726** ± 1724	**4260-8360**
Cr		20 ± 14	0-40	50 ± 14	30-70
Fe	W	348 ± 127	210-540	562 ± 287	260-**1030**
Cu	I	80 ± 93	10-**240**	192 ± 65	130-**310**
Ni	N	**140** ± 62	**60-210**	**1156** ± 244	**850-1430**
Pb	T	**105** ± 66	40-**210**	**300** ± 72	**190-410**
Zn	E	**158** ± 108	70-**340**	**934** ± 180	**590-1120**
Mn	R	373 ± 229	20-590	**7272** ± 1590	**5030-9020**
Cr		30 ± 25	0-60	**128** ± 27	90-**170**
Fe	S	503 ± 198	330-830	508 ± 228	300-910
Cu	P	100 ± 49	30-160	194 ± 90	80-**300**
Ni	R	**193** ± 174	**60-490**	**1060** ± 197	**810-1380**
Pb	I	**178** ± 118	90-**380**	**260** ± 70	**180-370**
Zn	N	**265** ± 153	**130-510**	**840** ± 168	**590-1090**
Mn	G	410 ± 307	40-890	**7008** ± 1389	**5270-8840**
Cr		43 ± 40	0-100	**111** ± 65	30-**220**

Table 4.11. Metal concentrations (µg/g) in the sediments of the Cowles and Nigel Dams at nine selected localities in the Blesbokspruit catchment during the four seasons of 1989. 1. The Cowles dam. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

METALS	CONCENTRATIONS (µg/g) DURING THE FOUR SEASONS			
	Summer	Autumn	Winter	Spring
	$\bar{x}$ ± S.D. n = 4	$\bar{x}$ ± S.D. n = 4	$\bar{x}$ ± S.D. n = 4	$\bar{x}$ ± S.D. n = 4
Fe Relative %	**33203,2** ± 19345,0 83,6 ± 10,4	**31865,9** ± 18878,0 82,3 ± 10,8	**29914,9** ± 18925,4 80,1 ± 12,0	**31084,6** ± 18915,5 93,7 ± 4,2
Cu Relative %	**874,1** ± 957,2 2,3 ± 2,0	**869,7** ± 1029,0 2,3 ± 2,1	**758,9** ± 853,6 2,2 ± 1,7	**540,4** ± 878,4 1,3 ± 1,9
Ni Relative %	**1389,8** ± 1288,0 4,1 ± 4,0	**1683,1** ± 1772,9 4,7 ± 4,3	**2118,6** ± 2562,8 5,8 ± 5,6	**705,8** ± 1089,1 1,8 ± 2,3
Pb Relative %	**106,2** ± 106,7 0,3 ± 0,2	**106,6** ± 106,1 0,3 ± 0,2	**107,6** ± 105,2 0,3 ± 0,2	**132,2** ± 134,7 0,5 ± 0,3
Zn Relative %	**596,3** ± 609,9 2,5 ± 2,6	**599,8** ± 612,7 2,6 ± 2,7	**605,2** ± 612,9 2,9 ± 3,0	**242,0** ± 178,0 1,1 ± 1,0
Mn Relative %	**1148,1** ± 1442,5 6,6 ± 10,0	**1180,6** ± 1488,4 7,2 ± 10,9	**1197,0** ± 1507,6 7,9 ± 12,2	323,3 ± 84,5 1,4 ± 0,8
Cr Relative %	**176,6** ± 120,0 0,6 ± 0,5	**178,4** ± 120,1 0,6 ± 0,6	**181,0** ± 120,6 0,7 ± 0,6	**73,6** ± 41,5 0,3 ± 0,1

Table 4.11 Continued. Metal concentrations (µg/g) in the sediments of the Cowles and Nigel Dams at nine selected localities in the Blesbokspruit catchment during the four seasons of 1989. 2. The Nigel dam. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

METALS	CONCENTRATIONS (µg/g) DURING THE FOUR SEASONS			
	Summer	Autumn	Winter	Spring
	$\bar{x}$ ± S.D. n = 5	$\bar{x}$ ± S.D. n = 5	$\bar{x}$ ± S.D. n = 5	$\bar{x}$ ± S.D. n = 5
Fe Relative %	**24219,5** ± 20084,8 88,9 ± 10,0	**19135,0** ± 16676,0 89,5 ± 7,7	**20865,5** ± 18538,4 87,9 ± 11,5	**22376,4** ± 19580,7 89,6 ± 7,1
Cu Relative %	97,6 ± 7,2 0,9 ± 0,8	62,8 ± 9,70 0,5 ± 0,3	18,8 ± 8,6 0,2 ± 0,3	37,2 ± 27,1 0,4 ± 0,5
Ni Relative %	**157,8** ± 55,3 1,3 ± 1,1	**90,4** ± 51,6 0,9 ± 0,9	**41,5** ± 17,2 0,4 ± 0,4	**282,4** ± 449,7 1,0 ± 0,7
Pb Relative %	13,0 ± 19,5 0,1 ± 0,2	12,7 ± 19,0 0,1 ± 0,2	12,2 ± 18,0 0,2 ± 0,2	50,3 ± 32,5 0,4 ± 0,3
Zn Relative %	**508,9** ± 171,4 3,7 ± 2,5	**520,9** ± 185,3 4,6 ± 3,3	**524,2** ± 190,2 4,8 ± 3,3	**220,2** ± 164,1 2,3 ± 2,2
Mn Relative %	281,6 ± 164,5 2,9 ± 4,0	286,3 ± 165,5 2,8 ± 3,2	287,7 ± 166,2 3,8 ± 5,2	**2046,8** ± 3728,8 5,3 ± 6,3
Cr Relative %	**219,2** ± 99,4 2,1 ± 2,6	**221,5** ± 97,9 1,6 ± 1,0	**221,9** ± 99,4 2,7 ± 3,4	**80,4** ± 21,4 0,9 ± 0,9

Table 4.12. Physical and chemical analyses of the water of the Florida Lake during bimonthly sampling periods at 8 randomly selected localities between February 1990 and December 1990. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

PHYSICAL AND CHEMICAL ANALYSIS	MEAN, STANDARD DEVIATION AND RANGE	MONTH OF SURVEY					
		February n = 8	April n = 8	June n = 8	August n = 8	October n = 8	December n = 8
Temperature (as ° C)	x ± SD	23,7 ± 2,1	20,1 ± 1,8	10,9 ± 0,4	15,3 ± 1,0	21,8 ± 2,2	23,2 ± 0,7
	Min-Max	20,0 - 26,0	17,0 - 22,0	10,0 - 11,0	14,0 - 17,0	17,0 - 24,0	22,0 - 24,0
pH	Min-Max	7,8 - 9,9	7,3 - 8,0	7,5 - 8,2	7,9 - 9,8	7,0 - 8,8	8,5 - 9,8
Dissolved oxygen (as mg/l)	x ± SD	6,3 ± 1,1	6,1 ± 0,7	8,3 ± 1,1	7,6 ± 0,7	7,3 ± 0,5	6,1 ± 0,6
	Min-Max	5,0 - 7,9	5,3 - 7,6	6,8 - 10,3	6,7 - 8,4	6,8 - 8,1	5,3 - 7,0
Conductivity (as µS/cm)	x ± SD	105,0 ± 11,0	115,0 ± 16,0	118,8 ± 15,5	112,5 ± 20,6	110,0 ± 17,7	157,5 ± 11,7
	Min-Max	85,0 - 120,0	90,0 - 130,0	80,0 - 150,0	80,0 - 150,0	80,0 - 140,0	130,0 - 170,0
Alkalinity (as mg/l $CaCO_3$)	x ± SD	55,0 ± 9,3	50,5 ± 7,8	55,5 ± 10,0	51,3 ± 12,5	58,8 ± 8,1	58,5 ± 8,0
	Min-Max	40,0 - 70,0	40,0 - 64,0	42,0 - 70,0	40,0 - 80,0	42,0 - 68,0	44,0 - 72,0
Total hardness (as mg/l $CaCO_3$)	x ± SD	77,5 ± 36,2	60,0 ± 9,0	72,0 ± 18,0	68,0 ± 11,1	67,9 ± 9,4	66,8 ± 7,8
	Min-Max	30,0 - 120,0	50,0 - 70,0	60,0 - 110,0	60,0 - 80,0	59,0 - 84,0	58,0 - 83,0
Ammonia (as mg/l NO_3)	x ± SD	0,037 ± 0,031	0,057 ± 0,118	0,657 ± 0,218	0,090 ± 0,244	0,209 ± 0,294	1,128 ± 0,975
	Min-Max	0,001 - 0,070	0,001 - 0,342	0,500 - 1,110	0,031 - 0,244	0,006 - 0,854	0,540 - 3,420
Nitrite (as mg/l NO_3)	x ± SD	0,025 ± 0,032	0,024 ± 0,028	0,036 ± 0,011	0,029 ± 0,005	0,037 ± 0,016	0,048 ± 0,014
	Min-Max	0,001 - 0,098	0,088 - 0,002	0,020 - 0,053	0,017 - 0,033	0,017 - 0,056	0,030 - 0,070
Nitrate (as mg/l NO_2)	x ± SD	7,7 ± 4,5	3,5 ± 1,4	4,8 ± 2,0	5,4 ± 7,0	3,0 ± 0,9	3,7 ± 1,2
	Min-Max	1,8 - 14,0	0,9 - 5,3	1,8 - 8,4	0,0 - 4,0	1,8 - 4,2	2,2 - 5,7
Orthophosphate (as mg/l PO_4)	x ± SD	0,836 ± 0,555	0,480 ± 0,741	2,814 ± 0,026	2,925 ± 0,085	1,088 ± 1,078	0,043 ± 0,017
	Min-Max	0,140 - 1,400	0,090 - 2,300	2,800 -2,860	2,800 - 3,000	0,200 - 2,800	0,020 - 0,070
Sulphate (as mg/l SO_4)	x ± SD	4,7 ± 2,8	12,0 ± 4,1	10,5 ± 6,7	9,2 ± 5,0	9,6 ± 4,8	38,0 ± 15,2
	Min-Max	2,0 - 10,0	7,0 - 18,0	5,0 - 22,0	4,0 - 21,0	1,0 - 17,0	23,0 - 60,0
Turbidity (as FTU units)	x ± SD	8,1 ± 7,8	7,9 ± 5,5	7,6 ± 3,1	5,8 ± 3,6	13,1 ± 6,6	6,4 ± 2,6
	Min-Max	2,0 - 18,0	3,0 - 20,0	8,0 - 12,0	2,0 - 10,0	5,0 - 22,0	3,0 - 10,0

Table 4.13. Concentration of selected metals in the water column and bottom sediments of the Florida Lake, Roodepoort, based on bimonthly samples taken at eight localities over a period of one year during 1990 - 1991. Values in bold indicate metal concentrations exceeding maximum limits laid down for the protection of aquatic life in river and dam water. (Kempster *et al.*, 1980)

METAL	CONCENTRATION IN µg/l (WATER) AND µg/g (SEDIMENT) ($\overline{X}$ ± S.D.)	
	Water (n = 56)	Sediment (n = 56)
Copper	22,9 ± 11,9	26,2 ± 3,5
Manganese	67,2 ± 33,4	134,7 ± 46,7
Iron	**1764** ± 956	**10282** ± 4503
Nickel	**76,6** ± 65,6	**108,4** ± 57,2
Zinc	**73,7** ± 71,6	**130,8** ± 49,1
Lead	**230,3** ± 46,2	**95,0** ± 48,9

5. METAL ACCUMULATION BY SOME OF THE NUMERICALLY DOMINANT AQUATIC AND SEMI-AQUATIC PLANTS

5.1 Introduction

The ability of aquatic weeds to absorb and accumulate various metals from the aquatic environment has been demonstrated by a number of research workers (Dean *et al.*, 1972; Dietz, 1973; Erikson and Mortimer, 1975). It has also been shown that aquatic macrophytes can be reliable indicators of metal pollution in freshwater ecosystems (Ray and White, 1976; Abo-Rady, 1980; Franzin and McFairlane, 1980; Kovacs *et al.*, 1984; Mortimer, 1985). Mortimer (1985) points out that the degree of metal uptake by some plants is largely dependent on the type of metal and the plant species involved. Guilizzoni (1975) showed that considerable variation in metal contents may occur between rooted submerged and rooted emergent aquatic weeds from the same environment. Whilst floating aquatic weeds may absorb metals mainly from the water column, rooted aquatic vegetation is capable of metal uptake through its roots, shoots and leaves (Gambrell *et al.*, 1976; Patrick *et al.*, 1977; Reddy and Patrick, 1977; Förstner and Wittmann, 1979). Investigations by Erikson and Mortimer (1975) showed that some submerged aquatic plants can absorb metals directly from the water column through their chlorophyll-containing leaves and stems and that the transfer of these metals can take place to the roots where they are stored.

It was mentioned earlier that the solubility of metals in water and their deposition in the substrate of lakes and rivers are largely pH dependent. It was also found that, in the case of the metals investigated in the present study with the exception of iron, their increased accumulation in the sediments is usually accompanied by their increasing uptake by rooted aquatic macrophytes. There is, however, strong evidence that the different organs of some plants, e.g. the roots, were better able to concentrate or store metals than others (Van der Merwe *et al.*, 1990).

In the present series of investigations, a number of emergent, floating leaf, submergent floating and mudplants (Venter, 1991), including the alga *Spirogyra* and the moss *Bryum* sp., were studied for their ability to absorb and accumulate metals from the mine- and industry-polluted aquatic environments concerned.

An initial exploratory, preliminary investigation was undertaken into the metal bio-accumulation ability of the commonly occurring semi-aquatic and aquatic plants in the flood plains of these streams, immediately adjacent to and in the affected streams . Of these plants (Table 5.1), some were investigated later in more detail (Tables 5.2 - 5.17). Because of their abundance in some areas of the wetlands, specific attention was given to the metal uptake by the Spanish reed *Arundo donax*, the bulrush *Typha capenses* and the water fern *Azolla filiculoides*.

5.2 Observations on metal uptake by algae

This part of the investigation only received scant attention. From the little information obtained on *Spirogyra* and *Nitella*, it appears that this group of plants may include representatives ideal in evaluating metal pollution in the aquatic environment, especially so if sessile species can be used.

5.2.1 *Spirogyra* sp.

Spirogyra usually develop dense mats on the substrate of pools in streams and in the lakes studied, especially during winter and early spring. Bimonthly investigations over a year on its metal uptake in Florida Lake showed that iron (x:2914 μg/g), which also occurred in the highest concentrations in the water and sediments of this lake, was accumulated most of all seven metals studied (Table 5.2). This is followed by manganese (781,9 μg/g and zinc (206,2 μg/g). The other metals, namely chromium (53,0 μg/g), copper (52,6 μg/g), lead (42,2 μg/g) and nickel (34,5 μg/g) were all accumulated in much lower concentrations than was the case with iron and zinc. Even though no strong seasonal tendency could be observed in the uptake of these metals by *Spirogyra*, copper occurred in the lowest concentrations in its tissues during winter (6,5 μg/g) in contrast to summer (89,4 μg/g) when this metal was accumulated by *Spirogyra* in much higher quantities. A reverse condition appears to exist for iron, nickel, chromium, zinc and lead which all appeared to increase in concentration during the colder months of March - July. Manganese was accumulated in much higher concentrations than in the water and sediments. The mean sediment concentration ratio (CR_s) for iron, nickel, chromium and lead was similar and much more consistent over the period of survey than was the case for that in the water column (CR_w).

5.2.2 *Nitella*

Nitella occurs in most stagnant waters of the mine-affected areas. It was collected from the littoral zone in Germiston Lake over four seasons in 1989. It was found to accumulate iron in relatively high but variable concentrations during the different seasons of the year (Table 5.3). This is also reflected by the sediment concentration ratio (CR_s) which fluctuated between a low of 18% (summer) and a winter high of 234%. Zinc was also accumulated by this alga in relatively high concentrations during all four seasons of the year with a maximum CR_s of 869% during winter. Although the sediment concentration of copper was relatively low compared to that of zinc, *Nitella* was able to bioconcentrate this metal in its tissues at much higher levels than those recorded in the lake sediments. This applied particularly to autumn (348,8 μg/g) and winter (151,4 μgh/g). Lead sediment concentrations varied between a mean of 80,5 μg/g during spring to 176,4 μg/g in summer. Of the seven metals under consideration, chromium fluctuated between a mean sediment concentration of 64,3 μg/g (summer) and 162,3 μg/g (autumn). This metal was also accumulated in higher concentrations than the sediments during the latter season. Chromium was, however, only accumulated in higher concentrations than in the sediments during autumn with a CR_s of 162,3%. In contrast, nickel was bioaccumulated in higher concentrations than in the sediment during all four seasons of the year with CR_s values ranging between 162,7% (spring) and 341,1% (winter). Magnesium was the best accumulated by *Nitella* of all four metals with particularly high levels during summer and autumn (Table 5.3).

5.3 Metal uptake by the moss *Bryum*

The ability of mosses which occur in metal-polluted aquatic habitats to absorb and accumulate metals in their tissues has been recorded in the literature by a number of research workers (Satake *et al., 1989*; Wehr *et al.*, 1983). Mosses have already been employed for decades as bio-indicators of metal pollution in the aquatic environment in countries such as Belgium (Empain *et al.*, 1980), France (Mouvet, 1980), America and England (Whitton *et al.*, 1982; Wehr *et al.*, 1981, 1983). The advantage of using aquatic mosses as bio-indicators of metal pollution in rivers has been demonstrated by Mouvet (1980). Advantages include the stability of colonies in areas where they are found, their uniform distribution and their high resistance to effects of toxic metals. It was against this background that *Bryum* sp. was included where it commonly occurs at two localities in the vicinity of Cowles Dam in the Blesbokspruit catchment area. Investigations were conducted during the moist spring and summer rainfall periods on moss which occurred at the in- and outflow areas of Cowles Dam (Table 5.4).

In addition to iron, the sediments at the sampling sites were characterised by abnormally high concentrations of copper (both seasons), nickel (spring), lead (summer) and zinc (both seasons). This is also reflected in their bioconcentration by *Bryum* (Table 5.4) which yield CR_s values much higher than in the sediment itself. It was only chromium which was taken up at much lower concentrations by the moss which suggests that it may be able to regulate the uptake of this particular potentially toxic metal in its tissues.

5.4 Metal uptake by some emergent aquatic vegetation

5.4.1 *Alisma plantago-aquatica*

The various organs of *A. plantago-aquatica*, including its flowers, were separately analysed for their metal content (Table 5.5). Iron, which was present in relatively high concentrations at the locality where it was sampled, was, along with copper, the best accumulated by the various organs of this plant. Compared to its sediment concentrations, copper was consistently accumulated to a greater extent by all the organs of *A. plantago-aquatica* than any of the other metals under consideration. Zinc was the only other metal which showed similar but slightly lower concentrations in the various organs of this plant than copper, exceeding the sediment zinc concentrations for all the organs analysed. Nickel, lead and manganese concentrations followed a similar tendency, where in most cases, excepting the roots, the metals were bioconcentrated at lower levels than in the stream sediments. Chromium, which occurred in 148 µg/g in the sediments, was, with the exception of the roots, either not detected or poorly represented in all the other organs of this plant.

5.4.2 *Polygonum lapathifolium*

Polygonum lapathifolium is an annual mud plant which reaches maturity, flowers and dies within the same year. It was collected for analysis from the Blesbokspruit during the summer of 1988-1989. Extremely high concentrations of iron, copper and manganese were measured in its organs (Table 5.6). In the case of copper and manganese, much higher concentrations were recorded in its organs than in the sediments where it grew. The roots, green stems and leaves as well as the white stems were the major sites of metal accumulation. Lead, followed by chromium, was the least accumulated.

5.4.3 *Schoenoplectus lacustris*

Schoenoplectus lacustris was collected at two localities from the Blesbokspruit during the summer of 1988 - 1989 (Table 5.7). Metal concentrations were consistently the highest in the roots and white stems. Copper was best accumulated, exceeding the sediment concentration in most organs. This was followed by zinc and nickel. Lead occurred in much lower concentrations with chromium being the least taken up by this plant.

5.4.4 *Phragmitis australis*

Bimonthly whole plant analyses were made on material collected at the Florida Lake during the period March 1990 - March 1991 (Table 5.8A). Material collected at five localities in the Blesbokspruit during the summer growing season of 1989 was analysed in more detail (Table 5.8B). In the case of *P. australis* from the Florida Lake, iron was clearly the best accumulated of all seven metals investigated with a mean concentration for the period of 2 679 µg/g. This was followed by manganese (150,4 µg/g) and zinc with 86,7 µg/g. All the other metals occurred, with some exceptions, in *P. australis* in concentrations of less than 50 µg/g. Copper (18,4 µg/g), followed by chromium (30,1 µg/g), was accumulated in the lowest concentrations at this particular locality. This is almost the only case of the various plant species analysed where copper does not occur in relatively high concentrations compared to the values found for this metal in the sediments. More detailed investigation not reported here (Venter, 1991) showed that the roots of *P. australis*, followed by the flowers, were the organs where the highest concentration for all the metals occurred. The stems and rhizomes were usually found to contain the lowest metal concentrations. In the case of *P. australis* from the Blesbokspruit (Table 5.8B), where metal concentrations were investigated in the various organs, iron was also found to be the best bioconcentrated. Copper and manganese were the other two metals best taken up from the sediments by this plant. Nickel, zinc and lead occurred in significantly lower concentrations in the different organs analysed. The roots were clearly the most efficient in the accumulation and storage of all seven metals with iron, manganese and copper concentration as in the Florida Lake, being higher in the plant than in the sediments. The white stems embedded in the substrate contained the second highest concentrations of the metals after the roots. The green stems and leaves were measurably less efficient in the storage of all seven metals.

5.4.5 Observations on concentrations of the metals zinc, manganese, nickel and iron in the water, in the sediments and in two aquatic macrophytes, *Typha capensis* and *Arundo donax* in sections of the Elsburgspruit affected by goldmine and industrial effluents

Introduction

Because of intensive gold-mining activities over many decades in the headwater region of this stream, its waters are at places severely contaminated by effluents from disused and active mines. Water pumped from these mines varies in pH and contains a variety of heavy metals. The volume released into the Elsburgspruit could amount to as much as 10 938 m^3/d (Wells, 1989), reaching the spruit directly, or indirectly through seepage. These volumes of low pH, highly saline effluent waters discharged into the Elsburgspruit were found to exert a marked influence on the biology of the affected streams (Schoonbee and Van der Merwe, 1989). Effluents from metal-processing industries containing Zn, Ni and Cu, as well as other metals, further complicated the presence and composition of certain of these metals in the water and in the stream sediments.

The influence mine effluent waters containing heavy metals on stream ecosystems has also received considerable attention elsewhere in the world (Eyres and Pugh-Thomas, 1978; Salomons and Mook, 1980; Burrows and Whitton, 1983; Burton *et al.*, 1983; Campbell and Stokes, 1985; Norris, 1986). From these and other related investigations it is evident that metals can not only have a deleterious effect on the composition and presence of certain stream biota (Wood, 1974), but that changes in the pH of the water can have a direct bearing on the water solubility as well as the depositing capacity of such metals in the substrata of standing and flowing water ecosystems (Förstner and Prosi, 1979).

In this study the concentrations of the heavy metals Zn, Mn, Ni and Fe in the water environment and stream sediments were investigated, as well as their accumulation by the two emergent aquatic plants, *Arundo donax* and *Typha capensis*, under acidic and alkaline water quality conditions at four selected localities in the Elsburgspruit catchment area. The investigation was carried out in summer during the active growth phase of both plants and before the onset of senescence.

Selection of sampling sites

The four different sampling localities on the Elsburgspruit and on one of its tributaries (Fig. 5.1) were selected mainly on the basis of prevailing pH conditions in the streams, but also for the reasons that follow. Sampling locality 1 was used to evaluate the effects of the acidic mine waters on the metal contents of the water, the sediments and the two aquatic plants just before it enters the main stream of the Elsburgspruit. Previous investigations (Schoonbee and Van der Merwe, 1989) had shown that the water of the Elsburgspruit at that point contained abnormally high concentrations of various metals and that the pH of the water was very low, fluctuating between 3,6 and 6,6. Sampling locality 2 was situated approximately 2,5 km downstream from locality 1 where the pH of the water showed some improvement, varying mainly between 4,4 and 6,9. Locality 3, situated on a tributary which also receives acidic mine effluent waters, was also shown to receive fluxes of relatively high concentrations of the metals concerned (Schoonbee and Van der Merwe, 1989). Sampling locality 4 was situated on the main stream again in a predominantly wetland area after the stream had flowed through densely-vegetated *T. capensis* and *A. donax* communities for more than 6 km. At this point the pH of the water already showed some recovery by fluctuating between 6,1 and 8,8.

Materials and methods

Selected chemical and physical analyses of the water according to APHA (1971) were done monthly during the survey. The two aquatic macrophyte species involved were collected in the streams at each of the four localities, a garden fork being used to dislodge the plants intact from the stream bed. The plants were then thoroughly washed to remove sediments from the roots. Each plant was separately dried for the same period and at the same temperature as specified for the detritus, and then macerated. Samples of approximately 1 g were digested and prepared for
metal analysis.

Results

Physico-chemical conditions of the water

The effects of the large volumes of acidic effluents from mines on the pH of the main stream and on its tributary can be observed clearly at localities 1, 2 and 4 (Table 5.9A). There were, however, definite signs of a recovery to normal alkaline stream conditions, with pH values exceeding 7 at locality 3 during most months of the year, except during March (6,2) and June (6,1). Values for conductivity also indicated the considerable contribution of the solids dissolved in the effluents from the mines towards the mineral loads of the stream at all four localities, with values fluctuating between 55 and 303 mS/m. The acidic conditions resulted in low values for alkalinity, which largely prevailed at localities 1, 2 and 4. Increased values for alkalinity, which fluctuated between 86 and 170 mg/l at locality 3, coincided with expected higher pH values. Values for hardness and sulphates were exceptionally high and followed approximately the same pattern at localities 1, 2 and 4, as was the case for conductivity, showing the marked influence of mine effluent waters on the water chemistry of the stream system. Acidic conditions, accompanied by the release of sulphates in the water, are usually caused by the oxidation of sulphur found in a pyritic form, e.g. iron pyrite ($Fe\ S_2$) in the mine rock formations (Koryak *et al.*, 1972). During the mining process, water and sulphur oxidising bacteria, when exposed to air, will convert the inorganic sulphur to a water soluble sulphate and sulphuric acid according to the chemical reaction:

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4$$

Although concentrations for nitrates were not exceptionally high at all four stations, phosphates were present in much lower concentrations. Even so, values for chemical oxygen demand (COD) together with those for nitrates and phosphates, suggested the presence of some organic enrichment of the stream water which cannot be accounted for by the effluents from the mines alone.

Metal concentrations in the water, stream sediments and aquatic plants

A comparison of the concentrations of the different metals (Table 5.9B) in the water medium, in the sediments and in the plant species clearly showed that changes in the concentrations of zinc, manganese and nickel in the various constituents analysed were closely associated, and possibly determined by the pH regime at the different localities. For instance, Zn, Mn and Ni had the highest concentrations in the water where the lowest pH conditions prevailed, with a definite, substantial decline of the metal concentrations in the water at locality 3 under generally alkaline environmental water conditions. Changes in pH from acidic to alkaline conditions in turn coincided in most cases with marked increases of the three metals in the sediment. At the same time, the concentrations of these metals in the two plants also increased significantly under more alkaline conditions such as those experienced at locality 3, suggesting that the capacity of both plants to accumulate these metals may be largely pH-dependent. The relatively high values for Zn in *A. donax* at locality 3 contradicted the findings for the main stream somewhat. This tributary showed fluxes of severe mine effluent pollution when the pH of the water may have been as low as 4,4, but stream conditions there were also found to recover rapidly, with the pH exceeding 7, during which period this plant may well be able to accumulate some of the deposited Zn from the sediments. When the situation of Fe is compared with the other metals in the stream ecosystem at the different pH levels, indications are that the uptake of this metal by the plants may be less affected by the pH of the water environment.

The results of the lowered concentrations of the heavy metals in the water, sediments and plants obtained for locality 4, where the pH was generally below 6 during most months of the year, substantiated the findings recorded at localities 1 and 2 regarding the water solubility and rate of deposit of the various heavy metals in the sediment under acidic environmental conditions.

