Heavy metal content in organs of the African sharptooth catfish, *Clarias gariepinus* (Burchell), from a Transvaal lake affected by mine and industrial effluents. Part 1. Zinc and copper

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Abstract

An investigation was made into the bioconcentration of the heavy metals zinc and copper in the intestine, spleen, liver, kidney, body fat, gonads, heart, muscle, brain, vertebrae and gills of the sharptooth catfish, *Clarias gariepinus*, in a lake contaminated by effluents and seepage water from mines and metal processing industries. Results showed marked differences in the bioconcentration capacity of the various organs. Compared to the concentrations of the metals in the lake sediments, the bioconcentration in the organs of this fish was shown to be highest for copper.

Introduction

With the establishment of gold and coal mines in South Africa during the past 80 years, several industrial zones surrounding the major cities were created to support the mining industry. Many of these consisted of heavy metal processing factories. Processed and unprocessed effluents and seepage waters from both the mines and the metal industries discharged into streams were found to contain variable concentrations of heavy metals (Wittmann and Förstner, 1976 a-c, 1977 a-b; Kempster *et al.,* 1980; Van der Merwe *et al.,* 1989).

Pollution from mines in particular leads to the severe acidification of some streams (Harrison, 1958) and lakes (Schoonbee *et al.,* 1985) in the Transvaal. One such lake is the Germiston or Victoria lake near Johannesburg which had been severely polluted for decades by mine and industrial effluents. However, removal of the major sources of pollution in this lake over a period of approximately 15 years has led to a rapid recovery (Vermaak, 1972; Schoonbee *et al.,* 1985) so that fish were introduced between the period of 1972 to 1976. These fish, which included the European common carp, *Cyprinus carpio* L., the cichlid, *Tilapia sparrmanii* (Smith) and the African sharptooth catfish, *Clarias gariepinus,* adapted well to their new environment and have spawned annually over the years since their introduction into the lake (Schoonbee *et al.,* 1985).

Although a significant improvement in water quality of the lake could be observed with pH values increasing from as low as 3,5 to 7-8, accompanied by a decline in the concentrations of mineral solutes in the water over the years (Schoonbee *et al.,* 1985), appreciable quantities of heavy metals still remained in the soft bottom substrate of the lake in concentrations which potentially might pose a threat to the aquatic life, including fish life. Since the lake has been used during the past few years for angling purposes, it was considered important to establish the extent of metal uptake by the various fish species in the lake. Although much work has been done in overseas countries on the exposure of fish to high levels of heavy metals in lake and river waters (Brown *et al.,* 1970; Atchison *et al.,* 1977; Koli *et al.,* 1978) no information is as yet available on the effects of heavy metals on our local fish fauna.

The present series of investigations of which this paper is the first, deal with the occurrence and accumulation of the heavy metals Zn, Cu, Pb, Cr, Ni, Fe and Mn in organs of 4 to 8 year old specimens of the sharptooth catfish caught in the lake as well as from the sediments of the lake itself. The fish analysed are all confined to the lake itself and because of the barriers and stream conditions below the lake, no recruitment can take place from outside the lake area. All fish, irrespective of age, are therefore totally confined to the lake and accumulation of heavy metals in the fish analysed is therefore exclusively from the lake itself.

In **Part 1** the concentrations of the heavy metals zinc and copper in the lake sediments are compared with values obtained in the intestine, spleen, liver, kidney, body fat, gonads, heart, muscle, brain, vertebrae and gills of *C. gariepinus.* **Part 2** deals with the heavy metals lead, chromium and nickel, whilst **Part 3** contains information on the presence and concentration of iron and manganese in both the sediments and the organs of this fish.