Discussion

A number of research workers have shown that pH changes in the water can have a direct bearing on the solubility and/or precipitation of certain metals in a freshwater aquatic environment (Shaw and Brown, 1974; Wood, 1974; Campbell and Stokes, 1985; Campbell and Tessier, 1985), affecting also the degree of toxicity of such metals (Whitley, 1968; Stumm and Morgan, 1970). The present study substantiates these findings. In the case of *A. donax* and *T. capensis*, the concentrations of Zn, Mn and Ni in the sediments of the Elsburgspruit had a direct bearing on the increased uptake of these metals by the plants. This tendency coincided with an increase in the pH of the water, from mainly acidic to alkaline conditions. Mortimer (1985) suggested that differences in the uptake rate of metals by aquatic plants could depend upon factors such as the species of the plant and changes in seasonal growth rates, as well as the physical age of the plant and the metal ion involved in the absorption process. The data showed that there were no clear-cut similarities in the absorption capacity of the metals between the two plants, with Zn and Fe being better accumulated under alkaline conditions by *T. capensis*, but with the reverse being the case for *A. donax*, where the absorption of Ni and Mn was more successful. Even though extremely high concentrations of Fe were recorded in the water environment at all four localities, this metal appeared not to be detrimental to either *T. capensis* or *A. donax*, as both plants thrived under both acidic and alkaline conditions at the different localities. In contrast with Mn, Ni and Zn, Fe was more readily absorbed by both plants at lower pH levels (locality 1). With the exception of locality 2, *T. capensis* was able to accumulate more Fe per mass in its tissues than was the case with *A. donax*.

From the results obtained it is evident that significant quantities of various metals are deposited in the stream sediments, particularly the wetland areas from where they are taken up into the food chain. Estimates of the density and distribution of *A. donax* in the Elsburgspruit wetland region showed this species to cover a surface area of approximately 43,5 ha, which at a peak summer standing may yield an estimated 3,75 kg dry mass plant material per m^2 area. This amounts to an estimated total summer yield of 1 630 t of *A. donax*, expressed as dry mass. Based on the heavy metal analysis, this tonnage of *A. donax* contained in its tissues an estimated 0,639 t of zinc, 6,561 t of manganese, 0,947 t of nickel and 2,033 t of iron. Should this plant be cropped regularly during the summer growing season, a substantial portion of these heavy metals present in the plants, and thus in the ecosystem, could effectively be removed. At present, *A. donax* is burned occasionally in the wetland regions of this stream during the dry winter months, with much of the ash and therefore the metals being returned directly into the stream.

5.4.6 *Typha capensis* in the Cowles and Nigel dams

Investigations on the metal accumulation by *Typha capensis* in the Cowles and Nigel dams were conducted at nine sampling localities during summer and spring of 1988 to 1989. These results are summarized in Tables 5.11A and 5.11B. Iron was accumulated best of all seven metals by the roots of *T. capensis* during both seasons. All the other organs were distinctly less efficient in the bioconcentration of this metal. The white parts of *T. capensis* contained the second highest concentration of iron, namely 674 µg/g in summer and 479 µg/g during spring, followed by concentrations in the green stems and leaves. Sediment concentration ratios for iron in the roots varied between 26,2% (summer) and 17,3% (spring) with values for the green stems and leaves being lower. A similar tendency existed for copper with the roots again being the major storage organ. The sediment concentration ratios for copper in the different organs were, however, markedly higher than those for iron. The sequence in efficiency of *T. capensis* to accumulate copper in its various organs followed the same pattern as for iron, namely roots > white stems > green stems > green leaves. The concentrations for nickel in the organs of *T. capensis* during both seasons again confirm the ability of the roots to accumulate and store metals. The sequence of importance in the accumulation of nickel in the other organs was the same as for iron and copper. This also applied to results obtained on the concentration ratios. Summer and winter concentrations of lead in the roots of *T. capensis* were almost the same, namely 60 µg/g and 59 µg/g respectively, with values in the other organs being much lower. This was also reflected by the different concentration ratios. Values for zinc were comparable with those obtained for nickel. Manganese was in terms of concentration the second most important metal in the tissues of *T. capensis* with the roots remaining the most important storage organ for this metal as well. Chromium was the only metal which was accumulated in extremely low concentrations in all the organs of *T. capensis* with the roots being the most efficient in the bioconcentration of this metal.

5.5 Metal uptake by submerged aquatic weeds

5.5.1 *Lagarosiphon* species

The submerged weeds *Lagarosiphon major* and *muscoides* usually occur together and were sampled in the Florida Lake where they covered open patches in the littoral zone of the lake. These plants were collected and analysed together during a bimonthly sampling program during 1990 - 1991 (Table 5.11). A summary of the results showed that manganese and iron were the metals best accumulated by *Lagarosiphon*. In the case of the other metals, nickel with a mean for the year of 105 µg/g and zinc, with a mean for the year of 150,5 µg/g, were the only other metals accumulated in quantities of more than 100 µg/g. Chromium with 83,0 µg/g was surprisingly high compared with the concentrations of this metal in all the other plants investigated. Lead (53,2 µg/g) and copper (19,8 µg/g) were accumulated in the lowest concentrations. With the exception of iron, the most active uptake of the other metals by *Lagarosiphon* took place during the spring and summer months. The absorption of copper, however, did not follow the same pattern in this submerged plant as that recorded for most of the other plants.

5.5.2 *Potamogeton pectinatus*

This submerged weed was mainly investigated in the Florida Lake because of its excessive growth there, which interfered with the recreational use of the lake (Venter and Schoonbee, 1991). Bimonthly sampling for metal analysis took place during March 1990 - March 1991. Results are summarized in Table 5.12. Iron, with a mean value for the year of 4 895,8 µg/g, occurred in the highest concentrations in the tissues of this submerged weed. This was followed by manganese (1 392,7 µg/g). The rest of the metals were present in the plant in much lower concentrations. Zinc was the only metal after iron which exceeded 100 µg/g in *P. pectinatus* during some of the bimonthly sampling periods. The mean concentrations of copper, nickel, lead and chromium were all below 51 µg/g. Although there was no clear seasonal pattern discernible in the uptake of the various metals by *P. pectinatus*, results suggest that the highest concentrations occurred during winter with lowest values being recorded during the warm summer months of the year.

5.6 Metal uptake by the rooted, floating leaved aquatic plant *Nymphaea* cf *spectabilis*

Nymphaea spectabilis was the only rooted floating leaved aquatic plant investigated during the survey. It was collected in the Florida Lake during the already mentioned bimonthly survey in March 1990 - March 1991. A summary of the results of metal uptake by this plant is included in Table 5.13. Iron, with a mean concentration of 5 478,9 µg/g, was clearly the metal best accumulated by this plant. Concentrations for chromium (91,6 µg/g) were surprisingly high compared with those of zinc (78,9µg/g) and lead (61,4 µg/g). In contrast to most of the other aquatic plants analysed, copper with a mean for the survey period of 10,6 µg/g occurred in the lowest concentration for all the metals in *N. spectabilis*.

5.7 Metal uptake by two floating aquatic weeds

5.7.1 Metal uptake by the duckweed *Lemna gibba*

Lemna gibba commonly occurred in the wetlands of the Blesbokspruit and in particular during summer at the Cowles Dam. Results on the metal uptake by this floating weed are summarized in Table 5.14. *Lemna gibba* does not appear to differ much from any of the submerged or emergent plants in its ability to accumulate high concentrations of iron and manganese in its tissues. Chromium was the metal accumulated in the smallest quantities of all seven metals. There were considerable differences in the concentrations of all the metals except for iron and magnesium in the sediments between the upstream and downstream localities where *Lemna* was collected, reflecting the history of metal pollution at these sites. Higher sediment metal concentrations were clearly affected for these different metals by their concentrations in the tissues of *Lemna*. It was only in the case of iron and chromium that a sediment concentration ratio of more than 100% could not be realized. The highest CR$_s$, namely 1 557%, was obtained for manganese.

5.7.2 Bioaccumulation of metals by the water fern, *Azolla filiculoides* in the Blesbokspruit wetland ecosystem affected by sewage, mine and industrial pollution

Introduction

It is known that floating aquatic weeds can be used to remove toxic metals from polluted waters (Muramota and Oki, 1983; Abbasi and Nipaney, 1985; Jain *et al.*, 1989). According to the literature, at least two species of *Azolla*, namely *A. pinnata* R. Br. and *A. filiculoides* Lam. have been investigated elsewhere under laboratory and natural environmental conditions for their capacity to absorb certain heavy metals. Sarkar and Jana (1987), who investigated the Hill activity of the chloroplasts of *A. pinnata* when exposed to various concentrations of the heavy metals Hg, As, Pb, Cu, Cd and Cr under laboratory conditions, showed the tolerance of this species towards certain concentrations of these metals. Jain *et al.* (1989), who studied the experimental uptake rate of Fe and Cu by *A. pinnata*, indicated that the presence of one metal in solution may affect the uptake rate of another metal ion present in the same solution. Mishra *et al.* (1987) showed that the mercury uptake by *A. pinnata* from a culture medium was both concentration- and time-dependent and that in addition to Hg accumulation, *A.pinnata* showed a significant decline in growth with increase in concentration of and exposure period to the metal.

According to Ashton and Walmsley (1984), there are three species of *Azolla* present in southern Africa namely, *A. pinnata* R. Br. var. *pinnata*, *A. nilotica* Decne. and *A. filiculoides* of which *A. pinnata* var. *pinnata* is the only indigenous species. *A. pinnata* was recorded from small sheltered pans in the Pongola floodplain, extending northwards into the coastal area of Mozambique (Wild, 1961) and into Zambia, the Caprivi and northern Botswana. The latter authors also showed *A. nilotica* to be present in the lower Zambezi River, and the Mozambique coastal plain as well as in the Cahora Bassa dam on the Zambezi River where it was found in sheltered backwaters. The third species, *A. filiculoides* was, according to Ashton and Walmsley (1984), introduced from South America (Moore, 1969; Ashton, 1977; Ashton and Walmsley, 1984; Wells *et al.*, 1986) and has until recently been restricted in its distribution in southern Africa (Jacot-Guillarmod, 1979). This water fern was originally recorded from the Oorlogspoort Stream (Oosthuizen and Walters, 1961; Jacot-Guillarmod, 1979) in the vicinity of Colesberg (northern Cape) (Oosthuizen and Walters, 1961; Wild, 1961) and at Upington (Jacot-Guillarmod, 1978) and the Hendrik Verwoerd Dam in the Orange River (Twyman and Ashton, 1972; Ashton, 1982; Ashton and Walmsley, 1984). However, *A. filiculoides* has been observed as recently as 1984 (De Wet, personal observations) to occur in small isolated patches in the Blesbokspruit wetland ecosystem near Springs and as recently as October 1989 in the Vaal River near Standerton (De Wet and Bezuidenhout, personal observations). Now, five years later, this weed has invaded much of the previously open areas in this wetland, causing a major problem in the ecology of this particular ecosystem, especially so in the Marievale bird sanctuary which serves as an important breeding ground for as many as 280 aquatic and semi-aquatic species of water birds, including some endangered species listed in the south African Red Data Book (Brooke, 1984). Birds affected include the greater flamingo, *Phoenicopterus ruber*, the lesser flamingo, *Phoenicopterus minor* and the Goliath heron, *Ardea goliath* which have frequented this sanctuary during recent years (Maclean, 1985).

Although the uptake and mobility of Cu, Cd and U by the roots and shoots of *A. filiculoides* have been demonstrated in Israel by Sela *et al.* (1988), no research has yet been done in South Africa on the potential of this species to remove these and other metals from the aquatic environment, especially under those eutrophic conditions currently existing in the Blesbokspruit. The Blesbokspruit, which traverses a major gold-mining and industrial area on the Witwatersrand, has for the past eighty years received effluents and seepage waters from surrounding mines and industries as well as discharges of nutrient-rich treated sewage effluent. These effluents contain variable concentrations of heavy metals such as Fe, Cu, Ni, Zn, Mn, Pb, Cr, Au and U (Viljoen, 1974; Wessels, 1974; Wittman and Förstner, 1976 a to c; 1977 a to b; Henzen and Pieterse, 1978; Murray, 1987).

The present study deals with the results obtained on the bioaccumulation of the heavy metals Fe, Cu, Ni, Zn, Pb, Mn and Cr by *A. filiculoides* where it occurs at four localities in the Blesbokspruit wetland ecosystem. A comparison is also made between the concentrations of these metals in *A. filiculoides* and in the sediments directly underneath the mats of this plant at the same localities.

The main reason why the comparison was made between the bioaccumulation of the metals by *Azolla* and their concentrations in the bottom sediments, and not with the metals in the water itself, was that the accumulated metals in both *Azolla* and the sediments reflected the history of metal contamination over an extended period of time more accurately until the day of sampling. The analysis of snap samples of water collected on the day of sampling on the other hand would only reflect the metal concentrations in the water at the specific moment of collection, be it high or low. It was not possible during the present investigation to obtain representative composite samples of water at the four localities for metal analysis over an extended period of time.

Materials and methods

During the present investigation, four localities were chosen covering the most important possible sources of metal pollution in the Blesbokspruit wetland ecosystem where *A. filiculoides* occurs (Fig. 5.2). Water samples for limited chemical analyses were collected at each locality, and the temperature, pH and dissolved oxygen recorded during the collection of *A. filiculoides*. The samples were analysed in the laboratory for ammonia, nitrate, nitrite, phosphate, sulphate and turbidity according to standard procedures.

Sampling of *A. filiculoides* took place during June 1989 at the beginning of winter when active growth of the fern was still observed. This formed part of an ongoing programme on the study of *A. filiculoides* in the Blesbokspruit wetland ecosystem. For the determination of biomass, five random samples of *A. filiculoides* were taken at each locality using a 20x20 cm plastic frame. All *A. filiculoides* material within the frame was carefully collected. Excess moisture was removed using a paper towel before the wet mass of each sample was determined. The mean wet mass of *A. filiculoides* from each locality was then determined per m^2 surface area, after which the total estimated biomass of *A. filiculoides* was calculated in metric t/ha. Another five subsamples of the water fern were also collected at each locality for the determination of heavy metal concentrations. Wet mass determinations of each sample were made. Samples were then individually dried in an oven at 90°C for 48 to 72 h. The dry mass of each sample was then determined.

Core samples of the upper 5 to 10 cm layer of the sediments were collected from the littoral zone of the wetland at each of the four different localities at the same time at which *A. filiculoides* was collected. These samples were also dried at 90°C for at least an electronic balance, approximately 1 g portions of each sample were then accurately weighed to the nearest mg. The samples were then digested according to standard procedures (Anderson, 1974; Agemian and Chu, 1976; Van Loon, 1980) by adding a 10 ml 1:1 concentrated perchloric and concentrated nitric acid mixture to the plant and sediment samples. The acid digestion was done in thoroughly cleansed metal-free 250 ml pyrex glass beakers covered with watch-glasses on a hotplate at a temperature of approximately 200 to 250°C.

The time of digestion lasted for at least 4 h during which period the total digestion and clearing of the sample occurred. Each digested sample was then separately filtered using a Millipore 6 μm paper filter and a vacuum pump. The filter was finally rinsed with distilled water to remove all traces of the dissolved heavy metals from the filter paper and the volume of each solution then made up to 100 ml in a volumetric flask. These solutions were individually transferred to clean glass storage bottles for later analyses of the different heavy metals.

To determine the organic content of the sediments collected at each locality, approximately 6 g of the homogenised dry material from each sample was accurately weighed and ashed in a Labcon muffle furnace Type RM4 at 550°C according to the procedure described by Nalewajko (1966).

A Varian atomic absorption spectrophotometer, Series No. 875, was used to measure the concentrations of the metals in each sample. During the atomic absorption analysis, use was made of Fe, Cu, Ni, Zn, Pb, Mn and Cr analytical standards. Values obtained, expressed as mg/g, were in turn recalculated to μg/g, using the initial dry mass of each plant or sediment sample before digestion. In the case of water samples results were expressed in μg/l.

A bio-accumulation ratio (CR) calculated for the various metals (also expressed as %) was determined by using the formula:

$$\mathrm{CR} = \frac{\text{Concentration of the metal in } \textit{A. filiculoides}\ (\mu\text{g / g dry mass})}{\text{Concentration of the metal in the sediments}\ (\mu\text{g / g dry mass})}$$

This formula corresponds with the Goldschmidt enrichment principle (Goldschmidt, 1937) as used by Hutchinson (1943) and Cannon (1960). The reason why the metal concentrations in the sediment were used, but not those in the water, has already been explained earlier.

Statistical evaluation of the results was made using Lotus 123 and Harvard Graphics software packages.

Results

Results on the selected physical and chemical parameters analysed for at the four localities are summarised here. Winter water temperatures, measured between 12:00 and 15:00, were found to be fairly constant, fluctuating between 12°C and 13°C. Although the water of the wetland was clearly affected by effluent and seepage waters from mines, as reflected by the mineral loads in the water at the various localities, pH values had recovered and were constantly above seven at all four localities, exceeding 8 at locality 1. Values for dissolved oxygen varied between 4,7 mg/l (locality 1) and 6,6 mg/l (locality 3). The concentrations of ammonia, nitrate, nitrite and orthophosphate showed the effects of the treated sewage effluents discharged into the Blesbokspruit wetlands upstream from localities 1 and 2 and in the vicinity of locality 4.

The effect of mine effluents on the water chemistry is reflected to some extent by the concentration of sulphates in the water which equalled or exceeded 300 mg/l at three of the four sampling localities. A relatively high turbidity value of 15 FTU at locality 1 can be ascribed to organic fibre in the water originating from the effluent of a paper and pulp factory.

The concentrations of the metals Fe, Cu, Ni, Zn, Pb, Mn and Cr from the sediments at the four localities in the Blesbokspruit wetlands (expressed as µg/g) are listed in Table 5.15. According to these findings the sequence in concentrations of the metals in descending order was Fe > Zn > Mn > Ni > Cr > Cu > Pb. Extremely high values for iron occurred in the sediments at all four localities, varying between 7 528 µg/g (locality 4) and 49 498 µg/g (locality 1). These high concentrations are directly related to the pyrite (FeS_2) occurring in the unearthed gold-bearing ore and which, when exposed to atmospheric oxygen and moisture, results in the oxidation of the sulphidic components (S_2^{2-}) in pyrite to sulphate (SO_4^{2-}) as a result of which acidic H^+ and Fe^{2+} (aq) are released into the aquatic environment (Harrison, 1958; Berner, 1970; Thompson, 1980; Förstner and Wittmann, 1979; Murray, 1987). The concentrations of Cu in the sediments were reasonably similar for all four localities, fluctuating between 89 µg/g (locality 3) and 97 µg/g (locality 4). Although Ni exceeded 100 µg/g in the sediments of all four localities, downstream there was a progressive increase of this metal in sediments at the successive sampling localities, with a significantly higher concentration at locality 4 (232 µg/g). This can possibly be ascribed to effluents originating from a metal-processing factory in the vicinity of this locality. In contrast, values for Pb in the sediments, which fluctuated considerably between the various sampling stations, showed an overall decline downstream in the sediments. A maximum of 51 µg/g was obtained for Pb at locality 1, followed by 39 µg/g at locality 2 and 6 µg/g at locality 3. Pb concentrations in the sediment at locality 4 were so low that they could not be measured. The concentration for Zn, in contrast with Pb, increased at the sampling localities downstream from Locality 1, reaching a maximum of 613 µg/g at locality 4. The concentration of this metal at locality 1 was also markedly lower (66 µg/g) than those found at sampling localities 2 to 4. As was the case for Ni, the increase of Zn at locality 4 can perhaps also be ascribed to the effect of effluents from the above-mentioned metal-processing factory, but some contribution certainly originated from the surrounding mining areas, as Zn was used in the past in the gold-extraction processes

(Murray, 1987). The higher values for Zn at localities 2 and 3 may have originated from the effluent of a sewage purification plant which entered the wetlands upstream from these localities. Values for Mn in the sediments showed a progressive decline from a maximum of 290 µg/g (locality 1) to 143 µg/g (locality 4; Table 5.15). Like Fe, Mn mainly originated from the ore of surrounding gold mines (Murray, 1987). Cr concentrations were the highest in the sediments at localities 1 and 2, and in all cases exceeded 128 µg/g. Values declined to below 100 µg/g at sampling stations 3 and 4.

Values obtained for the concentration ratio of the different metals by *A. filiculoides* at the four localities in the Blesbokspruit wetlands are listed in Table 5.15. The CR of Fe by *A. filiculoides* at the four localities showed variable levels, fluctuating between 4% at locality 1 and 63% at locality 4, with a mean value of 23% for all four stations. Even so, this metal, as with Mn, clearly occurred in the highest concentrations of all metals analysed for in *A. filiculoides* with a maximum of 7 906 µg/g at locality 2. *A. filiculoides* showed an even better ability to accumulate Mn in its tissues with a CR fluctuating between 950% (locality 1) and 3 814% (locality 4). Of interest was the downstream increase in concentration of Mn in *A. filiculoides* with peaks at localities 3 and 4, whereas the concentrations of this metal in the sediments gradually declined at the successive downstream sampling stations. The actual concentration of Cu in *A. filiculoides*, which varied between 37 µg/g (locality 4) and 93 µg/g (locality 1), showed a close relationship with the CR, varying between 102% (locality 1) and 42% (locality 3), respectively.

The bio-accumulation of Ni by *A. filiculoides* exceeded the concentrations of this metal in the sediments in two cases (localities 2 and 3). At localities 1 and 4, however, the Ni bio-accumulation values of *A. filiculoides* were lower. The ability of A. *filiculoides* to accumulate Pb from the aquatic environment appeared to be very good, and exceeded in all cases the concentrations of this metal in the sediments (Table 5.15). A fairly consistent level of bio-accumulation was recorded at localities 1 to 3 and even at locality 4, where the concentration of Pb could not be measured in the sediments, comparatively high values were recorded in *A. filiculoides*, with the concentration of Pb in the tissues of *A. filiculoides* still exceeding 109 µg/g. Although there was a tendency for *A. filiculoides* to bio-accumulate Zn in relatively large quantities at the localities downstream from locality 1, the accumulation of Zn, relative to the concentration of this metal in the sediments, gradually declined to 20% (locality 4), compared to 71% at locality 1. Cr occurred in the lowest concentrations of all seven metals in the tissues of *A. filiculoides*, varying between 4 µg/g (locality 4) and 14 µg/g at locality 1, despite appreciable concentrations recorded for this metal in the sediments (Table 5.15).

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Table 5.1 Preliminary findings on the ability to accumulate metals by some of the numerically dominant aquatic and semi-aquatic plants in the catchment areas of the mine and industrially polluted Elsburgspruit-Natalspruit, Blesbokspruit and Klipriver systems.

Plant name	Plant type	Iron	Manganese	Zinc	Lead	Nickel	Copper	Chromium
Marsilea macrocarpa	RFA	+	+	++	+		++	+
Isolepis costata	EA	++	++	+	+	+	+	++
Bromus inermus	MP	+	++	+	++	+	++	+
Avena sp.	MP	+					++	
Typha capensis	EA/MP	++	++	++	+	+	+	++
Arundo donax	EA/MP		+	+		+	++	+
Cotula coronopifolia	EA/MP	++	+		++	+		+
Polygonum lapathifolium	MP	+	++	++	++	+	+	+
Juncus lomatophillus	EA/MP	+	+	+				
Sonchus oleraceus	MP	+	++	+			+	
Alisma plantago-aquatica	EA/MP	+	++	+	++	++	++	+
Polypogon monspiliensis	MP	+		+	++		+	+
Potamogeton pectinatus	SA	+	++	++	++	++	+	+
Oenothera rosea	MP	+		+			+	
Veronica anagalis-aquatica	MP		++	+		++	++	
Agrostis lachnantha	MP							
Plantago lanceolata	MP	+	++	+	+	+	++	+
Chenopodium album	MP			+				
Schoenoplectus corymbosus	EA/MP	++	+	+		++		
Rumex conglomeratus	MP							+
Rapistrum rugosum	MP	+						+

EA: Emergent aquatic. FA: Floating aquatic. SA: Submerged aquatic. MP: Mudplant. RFA: Rooted floating aquatic (After Venter, 1991).

Footnote on symbols in table : no symbol = no analysis done; + = dry mass metal values in plant usually as high as those in sediments; ++ = dry mass metal values usually higher than those in sediments

Table 5.2 Bimonthly mean metal concentration ($\bar{x} \pm$ SD) and concentration ratios for the water (CR$_w$ %) and sediments (CR$_s$ %) of *Spirogyra* sp. in the Florida Lake, Gauteng, during March 1990 - March 1991. Results expressed as µg/g dry mass (sediments) or µg/l (water).

Metal		Period of survey and mean metal concentration ($x \pm$ SD) expressed as µg/l (water) or µg/g dry mass (sediment)			
		March	May	July	September
		n = 6	n = 6	n = 6	n = 6
Cu	$\bar{x} \pm$ SD	59,7 ± 8,1	40,6 ± 7,9	6,5 ± 0,7	19,3 ± 6,7
	CR$_{water}$	417,5	253,8	21,7	48,3
	CR$_{sediment}$	158,4	100,5	15,7	50,4
Mn	$\bar{x} \pm$ SD	970,1 ± 97,4	430,3 ± 210,6	1225,5 ± 0,7	897,5 ± 349,3
	CR$_w$	992,9	430,3	12255,0	1954,4
	CR$_s$	874,8	421,9	1462,4	720,9
Fe	$\bar{x} \pm$ SD	3435,8 ± 1069,3	3261,7 ± 966,4	2852,0 ± 5,7	1974,0 ± 1414,9
	CR$_w$	153,0	197,1	178,8	292,4
	CR$_s$	29,1	18,5	17,8	38,1
Ni	$\bar{x} \pm$ SD	36,5 ± 1,7	39,9 ± 0,7	37,0 ± 0,0	32,8 ± 8,8
	CR$_w$	42,3	27,5	19,0	93,7
	CR$_s$	39,1	49,0	47,0	64,6
Cr	$\bar{x} \pm$ SD	60,7 ± 30,6	58,4 ± 27,4	52,0 ± 0,7	47,5 ± 21,9
	CR$_w$	46,5	30,0	17,6	2375,0
	CR$_s$	27,5	27,0	51,8	31,0
Zn	$\bar{x} \pm$ SD	296,7 ± 49,7	300,4 ± 160,1	264,0 ± 7,1	175,0 ± 107,5
	CR$_w$	960,2	1201,6	1320,0	205,9
	CR$_s$	91,3	96,9	134,0	113,4
Pb	$\bar{x} \pm$ SD	58,3 ± 1,7	36,5 ± 10,9	24,0 ± 3,5	31,3 ± 1,8
	CR$_w$	30,7	15,5	8,1	14,2
	CR$_s$	41,5	16,0	8,2	25,5

Table 5.2 Continued

Metal		November	January	March	x ± SA
		n = 6	n = 6	n = 6	n = 42
Cu	$\bar{x}$ ± SD	76,0 ± 62,2	89,4 ± 1,4	76,6 ± 20,7	52,6 ± 31,4
	CR_{water}	217,1	311,5	418,6	241,2 ± 160,0
	$CR_{sediment}$	298,0	271,7	195,9	155,8 ± 107,3
Mn	$\bar{x}$ ± SD	549,5 ± 249,6	690,7 ± 130,7	709,8 ± 104,7	781,9 ± 269,8
	CR_u	999,1	1136,0	991,3	2679,9 ± 4246,1
	CR_s	341,3	459,2	531,7	673,2 ± 355,2
Fe	$\bar{x}$ ± SD	2885,0 ± 1989,8	2969,4 ± 30,7	3019,2 ± 167,4	2914,0 ± 464,9
	CR_w	730,4	303,1	195,7	292,9 ± 201,1
	CR_s	44,0	34,1	30,6	30,3 ± 9,7
Ni	$\bar{x}$ ± SD	31,5 ± 20,2	29,5 ± 0,7	34,3 ± 1,9	34,5 ± 3,6
	CR_w	103,3	89,7	92,0	66,8 ± 35,7
	CR_s	16,3	22,7	35,2	39,1 ± 16,4
Cr	$\bar{x}$ ± SD	48,7 ± 17,4	50,1 ± 4,9	53,7 ± 0,7	53,0 ± 5,0
	CR_w	27,5	55,2	48,7	371,5 ± 883,6
	CR_s	30,9	29,5	29,7	32,5 ± 8,7
Zn	$\bar{x}$ ± SD	129,0 ± 97,6	130,6 ± 10,7	147,8 ± 97,4	206,2 ± 78,0
	CR_w	32,3	199,1	321,3	605,8 ± 536,3
	CR_s	107,5	72,4	70,1	97,9 ± 22,7
Pb	$\bar{x}$ ± SD	42,5 ± 7,8	48,0 ± 30,0	54,6 ± 17,4	42,2 ± 12,4
	CR_w	15,7	25,2	29,3	19,8 ± 8,6
	CR_s	34,7	36,9	35,5	28,3 ± 12,3

Table 5.3. Mean metal concentrations (μg/g dry mass) in the wort *Nitella* sp. and in the bottom sediments of Germiston Lake during the four seasons of 1989 with an indication of sediment metal concentration ratio (CR %).

Metal	Parameters	Sampling season, metal concentration (μg/g) and concentration ratio %			
		Summer (n = 1) $\bar{x}$	Autumn (n = 2) $\bar{x}$	Winter (n = 2) $\bar{x}$	Spring (n = 3) $\bar{x}$
Iron	Sediment μg/g	18506	14755	11584	14396
(Fe)	*Nitella* μg/g	3410,3	25535,6	27297,2	13811,5
	CR_s,%	18,4	171,1	234,0	99,5
Zinc	Sediment μg/g	98,0	751,8	144,1	195,8
(Zn)	*Nitella* μg/g	328,1	1868,7	1221,8	427,9
	CR_s,%	334,8	244,2	868,7	246,5
Copper	Sediment μg/g	20,5	115,5	33,7	51,1
(Cu)	*Nitella* μg/g	76,9	348,8	151,4	98,2
	CR_s,%	375,2	298,7	437,2	224,0
Lead	Sediment μg/g	176,4	108,6	113,9	80,5
(Pb)	*Nitella* μg/g	144,6	160,6	24,3	210,2
	CR_s,%	82,0	-	24,4	258,7

Table 5.3 Continued

Metal	Parameters	Sampling season, metal concentration (µg/g) and concentration ratio %			
		Summer (n = 1) $\bar{x}$	Autumn (n = 2) $\bar{x}$	Winter (n = 2) $\bar{x}$	Spring (n = 3) $\bar{x}$
Chromium	Sediment µg/g	64,3	162,3	65,4	65,6
(Cr)	*Nitella* µg/g	32,7	87,4	77,7	38,2
	CR_s%	50,8	55,0	116,5	57,8
Nickel	Sediment µg/g	61,1	153,7	62,4	106,4
(Ni)	*Nitella* µg/g	142,7	295,7	188,7	138,6
	CR_s%	233,5	187,7	341,1	162,7
Manganese	Sediment µg/g	212,9	901,1	328,6	246,3
(Mn)	*Nitella* µg/g	3760,2	9853,2	2367,6	860,7
	CR_s %	1766,2	1101,8	1964,7	625,2

Table 5.4 Metal concentrations in *Bryum* sp. and the stream sediments (mg/g dry mass) at two localities in the Blesbokspruit during the summer and spring of 1989-1990.

Metal	SUMMER 1988-1989 (n = 10)				SPRING 1989 (n = 10)			
	Metal concentrations in *Bryum* (μg/g)		Metal concentrations in sediments (μg/g)	Concentration ratio (CR$_s$, %)	Metal concentrations in *Bryum* (μg/g)		Metal concentrations in sediments (μg/g)	Concentration ratio (CR$_s$, %)
	$\bar{x}$ ± SD	Range			$\bar{x}$ ± SD	Range		
Fe	7348 ± 219,4	5849 - 8206	51439	14	5907,7 ± 393,1	4739 - 7016	48561	12
Cu	534,2 ± 23,6	358,4 - 655,7	126,5	422	204,5 ± 10,6	153 - 245	104,8	195
Ni	3176,1 ± 919,5	2055 - 6935	1506,5	210	1089,9 ± 51,0	862 - 1410	1336	81
Pb	649,4 ± 12,7	90 - 141	170,5	380	122,5 ± 7,3	81 - 161	199	62
Zn	1596,4 ± 413,2	1034 - 3165	168,5	940	454,6 ± 16,2	419 - 489	179	253
Mn	5517,4 ± 783,6	4207 - 9139	306,5	1800	2746,5 ± 133,8	2152 - 3469	384,5	714
Cr	59,7 ± 5,7	46 - 71	132,3	45	58,8 ± 4,4	39 - 81	98	60

Table 5.5 Concentrations (μg/g dry mass) of metals in the organs of *Alisma plantago-aquatica* and stream sediments of the Blesbokspruit and their concentration ratios (CR) expressed as percentage of the sediment metal concentrations.

Plant organs and sediment concentration ratios (%)	Metals in plant organs and sediments (μg/g) and their concentration						
	Fe	Cu	Ni	Pb	Zn	Mn	Cr
Roots (RT)	2065	1418	2722	243	610	3512	93
White stem (WS)	676	887	60	24	239	97	0
Green stem (GS)	264	243	21	8	70	132	0
Leaves (LF)	769	684	124	24	179	362	2
Flowers (FL)	529	663	91	16	132	756	3
Sediment	59598	91	118	51	66	391	148
CR_{r} (%)	3,5	1558	2306	476,4	924,2	898,2	62,8
Cr_{ws} (%)	1,1	974,7	50,8	47,0	362,1	24,8	0
Cr_{gs} (%)	0,4	267,0	17,8	15,6	106,1	33,8	0
CR_{lf} (%)	1,3	751,6	105,1	47,0	271,2	92,6	1,4
CR_{fl} (%)	0,9	728,6	77,1	31,4	200,0	193,4	2,2

Table 5.6. Mean concentrations (x̄ ± SD) (µg/g dry mass) metals in organs of *Polygonum lapathifolium* during the summer of 1988-1989 at four localities on the Blesbokspruit and their sediment concentration ratios (CR) expressed as percentage of the sediment metal concentration.

Plant organs and their respective sediment concentration ratios	Metal concentrations in plant organs and sediments (µg/g) and their sediment concentration ratios (CR %)					
	Iron (n = 4)		Copper (n = 4)		Nickel (n = 4)	
	x̄ ± SD	Range	x̄ ± SD	Range	x̄ ± SD	Range
Roots (RT)	5259,7 ± 1332,8	3745 - 7085	3398,7 ± 810,2	2128 - 4170	337,3 ± 186,0	119 - 559
White stems (WS)	854,5 ± 713,7	149 - 1718	683,5 ± 456,0	127 - 1166	100,0 ± 80,4	22 - 221
Green stems (GS)	1672,4 ± 1561,0	68 - 3525	1030,2 ± 1071,4	202 - 2827	65,0 ± 42,9	14 - 124
Leaves (LF)	1638,4 ± 1125,2	620 - 3457	2289,0 ± 1147,2	750 - 3764	74,1 ± 33,4	48 - 131
Sediments (SED)	26773,0 ± 21716,9	2919 - 51341	100,2 ± 5,5	93 - 106	171,4 ± 53,9	107 - 232
CR_{rt} (%)	19,6		3391,9		196,6	
CRws (%)	3,2		682,1		58,3	
CR_{gs} (%)	6,3		1028,1		37,9	
CR_{lf} (%)	6,1		2284,4		43,2	

able 5.6 Continued

Plant organs and their respective sediment concentration ratios	Lead (n = 4)				Zinc (n = 4)				Manganese (n = 4)			
	x̄	±	SD	Range	x̄	±	SD	Range	x̄	±	SD	Range
Roots (RT)	43,1	±	6,8	37 - 454	600,6	±	194,4	412 - 862	2243,1	±	1667,7	535 - 4608
White stems (WS)	20,4	±	12,0	11 - 41	259,7	±	203,5	64 - 601	2103,7	±	2958,8	205 - 7224
Green stems (GS)	14,4	±	4,1	11 - 21	229,5	±	70,1	134 - 330	1450,0	±	1154,0	573 - 3391
Leaves (LF)	23,8	±	3,2	19 - 28	296,9	±	91,0	184 - 413	1731,9	±	692,0	587 - 2414
Sediments (SED)	3,6	±	6,2	0 - 14	529,6	±	186,0	259 - 766	325,9	±	155,0	143 - 505
CR_{rt} (%)	1197,2				113,4				688,3			
CR*ws* (%)	566,7				49,0				645,5			
CR_{gs} (%)	400,0				43,3				444,9			
CR_{lf}	661,1				56,0				531,4			

Plant organs and their respective sediment concentration ratios	Chromium (n = 4)			
	x̄	±	SD	Range
Roots (RT)	13,3	±	7,3	7 - 25
White stems (WS)	12,7	±	20,9	0 - 49
Green stems (GS)	3,1	±	3,0	1 - 8
Leaves (LF)	3,5	±	2,9	1 - 9
Sediments (SED)	237,5	±	103,4	93 - 363
CR_{rt} (%)	5,6			
CR*ws* (%)	5,3			
CR_{gs} (%)	1,3			
CR_{lf} (%)	1,5			

Table 5.7 Concentrations (µg/g dry mass) of metals in the organs of *Schoenoplectus lacustris* and stream sediments of the Blesbokspruit and their concentration ratios (CR) expressed as percentage of the sediment metal concentrations.

Plant organs and sediment concentration ratios (%)	Locality	Metals in plant organs and sediments (µg/g) and their concentration						
		Fe	Cu	Ni	Pb	Zn	Mn	Cr
Roots (RT)	1	5949	4170	480	37	415	535	7
White stem (WS)		1718	1099	124	11	193	476	1
Green stem (GS)		2910	870	87	11	213	583	3
Leaves (FL)		1684	1721	63	19	184	587	1
Flowers (FL)		1439	994	105	22	231	635	3
Sediment		59598	91	118	51	66	391	148
CR$_{rt}$ (%)		10,0	4582,4	406,7	72,5	628,8	136,8	4,7
CR$_{ws}$ (%)		2,9	1207,6	105,0	21,6	292,4	121,7	0,7
CR$_{gs}$ (%)		4,9	956,0	78,3	21,6	322,7	149,1	2,0
CR$_{lf}$ (%)		2,8	1891,2	53,4	37,3	278,8	150,1	0,7
CR$_{fl}$ (%)		2,4	1092,3	89,0	43,1	350,0	162,4	2,0
Roots (RT)	9	3745	2128	559	45	862	4608	25
White stem (WS)		149	127	22	18	64	205	1
Green stem (GS)		186	222	35	14	242	1252	1
Leaves (LF)		620	3764	48	28	413	1837	2
Flowers (FL)		-	-	-	-	-	-	-
Sediment		45307	106	217		481	505	192
CR$_{rt}$ (%)		8,3	2007,5	257,6		179,2	912,5	13,0
CR$_{ws}$ (%)		0,3	119,8	10,1		13,3	40,6	0,5
CR$_{gs}$ (%)		0,4	209,4	16,1		50,3	247,9	0,5
CR$_{lf}$ (%)		1,4	3550,9	42,1		85,9	363,7	1,0
CR$_{fl}$ (%)		-	-	-		-	-	-

Table 5.8A Bimonthly mean metal concentration (µg/g dry mass) ($\bar{x}$ ± SD) of *Phragmitis australis* in the Florida Lake, Gauteng, during March 1990 - March 1991.

Metal in µg/g	Sampling periods and metal concentrations (µg/g dry mass)											
	March			May			July			September		
	n = 8			n = 8			n = 8			n = 8		
	$\bar{x}$	±	SD	$\bar{x}$	±	SD	$\bar{x}$	±	SD	$\bar{x}$	±	SD
Cu	16,9	±	117	15,6	±	6,4	12,3	±	4,5	19,9	±	16,2
Mn	143,3	±	153,8	163,6	±	183,6	192,1	±	237,4	149,4	±	26,4
Fe	2294,4	±	3743,6	3973,1	±	7234,3	4171,7	±	7177,2	1822,1	±	2671,5
Ni	61,5	±	37,0	59,0	±	36,3	57,8	±	40,9	39,4	±	61,8
Cr	35,8	±	12,1	30,2	±	19,5	18,1	±	8,7	18,3	±	30,6
Zn	42,5	±	20,0	46,6	±	19,8	46,9	±	19,7	120,1	±	104,3
Pb	48,7	±	15,1	49,7	±	15,7	13,5	±	5,7	63,7	±	59,3

Metal in µg/g	Sampling periods and metal concentrations (µg/g dry mass)											
	November			January			March			September		
	n = 8			n = 8			n = 8			n = 8		
	$\bar{x}$	±	SD	$\bar{x}$	±	SD	$\bar{x}$	±	SD	$\bar{x}$	±	SD
Cu	20,8	±	14,3	22,4	±	12,5	21,2	±	11,6	18,4	±	3,6
Mn	154,6	±	24,8	134,0	±	13,7	115,8	±	49,1	150,4	±	24,0
Fe	2561,0	±	2885,3	2541,9	±	3035,3	1388,9	±	1687,0	2679,0	±	1039,4
Ni	41,2	±	61,4	38,7	±	40,4	44,9	±	16,7	48,9	±	10,1
Cr	21,6	±	31,4	42,0	±	55,4	45,0	±	33,8	30,1	±	11,2
Zn	142,6	±	91,5	113,4	±	78,8	94,7	±	60,8	86,7	±	41,2
Pb	65,6	±	60,7	56,7	±	39,3	56,5	±	23,6	50,6	±	17,6

Table 5.8B Mean concentrations (µg/g dry mass) (x̄ ± SD) of metals in the organs of *Phragmitis australis* at five localities on the Blesbokspruit and their concentration ratios (CR) expressed as percentage of the sediment metal concentration, during the summer growing season of 1989.

Plant organs and their respective sediment concentration ratios	Metal concentrations in plaent organs and sediments (µg/g) and their sediment concentration ratios (%)											
	Iron (n = 4)				Copper (n = 4)				Nickel (n = 4)			
	x̄	±	SD	Range	x̄	±	SD	Range	x̄	±	SD	Range
Roots (RT	3629,5	±	2232,7	139 - 6733	1028,9	±	1147,7	108 - 3268	248,9	±	368,9	
White stems (WS)	490,0	±	466,3	97 - 1365	142,8	±	771,1	64 - 282	31,4	±	34,1	
Green stems (GS)	252,9	±	317,8	40 - 877	63,8	±	56,1	14 - 167	14,1	±	16,4	
Leaves (LF)	244,3	±	334,2	41 - 903	70,9	±	63,8	9 - 184	13,6	±	12,9	
Sediments (SED)	21788,2	±	15827,9	6904 - 43057	443,6	±	809,6	10 - 2062	792,2	±	998,8	
CR_{rt} (%)	16,7				231,9				31,4			
CR_{ws} (%)	2,2				32,1				4,9			
Cr_{gs} (%)	1,2				14,4				1,8			
Cr_{lf} (%)	1,1				16,0				1,7			
Plant organs and their respective sediment concentration ratios	Lead (n = 4)				Zinc (n = 4)				Manganese (n = 4)			
	x̄	±	SD	Range	x̄	±	SD	Range	x̄	±	SD	Range
Roots (RT	46,2	±	32,5	12 - 104	113,2	±	100,6	20 - 288	2230,8	±	2465,3	78 - 5922
White stems (WS)	27,7	±	24,6	9 - 76	56,8	±	45,1	9 - 134	872,0	±	889,4	69 - 2382
Green stems (GS)	11,7	±	5,6	8 - 23	33,9	±	25,7	5 - 76	304,5	±	265,3	17 - 717
Leaves (LF)	5,2	±	3,5	1 - 12	20,2	±	18,9	2 - 54	258,8	±	247,4	3 - 693
Sediments (SED)	124,0	±	123,9	17 - 364	214,2	±	161,4	29 - 480	2082,8	±	3710,8	44 - 9502
CR_{rt} (%)	37,2				52,9				107,1			
CR_{ws} (%)	21,8				26,5				41,9			
Cr_{gs} (%)	9,4				15,8				14,6			
Cr_{lf} (%)	4,1				9,4				12,4			

Table 5.8B Continued

Plant organs and their respective sediment concentration ratios	Chromium (n = 4)	
	$\bar{x}$ ± SD	Range
Roots (RT)	15,2 ± 10,5	03,3 - 33
White stems (WS)	1,1 ± 0,8	0,2 - 2
Green stems (GS)	0,7 ± 0,7	0,1 - 2
Leaves (LF)	0,2 ± 0,3	0 - 1
Sediments (SED)	84,5 ± 40,8	26 - 139
CR_{rt} (%)	17,9	
CR_{ws} (%)	1,3	
Cr_{gs} (%)	0,8	
Cr_{lf} (%)	0,2	

Table 5.9A **Mean values for selected chemical and physical parameters reflecting conditions in the water of the Elsburgspruit (localities 1, 2 and 3) and on a tributary (locality 4) during the different seasons of 1988.**

	SAMPLING LOCALITIES AND SEASONS							
	1				2			
Analysis	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**
Temperature °C	19	7	14	20	19	5	15	20
pH	4,2-5,0	5,0-5,4	3,9-6,6	3,6-3,7	4,4-6,0	5,0-6,9	5,0-6,5	4,8-6,9
Conductivity µS/cm	283	300	303	230	267	230	250	163
Alkalinity as $CaCO_3$ mg/ℓ	5,33	7,17	7,7	0	10,7	32,3	7,6	20,3
Total hardness as $CaCO_3$ mg/ℓ	2076	2137	2167	1503	1909	1435	1653	950
Sulphates (SO_4) mg/ℓ	2210	2227	1990	1527	1997	-	1480	970
Nitrate (NO_3) mg/ℓ	3,6	3,5	4,0	2,8	3,6	5,85	4,87	3,27
Total phosphate (PO_4) mg/ℓ	0	2,11	0	0,13	0,11	0,24	0,15	0,11
COD mg/ℓ	5	4	18	9,7	5	105	25,3	29,3
	3				4			
Analysis	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**
Temperature °C	16	4	11	18	18	5	15	20
pH	6,2-8,1	6,1-7,5	6,8-8,2	8,2-8,8	5,1-6,8	4,7-4,9	4,4-5,6	5,2-7,1
Conductivity µS/cm	136	203	173	55	166,7	208	208	153
Alkalinity as $CaCO_3$ mg/ℓ	94	86	86	170	8,17	2,33	2,17	11,5
Total hardness as $CaCO_3$ mg/ℓ	833	1225	1163	238	1033	1330	1317	930
Sulphates (SO_4) mg/ℓ	718	-	105	87	1040	1360	1445	930
Nitrate (NO_3) mg/ℓ	1,93	3,65	3,03	1,52	3,8	7,6	7,2	4,0
Total phosphate (PO_4) mg/ℓ	0,22	0	0,12	0	0,54	0,13	0	0,12
COD mg/ℓ	12	14	20	11	12	0	6,7	1,5

Table 5.9B Metal concentrations in the water (μg/l), the sediments (μg/g dry mass), *Typha capensis* and *Arundo donax* (μg/g dry mass) at four selected localities in the Elsburgspruit system under various pH environmental conditions, with an indication of changes in the percentage thereof at the different sampling localities.

	Zn		Mn		Ni		Fe	
Parameter	**Conc.**	**%**	**Conc.**	**%**	**Conc.**	**%**	**Conc.**	**%**
Locality 1								
Water	11,0	70,7	4900	89,4	1700	98,8	3800	32,5
Sediment	66,3	4,26	30,6	0,56	8,28	0,5	257	2,2
T. capensis	313,4	20,1	351,3	6,4	7,6	0,4	4534	38,8
A. donax	76,6	4,92	197,7	3,6	4,6	0,27	2106,6	26,6
Locality 2								
Water	670	59,6	4900	89,2	1300	97,8	1200	37,3
Sediment	90,9	8,1	36,4	0,66	19,6	1,5	225,7	7,0
T. capensis	294,6	26,2	438,7	8,0	2,66	0,2	467	14,5
A. donax	68,6	6,1	120,3	2,2	6,02	0,5	1328,2	41,2
Locality 3								
Water	310	20,9	1800	17,2	720	44	1200	26
Sediment	261,8	17,7	949	9,1	252	15,4	323,6	7
T. capensis	519,5	35	3666	35,1	81,9	5,0	1848	40
A. donax	391,2	26,4	4024	38,5	581,8	35,6	1247	27
Locality 4								
Water	850	74,6	6400	88,3	2800	99,2	3300	56,8
Sediment	47,3	4,2	29,5	0,4	6,97	0,23	180,9	3,1
T. capensis	77,4	6,8	510,2	7,0	2,5	0,1	1254	21,6
A. donax	164,4	14,4	310,8	4,3	12,4	0,44	1079	18,6

Table 5.10A Mean concentrations (µg/g dry mass) (x̄ ± SD) of metals in the organs of *Typha capensis* in the Cowles and Nigel dams, Blesbokspruit catchment during spring (1989).

Plant organs and their respective sediment concentration ratios	Metal concentrations in plant organs and sediments (µg/g) and their sediment concentration ratios (%)											
	Iron (n = 9)				Copper (n = 9)				Nickel (n = 9)			
	x̄	±	SD	Range	x̄	±	SD	Range	x̄	±	SD	Range
Roots (RT	2786,1	±	-		552,0	±	498,4	44 - 1706	183,8	±	174,7	8 0 544
White stems (WS)	479,0	±	614,2	63 - 1989	110,6	±	89,4	18 - 335	32,4	±	35,8	4 - 98
Green stems (GS)	146,3	±	197,4	23 - 663	34,4	±	25,0	8 - 73	13,9	±	13,6	0,7 - 45
Leaves (LF)	80,8	±	78,4	17 - 282	21,8	±	26,7	3 - 89	7,0	±	6,8	0,1 - 22
Sediments (SED)	29189,8	±	19017,1	6904 - 58052	290,1	±	670,0	10 - 2062	532,9	±	862,3	37 - 2592
CR$_{rt}$ (%)		9,5				190,2				34,5		
CR$_{ws}$ (%)		1,6				38,1				6,1		
Cr$_{gs}$ (%)		0,5				11,9				2,6		
Cr$_{lf}$ (%)		0,3				7,5				1,3		
Plant organs and their respective sediment concentration ratios	**Lead (n = 9)**				**Zinc (n = 9)**				**Manganese (n = 9)**			
	x̄	**±**	**SD**	**Range**	**x̄**	**±**	**SD**	**Range**	**x̄**	**±**	**SD**	**Range**
Roots (RT	59,4	±	39,3	14 - 147	207,6	±	146,3	24 - 525	2149,3	±	2823,8	87 - 8377
White stems (WS)	24,3	±	12,2	10 - 44	78,4	±	49,6	11 - 167	1114,3	±	1664,5	46 - 5383
Green stems (GS)	14,9	±	8,6	4 - 29	56,2	±	44,1	6 - 135	857,2	±	1239,0	19 - 4009
Leaves (LF)	7,9	±	3,3	3 - 13	30,2	±	21,2	3 - 60	714,2	±	1051,9	10 - 3381
Sediments (SED)	94,4	±	105,3	17 - 364	244,4	±	175,8	29 - 500	1423,1	±	3055,4	44 - 9502
CR$_{rt}$ (%)		62,9				84,9				151,0		
CR$_{ws}$ (%)		25,7				32,0				78,3		
Cr$_{gs}$ (%)		15,8				23,0				60,2		
Cr$_{lf}$ (%)		8,4				12,4				50,1		

Table 5.10A Continued

Plant organs and their respective sediment concentration ratios	Chromium (n = 9)			
	$\bar{x}$	±	SD	Range
Roots (RT	5,2	±	5,1	1 - 15
White stems (WS)	0,7	±	0,5	0,1 - 2
Green stems (GS)	0,10			0 - 0,1
Leaves (LF)				
Sediments (SED)	77,3	±	33,9	26 - 139
CR_{rt} (%)		6,7		
CR_{ws} (%)		0,9		
Cr_{gs} (%)		0,1		
Cr_{lf} (%)				

Table 5.10B Mean concentrations (µg/g dry mass) (x ± SD) of metals in the organs of *Typha capensis* in nine localities on the Cowles and Nigel dams, Blesbokspruit catchment during spring (1989).

Plant organs and their respective sediment concentration ratios	Metal concentrations in plant organs and sediments (µg/g) and their sediment concentration ratios (%)											
	Iron (n = 9)				Copper (n = 9)				Nickel (n = 9)			
	x̄	±	SD	Range	x̄	±	SD	Range	x̄	±	SD	Range
Roots (RT	4747,1	±	1938,6	1918 - 7066	2298,9	±	812,2	1204 - 3407	267,0	±	147,5	47 - 528
White stems (WS)	674,7	±	777,2	85 - 2531	568,1	±	747,0	50 - 2391	41,9	±	35,2	6 - 130
Green stems (GS)	302,4	±	352,8	52 - 1011	163,2	±	192,6	15 - 640	33,7	±	26,5	6 - 99
Leaves (LF)	327,5	±	357,1	73 - 1236	158,4	±	84,1	70 - 323	34,1	±	13,8	9 - 57
Sediments (SED)	31374,0	±	19279,2	7528 - 59598	486,4	±	780,0	87 - 2440	780,2	±	1096,6	104 - 2895
CR_{rt} (%)		15,1				472,6				34,2		
CR_{ws} (%)		2.2				116,8				5,4		
Cr_{gs} (%)		1,0				33,6				4,3		
Cr_{lf} (%)		1,0				32,6				4,4		

Plant organs and their respective sediment concentration ratios	Lead (n = 9)				Zinc (n = 9)				Manganese (n = 9)			
	x̄	±	SD	Range	x̄	±	SD	Range	x̄	±	SD	Range
Roots (RT	60,2	±	34,7	13 - 139	442,6	±	230,1	168 - 857	2636,5	±	4568,5	321 - 14628
White stems (WS)	26,5	±	14,8	10 - 50	181,6	±	118,2	70 - 387	788,3	±	669,0	274 - 2416
Green stems (GS)	25,5	±	13,4	12 - 51	138,8	±	110,9	44 - 342	866,0	±	683,9	331 - 2483
Leaves (LF)	17,8	±	9,4	7 - 37	90,3	±	40,1	47 - 156	1087,1	±	860,7	414 - 3187
Sediments (SED)	59,4	±	90,1	0 - 289	583,8	±	441,3	66 - 1630	693,7	±	1122,2	105 - 3644
CR_{rt} (%)		101,3				75,8				380,1		
CR_{ws} (%)		44,6				31,1				113,6		
Cr_{gs} (%)		42,9				23,8				124,8		
Cr_{lf} (%)		30,0				15,5				156,7		

Table 5.10B Continued

Plant organs and their respective sediment concentration ratios	Chromium (n = 9)			
	$\overline{x}$	±	SD	Range
Roots (RT	8,9	±	9,2	2 - 29
White stems (WS)	1,4	±	1,4	0,1 - 5
Green stems (GS)	0,5	±	0,9	0,1 - 3
Leaves (LF)	0,4	±	0,3	0,1 - 1
Sediments (SED)	187,6	±	111,5	64 - 378
CR_{rt} (%)		4,7		
CR_{ws} (%)		0,7		
Cr_{gs} (%)		0,3		
Cr_{lf} (%)		0,2		

Table 5.11 Bimonthly mean metal concentration ($\bar{x}$ ± SD) (μg/g dry mass) and concentration ratios for the water (CR_w %) and sediments (CR_s %) of *Lagarosiphon* spp. in the Florida Lake, Gauteng, during March 1990 - March 1991.