Materials and methods

Eleven specimens of *Clarias gariepinus* were collected randomly in Germiston lake during the summer of 1988-89 using 110 and 130 mm gill nets. The fish were weighed and the total length of each individual specimen determined. The following organs were then removed from each fish for analysis: intestine, spleen, liver, kidney, body fat, gonads, heart, brain, muscle, vertebrae and gills. The pectoral fin and otoliths were also collected from each fish for age determinations. The wet mass of each organ was determined whereafter it was dried in an oven at 90°C for 48 to 72 h. The dry mass of each sample was then also determined.

Core samples of the upper 5 to 10 cm of the sediments were collected at nine sites from the littoral zone around the perimeter of Germiston lake (Fig. 1) during the summer season at the same time when the fish were collected. These were also dried at 90°C for a minimum period of 48 h.

Both the organs and sediment samples were separately homogenised. Approximately 1 g of each sample was then accurately weighed to the nearest milligram on an electronic Sartorius Model No. R200D balance.

The samples were then digested after adding a 10 mℓ 1:1 perchloric and nitric acid mixture according to standard procedures. This was done in a 250 mℓ pyrex glass beaker covered with a watchglass on a hot plate at a temperature of approximately 200°C. The time of digestion lasted for at least 4 h during which

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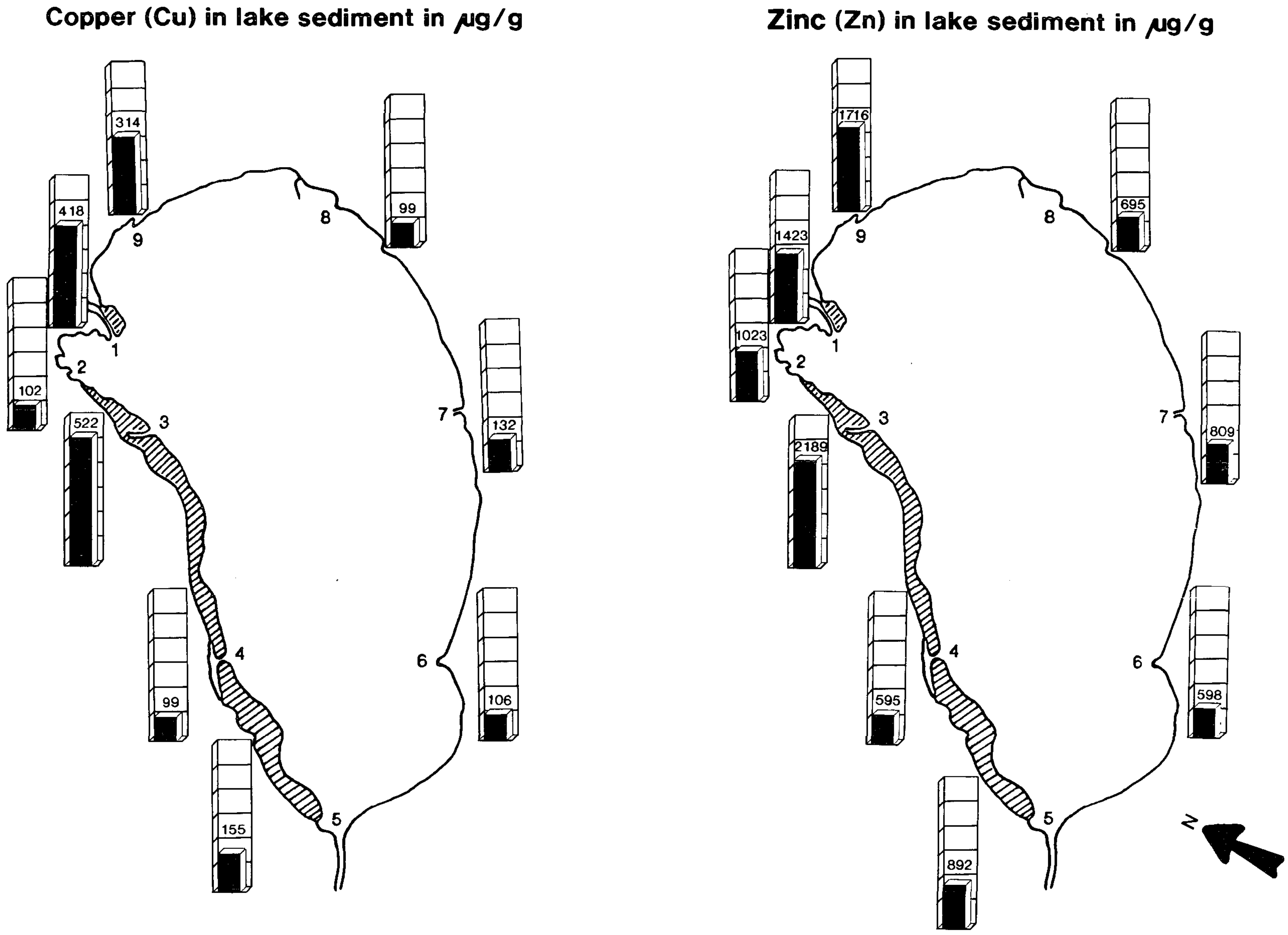