Metal		Period of survey and mean metal concentration ($\bar{x}$ ± SD) expressed as μg/l (water) or μg/g dry mass (sediment)			
		March	May	July	September
		n = 15	n = 15	n = 15	n = 15
Cu	$\bar{x}$ ± SD	14,6 ± 3,4	19,7 ± 7,0	15,7 ± 2,3	27,3 ± 13,1
	CR_w (%)	99,3	153,9	54,5	60,7
	CR_s (%)	51,0	66,3	54,5	98,6
Mn	$\bar{x}$ ± SD	1637,8 ± 973,3	2561,1 ± 366,9	1365,3 ± 22,4	1285,4 ± 152,7
	CR_w (%)	1623,2	2328,3	10922,4	2014,6
	CR_s (%)	1389,1	2561,1	574,6	1104,2
Fe	$\bar{x}$ ± SD	5186,9 ± 2274,9	8191,7 ± 4883,8	5193,3 ± 1605,9	4967,3 ± 2224,7
	CR_w (%)	220,2	247,5	275,9	555,8
	CR_s (%)	50,5	51,5	31,2	98,3
Ni	$\bar{x}$ ± SD	42,8 ± 10,5	72,1 ± 12,6	50,0 ± 5,6	49,3 ± 14,2
	CR_w (%)	84,6	51,0	25,8	74,4
	CR_s (%)	42,6	97,6	69,8	124,2
Cr	$\bar{x}$ ± SD	70,5 ± 26,9	52,1 ± 22,5	13,7 ± 8,4	84,0 ± 56,2
	CR_w (%)	58,4	27,9	5,0	413,8
	CR_s (%)	41,4	25,1	15,8	49,8
Zn	$\bar{x}$ ± SD	114,7 ± 19,3	163,9 ± 73,7	128,0 ± 19,0	198,0 ± 52,6
	CR_w (%)	361,8	596,0	408,9	193,2
	CR_s (%)	66,1	85,0	89,3	192,0
Pb	$\bar{x}$ ± SD	40,9 ± 4,5	70,9 ± 24,7	45,3 ± 6,6	93,0 ± 39,6
	CR_w (%)	20,6	31,9	14,3	36,8
	CR_s (%)	45,1	69,0	22,6	139,6

Table 5.11 Continued

Metal		November	January	March	x ± SA
		n = 15	n = 15	n = 15	n = 7
Cu	$\bar{x}$ ± SD	23,5 ± 11,9	20,4 ± 6,7	17,1 ± 7,0	19,8 ± 4,5
	CR$_w$ (%)	98,7	105,1	159,8	104,6 ± 40,8
	CR$_s$ (%)	111,9	94,0	64,5	77,3 ± 23,9
Mn	$\bar{x}$ ± SD	1207,3 ± 368,7	1477,8 ± 901,0	1709,4 ± 377,4	1606,3 ± 458,3
	CR$_w$ (%)	2541,7	2701,6	3481,5	3659,0 ± 3255,1
	CR$_s$ (%)	1063,7	1075,5	1419,8	1312,6 ± 616,6
Fe	$\bar{x}$ ± SD	6518,3 ± 1607,2	6373,9 ± 1207,4	6077,2 ± 333,7	6086,9 ± 1109,9
	CR$_w$ (%)	1694,8	354,7	622,4	567,3 ± 520,8
	CR$_s$ (%)	102,6	81,6	62,6	68,3 ± 26,7
Ni	$\bar{x}$ ± SD	321,3 ± 72,0	100,7 ± 14,2	98,7 ± 5,6	105,0 ± 98,3
	CR$_w$ (%)	1020,0	486,5	309,4	293,1 ± 362,3
	CR$_s$ (%)	149,9	73,1	81,8	91,3 ± 36,1
Cr	$\bar{x}$ ± SD	143,0 ± 98,3	127,4 ± 21,7	90,6 ± 7,7	83,0 ± 43,9
	CR$_w$ (%)	165,5	142,9	99,1	130,4 ± 137,9
	CR$_s$ (%)	136,6	93,4	60,8	72,9 ± 52,8
Zn	$\bar{x}$ ± SD	177,3 ± 27,4	150,3 ± 50,3	121,3 ± 37,4	150,5 ± 31,1
	CR$_w$ (%)	78,82	260,5	301,0	314,3 ± 165,2
	CR$_s$ (%)	241,2	215,6	75,8	137,9 ± 75,1
Pb	$\bar{x}$ ± SD	43,0 ± 3,7	41,7 ± 6,6	37,4 ± 4,3	55,2 ± 20,8
	CR$_w$ (%)	17,6	21,9	20,1	23,3 ± 8,1
	CR$_s$ (%)	73,6	62,0	47,4	65,6 ± 36,9

Table 5.12 Bimonthly mean metal concentration ($\bar{x}$ ± SD) and concentration ratios for the water (CR_w %) and sediments (CR_s %) of *Potamogeton pectinatus* in the Florida Lake, Gauteng, during March 1990 - March 1991. Results expressed as µg/l (water) and µg/g dry mass (sediment).

Metal		Metal concentrations (x ± SD) and concentration ratios (CR) expressed as %			
		March	May	July	September
		n = 20	n = 20	n = 20	n = 20
Cu	$\bar{x}$ ± SD	11,9 ± 2,3	20,9 ± 7,4	25,4 ± 8,1	23,0 ± 4,9
	CR_w (%)	80,9	163,3	88,2	51,1
	CR_s (%)	42,3	70,4	88,2	83,0
Mn	$\bar{x}$ ± SD	1175,0 ± 207,1	2422,5 ± 101,9	1516,0 ± 409,9	1244,4 ± 169,1
	CR_w (%)	1164,5	2202,3	12135,2	1950,5
	CR_s (%)	996,6	2422,5	638,4	1069,1
Fe	$\bar{x}$ ± SD	2597,5 ± 370,7	9434,4 ± 860,2	5883,0 ± 1259,7	5587,0 ± 536,4
	CR_w (%)	108,2	285,0	312,5	625,1
	CR_s (%)	24,8	59,3	35,3	110,5
Ni	$\bar{x}$ ± SD	39,5 ± 9,7	69,5 ± 17,7	44,1 ± 4,7	41,4 ± 9,7
	CR_w (%)	77,9	49,2	22,8	62,4
	CR_s (%)	39,4	94,0	61,6	104,3
Cr	$\bar{x}$ ± SD	76,1 ± 6,7	69,5 ± 17,7	18,7 ± 8,9	51,9 ± 25,9
	CR_w (%)	63,0	37,3	6,9	255,7
	CR_s (%)	44,7	33,5	21,6	30,8
Zn	$\bar{x}$ ± SD	82,5 ± 26,2	134,4 ± 58,6	241,2 ± 75,1	161,3 ± 63,6
	CR_w (%)	260,3	488,7	770,6	157,4
	CR_s (%)	47,6	69,7	168,3	156,5
Pb	$\bar{x}$ ± SD	31,9 ± 7,0	65,3 ± 21,9	61,6 ± 19,5	56,7 ± 9,7
	CR_w (%)	16,0	29,3	19,4	22,5
	CR_s (%)	35,2	63,5	30,7	85,1

Table 5.12 Continued

Metal		November	January	March	$\bar{x}$ ± SA
		n = 20	n =205	n = 20	n = 7
Cu	$\bar{x}$ ± SD	21,8 ± 7,9	19,7 ± 7,9	13,9 ± 3,4	19,5 ± 4,9
	CR_w (%)	91,6	101,5	129,9	100,9 ± 36,2
	CR_s (%)	103,8	90,8	52,5	75,9 ± 22,0
Mn	$\bar{x}$ ± SD	1039,1 ± 226,8	1147,9 ± 207,1	1204,3 ± 300,9	1392,7 ± 477,1
	CR_w (%)	2187,6	2098,5	2452,7	3455,9 ± 3848,7
	CR_s (%)	915,5	835,4	1000,2	1125,4 ± 589,2
Fe	$\bar{x}$ ± SD	4304,6 ± 1953,8	3741,3 ± 70,9	2470,5 ± 1134,7	5894,8 ± 2411,4
	CR_w (%)	1119,2	208,2	253,0	415,9 ± 348,9
	CR_s (%)	27,7	47,9	25,5	53,0 ± 30,1
Ni	$\bar{x}$ ± SD	41,7 ± 7,7	36,7 ± 9,7	40,1 ± 4,7	44,7 ± 11,2
	CR_w (%)	132,4	177,3	125,7	92,5 ± 54,4
	CR_s (%)	19,4	26,6	33,2	54,1 ± 33,6
Cr	$\bar{x}$ ± SD	37,7 ± 8,94	43,1 ± 10,4	56,4 ± 6,3	50,5 ± 19,5
	CR_w (%)	43,6	48,4	61,7	73,8 ± 82,4
	CR_s (%)	36,0	31,6	37,8	33,7 ± 7,1
Zn	$\bar{x}$ ± SD	69,3 ± 50,1	75,3 ± 31,7	79,2 ± 13,8	120,5 ± 63,6
	CR_w (%)	30,8	130,5	196,5	290,7 ± 255,0
	CR_s (%)	94,3	108,0	49,5	99,1 ± 48,6
Pb	$\bar{x}$ ± SD	42,4 ± 14,4	37,6 ± 3,1	34,8 ± 7,1	47,2 ± 13,7
	CR_w (%)	17,4	19,7	18,7	20,4 ± 4,4
	CR_s (%)	72,6	55,9	44,1	55,3 ± 20,0

Table 5.13 Bimonthly mean metal concentrations (x̄ ± SD) (µg/g dry mass) of *Nymphaea* cf *spectabilis* in the Florida Lake, Gauteng, during March 1990 - March 1991. Results expressed as µg/l (water) and µg/g dry mass (sediment).

Metal		Period of survey and mean metal concentrations (x ± SD) expressed as µg/g dry mass											
		March			May			July			September		
		n = 8			n = 8			n = 8			n = 8		
Cu	x̄ ± SD	13,5	±	9,4	11,6	±	11,0	9,3	±	11,1	9,4	±	3,9
Mn	x̄ ± SD	176,6	±	108,0	196,0	±	122,2	197,6	±	160,9	126,2	±	40,7
Fe	x̄ ± SD	7384,2	±	4819,6	7743,8	±	10038,3	6461,1	±	6171,9	2446,8	±	1130,2
Ni	x̄ ± SD	186,0	±	94,3	140,8	±	99,2	41,1	±	27,2	32,6	±	33,7
Cr	x̄ ± SD	144,9	±	159,6	147,8	±	190,7	47,7	±	8,8	15,3	±	13,0
Zn	x̄ ± SD	109,7	±	34,8	118,0	±	42,9	64,9	±	41,3	70,6	±	20,8
Pb	x̄ ± SD	68,3	±	37,3	60,4	±	37,1	110,8	±	27,4	33,8	±	8,9
		November			January			March			x		± SD
		n = 8			n = 8			n = 8			n = 7		
Cu	x̄ ± SD	8,2	±	3,2	11,2	±	9,0	10,9	±	8,9	10,6	±	1,8
Mn	x̄ ± SD	154,1	±	95,5	158,1	±	74,7	168,1	±	97,4	168,1	±	25,1
Fe	x̄ ± SD	2438,3	±	1754,5	4313,7	±	5165,9	7564,2	±	8177,0	5478,9	±	2375,9
Ni	x̄ ± SD	217,8	±	32,4	159,7	±	59,7	179,5	±	55,6	136,5	±	72,2
Cr	x̄ ± SD	14,5	±	5,0	124,0	±	110,5	147,3	±	142,7	91,6	±	63,0
Zn	x̄ ± SD	3,7	±	1,0	82,8	±	50,1	102,4	±	33,2	78,9	±	38,7
Pb	x̄ ± SD	26,7	±	5,6	61,6	±	38,7	68,1	±	34,2	61,4	±	27,4

Table 5.14 Metal concentrations in the tissues of *Lemna gibba* (μg/g dry mass), the stream sediments (μg/g) and water (μg/l) during the early summer of 1990 at two localities on the Cowles dam in the catchment of the Blesbokspruit with an indication of the metal concentration ratios for the sediments (CR_s %) and water (CR_w %).

Parameter	Metal concentrations in *Lemna* (μg/g), sediments (μg/g) and water (μg/l)													
	Fe		Cu		Ni		Pb		Zn		Mn		Cr	
	1	4	1	4	1	4	1	4	1	4	1	4	1	4
Lemna	722	3368	281	113	478	421	295	75	573	399	4803	7145	63	41
Sediment	39069	38051	2193	42	2231	87	368	61	373	48	308	468	139	63
CR_s %	1,8	5,8	12,8	265,4	21,4	485,1	80,0	123,1	183,1	837,2	1557	1526	45,6	64,7
Water	330	360	30	80	80	140	90	110	140	130	400	40	a*	100
CR_w %	218,8	935,4	938,1	140,9	597,9	300,9	327,5	68,1	409,5	306,6	1201	17863	0	40,6

Table 5.15 Values (µg/g dry mass) obtained for the different metals in *Azolla filiculoides* and the bottom sediments with concentration ratio (CR) values at the four different localities in the Blesbokspruit wetlands.

		Localities				
Metals	**Parameters**	**1**	**2**	**3**	**4**	**mean**
		$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD
Iron (µg/g)	*Azolla*	2 455 ± 68	7 906 ± 861	1 893 ± 115	4 710 ± 41	4 241 ± 2 364
	Sediment	59 598	45 650	23 898	7 528	34 169 ± 19 960
	CR (%)	4	17	8	63	23 ± 23
Copper (µg/g)	*Azolla*	93 ± 4	67 ± 9	37 ± 1	60 ± 4	64 ± 20
	Sediment	91	94	89	97	93 ± 3
	CR (%)	102	72	42	62	70 ± 22
Nickel (µg/g)	*Azolla*	80 ± 4	168 ± 15	189 ± 11	89 ± 6	131 ± 48
	Sediment	118	137	170	232	164 ± 43
	CR (%)	67	123	111	38	85 ± 26
Lead (µg/g)	*Azolla*	120 ± 14	119 ± 14	121 ± 13	109 ± 0,3	117 ± 5
	Sediment	51	39	6	0	24 ± 21
	CR (%)	237	308	1 883	0	607 ± 745
Zinc (µg/g)	*Azolla*	49 ± 2	109 ± 5	91 ± 11	120 ± 1	92 ± 27
	Sediment	66	376	415	613	368 ± 196
	CR (%)	74	29	22	20	36 ± 22
Manganese (µg/g)	*Azolla*	3 746 ± 100	3 679 ± 257	5 535 ± 13	5 459 ± 32	4 607 ± 893
	Sediment	390	307	242	143	271 ± 90
	CR (%)	959	1199	2 287	3 814	2 065 ± 1 127
Chromium (µg/g)	*Azolla*	14 ± 1	21 ± 3	7 ± 0,3	4 ± 0,2	12 ± 7
	Sediment	129	128	98	94	112 ± 16
	CR (%)	11	17	7	4	10 ± 5
Sediment organic	content (%)	0,8	6,9	1,2	3,6	3,1 ± 2,4

Table 5.16 Estimated densities of *Azolla filiculoides* in t/ha expressed as dry mass with an indication of the quantities of the different metals accumulated by this plant at the four localities in the Blesbokspruit wetlands.

Metals in kg per ton dry mass (a) : per hectare (b)																
Localities	g/m^2 dry mass	t/ha dry mass	Fe		Cu		Ni		Pb		Zn		Mn		Cr	
			a	b	a	b	a	b	a	b	a	b	a	b	a	b
1	655,85	6,56	2,45	16,10	0,09	0,61	0,08	0,52	0,12	0,79	0,05	0,32	3,75	24,57	0,01	0,09
2	77,00	0,77	7,91	6,09	0,07	0,05	0,17	0,13	0,12	0,09	0,11	0,08	3,68	2,83	0,02	0,02
3	224,90	2,25	1,89	4,26	0,04	0,08	0,19	0,42	0,12	0,27	0,09	0,21	5,54	12,45	0,007	0,02
4	968,10	9,68	4,71	45,60	0,06	0,58	0,09	0,86	0,11	1,06	0,12	1,16	5,46	52,85	0,004	0,04

6. BIO-ACCUMULATION OF METALS BY THE FRESHWATER CRAB *POTAMONAUTES WARRENI* FROM THE NATALSPRUIT WETLAND

6.1 Introduction

Numerous experimental studies have been made of the mechanisms involved in the bio-accumulation or regulation of heavy metals in marine decapods, e.g. *Cancer pagurus* (Guary & Negrel, 1980), *Carcinus maenas* (Miramand, *et al.*, 1981; Rainbow 1985; Zatta, *et al.*, 1985), *Scylla serrata* (Krishnaja, *et al.*, 1987), *Uca triangularis* and *U. annulipes* (Devi, 1987), *Palaemon elegans* (Rainbow & White, 1989) and *Lysmata seticaudata* (Miramand *et al.*, 1981). However, comparatively little information is available in published literature on metals in freshwater decapods. Research by Bryan (1966) on the metabolism of zinc by the freshwater crayfish *Austropotamobius pallipes pallipes* demonstrated the differences which may exist between marine and freshwater decapods in the absorption and accumulation of heavy metals.

According to Barnard (1950) there are at least ten species of the decapod *Potamonautes* inhabiting inland waters in southern Africa. All these species occur mainly amongst stones-in-current habitats and in pools where they may shelter under stones. Most of them appear to be scavengers, also taking live insects, amphibian larvae and small fish as food. In the wetland region of the Natalspruit (Figure 2.3), which receives nutrient-rich treated sewage effluents as well as effluents from mines and industries containing high loads of heavy metals, the freshwater crab *P. warreni* commonly occurs amongst the other benthic macro-invertebrate fauna, dominating these organisms in size and biomass. Investigations also showed that considerable quantities of heavy metals can be accumulated in the various components of the food chain (Van Eeden, 1989) in this particular wetland region.

Since it was necessary to obtain sufficient material to analyse for at least seven metals, use was made of the whole body of the crabs as a first phase of this ongoing investigation. This was followed at a later stage by a more detailed analysis of the accumulation of certain heavy metals by the various organs and tissues of this decapod (Du Preez *et al.*, 1993; Steenkamp *et al.*, 1993, 1994A,B). The approach of whole body analysis for heavy metals in aquatic organisms has, in fact, been advocated by Goodyear & Boyd (1972), Davis & Boyd (1978) and Vinikour, Goldstein & Anderson (1980), and provides, among other data, important information for the preparation of nutrient budgets and for the study of biochemical and pollutant cycles in a particular ecosystem. This approach also highlights information on the actual mechanisms of metal uptake as well as the bio-concentration and regulation of heavy metals by the bodies of whole organisms. The pooling of the crabs collected from each locality and the homogenization of the composite samples before analysis provided adequate material for replicate AAS analysis of the heavy metals. A more representative picture of the metal loads accumulated by *P. warreni* at the given localities in this wetland region was also obtained in this way.

6.2 Materials and methods

Six localities were selected for the collection of sediments and crabs. Locality 1 (Fig. 2.5) was situated near the upper end of an expanded wetland, stretching over a length of almost 8 km, covered mainly by the giant or Spanish reed *Arundo donax* and the bullrush *Typha capensis*. In the sections where stream flow was noticeable, the stream bed was usually covered by the submerged plants *Potamogeton pectinatus* and species of *Lagarosiphon*, which included *L. major* and to a lesser extent *L. muscoides*. Pools or slow-flowing backwaters found within the marshes were usually covered by dense mats of duckweed *Lemna gibba*. At this point the stream already contained metals originating upstream from effluents of mines and industries bordering the headwaters of the Elsburgspruit (Schoonbee & Van der Merwe 1989). In addition to the heavy metal loads, this water also contained discharges of treated sewage effluent from a nearby sewage purification works, upstream from this locality.

Localities 2A and 2B, which were approximately 120 m apart, covered both sides of the wetlands in the same area, but nearly 7 km downstream from Locality 1. In the case of Locality 2A the stream consisted mainly of the water flowing from Locality 1, whereas Locality 2B usually received intermittant discharges of partly treated sewage effluent used for irrigation along the west bank of the wetland as well as water from stormwater drains originating in the nearby Katlehong township, directly upstream from this locality. Locality 3 was situated nearly 200 m downstream from Localities 2A and 2B, after the confluence of their waters. Locality 4 was situated 1,3 km downstream from Locality 3, on the outflow canal from the last maturation pond of a second sewage purification works, just before it joins the stream. In this canal, which forms a natural riverbed, the highly eutrophic, but otherwise good quality effluent, flowed largely over stones-in-the-current, creating ideal habitats and feeding grounds for *P. warreni*. Locality 5 was situated approximately 300 m downstream from Locality 4, at the lower end of this wetland system where normal open riverine conditions prevailed. Habitat types such as stony runs and stickles (Allen, 1951) commonly occurred here, providing ample shelter and feeding grounds for the crabs.

In total, 22 crabs were randomly collected at the six different localities (Table 6.1). The mean wet mass of the 22 crabs analysed, fluctuated between 90,76 g and 323,89 g with corresponding dry mass values of 32,07 g and 116,10 g. The crabs were frozen directly after collection and kept frozen until analysed. Before analysis all crabs were rinsed in tap water to dislodge loose particles and commensal organisms such as leeches, which were at times found to be associated with the crabs. Moisture was carefully removed from the crabs using paper towels, before each specimen was weighed and individually oven-dried at 100°C until the dry mass of the material stabilized, after which the dry mass was determined. As mentioned, all the crabs collected at each locality were pooled and then pulverized using a plastic pestle and mortar. Approximately 1 g of each sample was accurately weighed and then digested at 200°C using a mixture of 1:1 v/v concentrated nitric and perchloric acid. Digestion took place for approximately 2 h. The individual solutions containing the digested material were allowed to cool, before being filtered through acid-resistant 0,45 μm filter paper. These filter papers were finally rinsed with hot distilled water to remove all traces of the heavy metals. The samples were accurately made up to 100 ml volumes using distilled water. Metal contents of the different samples were determined using a Varian Model AA 875 atomic absorption spectrophotometer.

The heavy metal concentrations in the stream sediments were considered to be a more representative reflection of the compounded effects of long-term metal contamination of the stream ecosystem at a given locality than the water samples, which were found to vary considerably in metal content, depending upon the time of sampling and the periodic release of metal-containing effluents into this stream from mines, industries and sewage purification works. For this reason only the metal content in the sediments was considered for comparison with the metal accumulated by *P. warreni* at each of the different localities.

Sediment samples were collected at each locality, using a "Perspex" core sampler to a mean depth of approximately 55 mm. These samples were oven-dried at 100°C for five days. The dried samples were then pulverized with a plastic pestle and mortar, after which a 1 g subsample was digested as described for the crabs. After thoroughly rinsing the filter paper following digestion, the undigested remains of each sample were dried, weighed, and the mass in each case subtracted from that of the pre-digested sample. This provided an assessment of the actual mass of the digested organic material present in each sample. Metal concentrations in mg/l were determined on the AAS. These results were transformed to µg/g dry mass for both the crab and sediment samples.

6.3 Results

The concentrations of the metals accumulated by the crabs and their respective concentrations in the sediments, as well as their bio-concentration ratio (CR - expressed as a percentage value), for all the metals under investigation, at each of the six localities, are presented in Table 6.1.

6.3.1 Sediments

Based on their respective concentrations in the sediments, the sequence of importance of the seven heavy metals can be arranged in the following order: Pb < Cu < Cr < Ni < Zn < Mn < Fe. The highest concentration of Pb in the sediments occurred at Locality 1 (210,7 µg/g). A decline in the concentrations of Pb were observed at Localities 2A, 2B and 3 (Table 6.1). At Locality 5, downstream of the effluent discharged from the second purification works (Locality 4, Fig. 2.3), the sediment concentration value of Pb rose to 48,9 µg/g. This increase can most likely be ascribed to the additional loads of Pb occurring in the effluents discharged into the stream at Locality 4, which had a sediment concentration of 45,6 µg/g. The highest concentration of Ni in the sediments also occurred at Locality 1 (1 073,6 µg/g), followed by a successive reduction in the Ni concentrations at the other downstream localities (Table 6.2).

Like the previous metals referred to, the highest concentration of Zn in the sediments again occurred at Locality 1 (1 047,1 µg/g). This metal showed a marked decline in concentration at Localities 2A, 2B and 3 (Table 6.1). An increase in the Zn concentration to 178,1 µg/g at Locality 5 could be ascribed to the possible cumulative effects of higher loads of Zn found in the effluents discharged from the second sewage purification works sampled at Locality 4 (Table 6.1, Fig. 2.3).

From a concentration of 134,4 μg/g at Locality 1, sediment-bound Cr increased further in concentration at Localities 2A and 2B. At Locality 3 there was a decline in the Cr concentration to 106,5 μg/g. However, higher concentrations of Cr were again found in the sediments sampled at Localities 4 and 5.

There was a marked decrease in the sediment-bound Fe concentration between Localities 1 and 2A. However, the Fe concentration was much higher at Localities 2B and 3. The dilution effects of the effluent from the second sewage purification works sampled at Locality 4 (Fig. 2.3), which contained a much lower Fe concentration in the sediments, may have resulted in the lower value of Fe found in the sediment sampled at Locality 5. Values for Mn in the sediments were generally high (Table 1). There was considerable fluctuation in the concentrations of this metal at the various localities. A marked decline in the concentration of Mn occurred in the sediments between Localities 1 and 2A and 2B, respectively. The further increase of Mn concentrations in the sediments downstream from Locality 3 cannot be explained, nor the relatively low concentration of 1 301,7 μg/g recorded at Locality 5, if the much higher Mn concentration found in the sediments at Locality 4 is taken into consideration. However, the origin of Fe and Mn can be related to the effects of the gold-mining activities on the stream water (Murray, 1987).

Cu also occurred in the highest concentration in the sediments at Locality 1 but declined towards Localities 2A and 2B (Table 6.1). There was an increase in the Cu concentration ion the sediments towards Locality 3. The sediments at Locality 4, receiving the effluent of the second sewage purification works (Fig. 2.3), had a Cu concentration value of 93,3 μg/g, and this value was higher than that found in the sediments of the stream itself, excluding Locality 1. Concentrations of this metal declined rapidly to 44,3 μg/g at Locality 5.

6.3.2 Crabs

Table 6.1 shows that all seven metals investigated occurred in the sediments in measurable but variable quantities. In *P. warreni* all seven metals also occurred in measurable quantities with the sequence of bio-accumulation by the crab being Cr < Pb < Ni < Cu < Zn < Fe < Mn.

The bio-concentration ratio (CR) for each metal found in the crab, expressed as a percentage of the concentration of the heavy metal deposited in the sediments, showed a CR value of approximately 7% for Pb at Localities 1, 2A, 2B and 4. At Localities 3 and 5, however, there was a dramatic increase in the CR of Pb of up to 30%. As with Pb, Ni was also bio-accumulated by the crabs in low concentrations at Localities 1 and 2B, resulting in comparatively low CR values (Table 6.1). The CR for Ni increased substantially to 80,5% at Locality 2A. At Localities 3 and 5 the Ni concentrations in the crabs exceeded the concentrations of this metal in the sediments, resulting in CR values larger than 100% (Table 6.1). Even in the effluent from the second sewage purification works (Locality 4), the concentration of Ni bio-accumulated by *P. warreni* approached that of the Ni concentration found in the sediments.

In the case of Zn, the lowest CR for this metal occurred at Locality 1 (13,2%). The CR values for zinc then increased at the other downstream localities with a peak of 97,5% at Locality 3 (Table 1), but then declined to 57% at Locality 5. However, at Locality 4, the concentration of Zn accumulated by the crab exceeded that in the sediments, resulting in a CR value of 108%. Cr showed the lowest CR of all the metals in the crab, with values of less than 7% recorded at Localities 1, 2A, 2B, 3 and 4, but reaching a high of 21% at Locality 5.

Despite the exceptionally high concentrations of Fe in the sediments and in the crabs, the CR values of Fe were the lowest for all the metals under investigation, fluctuating between 0,6% at Locality 2B and 3,5% at Locality 4. It must be pointed out, however, that the actual bio-accumulation of Fe by *P. warreni* was, with the exception of Mn, the highest of all the metals under investigation, with a maximum of 1 068,8 μg/g recorded at Locality 3 (Table 6.1). Mn occurred in appreciable concentrations in the sediments where it was bio-accumulated by *P. warreni* at high levels at most localities. The CR for this metal in the crab varied between 24% at Locality 1 and 151% at Locality 2A. Although the concentrations of Cu in the sediments at the different localities were never exceptionally high, the bio-accumulation of Cu by *P. warreni* appeared to be the highest in relation to the concentrations of this metal in the sediments (Table 6.1), exceeding the latter at three (2A, 2B, 5) of the six localities. The CR values for Cu varied between 75% (Locality 2) and 166% (Locality 2A), respectively.

With the exception of Cr and Cu, a poor correlation existed for all the other metals between the concentrations in the sediments and their corresponding CR values in *P. warreni*. Cr showed a negative correlation (r = -0,814), while in the case of Cu a positive correlation (r = 0,829) existed.

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Table 6.1 Bio-accumulation of metals ($\bar{x}$ ± SE; n = 10) by *Potamonautes warreni* compared with the metal concentrations ($\bar{x}$ ± SE; n = 10) in the sediments at six localities in the Natalspruit wetland area polluted by mine, industrial and sewage effluents. CR = concentration ratio.

Locality	Parameter	Lead (µg/g)	Nickel (µg/g)	Zinc (µg/g)	Chromium (µg/g)	Iron (µg/g)	Manganese (µg/g)	Copper (µg/g)
1	Crab	13,9 ± 0,06	58,6 ± 0,06	138,3 ± 0,01	5,0 ± 0,06	503,5 ± 0,17	1390,3 ± 0,01	99,3 ± 0,03
	Sediment	210,7 ± 0,04	1973,6 ± 0,05	1047,1 ± 0,05	134,3 ± 0,01	45519,3 ± 0,12	5693,1 ± 0,01	132,1 ± 0,01
	CR(%)	6,6	5,5	13,2	3,7	1,1	24,4	75,2
2A	Crab	4,0 ± 0,06	30,9 ± 0,06	73,3 ± 0,03	2,0 ± 0,01	158,7 ± 0,08	622,9 ± 0,06	76,9 ± 0,01
	Sediment	59,3 ± 0,07	38,4 ± 0,04	107,2 ± 0,01	185,7 ± 0,04	21768,1 ± 0,16	413,5 ± 0,01	46,3 ± 0,03
	CR(%)	6,8	80,5	68,4	1,1	0,73	150,6	166,1
2B	Crab	4,0 ± 0,05	19,9 ± 0,05	82,0 ± 0,05	3,0 ± 0,01	239,2 ± 0,07	568,0 ± 0,03	81,7 ± 0,02
	Sediment	55,6 ± 0,04	59,0 ± 0,06	259,9 ± 0,01	151,9 ± 0,06	38525,4 ± 0,13	1526,9 ± 0,02	63,1 ± 0,03
	CR(%)	7,2	33,7	31,6	2,0	0,62	37,2	129,5
3	Crab	11,0 ± 0,04	46,9 ± 0,07	103,1 ± 0,04	7,0 ± 0,01	1068,8 ± 0,16	1675,0 ± 0,02	84,7 ± 0,01
	Sediment	36,4 ± 0,04	33,1 ± 0,05	105,7 ± 0,04	106,5 ± 0,04	59808,1 ± 0,29	3830,6 ± 0,02	88,7 ± 0,04
	CR(%)	30,2	141,7	97,5	6,6	1,8	43,7	95,5
4	Crab	2,0 ± 0,06	34,6 ± 0,05	119,0 ± 0,02	3,0 ± 0,01	602,9 ± 0,14	1098,8 ± 0,01	81,2 ± 0,01
	Sediment	46,6 ± 0,03	36,3 ± 0,04	110,3 ± 0,02	124,7 ± 0,04	17166,5 ± 0,11	3223,2 ± 0,01	93,3 ± 0,05
	CR(%)	4,4	95,3	107,9	2,4	3,5	34,1	87,0
5	Crab	12,0 ± 0,03	52,0 ± 0,04	100,9 ± 0,06	23,0 ± 0,02	392,7 ± 0,08	1259,0 ± 0,02	69,9 ± 0,01
	Sediment	48,9 ± 0,04	35,2 ± 0,06	178,1 ± 0,01	110,8 ± 0,05	18917,7 ± 0,33	1301,7 ± 0,02	44,3 ± 0,02
	CR(%)	24,5	147,7	56,7	20,8	2,1	96,7	157,8

7. BIO-ACCUMULATION OF METALS BY THE SOUTHERN MOUTHBROODER *PSEUDOCRENILABRUS PHILANDER* AND THE EUROPEAN PERCH, *PERCA FLUVIATILIS* FROM TWO MINE POLLUTED WATERS IN THE GAUTENG PROVINCE

7.1 Introduction

Effluents from gold mines and metal-processing industries contributed greatly to the acidification and metal contamination of streams and lakes in the Gauteng Province, South Africa (Harrison, 1961; Schoonbee and Van der Merwe, 1989; Van der Merwe *et al.*, 1990). Once released into freshwater ecosystems, pH changes in the water may result in the deposition of metals in stream and lake sediments (Förstner and Prosi, 1979; Campbell and Tessier, 1985), from where they can affect the ecology of certain aquatic biota (Harrison, 1958; Nehring, 1976; Whitton *et al.*, 1981). Such metals may thus be transferred from aquatic plants and macroinvertebrate fauna to freshwater fish via several pathways in the food chains of such affected ecosystems (Heath, 1978). It has been demonstrated that fish which are present in such waters may obtain these metals by means of diffusion through gill and skin surfaces (Matthiessen and Brafield, 1977; Heath, 1987) or, from their natural food (Mathis and Cummings, 1973; Moore and Ramamoorthy, 1984; Villegas-Navarro and Villareal-Trevino, 1989).

Conflicting reports exist concerning the mechanism involved, and/or the ability of fish to bioconcentrate the various metals in their organs and tissues. Some researchers have found that a positive correlation exists between fish body mass and metal concentration (Phillips *et al.*, 1980; Mohamed et al., 1990), while others (Goodyear and Boyd, 1972; Johnson, 1987) recorded no correlation between these two parameters. In some cases, however (Chernoff and Dooley, 1979; Anderson and Spear, 1980; Memmert, 1987), a definite inverse relationship was found to exist between body mass and metal concentration.

In the present series of investigations attention was given to some aspects of the uptake of selected metals by the two fish species in two different metal-polluted waters, namely the Florida Lake (*Perca fluviatilus*) and the Spaarwater Dam (*Pseudocrenilabus philander*).

7.2 Bio-accumulation of metals by *Pseudocrenilabrus philander* in the Spaarwater dam

In the case of the mine-polluted Spaarwater pan (Fig. 2.4B), a number of fish species still occur, including the cichlid, *Pseudocrenilabrus philander*, also known as the southern mouthbrooder, which is mainly confined to the shallow littoral zone of the lake. This fish is known to feed actively on benthic macro-invertebrates, including freshwater crustaceans and insect larvae as well as small fish (Polling *et al.*, 1995; Schoonbee, Personal observations). A certain amount of detritus also occurs in the diet of this fish, reflecting its benthic feeding habits.

Investigations were made into the ability of *P. philander* to bio-accumulate the metals Fe, Mn, Zn, Cu, Ni and Pb from the aquatic environment of this impoundment which contains appreciable quantities of the six metals in both the sediments and the water. Investigations were also made to determine the ability of its smaller juveniles to accumulate these metals in higher concentrations per equivalent mass than the larger, older fish.

7.2.1 Materials and methods

Collection and preparation of samples

A modified Moore shocker (Moore, 1968) was used to collect 389 individuals of *P. philander* at four localities in the littoral zone of the Spaarwater pan. Water and sediment samples were also collected at these sites for various physical and chemical analyses, including metal analyses (Standard Methods, 1989). Fish were individually weighed and the total and standard lengths determined. All sediment and fish samples were individually dried in an oven at 90°C for a minimum period of 48 hours, and the dry mass determined. Due to the relatively small size of the juveniles, specimens below 0,5 g individual wet mass were pooled in certain successive mass groups to allow for sufficient material in each case for reliable AAS determination of the six metals. The smallest specimens (47 in total), ranging from 0,0200 g to 0,1799 g, were grouped in successive size classes with intervals of 0,04 g (30 specimens) which were pooled for successive 0,02 g mass intervals. The largest of the juvenile fish, ranging between 0,2500 and 0,4999 g in mass, were combined in successive groups of 0,01 g intervals. Fish larger than 0,5 g wet mass were large enough to be individually analysed following the drying process. A total of 264 fish samples, which included the composite (smaller) and the individual (larger) fish were thus prepared for whole-body AAS analysis. All dried samples, including the collected water, were separately digested according to standard procedures (Van Loon, 1980) using a 1:1 perchloric and nitric acid mixture. The period of digestion lasted for at least 4 h, during which time total digestion and clearing of the samples occurred. Each digested sample was then filtered separately using a 6 μm Millipore filter paper. Individually filtered samples were then made up to 100 ml with double distilled de-ionised, metal-free water and transferred to separate clean metal free labelled storage bottles.

Atomic Absorption analysis of samples

A Varian SpectrAA-10 Atomic Absorption Spectrophotometer was used to determine the concentration of Fe, Zn, Mn, Cu, Ni and Pb in the water, sediments and fish samples. The results obtained were recalculated to μg/g using the actual dry mass of each digested sediment and fish samples. In the case of the water, results are expressed in μg/l. Concentration ratios (CR) (Heath, 1987; Sorensen, 1991) were calculated for all the fish samples using the following equation:

$$\mathrm{CR} = \frac{[\mathrm{M}]_\mathrm{f}}{[\mathrm{M}]_\mathrm{s}}$$

Where: [M] = metal concentration, and where f and s refer to the fish and sediments, respectively. Results are not expressed as a percentage but as a ratio only. The data was statistically evaluated using Spearman's correlation coefficient and a t-test on a 99% scale of significance (Zar, 1984). For the t-test a t-value was calculated using the following equation:

$$t = r\frac{\sqrt{n-2}}{\sqrt{1-r^2}}$$

Where: r = correlation coefficient and
n = size of test sample.

The degrees of freedom (df) were 262. Data was graphically illustrated using Harvard Graphics.

7.2.3 Results

Results on the water chemistry showed the water of the impoundment to be alkaline, with a pH fluctuating between 7,6 and 8,1 and with a mean calculated pH of 7,9 (Table 4.8). The summer water temperature exceeded 22°C in all cases. The alkaline nature of the water was further demonstrated by values obtained for alkalinity and hardness. Conductivity values ranged between 850 µS/cm and 1300 µS/cm, with a mean of 1038 µS/cm. This, together with the sulphate values, which fluctuated between 150 mg/l and 200 mg/l, clearly reflects the effect of waste water seepage from the surrounding mine dumps into the dam. Ammonia, nitrite, nitrate and orthophosphate suggest some degree of organic contamination of the water. The turbidity of the water, at less than 5 FTU, is low.

The wet mass of all individuals of *P. philander* collected in the Spaarwater pan ranged between 0,048 g and 9,534 g and the length between 17,5 mm total length (14,5 mm standard length) and 81 mm total length (66 mm standard length). A mean moisture content of 78,5% was obtained for all fish specimens.

The mean metal content in the water, sediments and fish, as well as the fish/sediment BCR$_s$ values, are summarised in Table 7.1. Of the six metals, iron occurred in the highest concentrations for all six metals in both the water (µg/) and sediments (µg/g). Manganese (88 µg/l) showed the second highest concentration of all six metals in the water, followed by Pb (48 µg/g), Ni (37 µg/l), Zn (34 µg/l) and Cu (7 µg/l). In the case of the sediments, Mn with a concentration of 76 µg/g dry mass, showed the second highest concentration of all the metals, followed by Ni (41 µg/g), Pb (36 µg/g), Zn (33 µg/g) and Cu (7 µg/g). With the exception of Zn, which was considerably higher than in the sediments, concentrations of the metals in the fish followed almost the same pattern as those obtained for the sediments, namely Fe occurring in the highest concentrations (595 µg/g), followed by Zn (279 µg/g), Mn (99 µg/g) and Cu (7 µg/g). With the exception of Zn, which was considerably higher than in the sediments, concentrations of metals in the fish followed almost the same pattern as those obtained for the sediments, namely Fe occurring in the highest concentrations (696 µg/g), followed by Zn (279 µg/g), Mn (99 µg/g), Ni (80 µg/g), Pb (µg/g) and Cu (9 µg/g). With regard to the fish/sediment CR values, an exceptionally high concentration factor was obtained for zinc (8,54). By contrast, a very low CR value of 0,1 was recorded for iron, while the CR values for copper, manganese, nickel and lead were all approximately of the same order of magnitude, fluctuating between 1,23 and 1,96 (Table 7.1).

Overall, a relatively wide range in concentrations was obtained for all metals in the individual fish samples (Table 7.1 and Figs. 7.1-7.6), with the smaller fish clearly having higher concentrations of metals per equivalent mass compared to those of the larger specimens. The greatest difference between the highest and lowest concentrations obtained for specific metals per fish mass was that for nickel, ranging between 7 µg/g and 333 µg/g, and iron, ranging between 105 µg/g and 3246 µg/g. This was followed by lead (10 µg/g and 162 µg/g), manganese (41 µg/g and 549 µg/g), and copper (4 µg/g and 26 µg/g). The smallest difference between the highest and lowest concentration obtained for the fish for any metal was that for zinc, which varied between 154 µg/g and 549 µg/g.

An overall negative correlation for all metals examined was found to exist between the increase in mass of the individual fish and the metal concentration per equivalent mass. The highest negative correlation was obtained for lead, followed by copper, zinc and nickel. The inverse relationships between metal concentration and the individual mass of the fish for the total population of *P. philander* were highly significant for all the metals. According to the results obtained, the metal concentration:body mass ratios followed the contours of a power regression with the highest negative correlation occurring amongst the smaller fish between the mass groups of 0,008 g and 0,2 g. This tendency clearly declined with the increase in the mass and consequently the age of the fish beyond 0,4 g.

To summarise: An investigation was made into the bioaccumulatin of Fe, Mn, Zn, Cu, Ni and Pb by the cichlid *Pseudocrenilabrus philander* from a mine-polluted impoundment in the Transvaal. With the exception of Fe, all the other metals were accumulated in the tissues of the fish in higher concentrations than those in the sediments of the lake, with the highest bioconcentration of Zn being 8,54. Results also showed that there was an inverse relationship between metal concentration and body mass of the fish, with the smaller juvenile fish being better able to concentrate all the metals per equivalent body mass than was the case for the larger, adult fish. This phenomenon is linked to a superior bioregulation mechanism for metals by the larger older fish, as well as the relatively higher metabolic rate of the younger juvenile fish.

The investigation showed that *P. philander* appears to be reasonably tolerant to various concentrations of the metals Fe, Mn, Zn Cu, Ni and Pb in the Spaarwater dam and that a possible mechanism exists whereby concentrations of these metals are bioregulated, particularly so in the larger, older fish in the population. This may explain its abundance in the Spaarwater dam despite seepages into the dam of water from nearby mines.

7.3 Metal concentrations in the organs and tissues of the European perch *Perca fluviatilus* from the mine polluted Florida Lake

7.3.1 Introduction

During recent investigations into the biological control of submerged weeds in the Florida Lake (Fig. 2.5), using the Chinese grass carp, *Ctenopharyngodon idella* (Val.) as biological control agent (Venter and Schoonbee, 1991), results showed abnormally high concentrations of Cu, Mn, Fe, Ni, Zn and Pb in the water and sediments which could largely be traced to past gold-mining activities in the catchment area. Apart from analysing the concentrations of these metals in the water, sediments and aquatic weeds, the organs and tissues of the omnivorous European perch *Perca fluviatilis* L. which occur in the lake, were also analysed. This contribution deals with these findings.

7.3.2 Materials and methods

Selected physical and chemical analyses (including metals) of the water and sediments were made according to standard analytical procedures (Standard Methods, 1989). Bimonthly sampling, covering the entire area of the lake, took place during 1990/1991 at eight randomly selected sampling localities (Fig. 2.5). Metal analyses of the lake water and sediments at these localities were also done.

Eleven perch specimens, irrespective of age or sex, were randomly collected from the lake during the summer (October) 1990, using gill nets. At the laboratory the following organs and tissues were individually removed from each fish for analysis of heavy metal concentrations: muscle, gill, spleen, vertebrae, liver, gonads and digestive tract. Following dissection, fresh tissue samples were weighed, correct to the nearest milligram, and then oven dried at 60°C until a constant mass was obtained.

Dried fish tissue and sediment samples and, in the case of water, 100 ml quantities, were individually digested in a clean, metal-free 250 ml flask using a mixture of 10 ml of $HClO_4$ (60%) and 10 ml HNO_3 (55%). Acid digestion of the material was performed at approximately 200-250°C for at least 6-7 hours during which total digestion and clearing of the samples were achieved. Each digested sample was filtered under vacuum using 0,45 μm membrane filter paper. In each case the filter system was thoroughly rinsed with double-distilled water to remove all traces of any remaining dissolved metals. Samples were then individually made up to 100 ml with distilled water and stored in clean, metal-free glass containers for analyses of the different metals, using a Varian atomic absorption spectrophotometer. Analytical standards for Cu, Mn, Fe, Ni, Zn and Pb were prepared from standard Saarchem (Reg.) stock solutions. The metal concentrations in the organs, tissues and sediments were expressed in μg/g dry mass and in the case of the water samples in μg/l.

A concentration ratio (CR) of the metals in the tissues and organs of the fish, expressed as a percentage of that found in the lake water and sediments, were calculated using the formula of Wiener and Giesy (1979):

$$CR = \frac{Co}{Cw \text{ or } Cs}$$

where Co = dry massd concentration of a given metal in the tissue and Cw and Cs the mean concentration of the metal in the water (Cw) and lake sediments (Cs).

7.3.3 Results

The lake water was alkaline, with pH values fluctuating between 7,0 and 9,9 (Table 4.12). A mean conductivity, exceeding 121 µS/cm, indicated moderate concentrations of dissolved solids in the lake water. Mean values for alkalinity and total hardness were 52,2 mg/l and 64,2 mg/l, respectively. Moderate concentrations of ammonia, nitrate and orthophosphates were recorded, indicating slightly prevailing eutrophic conditions (Venter and Schoonbee, 1991). With the exception of Pb, where a mean concentration of 230 µg/l was recorded in the water column and 95 µg/g in the sediments, concentrations of all the other metals were comparatively higher in the sediments than in the water itself. This corresponds well with the conception that in alkaline water most metals normally tend to precipitate and accumulate in the sediments (Wood, 1974).

A comparison of the metal concentrations in the water and sediments indicates abnormally high values for Fe in both the sediments and water, but particularly so in the sediments (Table 7.2). Mean values for Cu exceeded 20 µg/l in the lake water and 20 µg/g in the sediments. Values for Mn, Ni and Zn, which were noticeably high in both the sediments and water column, can be related to the effect of the already mentioned previous gold mine activity.

Concentrations of Fe, Ni and Zn, on average, were the highest of all the metals analysed in the perch (Table 7.2). Comparatively high concentrations of Pb were recorded in most organs, especially the spleen (38,5 µg/g), vertebrae (39,6 µg/g) and gonads (34,0 µg/g). Concentrations of Cu were highest in the walls of the gut (38,3 µg/g), liver (27,6µg/g) and spleen (25,8 µg/g). Lowest concentrations of Cu were found in the muscle, gills and vertebrae. Manganese was highest in the wall of the gut, followed by the gills, with very low concentrations in gonad and muscle. As expected, the highest concentrations of Fe were found in the liver and spleen. Zn is bioconcentrated in the liver, spleen and gonads, with lowest concentration in the muscle. In comparing concentrations of the metals in the various organs and tissues of the perch, expressed as a concentration ratio (CR %), Ni (vertebrae: 586,3) appears to be the best bioconcentrated, followed by Zn (spleen: 349,7) (Table 7.3).

To summarise: The Florida Lake, which showed some signs of organic enrichment and eutrophication of its waters, had been polluted decades ago by gold-mining activities. Despite signs of recovery in its water chemistry, the lake water and sediments still contain excessive loads of certain metals originating from mine effluents deposited in its sediments from where they are leached into the water column or returned there by biological activity of the aquatic flora and fauna of the lake. The one exception is lead, much of which is washed into the lake from the streets via stormwater drains. Metal concentrations in the abiotic environment are reflected by the aquatic flora as well as by the organs and tissues of the European perch, *P. fluviatilis*, which showed highest concentrations of Fe, Ni and Zn in its gills, spleen, liver, bone tissue and gonads. Whole-body analysis of some fish would have provided a more balanced picture of the metal loads accumulated by the perch.

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Table 7.1 Metal concentrations in the water, sediments and *Pseudocrenilabrus philander* as well as the concentration ratios (CR) for the various metals in the fish and sediments in the Spaarwater pan. Results are based on dry mass values of the sediments and the whole-body dry mass analysis of the fish.

Parameters	**Mean SD and Range**	**Metals**					
		Fe	**Mn**	**Zn**	**Cu**	**Ni**	**Pb**
Water (µg/ℓ) n=4	$\bar{x}$	1 119	88	34	7	37	48
	SD	1 764	47	17	4	11	5
	CV %	109,0	53,0	49,6	52,5	29,4	11,2
	Range	263 - 4 650	53 - 169	20 - 63	5 - 13	28 - 56	43 - 56
Sediments (µg/g) n=4	$\bar{x}$	5 979	76	33	7	41	36
	SD	5 984,0	20,8	11,3	4,2	7,7	5,8
	CV %	100,1	27,2	34,6	57,0	18,5	15,9
	Range	1 720 - 16 210	49 - 97	21 - 51	4 - 14	34 -54	29 - 44
Fish (µg/g) n=264	$\bar{x}$	595	99	279	9	80	71
	SD	329,0	34,6	66,8	3,1	47,0	35,6
	CV %	55,0	35,1	23,9	34,8	59,1	50,0
	Range	105 - 3 246	41 - 291	154 - 549	4 - 26	7 - 333	10 - 162
Concentration Ratio (CR): Fish/sediment ratio (n=264)	$\bar{x}$	0,10	1,29	8,54	1,23	1,92	1,96
	SD	0,06	0,45	2,04	0,43	1,13	0,98
	CV %	55,0	35,1	23,9	34,8	59,1	50,0
	Range	0,02 - 0,54	0,54 - 3,83	4,67 - 16,64	0,57 - 3,71	0,17 - 8,12	0,28 - 4,50

Table 7.2 Analysis of selected metals in the water column (µg/l) and sediments (µg/g dry mass) of the Florida Lake, Gauteng, South Africa, based on a bimonthly sampling programme at eight localities during 1990-1991 (n=56; $\bar{X}$ ± SD)

Analysis	Metal concentration	
	Water column (µg/l) n=56	Lake sediments (µg/g dry mass) n=56
Temperature °C	22,9 ± 11,9	
pH	7,0 - 9,9	
Dissolved oxygen (mg/l)	6,8 ± 0,9	
Conductivity (µS/cm)	121,3 ± 17,8	
Cu	22,9 ± 11,9	26,2 ± 3,5
Mn	67,2 ± 33,4	134,7 ± 46,7
Fe	1 763,6 ± 955,6	10 282,2 ± 4503,0
Ni	76,6 ± 65,6	108,4 ± 57,2
Zn	73,7 ± 71,6	130,8 ± 49,1
Pb	230,3 ± 46,2	95,0 ± 48,9

Table 7.3 Metal concentrations (μg/g dry mass: x̄ ± SD) in the organs and tissues of the perch *Perca fluviatilis* as well as the respective calculated concentration ratios for the water column (CRw%) and lake sediments (CRs %) in the Florida Lake, Gauteng, South Africa

		CONCENTRATIONS IN ORGANS AND TISSUES IN μg/g DRY MASS						
Metal	Parameter	Muscle	Gills	Spleen	Vertebrae	Liver	Gonads	Gut wall
Cu	Conc	4,5±1,2	4,6±0,8	25,8±4,1	8,0±1,3	27,6±12,4	5,1±1,2	38,3±28,0
	CRw	19,6	20,1	112,7	35,0	121,0	22,3	167,2
	CRs	17,2	17,6	98,5	30,5	105,3	19,5	146,2
Mn	Conc	2,6±0,8	26,4±2,2	7,6±0,8	36,3±10,0	10,3±2,7	4,0±1,5	45,1±37,7
	CRw	3,9	39,3	11,3	54,0	15,3	6,0	67,1
	CRs	1,9	19,6	5,6	27,0	7,6	3,0	33,5
Fe	Conc	91,9±31,2	209,1±5,6	1089,6±88,9	180,4±14,2	1169,3±259,9	142,0±33,4	501,3±247,9
	CRw	5,2	11,9	61,8	10,2	132,6	8,1	24,4
	CRs	0,9	2,0	10,6	1,8	11,4	1,4	4,9
Ni	Conc	165,1±28,2	344,5±22,8	256,1±17,8	449,1±67,3	210,5±59,9	166,6±65,9	150,4±22,8
	CRw	215,5	449,7	334,3	586,3	274,8	217,5	196,3
	CRs	152,3	317,8	236,3	414,3	194,2	153,7	138,7
Zn	Conc	3,3±0,5	83,4±14,3	257,7±110,5	77,3±9,0	130,7±23,9	181,1±40,6	94,7±12,0
	CRw	4,5	113,2	349,7	104,9	177,3	245,7	128,5
	CRs	2,5	63,8	197,0	59,1	99,9	138,5	72,4
Pb	Conc	29,4±3,0	19,2±1,4	38,5±7,5	39,6±4,8	29,2±7,4	34,0±12,6	19,6±12,5
	CRw	12,7	8,3	16,7	17,2	12,7	14,8	8,5
	CRs	30,9	20,2	40,5	41,7	30,7	35,8	20,6

8. METAL UPTAKE BY THE ADULTS, LARVAE AND JUVENILES OF THE PLATANNA *XENOPUS LAEVIS* FROM THE MINE- AND INDUSTRY-POLLUTED NATALSPRUIT WETLANDS

8.1 Introduction

The species of *Xenopus* include the only South African amphibians of which the larvae and the adults are both completely aquatic. *Xenopus laevis*, also known as the platanna, has the widest distribution of all the species in this genus south of the Sahara (Nieuwkoop and Faber, 1956; Passmore & Carruthers, 1979). It is able to invade a wide variety of aquatic habitats and has a wide temperature tolerance (Brown, 1970; Prinsloo *et al.*, 1981). The frogs are able to migrate actively between water bodies and can survive dessication by burrowing in the mud. The larvae and tadpoles mainly feed upon phyto- and zooplankton organisms while the frogs may feed on a variety of aquatic and terrestrial organisms including freshwater snails, chronomidae larvae and the freshwater crab *Potamonautes*. Small fish and even its own larvae and juveniles are taken as food (Schoonbee *et al.*, 1992). *Xenopus laevis* have already been used in bio-essay experiments to evaluate various pollutants and to determine its ability to bio-accumulate metals such as lead from its diet (Ireland, 1977).

The presence of *X. laevis* in the metal-polluted Natalspruit wetlands (Fig. 8.1) provided the opportunity to examine its adult, larvae and juvenile stages for their ability to accumulate metals from this particular ecosystem. Attention was also given to the metal accumulation by stage 1 larvae of the river frog *Rana angolensis* collected from this same locality.

8.2 Materials and methods

X. laevis frogs were collected using baited traps. Handnets were employed to obtain the larval and tadpole stages of the two frog species. Whole-body metal analysis as well as that of selected organs were made on the frogs while whole-body analysis only was made on the larvae and juveniles of *X. laevis* and *R. angolensis*. The very practical approach of using whole-body analysis of fish has been followed with success by authors such as Goodyear and Boyd (1972) and Davis and Boyd (1978). Organs and tissues of *X. laevis* frogs analysed for their metal contents included the heart, liver, lungs, kidneys, gonads, thigh muscle, fat bodies, spleen, gall bladder, intestine, stomach and skin.

Tadpoles were divided into the different tadpole and morphological developmental stages according to Schoonbee *et al.* (1992) and Nieuwkoop and Faber (1956), respectively (Table 8.1).

All results on metal concentration in the sediments, whole-body and organs are based on dry mass determinations. Standard laboratory and analytical procedures were

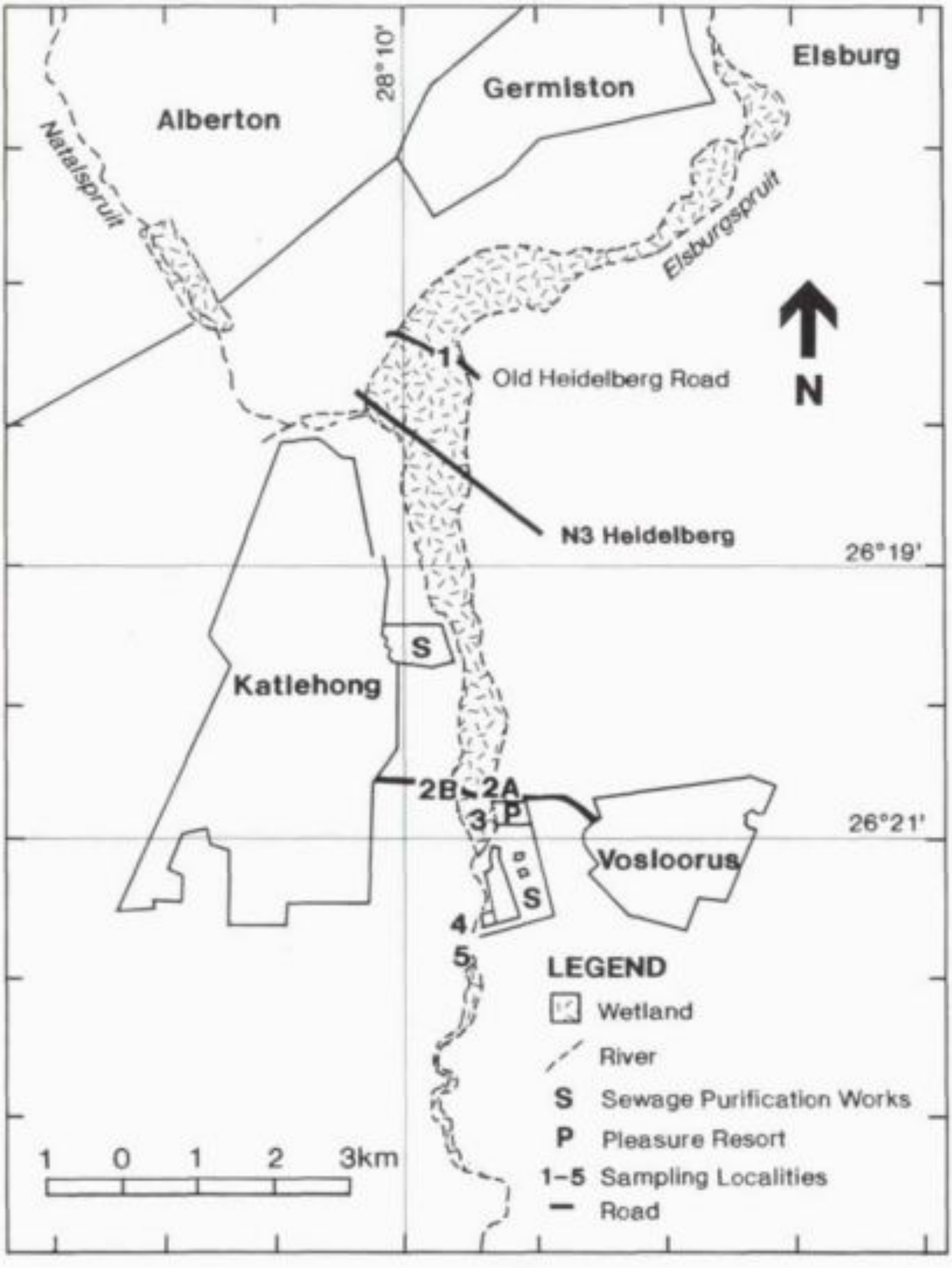

Figure 8.1: The Natalspruit wetland indicating the localities where collections of *Xenopus laevis* and *Rana angolensis* were made.

followed in the preparation and metal analysis of samples (APHA, 1989; VARIAN, 1989). Results are expressed as µg/l in the case of the water.

Table 8.1 Tadpole and juvenile developmental stages in the life cycle of the platanna, *X. laevis* according to Schoonbee *et al.* (1992) and Nieuwkoop and Faber (1956).

Stage	Morphological characteristics	Developmental stage
1	Tadpoles without limbs.	Premetamorphose 1-45
2	Tadpoles with hind limbs only.	Premetamorphose 46-55
3	Tadpoles with fore and hind limbs.	Premetamorphose 56-59
4	Tadpoles with well-developed fore and hind limbs and the head-shape changes to that of a juvenile frog. During this stage the tail shortens.	Climax 60-65
5	Immature frogs the tails of which are completely reabsorbed.	Postclimax 66+

Table 8.2 Mean metal concentration (µg/g dry mass) in organs and tissues of the platanna *Xenopus laevis* (n = 9)

Organ	Metal and concentrations in µg/g						
	Pb	Mn	Fe	Ni	Cr	Zn	Cu
Heart	1,3	10,0	1907,3	22,6	18,6	459,1	29,5
Liver	0,9	8,8	10376,6	13,2	7,5	188,3	378,6
Lung	0,9	7,0	2127,1	0,9	11,6	276,4	30,0
Kidney	0,9	9,2	2263,4	11,0	3,7	309,4	25,3
Testes	9,6	83,7	5466,8	9,6	77,0	1252,4	169,4
Ovary	4,7	14,4	549,0	3,9	3,9	377,7	17,5
Intestine	10,5	146,2	12526,3	37,9	23,1	1083,3	44,0
Muscle	0,8	5,0	540,1	0,8	6,5	155,0	15,1
Fatbodies	0,2	2,4	254,2	7,7	0,2	611,8	10,5
Stomach	1,1	115,0	5421,1	17,2	16,1	513,1	38,2
Gallbladder	14,3	41,4	7928,6	42,9	114,3	1822,9	250,0
Spleen	11,6	63,6	8208,1	46,2	80,9	1587,3	181,5
Skin	0,9	9,8	622,7	0,9	8,2	591,6	30,0

8.3 Results

8.3.1 Metal concentrations in the organs and tissues of *X. laevis* frogs (Tables 8.2 and 8.3)

Metals in organs

Iron, followed by zinc, were the two metals with the highest overall concentrations in the various organs and tissues of *X. laevis*. Copper concentrations were the third highest, ranging from 10,5 µg/g (fatbodies) to 378,6 µg/g in the liver. Although concentrations of nickel and chromium were generally lower than those of the metals already discussed, occasional high values were still recorded for some organs and tissues. Concentrations in the spleen and gallbladder were two examples. Concentrations of lead were clearly the lowest in the organs, the highest values for this metal being in the gallbladder (14,3 µg/g) and spleen (11,6 µg/g). Metal concentrations of less than 0,9 µg/g were found in the liver, lungs, kidneys, muscle and skin. The fatbodies which had amongst the lowest concentrations of all metals, contained only 0,2 µg/g lead.

Whole-body metal concentrations

The whole-body metal analysis of *X. laevis* frogs (Table 8.3) provided a somewhat different but more representative picture of the metal concentrations recorded in the aquatic environment with the concentrations of iron (411,1 µg/g) and zinc (220,1 µg/g) still being the highest of the metals but considerably lower than values recorded for some of the individual organs, putting the mass dominance and metal concentrations of the muscles into better perspective. Manganese, with a mean value of 43,3 µg/g, was the third most important metal in terms of concentration, followed by copper (22,9 µg/g). The relative importance of lead increased with a mean of 11,2 µg/g, being higher than metal with 8,1 µg/g and chromium with a mean of 3,6 µg/g, the lowest concentration of all seven metals.

Table 8.3 Wet and dry mass, moisture content and mean concentrations of seven metals (as µg/g dry mass) in whole bodies of the platanna *Xenopus laevis* (n = 4).

Analysis	$\bar{x}$ ±	SD	Min	- Max	CV %
Wet mass (g)	59,8	52,9	6,7	- 106,2	88,5
Dry mass (g)	16,9	15,8	1,2	- 31,1	95,2
Moisture (%)	75,8	5,3	70,7	- 81,7	7,0
Lead	11,2	6,4	7,4	- 20,9	57,1
Nickel	8,1	3,5	4,7	- 12,7	43,2
Zinc	220,6	43,2	189,4	- 284,5	19,6
Chromium	3,6	2,8	0,9	- 7,5	77,8
Iron	411,1	195,9	209,4	- 616,3	47,7
Manganese	43,3	52,2	0,4	- 117,0	120,6
Copper	22,9	11,7	14,5	- 39,4	51,1

CV: Coefficient of variability

8.3.2 Metal concentrations in the four developmental stages of *X. laevis* larvae and juveniles (Table 8.4).

Although individual values obtained for the different metals varied, there was no clearcut difference in metal concentrations between the developmental stages with iron and manganese being the two exceptions. These two metals occurred in much higher concentrations in the stages 1 and 2 larvae. Copper was consistently higher than 500 µg/g for all stages. Values for zinc, which varied between 332,4 µg/g (stage 1) and 404,3 µg/g (stage 4) as well as nickel with a highest value of 108,8 µg/g (stage 1) followed a similar trend. Mean values for chromium (23,9 - 37,0 µg/g) and lead (7,5 - 13 µg/g) were the lowest.

Table 8.4 Mean metal concentrations (µg/g dry mass) in four developmental stages of *Xenopus laevis* larvae and juveniles from the mine- and industry-polluted Natalspruit wetland ecosystem.

METAL	Developmental stages and mean metal concentration (µg/g)			
	1 (n = 43)	3 (n = 37)	4 (n = 41)	5 (n = 53)
Lead	13,0	10,5	10,4	7,5
Nickel	108,8	98,7	88,6	98,8
Zinc	332,4	344,7	404,3	378,5
Chromium	37,0	24,7	26,7	23,9
Iron	7195,9	3990,4	355,2	518,4
Manganese	673,4	434,0	42,6	63,6
Copper	525,9	533,8	536,7	594,7

8.3.3 Metal concentrations in *Rana angolensis* stage 1 tadpoles

An almost similar but more variable picture emerged from the analysis results on the individual metals obtained for *Rana angolensis* (stage 1) larvae (Table 8.5). The mean values for iron and manganese were clearly the highest, followed by copper (733,1 µg/g), zinc (532,7 µg/g) and nickel (226,8 µg/g), with values for chromium (71,1 µg/g) and lead (24,2 µg/g) being the lowest.

Table 8.5 Mean metal concentrations (expressed as µg/g dry mass) in stage 1 *Rana angolensis* larvae from the metal-polluted Natalspruit wetland ecosystem (n = 136).

METAL	Concentration in µg/g dry mass	
	Mean	Range
Lead	24,2	10,6 - 29,7
Nickel	226,8	62,6 - 691,0
Zinc	535,7	234,1 - 1725,5
Chromium	71,1	23,5 - 230,3
Iron	3055,9	276,9 - 7845,9
Manganese	2140,0	99,9 - 5676,7
Copper	733,1	22,9 - 3781,2

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9. COMPARATIVE METAL CONCENTRATIONS IN THE ORGANS AND TISSUES OF THE REDKNOBBED COOT *FULICA CRISTATA*, REED CORMORANT *PHALACROCORAX AFRICANUS*, AND SACRED IBIS *THRESKIORNIS AETHIOPICUS* FROM THE METAL-POLLUTED NATALSPRUIT WETLAND

9.1 Introduction

The eastern half of Johannesburg, commonly known as the East Rand, is the most highly developed industrial region on the Witwatersrand and probably in South Africa. It is drained by tributaries of the Elsburgspruit, Natalspruit and Blesbokspruit river systems, among others, and these systems also include extensive natural wetlands. More than 1800 industries, ranging from large chemical industries to small one-man engineering firms, occur in this area (Viljoen *et al.*, 1985). The bulk of the water flowing in most of these streams consists of effluents and seepage waters originating from old mines, slimes dams, ash heaps, industries, sewage purification works, suburban areas and agricultural practices. Such effluents comprise the major sources of a variety of pollutants, including dissolved metals (Viljoen *et al.*, 1985; Jones *et al.*, 1989; Schoonbee and Van der Merwe, 1989). It is well documented that the processing of these metals by industries is also of strategic importance to the entire industrial world (Greenwood and Earnshaw, 1984). However, it is unfortunate that some metal-processing industries were and in many cases still are responsible for various forms of environmental pollution, resulting in serious hazards to living aquatic flora and fauna, including birds, when these liquid or solid wastes are released into the river and wetland ecosystems (White and Kaiser, 1976; Kempf and Sittler, 1977; Bull *et al.*, 1983; Ohlendorf *et al.*, 1986).

The wetlands occurring in the Witwatersrand, including those in the East Rand, provide habitats for a wide variety of aquatic and semi-aquatic birds (Tarboton *et al.*, 1987; Ryan and Isom, 1990; Tarboton, 1993). Many of these bird species are either typical of wetlands or are dependent on the specialized habitats created by the wetlands. Mismanagement and pollution of wetlands may mean a loss of habitat for a number of bird species. Furthermore, some wetlands may contain endangered biota, including certain bird species recorded in the South African Red Data Book on Birds (Brooke, 1984). A number of provincial parks and bird sanctuaries are located within the boundaries of the Witwatersrand such as the Rondebult, Florence Bloom, Melrose, Carlos Rolfes, Korsman, Springs and Marievale Bird Sanctuaries (Ryan and Isom, 1990). There are also several aquatic environments, of which the Vlakplaats Water Pollution Control Works (WPCW) is one, which do not receive any formal protection from conservation bodies. This particular area is relatively rich in bird life with a total of 139 species recorded here (Whitehouse and Whitehouse, 1978; Van Eeden, personal observations). The more commonly occurring aquatic birds associated with these sites include cormorants, ibises, coots, ducks, geese and other waterfowl as well as waders (Ryan and Isom, 1990). However, these sites, which are constantly polluted by metal-containing effluents, seepages and/or surface runoff water, simultaneously provide metal-contaminated food and drinking water for these birds.

The association between aquatic birds and the industrial and mining activities of man prompted investigations to be carried out on the potential of some bird species, eg. gulls (Munoz *et al.*, 1976), as biomonitors of metal pollution in aquatic environments. Any bird which is exposed to a metal-contaminated environment can be used to quantify relative levels of metal pollution by the measurement of the metal in a number of organs and tissues which may sequester metals from the body of the bird. However, a potential bird species to be used as an indicator organism needs to comply with various requirements in order to be a reliable and continuous biomonitor (Hahn *et al.*, 1985; Weyers *et al.*, 1985; Ellenberg *et al.*, 1985). The most important requirement is that birds from any trophic level should be able to accumulate a variety of metals without being killed by the potentially large concentrations encountered. Preference will also be given to those bird species that have a relatively sedentary home range behaviour and are abundant in the various study regions. To study the transfer of metals in a foodchain the diet of each bird should also be known.

The aquatic environments occurring in highly developed areas such as the East Rand are continuously under the threat of metal-containing effluents from industries, mines, sewage purification works, suburban areas and agricultural practices. The potential threat that these metal-containing effluents possess was aptly demonstrated by the deaths, low breeding success and deformed chicks of various aquatic birds from the Kesterson National Wildlife Refuge, which is seriously polluted by selenium and boron originating from irrigation drainage waters (Ohlendorf *et al.*, 1986; Hothem and Ohlendorf, 1989). However, most metal-containing effluents exert an often imperceptible and long-term impact on the aquatic environment, including the aquatic avifauna. These considerations led to the present investigation which was conducted at the Vlakplaats WPCW. This particular site, which borders on the extensive Natalspruit wetlands, is known to contain metals in the abiotic and biotic compartments (Van Eeden, 1990; Van Eeden and Schoonbee, 1991, 1992, 1993; Adendorff, 1992; Fleischer, 1993; Steenkamp *et al.*, 1993). Among the various aquatic and semi-aquatic avifauna, the Redknobbed Coot *Fulica cristata*, Reed Cormorant *Phalacrocorax africanus* and Sacred Ibis *Threskiornis aethiopicus* are the most numerous. These three species occupied clearly delineated trophic levels in the sampling area. Reed Cormorants are mainly piscivorous (Brown *et al.*, 1982), Sacred Ibises are omnivorous (Clark and Clark, 1979; Clark, 1979; Brown *et al.*, 1982; Ginn *et al.*, 1989) and Redknobbed Coots are herbivorous (Fairall, 1981; Brown *et al.*, 1982). In view of these facts, the primary aim of this investigation was to determine if possible variations in cadmium, copper, nickel and lead concentrations in the liver, kidney, bone and blood of these three bird species could be related to either their particular trophic status or their specific diets at this locality.

Surveys of the available literature indicated that little information exists on the concentration ranges of metals accumulated in the organs and tissues of the Redknobbed Coot, Reed Cormorant and Sacred Ibis from metal-contaminated aquatic environments. However, this problem was alleviated by recent research concerning the concentrations of metals in the organs and tissues of these three bird species (Van Eeden, 1990; Van Eeden and Schoonbee, 1992; 1993). The second aim of this investigation was to gain further knowledge concerning the concentration ranges of cadmium, copper, nickel and lead in selected organs and tissues of these three bird species sampled from the same aquatic environment known to be subjected to metal pollution.

9.2 Description of the study area

Detailed descriptions of the Natalspruit wetland area, including the drainage basin, water flow and composition, representative flora of the area as well as the occurrence and abundance of birds species from this area can be found elsewhere in the report and in Whitehouse and Whitehouse (1978), Viljoen *et al.* (1985), Jones *et al.* (1989) and Van Eeden and Schoonbee (1991, 1992).

9.3 Materials and methods

Samples of the Redknobbed Coot, Sacred Ibis and Reed Cormorant were collected at the study area with the landowner's permission and with the regional permit procured from the relevant Provincial Authority. Use was made of various methods of collection, but in most cases shooting (0.22 and shotgun) was used to collect virtually all bird specimens since this method proved to be the most successful.

A brief description of the methods used is provided. However, a more detailed description of the methods and materials used for this investigation can be found in Van Eeden (1994). Precautionary steps were taken in order to prevent possible metal contamination during the various procedures of laboratory analysis. The liver, kidney and tibio-tarsus (taken as representative of the skeleton and further referred to as "bone") were dissected out, whilst a blood sample was carefully scooped from the body cavity of each bird with the aid of a clean stainless steel spoon. Dried material was used in this study because it has been demonstrated that the variations in the metal concentrations of a particular organ can be ascribed in large measure to the varying moisture contents of that organ (Adrian and Stevens, 1979). In all cases the entire organs, tissues or blood samples were used for metal analysis. The acids used for wet ashing the samples were a 1:1 (v/v) mixture of concentrated nitric acid (55% Saarchem) and concentrated perchloric acid (60% Labchem). The concentrations of metals in all the digestates were determined by air-acetylene flame atomic absorption spectrophotometry (AAS) using a Varian SpectrAA 10 series AAS according to standard operational procedures (Varian, 1989).

The results of this investigation were summarized by determining the minimum and maximum metal concentration values and by calculating the mean concentration ($\overline{X}$), the standard deviation (SD) and the coefficient of variation (CV%). Any concentration value which was larger than $\overline{X}$+2SD was not included for statistical analysis. Selected statistical methods were also used to determine the possible significance of the results. Use was made of the nonparametric analysis of variance by ranks method, namely the Kruskal-Wallis Test (Hassard, 1991), after which the Newman-Keuls Test was used in order to locate a mean which differed from others of the same group (Zar, 1974; Hassard, 1991). The significance level used throughout this test was $p<0.05$. The statistical analysis was conducted with the aid of the KwikStat ShareWare Package distributed by TexaSoft.

9.4 Results

9.4.1 Variations in organ and tissue metal concentrations in the three bird species.

The mean cadmium concentrations in all four organs and tissues from the three bird species analysed were the lowest of the four metals investigated (Tables 9.1 to 9.4, Figure 9.1). Generally, the highest mean cadmium levels occurred in the kidneys of all three species, especially in the case of the Sacred Ibis (maximum 13 μg/g, Table 9.1) and the bone, especially in the case of the Redknobbed Coot (maximum 18 μg/g; Table 9.2). The liver and blood of the three species contained relatively smaller mean cadmium levels, although a maximum blood cadmium concentration of 16 μg/g was recorded for a Reed Cormorant (Table 9.3).

The extent of variability in the metal concentrations within the organs and tissues of the three bird species can be compared by calculating the coefficient of variation (CV%). The effectiveness of this statistic when employed in this manner was demonstrated by Muirhead and Furness (1988) for seabirds and Cosson *et al.* (1988) for aquatic birds. Doing this showed that the metal levels had relatively high degrees of variability within the three species and also among the species. For instance, a wide range in metal variability was found for the non-essential and potentially toxic metals cadmium (11% in Reed Cormorant bone to 123% in Reed Cormorant blood) and lead (12% in Reed Cormorant bone to 88% in Redknobbed Coot blood) compared to the relatively smaller range in metal variability for the essential metal copper (9% in Sacred Ibis bone to 73% in Redknobbed Coot liver; Tables 9.1 to 9.4). A range of 22% in Sacred Ibis bone to 105% in Reed Cormorant blood was calculated for nickel, which may be an essential metal.

Copper, nickel and lead were found in much higher concentrations in the various organs and tissues when compared to cadmium (Figure 9.1). Of the four metals, copper was the dominant metal ion present in the liver of all three bird species with its concentration being approximately three to four times higher than those of liver cadmium, nickel and lead (Table 9.4 and Figure 9.1). The range and variation in liver copper levels for the Sacred Ibis and Redknobbed Coot were approximately the same (14-85 μg/g; 73%) even though their respective means differed by about 5 μg (Table 9.4). Although the observed mean liver copper concentrations of the Reed Cormorant compared well with those of the other two species, its range (24-37 μg/g) and its variability (16%) were much smaller than those recorded for either the Sacred Ibis or the Redknobbed Coot (Tables 9.1 and 9.4).

Compared to the liver, copper occurred in relatively smaller mean concentrations in the kidneys (12-21 μg/g) and blood (4-26 μg/g) of the three species (Tables 9.1 and 9.3) and a clear pattern was found for these particular data (Figure 9.1). Accordingly, the kidney and blood copper levels of the three bird species can be arranged in the following order of increasing concentrations: Sacred Ibis < Reed Cormorant < Redknobbed Coot. The mean copper concentrations in the bone (<10 μg/g) of the three bird species were quite low compared to copper values found in the other organs and tissues (Table 9.2).

Nickel and lead were the dominant metals in the bone of all three species (Figure 9.1) and their concentrations were approximately one to six times higher than those of bone cadmium and copper (Table 9.2 and Figure 9.1). However, no clear pattern could be found for bone nickel and lead levels. Of the three bird species, the Reed Cormorants had the highest bone lead concentrations (mean 59 μg/g) while the highest mean bone nickel values were found for Reed Cormorant (34 μg/g) and Redknobbed Coot (36 μg/g) although a single value of 110 μg Ni/g was recorded in the bone of one coot specimen (Table 9.2).

The mean concentration range of nickel and lead in the blood and the kidneys of the three bird species was approximately two to three times that found in their liver, which contained <10 μg/g nickel and lead respectively (Figure 9.1). However, quite large variations were found in the mean blood lead levels (13 μg/g in Sacred Ibis to 32 μg/g in Reed Cormorant; Table 9.3) and mean kidney nickel concentrations (10 μg/g in Sacred Ibis to 23 μg/g in Reed Cormorant; Table 9.1). On the other hand, the mean concentrations of blood nickel and kidney lead concentrations among the three bird species were comparable (Figure 9.1). However, the variability in blood nickel levels was quite high among the three species (60% for Redknobbed Coot to 105% for Reed Cormorant; Table 9.3) compared to values found for kidney lead (27% for Reed Cormorant to 32% for Sacred Ibis; Table 9.1).

A few individual organ or tissue samples contained metal concentrations which were found to be exceptionally high and which exceeded the $\bar{X}$+2SD. For instance, from the Sacred Ibis, one liver sample had a copper content of 255,9 μg/g while some kidney samples had values of 45,6 and 54,2 μg Cd/g and 112,2 μg Pb/g respectively. The Reed Cormorant sample contained three blood samples with 133,3 μg Cu/g, 251,1 μg Ni/g and 162,9 μg Pb/g as well as a liver sample with a value of 274,6 μg Cu/g. High lead values in the kidney (237,7 μg/g), bone (115,9 μg/g) and blood (305,4 μg/g) were obtained for the Redknobbed Coot sample. The high metal concentrations found in these samples may indicate that some of the birds were naturally more contaminated by metals, lead in particular, than the majority of the other birds analysed. These exceptional values, falling well outside the normal distribution of the data, were excluded from the results used in the statistical evaluation of the data.

The percentage moisture content for the liver, kidney, bone and blood is provided in order to compare the dry weight metal concentrations presented here with the predominantly wet weight metal concentrations found in the literature. The percentage moisture for the liver, kidney, bone and blood of the three bird species are, respectively: Sacred Ibis (72,3; 79,0; 19,5; 72,7), Reed Cormorant (73,3; 79,0; 20,5; 75,6) and Redknobbed Coot (79,0; 80,6; 14,2; 77,2).

9.4.2 Statistical evaluation of the metal concentrations in the organs and tissues of the three bird species

From Figure 9.1 it can be seen that definite species-specific trends occurred for most of the organs and metals. Selected statistical methods were therefore used to determine the significance of these results. The concentration of cadmium in the livers of the three bird species was the only case (1 out of 16) in which no statistically significant ($p<0,05$) differences were found. In all the other cases significant differences occurred ($p<0,05$) for a specific metal between at least two of the three species (Table 9.5). In eight cases no significant differences were observed between two of the three species. For instance, no differences were observed between the mean liver copper, kidney cadmium and lead as well as blood cadmium levels of the Sacred Ibis and the Redknobbed Coot, although significant differences were detected in these cases between the aforementioned two species and the Reed Cormorant (Table 9.5). Similar trends were found for liver nickel and lead, femur nickel and blood nickel (Table 9.5). However, in nearly 50% of all the cases (7 out of 16) statistically significant differences ($p<0,05$) were detected among the mean metal concentrations for a particular organ/tissue of all three bird species (Table 9.5). For example, the mean concentrations of kidney copper, bone cadmium and blood copper differed significantly among the three species and in the following order of increasing concentrations: Sacred Ibis < Reed Cormorant < Redknobbed Coot. The mean concentrations of kidney nickel, bone copper and blood lead differed significantly among the three species and in the following order of increasing concentrations: Sacred Ibis < Redknobbed Coot < Reed Cormorant. The mean concentration of bone lead differed significantly among the three species and in the following order of increasing concentrations: Redknobbed Coot < Sacred Ibis < Reed Cormorant (Table 9.5).

To summarise : The concentrations of cadmium, copper, nickel and lead in the liver, kidney, bone and blood of the herbivorous Redknobbed Coot *Fulica cristata*, the piscivorous Reed Cormorant *Phalacrocorax africanus* and the omnivorous Sacred Ibis *Threskiornis aethiopicus* were determined. It was anticipated that these three bird species may be useful indicator organisms of cadmium, copper, nickel and lead considering their particular trophic status and their specific diets. This study was conducted on samples collected from the Natalspruit wetland, which is known to be seriously polluted by these metals. Use was made of dried material as well as standard flame atomic absorption spectrophotometer techniques. For all three bird species the cadmium levels were consistently lowest in the four organs and tissues analysed. Highest concentrations of cadmium occurred in the kidneys, especially in the case of the Sacred Ibis (3,4 μg/g) and interestingly in the bone tissues of the Redknobbed Coot (5,1 μg/g). The maximum blood cadmium values (4,7 μg/g) were recorded for the Reed Cormorant. Copper, nickel and lead were found in much higher concentrations in the four organs and tissues of all three species. Copper was clearly the dominant metal by mass in the livers of all three bird species. Nickel and lead occurred in the highest concentrations in the bone of all three species with concentrations being at least six times higher than those of cadmium and copper. The Reed Cormorants generally exhibited the highest bone lead concentrations (59,0 μg/g). Nickel and lead values for blood and kidneys were approximately 1-3 times higher than those in the bone tissues. With the exception of liver, significant differences ($p<0,05$) were recorded for cadmium in all the other organs and tissues among the three species. In the case of the other metals, significant differences were recorded between at least two of the three species. It was concluded that the dietary preferences of these three bird species as well as the metal contents in their diets all play an important role in the actual uptake and bio-accumulation of lead, nickel, copper and cadmium.

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Table 9.1 Metal concentrations in the kidney of three bird species from the natalspruit wetlands. Results are expressed as sample size, $\bar{x} \pm$ SD (µg/g dry mass), coefficient of variation (CV%) and range (minimum - maximum)

Metal	Sacred Ibis	Reed Cormorant	Redknobbed Coot
Cadmium	n=14 3,4 ± 3,8 111,8% 0,72 - 13,3	n=6 3,2 ± 1,7 53,1% 0,83 - 5,8	n=12 1,9 ± 1,2 36,2% 0,41 - 4,2
Copper	n=16 11,9 ± 3,3 27,7% 6,9 - 20,3	n=6 16,2 ± 2,6 16,0% 12,5 - 19,3	n=12 20,5 ± 8,5 41,5% 2,1 - 34,5
Nickel	n=16 10,1 ± 3,6 35,6% 4,1 - 17,2	n=6 22,8 ± 10,1 44,3% 3,4 - 31,5	n=12 19,7 ± 11,3 57,4% 9,3 - 44,2
Lead	n=15 10,6 ± 3,4 32,1% 4,7 - 19,1	n=6 12,4 ± 3,4 27,4% 7,6 - 16,6	n=11 9,5 ± 2,8 29,5% 6,0 - 15,5

Table 9.2 Metal concentrations in the bone tissue of three bird species from the Natalspruit wetlands. Results are expressed as sample size, $\bar{x} \pm$ SD (µg/g dry mass), coefficient of variation (CV%) and range (minimum - maximum)

Metal	Sacred Ibis	Reed Cormorant	Redknobbed Coot
Cadmium	n=16 2,5 ± 0,29 11,6% 2,1 - 3,0	n=6 4,9 ± 0,54 11,0% 4,3 - 5,8	n=12 5,1 ± 4,2 82,4% 2,5 - 18,3
Copper	n=16 4,0 ± 0,36 9,0% 3,3 - 4,4	n=6 8,2 ± 1,5 18,3% 6,0 - 10,6	n=12 6,6 ± 4,4 66,7% 3,9 - 20,3
Nickel	n=16 10,5 ± 2,3 21,9% 7,8 - 15,5	n=6 34,2 ± 19,5 57,0% 15,6 - 58,1	n=12 36,1 ± 27,4 75,9% 12,1 - 110,0
Lead	n=16 37,9 ± 15,5 40,4% 20,3 - 72,3	n=6 59,0 ± 6,8 11,5% 51,3 - 68,9	n=11 32,4 ± 10,1 31,2% 21,1 - 58,2

Table 9.3 Metal concentrations in the blood of three bird species from the Natalspruit wetlands. Results are expressed as sample size, $\bar{x}$ ± SD (µg/g dry mass), coefficient of variation (CV%) and range (minimum - maximum)

Metal	Sacred Ibis	Reed Cormorant	Redknobbed Coot
Cadmium	n=16 1,7 ± 0,91 53,5% 0,74 - 3,8	n=6 4,7 ± 5,8 123,4% 1,2 - 15,7	n=12 1,8 ± 1,3 72,2% 0,55 - 4,4
Copper	n=16 4,2 ± 1,9 45,2% 1,9 - 8,8	n=5 12,2 ± 8,2 67,2% 5,2 - 25,7	n=12 26,1 ± 15,7 60,2% 6,5 - 57,3
Nickel	n=16 18,4 ± 14,1 76,6% 6,6 - 62,7	n=5 21,0 ± 22,0 104,8% 3,7 - 51,4	n=12 20,9 ± 12,5 59,8% 7,4 - 42,9
Lead	n=15 13,3 ± 9,0 67,7% 4,4 - 40,6	n=5 31,6 ± 23,9 75,6% 12,7 - 58,9	n=11 23,8 ± 21,0 88,2% 6,2 - 77,2

Table 9.4 Metal concentrations in the liver of three bird species from the Natalspruit wetlands. Results are expressed as sample size, $\bar{x}$ ± SD (µg/g dry mass), coefficient of variation (CV%) and range (minimum - maximum)

Metal	Sacred Ibis	Reed Cormorant	Redknobbed Coot
Cadmium	n=14 1,4 ± 1,7 121,4% 0,43 - 7,0	n=6 1,0 ± 0,24 24,0% 0,65 - 1,3	n=12 1,1 ± 0,46 41,8% 0,68 - 2,1
Copper	n=13 33,3 ± 23,3 70,0% 13,8 - 84,8	n=5 29,4 ± 4,8 16,3% 24,3 - 37,3	n=12 27,6 ± 20,1 72,8% 14,2 - 84,3
Nickel	n=14 7,2 ± 1,7 23,6% 4,6 - 11,4	n=6 9,0 ± 2,8 31,1% 7,1 - 14,4	n=12 9,1 ± 3,3 36,3% 5,4 - 17,1
Lead	n=14 5,3 ± 2,9 54,7% 1,8 - 12,4	n=6 8,8 ± 1,5 17,0% 6,8 - 10,1	n=12 9,3 ± 5,5 59,1% 3,2 - 22,9

Table 9.5 Analysis of variance (H) and multiple comparisons analysis of the concentrations of four metals in organs and tissues of three bird species from a mine-polluted wetland. Mean metal concentrations within a row sharing a common superscript are not significantly different at the p<0,05 level of significance

Organ/Tissue	Metal	H-Value	Bird species and mean metal value		
Liver	Cd	0,60	SI 1,4^a	RC 1,0^a	BH 1,1^a
Liver	Cu	2,44	SI 33,3^a	BH 27,6^a	RD 29,4^b
Liver	Ni	3,23	SI 7,2^a	RC 9,0^b	BH 9,1^b
Liver	Pb	8,37	SI 5,3^a	RC 8,8^b	BH 9,3^b
Kidney	Cd	2,18	SI 3,4^a	BH 1,9^a	RC 3,2^b
Kidney	Cu	14,37	SI 11,9^a	RC 16,2^b	BH 20,5^c
Kidney	Ni	12,53	SI 10,1^a	RC 22,8^b	BH 19,7^c
Kidney	Pb	3,35	SI 10,6^a	BH 9,5^a	RC 12,4^b
Bone	Cd	23,07	SI 2,5^a	RC 4,9^b	BH 5,1^c
Bone	Cu	23,19	SI 4,0^a	RC 8,2^b	BH 6,6^c
Bone	Ni	23,87	SI 10,5^a	RC 34,2^b	BH 36,1^b
Bone	Pb	10,04	SI 37,9^a	RC 59,0^b	BH 32,4^c
Blood	Cd	1,79	SI 1,7^a	BH 1,8^a	RD 4,7^b
Blood	Cu	21,70	SI 4,2^a	RC 12,2^b	BH 26,1^c
Blood	Ni	2,55	SI 18,4^a	RC 21,0^a	BH 20,9^b
Blood	Pb	4,39	SI 13,3^a	RC 31,6^b	BH 23,8^c

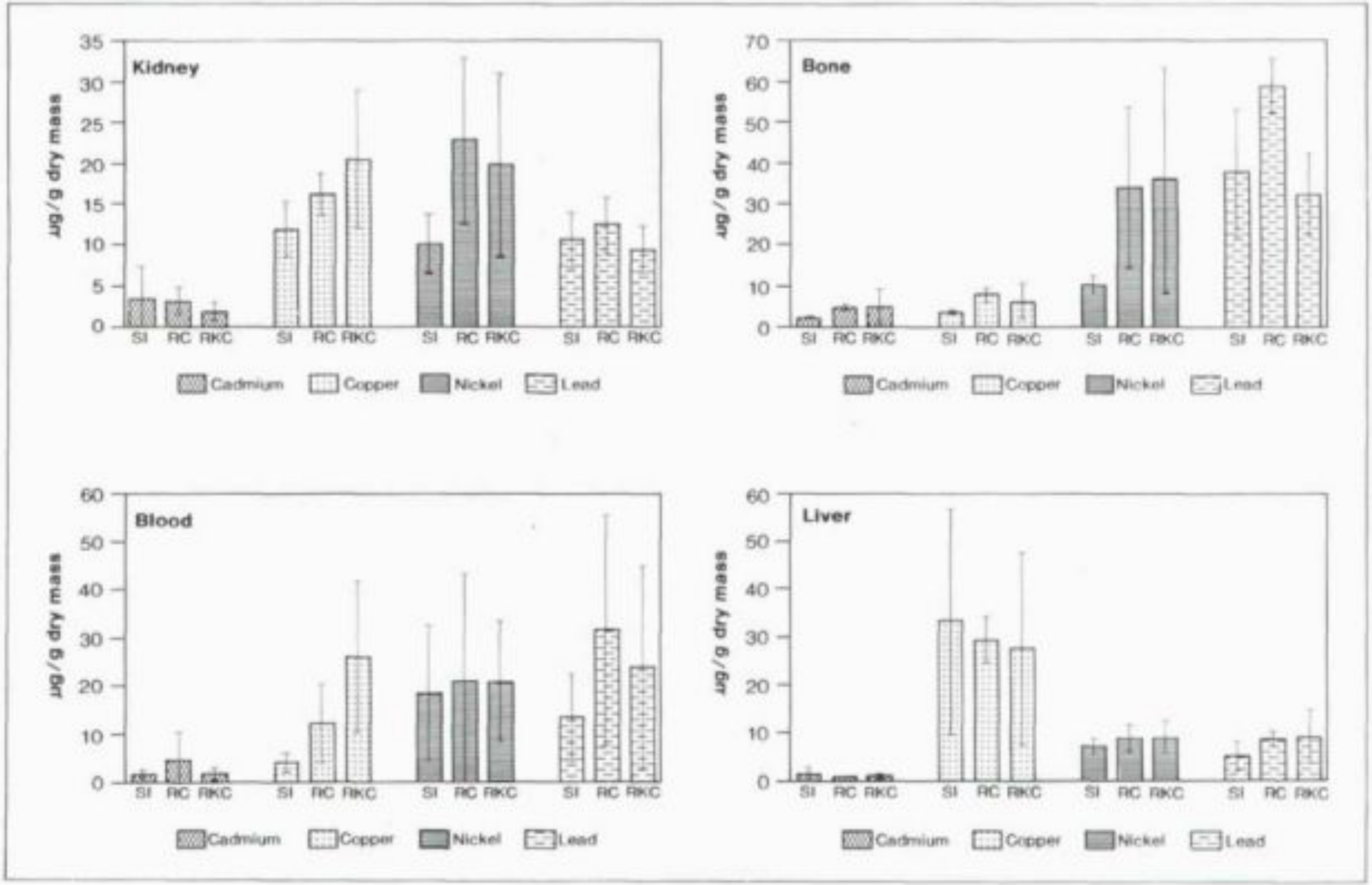

Figure 9.1: The concentrations (mean ± SD expressed as µg/g dry mass) of cadmium, copper, nickel and lead in organs and tissues of the Sacred Ibis (SI), Reed Cormorant (RC) and Redknobbed Coot (RKC) sampled from the metal-polluted Natalspruit wetlands and Vlakplaats WPCW area.

10. GENERAL DISCUSSION

10.1 Physical and chemical conditions of the water and sediments

Based on the findings of the physical and chemical conditions at the different localities during the various surveys, a picture emerged concerning the water quality conditions and, in particular, metal concentrations which prevailed.

The Germiston Lake had clearly recovered substantially from the original effects of mine pollution which occurred prior to 1970. In 1988 - 1989 there was a further continuous decline in mineral loads of the lake water. However, metal concentrations in the lake sediments showed that considerable quantities of the seven metals and in particular, iron and manganese, are trapped in the thick layers of sediments, where their release, under clearly alkaline conditions will probably be a slow process facilitated mainly by factors such as metal speciation and the biological availability of a small fraction of the total metal loads present.

The water of Germiston Lake overflows during the rainy season into the Elsburgspruit and further contributes to the existing burden of minerals and metals derived from effluents and seepage waters from mines, and the industrial, refuse and ash dumps which encroach on the banks of this stream. Investigations by Van der Merwe *et al.* (1990) showed that the expanded growths of emergent and submerged aquatic vegetation through which the water of the Elsburgspruit and later, the Natalspruit flows, play an important role in trapping some of these metals during the active summer growing season. The considerable metal loads carried by the water of the river system do not appear to be reduced significantly by the wetland ecosystem. There was, however, clear indications that the organic pollution by sewage effluents discharged into the wetlands in both the Elsburgspruit and Blesbokspruit rivers, are contained to a large extent, with the nutrients being well utilized by the luxuriant growths of the aquatic vegetation. Iron and manganese were found to be the major constituents in terms of concentrations in both the water column and the stream sediments of the affected rivers with concentrations of zinc and nickel being prominent throughout the periods of investigation. Lead and copper were always present in relatively low concentrations with highest quantities in the sediments.

Both the Cowles and Nigel dams contained appreciable loads of most of the metals investigated. Effluents from sewage purification works and from a paper factory (Cowles dam) contributed significantly to the eutrophication of both impoundments. As was the case with all the other water bodies investigated, iron and manganese again featured prominently in the water and sediments of both dams. This was followed by lower concentrations of zinc, nickel, copper and lead, with the concentrations for chromium clearly the lowest.

Conditions in the Florida Lake reflect the long-term effects of metal pollution by mines on freshwater ecosystems. The water of this lake was generally low in dissolved solids and yet the concentrations of iron were still high. Lead concentrations were particularly high in the water column, pointing towards a major contribution by the effects of motor vehicle emissions and surface runoff water from the streets into the lake. Manganese, nickel and zinc occurred in lower, but comparable, quantities in the lake water and sediments with copper concentrations being the lowest. Results on this particular survey suggest that, with the exception of lead, the lake sediments may still remain the major source of metal pollution in Florida Lake.

10.2 Bioaccumulation of metals by the aquatic and semi-aquatic vegetation

Of the seven metals analysed for, Mn and Fe are the two metals with the highest bioconcentrations in floating aquatic weed *A. filiculoides* (Table 5.15) in terms of µg/g dry mass. Comparing the CR values, Mn (2 065%) followed by Pb (607%), Ni (85%) and Cu (70%) were the best absorbed by *A. filiculoides*, with the CR for Cr (10%) being the lowest of all the metals.

Of the metals investigated in the present study Fe, Cu, Mn and Zn, which occurred in appreciable quantities in both the stream sediments and in *A. filiculoides*, are considered to be essential elements (Hutchinson, 1943; Cannon, 1960) for plant growth and metabolism. According to Goldschmidt (1937) and Brooks (1972), the extent of accumulation of specific metals by terrestial plants allows them to be classified into biogenic, intermediate and non-biogenic elements. The non-biogenic elements are also considered as ballast elements (Frye-Wyssling, 1935). These metals are accumulated without any known physiological requirements for them by plants. Biogenic metals, on the other hand, play important physiological roles in plants such as in protein synthesis (Zn) (Devlin, 1975), as enzyme activators (Cu and Mn) (Devlin, 1975; Ting, 1982), as metalloenzymes (Zn) (Brooks, 1972; Ting, 1982) and as prosthetic groups in respiratory enzymes (Fe) (Brooks, 1972). Elements with an enrichment coefficient (Goldschmidt, 1937) or CR greater than 10% are, with the exception of ballast elements, considered as biogenic, whilst those with coefficients less than 1% are considered as non-biogenic.

Judged by present results obtained on the CR by *A. filiculoides* of Cu, Ni, Pb and Zn, these metals clearly fall within the framework of biogenic metals as defined by Hutchinson (1943) and Cannon (1960) for terrestrial plants, with Fe being the best placed as an intermediate element, even although a relatively high CR value was recorded for Fe in *A. filiculoides* at locality 4 (Table 5.15). The CR for Cr, on the other hand, fits in with the classification by the latter authors for this element as non-biogenic. From these results it is clear that, in terms of the bio-accumulation of the various metals, the aquatic weed *A. filiculoides* behaved in a way similar to that described for terrestrial plants (Hutchinson, 1943; Cannon, 1960).

Looking at the estimated concentrations of the metals in the standing crop of *A. filiculoides* in the Blesbokspruit wetland ecosystem (Table 5.16) and taking into consideration the massive growths and vegetative reproduction that this plant is capable of throughout the year, even during the winter, the total potential mass of metal that can be bio-accumulated from the aquatic environment by this plant may be considerably higher than the calculated figures given in Table 5.16. It appears that *A. filiculoides*, having a comparatively short life cycle (Ashton, 1982; Ashton and Walmsley, 1976; Ashton, 1977), rapidly grows to maturity and eventually dies off, sinking to the bottom where the metals can be trapped in the sediments from where they may eventually be released again into the aquatic environment during the process of decay. Alternatively, *A. filiculoides* and the bio-accumulated metal loads can be washed downstream during floods and deposited in the sediments of streams and rivers great distances from where the metal loads originated. However, if an active cropping programme of *A. filiculoides* can take place throughout the year, substantial quantities of these metals concerned can effectively be removed with the plant material from the aquatic environment. Some of the metals can then be reclaimed (Tel-Or, undated report; Clark, 1984).

Some emergent, submerged and floating aquatic weeds, which occurred in metal-containing mine- and industry-polluted waters of streams on the Witwatersrand, were investigated for their ability to absorb and accumulate a selected number of metals in their organs and tissues. Iron, manganese and zinc, which occurred in the highest concentrations in the water and sediments of the affected streams, were usually accumulated most in the organs and tissues of the affected plants. When it comes to the extent of bioaccumulation of the various metals compared to their concentration in the abiotic environment, copper and zinc featured prominently in the organs and tissues of these aquatic weeds. Some seasonal tendencies observed in the uptake of metals by some plants were largely obscured by the variable metal contents of the effluents and seepage waters from mines and industries discharged into the streams. Chromium appeared to be the least absorbed and accumulated and indications are that aquatic plants may possess a mechanism to selectively inhibit the uptake of potentially toxic metals from the aquatic environment. This, however, is not necessarily the case with lead. The findings of the present survey indicate that, if a choice has to be made of aquatic weeds to be used as biomonitors of metal contamination of the aquatic environment, a combination of typically mud plants (*Bryum* sp.), emergent aquatic (*T. capensis*, *A. donax* and *Phragmitis australis*) submerged (*P. pectinatus*) as well as floating aquatic weeds (*Azolla filiculoides*) should ideally be combined to evaluate and integrate the extent of metal pollution of a particular water body. In view of the present findings, it is recommended that much more detailed studies be made to evaluate some of these and other commonly occurring aquatic plants in man-made wetland ecosystems to find out if they can be employed as potential biofilters in the reduction and possible elimination of potentially harmful metals from industrial effluents and from streams affected by these pollutants.

10.3 Metal uptake by the fresh water crab, *Potamonautes warreni*

The concentrations in which the metals occurred in the sediments of the Natalspruit wetlands and their bio-accumulation by *P. warreni* placed these metals roughly in four different categories: those which occurred in high concentrations in the sediments but with a relatively low CR, namely Fe; those with high concentrations in the sediments and also with a high CR, namely Mn and Zn; those with a low concentration in the sediments but with a comparatively high CR, namely Cu and Ni, and those metals which not only occurred in low concentrations in the sediments but also with a relatively low CR, namely Pb and Cr. Evidence exists that marine and freshwater invertebrates, and notably decapod crustacea, have evolved mechanisms to regulate bodily concentrations of essential but potentially toxic metals (Bryan, 1964, 1976; Bryan & Ward, 1965; Martin, 1974; Rainbow, 1985). With regard to the bio-accumulation of the various metals by *P. warreni* in this study, indications are that the accumulation of the metals Pb, Cr and Fe in the body of this freshwater crab may still be regulated successfully where it occurred in the polluted waters of the Natalspruit wetlands.

Regulating mechanisms may take the form of biochemical detoxification processes (Simkiss, 1981), the production of physiologically inert, insoluble or granular deposits (Walker, Rainbow, Foster & Holland, 1975; Walker, 1977) or by means of metal-binding proteins (Cherian & Goyer, 1978; Guary & Negrel, 1980). However, beyond certain levels of bio-accumulation the regulatory processes for some metals may break down after which net accumulation commences (White & Rainbow, 1982, 1984). This phenomenon applies particularly to Cu and Zn (Rainbow, 1985). When considering the accumulation of Zn, Mn and Ni by *P. warreni*, the regulatory processes appear to be replaced by that of active accumulation. This can only be substantiated by further and more detailed investigations.

It was only for the metals Cu and Cr that a reasonable correlation existed between their respective concentrations in the sediments and in the body of *P. warreni*, with Cr being excreted at a faster rate or accumulated in comparatively smaller quantities by the crab as its concentration increased in the sediments. Cu, on the other hand, was bio-accumulated most successfully, exceeding the concentrations for this metal in the sediments at three localities. This phenomenon might be ascribed to the active accumulation of this metal by *P. warreni*.

Guary and Negrel (1980) suggest that the hemolymph in crabs may act as a common carrier protein for Fe. Crustacean hemocyanin, which is a copper-containing protein, has been shown to act as a nonspecific metal carrier in binding Fe (Ghidalia, Fine & Marneux, 1972) and Zn (Martin, Van Wormhoudt & Ceccaldi, 1977). In studying the absorption of radioactive and non-radioactive Mn by the lobster *Homarus vulgaris*, Bryan & Ward (1965) showed that losses of bio-accumulated Mn largely took place across the body surface and also by urinary and faecal excretion. In his investigations into the metabolism of Zn and ^{65}Zn in marine crabs, lobsters, and in the freshwater crayfish *Austropotamobius pallipes pallipes*, Bryan (1966) showed that marked differences do exist between marine and freshwater decapods, in both the absorption and excretory processes for metals. Bryan (1966) also showed that the exoskeleton of freshwater decapods may be relatively impermeable to Zn, both in terms of absorption and excretion. As in marine decapods, the hepatopancreas of the freshwater crayfish *A. p. pallipes* also plays an important role in the regulation of the body-Zn concentration. As regards excretion, Zn (and probably other metals as well) is mainly eliminated via the faeces of freshwater crayfish, in contrast to marine decapods where elimination of metals occurs via the body surface, gills and urine. In their studies on the Pb uptake and loss in the freshwater field crab, *Barytelphusa guerini*, Tulasi *et al.* (1987) showed that Pb was absorbed through the gills and from there distributed by the hemolymph to the hepatopancreas, muscles and exoskeleton. However, ongoing research at our laboratory will shed more light on the role of certain organs of the freshwater decapod, *P. warreni*, in the accumulation, bio-concentration and regulation of these and other metals.

Potamonautes warreni was shown to be a very useful benthic macroinvertebrate organism to evaluate metal pollution in the affected aquatic ecosystems affected by effluents from mines and industries. It is recommended that further investigations be made on its potential as a reliable indicator of metal pollution in aquatic ecosystems.

10.4 Metal uptake by fish

Based on observations on its abundance in the Spaarwater dam, *P. philander* does not appear to be detrimentally affected by the metal loads in the lake. This species is a benthic feeder and probably obtains most of its metals via the food, but also from the water environment where the metals in their ionic form can be absorbed through the gills and to a lesser extent through the skin surfaces of the fish (Villegas-Navarro & Villarreal-Trevino, 1989; Heath, 1987). The benthic macro-invertebrate fauna, its major food source, is known for its ability to accumulate large quantities of metals from the sediments (Cover and Wilhm, 1982). According to Heath (1987), a limited control mechanism for the uptake of essential metals occurs amongst fish, and elimination rates may be more dependent upon uptake rates (Bryan, 1964, 1967) than is probably the case for non-essential metals such as lead. With the exception of Pb, the other metals examined here are all essential for various respiratory and metaloenzymatic processes (Mohamed *et al.*, 1990). The comparatively low CR value obtained for Fe could partially be explained on the basis of this control mechanism. CR values obtained for all the other metals, which were present in much lower concentrations than Fe in both the water and sediments, were relatively higher, fluctuating between 1,23 (Cu) and 8,54 (Zn). Lead is known to be a cumulative toxic metal with no beneficial or nutritional qualities for fish or other vertebrates (McKee & Wolf, 1963). However, lead in high enough concentrations can cause the inhibition of ALA-D activities in fish which results in anaemia (Hemberg *et al.*, 1972; Johansson-Sjobeck & Larsson, 1979; Tewari *et al.*, 1987). This metal also impairs the functions of the liver, kidney and spleen (Haider, 1964) and may cause a decrease in bone Ca (Dwyer *et al.*, 1988) which may result in spinal deformities (Davies & Everhart, 1973) and even the death of the fish (McKee & Wolf, 1963). The CR value obtained for Pb, which was in the same order of magnitude as some of the essential metals examined, can possibly be explained by the fact that the main deposition site of this metal is bone, where it can substitute Ca (Settle & Patterson, 1980; Miyahara *et al.*, 1983; Sorensen, 1991). Lead uptake by the fish might, however, be restricted by the fact that this metal is largely absorbed through the gills and skin (Varanasi & Gmur, 1978; Moore & Ramamoorthy, 1984), and in particular with increasing dietary or aqueous Ca where Ca suppresses the synthesis of Ca-binding protein in the gut, resulting in reduced uptake of both Ca and Pb (Varanasi & Gmur, 1978).

Looking at the juvenile fish's ability to accumulate higher concentrations of the metals compared to larger individuals, *P. philander* clearly displays a higher negative correlation between body mass and metal concentrations per mass unit amongst the smaller fish. These findings confirm those of O'Rear (1971) and Matthiessen and Brafield (1977) for the fish species *Morone saxatilis* (Cu and Zn) and *Gasterosteus aculeatus* (Zn). The distinctly negative correlation between body size and metal uptake amongst the smaller fish, especially those below 0,2 g in mass, can most likely be related to the relatively higher oxygen consumption and metabolic processes amongst the smaller juvenile fish (Matthiessen & Brafield, 1977; Anderson & Spear, 1980; Gobas & Mackay, 1987; Clark *et al.*, 1990). The highest negative correlation between body mass and metal concentrations was obtained for Pb. Lead uptake under natural conditions might be directly linked to the oxygen consumption in fish as was shown for Zn-65 by Matthiesen and Brafield (1977). Furthermore, smaller fish have a disproportionately large mass-specific lamellar surface to volume ratio (Muir, 1969; Hughes, 1970), so that the juvenile *P. philander* may be able to bioconcentrate relatively more of the metal. In the case of the other metals examined, where uptake is to a large extent also dependent on feeding behaviour, more variation can be expected to occur because of feeding preferences and the health status of the individual fish.

Regulation mainly exists in the ability of the fish to excrete the metals (Heath, 1987). It is possible that the inverse relationship between mass and metal concentrations that was found to exist for *P. philander* is also a reflection of the fish's ability to bioregulate metals; i.e. larger individuals are better able to bioregulate or excrete the metal than smaller individuals. Somewhat contradictory findings exist in the literature in this respect. Anderson and Spear (1980) showed that a disproportionate decrease in Cu clearance rate occurs with increasing body mass in fish, so that smaller fish are potentially better able to excrete the metal than larger individuals. This was also found by Sharpe *et al.* (1977) for methylmercury clearance in goldfish *Carassius auratus*. Matthiessen and Brafield (1977), however, showed in their uptake rate experiments of Zn by *Gasterosteus aculeatus* that the bioregulation mechanisms are triggered sooner in the larger specimens than in the smaller individuals, which might explain the higher build-up in concentrations of Zn and all the other metals among the smaller fish of *P. philander*.

Heath (1987) suggests that metals may reach high concentrations in larger omnivorous fish. This tendency may be due to magnification of particular metals in the food chain and to old age of such fish. The natural food of the european perch, *P. fluviatilis* in the Florida Lake consists mostly of macro-invertebrates and small fish (McCormack, 1970; Giles *et al.*, 1990; Venter and Schoonbee, personal observations). As a rule, aquatic invertebrates are able to accumulate appreciably higher concentrations of metals than fish under similar environmental conditions (Goodnight, 1973; Bargos *et al.*, 1990). Thus, predators of these aquatic invertebrates may attain a considerable burden of bioavailable metals via this specific food chain. This phenomenon might also explain the comparatively high values recorded for Cu, Mn and Fe in the walls of the gut of the perch from the Florida Lake (Table 7.2). Once these accumulated metals are transferred to the circulatory system of the fish, the degree of concentration in the various organs and tissues will then depend on the binding of the metal to specific ligands. It is to be expected that organs such as the liver, which secrete specific metal-binding proteins as part of normal metal regulation processes and as a detoxification mechanism, and other organs which are the target of toxicant action (e.g., the spleen) may accumulate metals to significantly higher levels than in the rest of the other organs and tissues (Stagg and Shuttleworth, 1982). Yamamoto *et al.* (1978) described the liver of the common carp *Cyprinus carpio* as containing low molecular mass proteins including many sulfhydryl groups due to the large amount of cysteine in the molecule (methallothioneins). These sulfhydryl groups are able to bind to various metals and may thus render them less harmful to cellular structures. According to Heath (1987) there might be several other organs where methallothioneins are functional in fish. With the tendency for the liver of the perch to accumulate several metals, as in this survey, the presence of large amounts of methallothioneins in this organ is therefore not surprising. This phenomenon might possibly explain the comparatively high concentrations of Cu, Fe, Ni and Zn in the liver of the perch in the lake, which is probably the result of the binding of metals as part of a process of their detoxification in the body of the fish.

Contrary to the findings of Holcombe *et al.* (1976) which showed that the gills, liver and kidney tissues of the brook trout, *Salvelinus fontinalis*, are the main target organs of Pb, the present study shows that the vertebrae and the spleen are the major tissues to accumulate this metal in the perch. These findings correlate with those reported on by Heath (1987) which indicated that the spleen in estuarine fish may also be the target organ for Pb accumulation.

The comparatively high Zn concentrations in the gonads of the perch might explain the fact that the Zn requirements of the gonads of fish increase during the spawning period (Fletcher *et al.*, 1975). The present survey was conducted during the summer when, according to observations, natural spawning of the perch normally occurs in the lake. Fletcher and King (1979) suggest that female Sockeye salmon (*Oncorhynchus nerka*) are capable of storing Zn^{2+} during the summer feeding period, using the stored metal for gonadal development during winter. One possible site of Zn storage during this time was the liver. The present study on the perch shows that, with the exception of the spleen and the gonads, the liver is also a major storage organ for Zn. As with the liver, the spleen might likewise act to detoxify this and other potentially toxic metals from the aquatic environment.

The european perch, like the aquatic weeds and other organisms analysed for metals in the Florida Lake, reflects the metal loads in the water and sediments of the abiotic environment and for this reason proved to be a useful organism in reflecting this particular form of pollution of the aquatic environment. However, the differences in metal loads in the various organs and tissues of this fish suggest different pathways of absorption, transport, storage, excretion and also bioregulation of potentially toxic metals. For the purpose of this study whole-body analysis might have provided more useful data on the actual interpretation of the effect of metals in the abiotic environment on the whole organism as such.

10.5 Metal accumulation by the platanna *Xenopus laevis*

A comparison of the data obtained on metal concentrations in the whole-body and in the individual organs of *X. laevis* provided an opportunity to assess the value of each in terms of the environmental effect of metal pollution on this amphibian. Although extremely interesting in providing a much clearer picture of the possible sites of metal absorption, accumulation, storage and excretion in the different organs and tissues, the whole-body metal analysis had the advantage of balancing out their individual contributions in terms of the body mass it represents. This particularly applies to metals in, for instance, the body muscle tissue. Whole-body metal analysis also enables a more realistic assessment of the metal concentrations in the frog, tadpole and larval stages and those in the surrounding metal-polluted aquatic environment. The information thus obtained lends support to the findings and approach of authors such as Davis and Boyd (1978) concerning whole-body metal analysis of organisms.

Investigations on the uptake of selected metals by the frog and tadpole stages of the platanna *Xenopus laevis* showed that certain organs and tissues had much higher concentrations of some of the metals than others. This can be linked to sites of uptake, storage and excretion as well as the possible processes of metal bioregulation and does not necessarily reflect the extent of metal pollution in the aquatic environment. Whole-body metal analysis of the frog and of the larval stages of both the platanna and *Rana angolensis* yielded data which more closely approximated metal concentration ratios in the water and sediments of the areas of study. Preliminary conclusions are therefore that both types of metal analysis would be extremely useful especially as they would provide more information on the ability of *X. laevis* and, in fact, other amphibia to bioregulate these metals, but that whole-body analysis provided a more reliable estimate of the effects on the entire body of these aquatic lower vertebrates.

10.6 Metal uptake by aquatic and semi-aquatic birds

Of the four metals studied in this investigation, cadmium occurred in the smallest concentration in the liver, blood, kidney and bone of all three bird species. In this study kidney cadmium levels exceeded liver cadmium levels by a factor of 2 to 3 (Tables 9.1 and 9.4). Similar trends were also recorded for oceanic sea birds such as albatrosses, petrels, prions and penguins (Lock *et al.*, 1992), near-shore sea birds such as gulls (Nicholson, 1981; Reid and Hacker, 1982) as well as inland birds such as herons and egrets (Hulse *et al.*, 1980; Cheney *et al.*, 1981). Such a situation may well indicate the existence of a chronic, low-level exposure to cadmium in the Natalspruit wetlands rather than an acute cadmium toxicity which would, according to Scheuhammer (1987), result in higher cadmium levels in the liver than in the kidney. Toxic effects of cadmium only occur in humans and other mammals when kidney cadmium levels reach about 100 µg/g wet mass (Scheuhammer, 1987), which was well above the concentrations recorded for the birds in this study. Thus, when considering the indicator ability of the coot, ibis and cormorant it can be assumed that the current low levels of cadmium found in the Natalspruit wetlands (Van Eeden, 1994) and surrounding areas poses no immediate threat to these and other avifauna inhabiting or frequenting these aquatic environments.

During this study it was found that large concentrations of cadmium, nickel and lead occurred in the bone tissue of all three bird species (Figure 9.1). These concentrations generally exceeded the liver cadmium, nickel and lead concentrations by factors of 2-5, 1-4 and 4-7, respectively (Tables 9.2 and 9.4). The relatively small variations found in the bone metal concentrations of the three bird species (Figure 9.1) may be due to their low availability for redistribution or excretion in the bird's body once these metals are physiochemically bound in the bone matrix. It may thus be that bone would be a poor indicator tissue of acute, short-term pollution but may be a more reliable indicator of historical exposure due to its ability to sequester metals over a period of time from the body of those birds exposed to long-term chronic metal pollution. What is of real importance is the comparatively high concentrations of lead found in the bone of all three species during this investigation. Bone lead levels of >10 μg/g and even >20 μg/g (Amiard-Triquet *et al.*, 1992) dry mass are considered as elevated or threshold values. In this study the mean and even the ranges of bone lead concentrations were consistently higher than 20 μg/g (Table 9.2).

The coefficient of variation, as used by Muirhead and Furness (1988) and Cosson *et al.* (1988), recorded in this study suggests that the concentrations of all four metals were not well regulated in most of the organs and tissues. On average for the three species combined, the variability in metal concentrations for bone was 37%, followed by the kidney (43%) and liver (47%). However, a much larger variability of 75% was found for blood (Table 9.4). These results might suggest that the ability of all three bird species to regulate the concentrations of essential (copper and nickel) as well as non-essential metals (cadmium and lead) is not necessarily adequate, particularly in the case of the blood. The high percentage values may further suggest an inadequate ability to effectively regulate the concentration of a metal due to the presence and number (or lack thereof in the case of blood) of metal-binding sites in the kidney and liver (Chen and Ganther, 1975). However, a naturally high variability in organ and tissue metal content and a high metabolic turnover should not be ruled out. The fact that these three species of bird are exposed to high metal levels in their food and drinking water should be taken into consideration. These high metal levels originate from the Natalspruit wetland and adjacent Vlakplaats sewage purification works which is known to be seriously polluted by metals (Van Eeden, 1994).

Literature surveys indicated that little information is available on the concentrations of metals in the organs and tissues of the Redknobbed Coot. No known comparative information is available for the Sacred Ibis apart from that recorded by Van Eeden (1990) on birds sampled from the Natalspruit wetlands during 1989. Van Eeden (1990) recorded mean dry mass concentrations of copper (13 μg/g) and nickel (13 μg/g) in the kidneys, lead (37 μg/g), nickel (13 μg/g) and copper (5 μg/g) in the sacral vertebrae and lead (4 μg/g) and nickel (11 μg/g) in the liver which were comparable with those found in the present study (Tables 9.1, 9.2 and 9.4). The dry mass concentrations of 11 μg Ni/g and 38 µg Pb/g in the bone of the Glossy Ibis (*Plegadus falcinellus*) sampled from the highly industrialised Acre Valley area in Israel (Fishelson *et al.*, 1994), matched those values recorded for the Sacred Ibis in the present study (Tables 9.2 and 9.4) and also those recorded by Van Eeden (1990). However, the mean dry mass concentrations of kidney lead (5 µg/g) and liver copper (26 µg/g) concentrations found in 1989 (Van Eeden, 1990) increased to 11 µg Pb/g and 33 µg Cu/g when compared to values found during the present study. The fact that these increases were noted in organs such as the liver and kidney, which are generally good indicators of short-term metal exposure, would seem to indicate that the environmental levels to which the Sacred Ibis was exposed, have increased slightly over the last five years (Van Eeden, 1994). The origin of these metals can most probably be related to the specific dietary preferences of the ibises found at the Vlakplaats WPCW. It is a common practice at the Vlakplaats WPCW to dispose of the sewage screenings and other untreated debris in heaps which are only periodically covered with soil. In such cases ibises tend to feed primarily on the sewage screenings (Van Eeden, personal observations) which, by virtue of their origin, might contain higher and more variable concentrations of metals. A similar reason might be applicable in the case of the nickel and lead concentrations recorded in the liver (13 µg Ni/g and 33 µg Pb/g) of the Glossy Ibis (Fishelson *et al.*, 1994) which were 1-2 (Ni) and 6-7 (Pb) times higher than those values recorded for the Sacred Ibis in the present study and by Van Eeden (1990). This was particularly apparent in the case of the liver lead concentrations. Hall and Fisher (1985) found that the White-Faced Ibis (*Plegadus chihi*) is capable of ingesting spent lead shot whilst probing the mud for food and in the process might be exposed to acute levels of lead, which can result in death due to lead toxicosis (Amiard-Triquet *et al.*, 1992).

During a preliminary survey on the concentrations of metals on a number of birds species from the Natalspruit wetlands, dry mass metal values in the liver (1 µg Pb/g, 24 µg Ni/g, 70 µg Cu/g), kidney (1 µg Pb/g, 10 µg Ni/g, 46 µg Cu/g) and sacral vertebrae (1 µg Pb/g, 21 µg Ni/g, 19 µg Cu/g) of a single Reed Cormorant was recorded by Van Eeden (1990). Similar values were also observed for a single White-Breasted Cormorant *P. carbo* collected from the same locality (Van Eeden, 1990). However, these values do not compare well with those found for the Reed Cormorant during this investigation, most probably due to the analysis of single specimens. However, Greichus *et al.* (1977) recorded dry mass concentrations of 0,6 µg Cd/g, 5 µg Cu/g and 2 µg Pb/g in three Reed Cormorant carcasses collected from the Hartbeespoort Dam which was considerably lower than the organ and tissue metal levels found for the cormorant during this study (Tables 9.1 to 9.4), especially in the cases of lead and cadmium. This can be attributed to dilution through mere body volume in contrast to organs or tissues that are capable of sequestering metals in high concentrations from the body of the bird.

Various studies on the concentrations of a number of metals in a variety of bird species have suggested that the diets of birds were most probably the major source of these metals (Hulse *et al.*, 1980; Cheney *et al.*, 1981; Nicholson, 1981; Reid and Hacker, 1982; Lock *et al.*, 1992). These authors also suggested that the specific dietary preferences among different bird species as well as among individuals of the same species could have a pronounced effect upon the concentrations of these metals in the bird's organs and tissues. It would also appear that the effects of diet are greater than taxonomic differences in evaluating organ and tissue metal burdens. Reed Cormorants might exhibit higher metal levels as a consequence of their specific diet rather than as a result of any intrinsic function of being a Reed Cormorant. The dietary preferences as well as the occurrence and concentrations of cadmium, copper, nickel and lead in various food items of each of the three bird species is known (Van Eeden, 1994). This discussion may throw more light on the role of diet on the possible transfer of metals from the food to the birds concerned.

It can be assumed that some of the coots, cormorants and ibises (or for that matter any other aquatic bird) collected from the Natalspruit wetlands during the survey period in 1991-1993, may have originated from other more or less polluted habitats, most probably due to the extensive droughts experienced over the last couple of years. Rainfall, which has a definite seasonal trend in South Africa, will determine the stability of a water body, which in turn will influence the availability of, among other things, food, shelter and drinking water, and therefore the number and species of aquatic birds occurring there. However, rainfall can also dilute a metal-polluted water body and at the same time leach metals from soils as well as refuse and mine dumps into the same water body, thus creating a more or less constant source of metals in the affected ecosystems, a situation from which aquatic birds are then unable to escape.

10.7 Construction of the metal transfer of a simplified food web for the sacred ibis *Threskiornis aethiopicus* from the Natalspruit wetland ecosystem.

Using the concentrations of Pb, Mn, Ni and Cr recorded in the water, sediments and in the major dietary items of 16 birds of the sacred ibis collected in the Natalspruit wetlands, the possible routes of accumulation and transfer of these metals from the water and sediments through the biotic components of its major dietary constituents to the ibis was constructed. (Figures 10.1-10.4). The benthic macro-invertebrate fauna effectively bioconcentrated Pb and Ni and to a lesser extent Cr, but did not appear to be able to utilize effectively Mn from the sediments. (Figure 10.4). Metal concentrations in the crab, *P. warreni* were even lower. This also applied to the platanna, *X. laevis* which prey upon these benthic macro-invertebrates, including the crab. (Schoonbee *et al.*,1992). Looking at the concentrations of the four metals in some organs and tissues of the ibis, results suggest that Mn (all organs) and Pb (liver) were both poorly bioaccumulated while Ni (liver) and Pb (kidney) were present in compareble concentrations than in the food items of the bird. Only Cr (all organs) and Pb (bone tissue) appeared to be effectively bioconcentrated from the dietary items of the bird.

To summarise : The results suggest that an efficient mechanism exist in benthic macro-invertebrate organisms (including the crab) to accumulate metals such as Pb, Ni and Cr from the abiotic environment thereby biomagnifying these metals in the food chain of the ibis. However birds such as the ibis at the top of the food web are effective in bioregulating potentially toxic metals such as Pb and Ni in its organs and tissues.

10.8 Concentration ratios

Although CR values were recorded for both the water and sediments, the CR_s values for the sediments (expressed as dry mass) were preferred to evaluate the ability (or potential) of the aquatic flora and fauna to bioaccumulate metals analysed for at the various localities. Bain *et al.* (1994) discussed the merits and weaknesses of the concentration ratio and its use to demonstrate the possible transfer of metals in a food chain system with specific reference to the transfer of radium from irrigation water to vegetable crops. A serious weakness is the unqualified use of CR_s and CR_w as such without taking into consideration the speciation of metals and the bioavailability of only a fraction of the total metal concentration of a given metal measured in the water and in the sediments of aquatic ecosystems. This may lead to a distortion of the actual ability of aquatic flora and fauna to accumulate metals in their organs and tissues. It also influences the entire interpretation of the possible biomagnification of metals in organisms and the transfer of potentially toxic metals within the food web of aquatic ecosystems.

10.9 Water quality criteria for metals

According to the water quality criteria aimed at the protection and maintenance of aquatic life in rivers and dams (Kempster *et al.*, 1980), all the metals investigated during the present survey had concentrations in both the water and the sediments which exceeded the proposed maximum limits. This means that all the wetland ecosystems investigated during the present survey are under constant threat of potentially toxic metal loads which exceeded the proposed limits laid down in the world literature for the preservation of aquatic life. In the present study, no attention was given to the actual effects of metals on the morphology, histology and physiology of any of the aquatic flora and fauna investigated for their metal uptake during the ecological survey of the present study. It is recommended that in future follow-up projects, specific studies be undertaken on metals in the aquatic environment to evaluate the physical damage done to organs and tissues of both the affected aquatic plants and animals.

10.10 Recommendations

From the results obtained, it is evident that significant quantities of the various metals released into the aquatic environment can be retained by and recirculated in the wetland ecosystems. It was also shown that emergent, submerged and floating aquatic weeds are extremely successful in the bioconcentration of these metals in their roots, shoots and foliage. These metals are, however, again released into the abiotic environment following the die-off and subsequent decay of the organic material. In view of the findings of the present investigation concerning certain problems encountered with the handling and interpretation of data on the fate of metals in these wetland ecosystems, the following recommendations are made :

- **Aquatic weeds efficient in the absorption and accumulation metals which occur in these and other metal polluted wetlands should be properly documented and further investigated for possible future use in artificial wetlands aimed at the removal of potentially toxic metal loads from effluents and seepage waters from mines, industries and refuse disposal sites.**

- **Rehabilitation of existing mine- and industry-polluted rivers and lakes, so that these water bodies can be utilized for recreational and other purposes by the evergrowing population in the Gauteng province and elsewhere.** The success of the rehabilitation of the Germiston Lake from mine- and industrial pollution and the biological control of excessive growths of the submerged aquatic weeds *Potamogeton pectinatus* and *Lagarosiphon* species in both Germiston Lake and in the Florida Lake, Roodepoort, using the Chinese grass carp, *Ctenopharyngodon idella*, serve as a good example of the recovery potential of such water bodies from pollution.

- **Investigations into metal speciation and into the bioavailable components of the various metals should be conducted.** Such information will throw more light on the fractions of the total concentrations of metals which can effectively be utilized by aquatic organisms. This will also enable the research worker to reasses the use of concentration ratios of metals in the aquatic environment as used at the present stage by research workers and, to be able to come forward with a modification of this ratio.
- **Investigations into the direct and indirect effects of the various potentially toxic metals on the organs and tissues of target organisms in the aquatic environment.** This must include research on physiological, morphological and histological effects and will to a large extent involve the correlation of field data with that of laboratory studies.

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Figure 10.1: Lead in the water, sediments, benthic macro-invertertebrate fauna, crabs, platanna and organs of the Sacred Ibis from the Natalspruit wetland. Metal concentration in µg/g dry mass or µg/l.

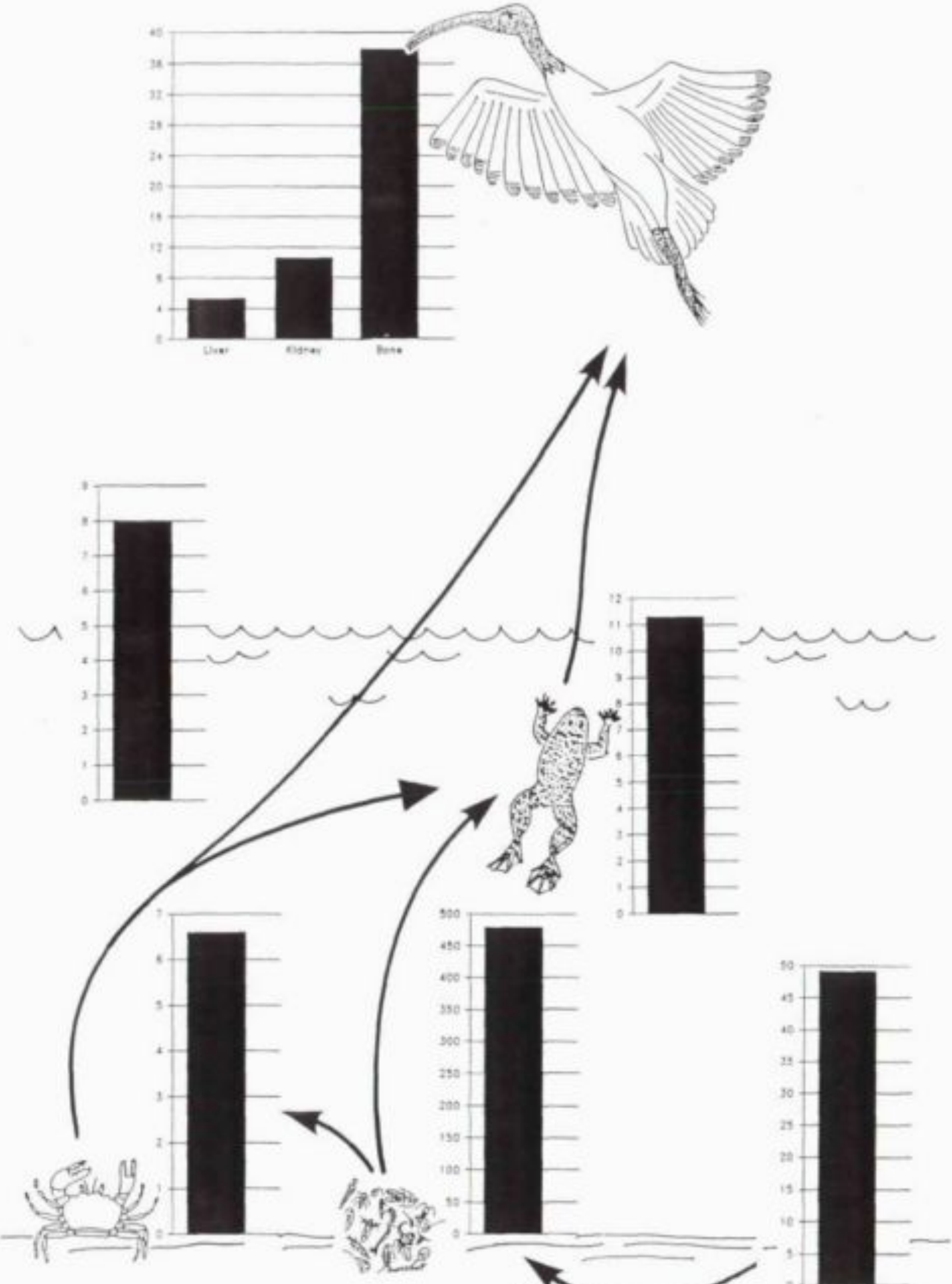

Figure 10.2: Manganese in the water, sediments, benthic macro-invertertebrate fauna, crabs, platanna and organs of the Sacred Ibis from the Natalspruit wetland.
Metal concentration in µg/g dry mass or µg/l.

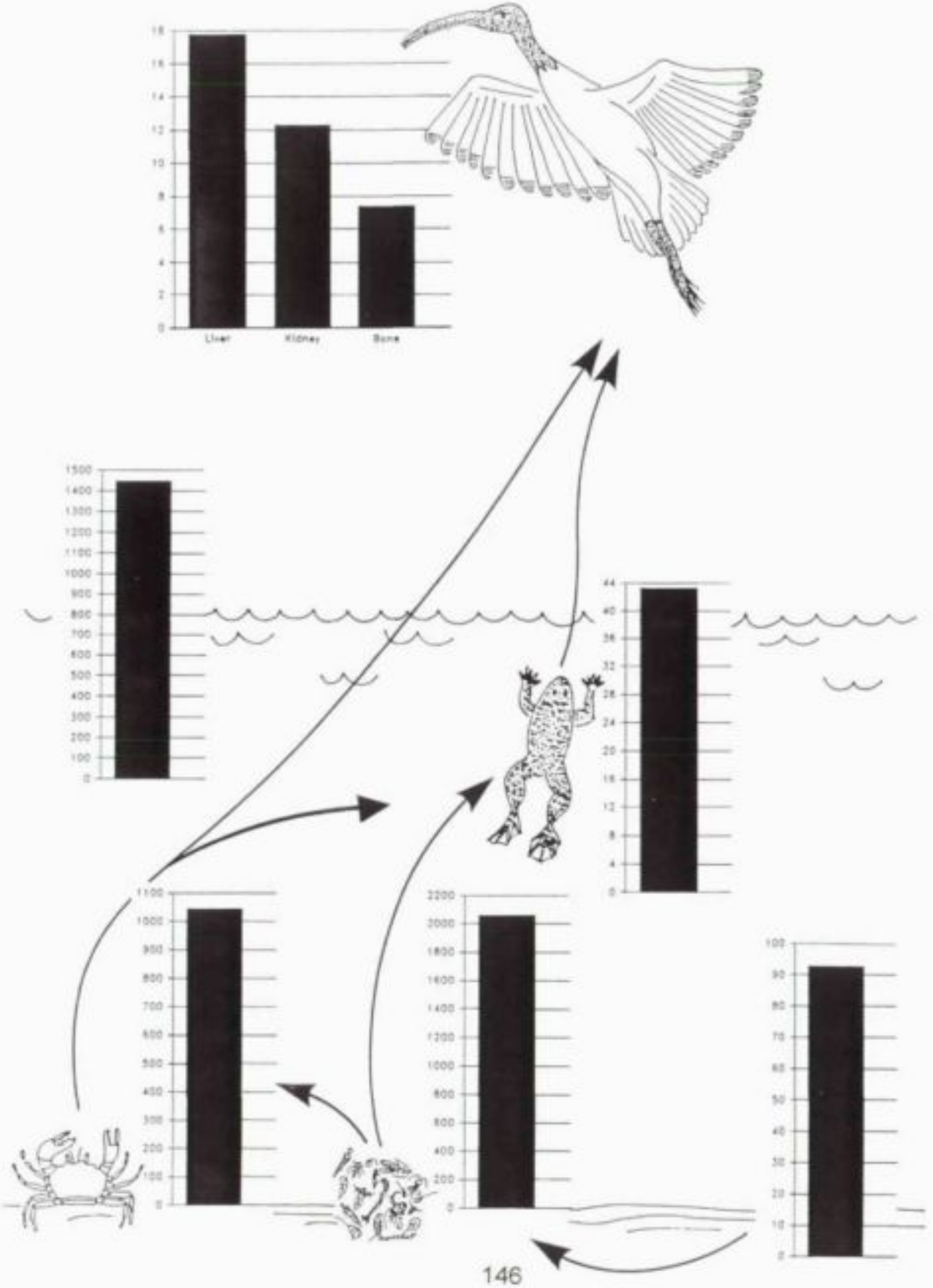

Figure 10.3: Chromium in the water, sediments, benthic macro-invertertebrate fauna, crabs, platanna and organs of the Sacred Ibis from the Natalspruit wetland. Metal concentration in µg/g dry mass or µg/l.

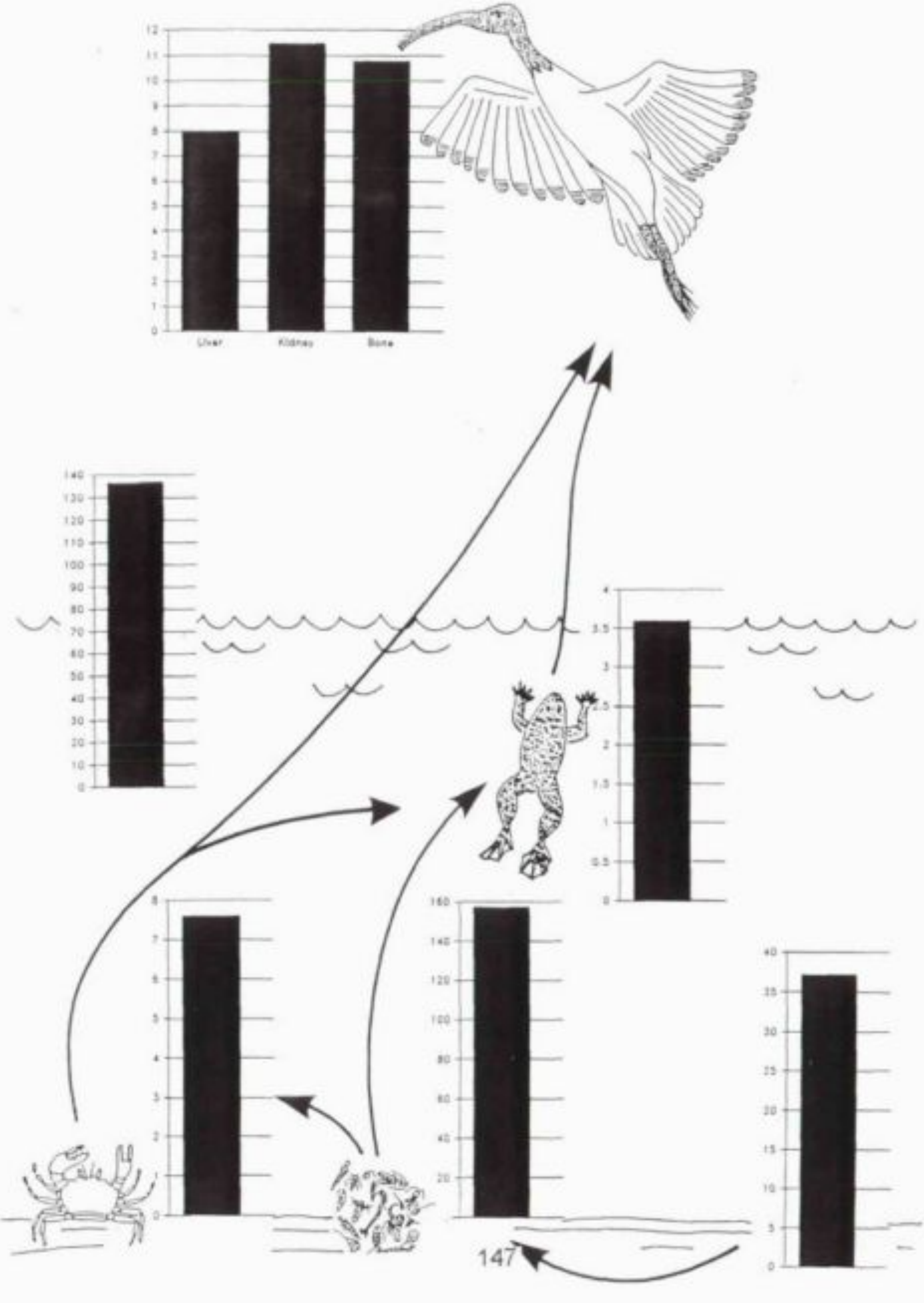

Figure 10.4: Nickel in the water, sediments, benthic macro-invertertebrate fauna, crabs, platanna and organs of the Sacred Ibis from the Natalspruit wetland. Metal concentration in µg/g dry mass or µg/l.