Figure 1
Diagram of Germiston lake with sampling localities and concentrations ($\mu g.g^{-1}$) of Cu and Zn in the lake sediments at each site during the summer of 1988-1989.

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 metals and the volume of each solution was then made up to 100 mℓ in a volumetric flask. These solutions were individually transferred to clean glass storage bottles for later analysis of the different heavy metals.

To determine the organic contents of the sediments from each locality, approximately 1 g of homogenised dry material was accurately weighed out and ashed in a Labcon muffle furnace Type RM4.

A Varian atomic absorption spectrophotometer, Series No. 875 was used to determine the concentrations of the metals in the samples. During the atomic absorption analysis use was made of a zinc analytical standard stock solution (1 000 ppm) of Holpro, Catalogue No. 38945, diluted to a range of 0,5 to 2,0 ppm, while copper analytical standard stock solution (1 000 ppm), Catalogue No. 38564, diluted to a range of 2 to 10 ppm was used for the determination of copper in the samples. Values obtained, expressed as mg.ℓ^{-1}, were in turn recalculated to μg.g^{-1} using the actual dry mass of each digested sample, i.e. values are expressed as μg element per gram fish on a dry weight basis.

Results were analysed statistically using Lotus 123, Harvard Graphics and Statgraphics software packages.

Results

Results on the values obtained for zinc and copper in the sediments at the nine different localities are summarised in Table 1. Values for zinc fluctuated between 595 μg.g^{-1} (Locality 4) and 2 189 μg.g^{-1} (Locality 3), with the second highest concentration for this metal occurring at Locality 9 (1 715 μg.g^{-1}). The mean value of zinc for all localities in the lake exceeded 1 100 μg.g^{-1}. The concentracion for copper in the sediments were generally much lower at the different localities than those recorded for zinc, with a mean value of 216 μg.g^{-1} for all localities. Highest values for copper again occurred at Localities 1 (418 μg.g^{-1}) and 3 (522 μg.g^{-1}) which corresponded with areas where peak values were also obtained for zinc in the lake.

TABLE 1
ANALYSIS OF THE BOTTOM SEDIMENTS AT NINE SAMPLING LOCALITIES IN GERMISTON LAKE FOR ZINC AND COPPER WITH AN INDICATION OF THE ORGANIC CONTENTS OF THE SEDIMENTS AT EACH SITE

Sampling localities	Zinc (μg/g)	Copper (μg/g)	Organic content (%)
1	1423	418	24
2	1023	102	20
3	2189	522	79
4	595	99	16
5	892	155	35
6	597	106	8
7	808	132	15
8	695	99	23
9	1715	314	56
$\overline{X}$	1104	216	31
SD.	524,4	151,8	22,9
C.V.	48	70	75

$\overline{X}$: 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

Age determination of the *C. gariepinus* specimens collected for heavy metal analysis showed the fish to vary between 4 and 8 years in age with individual mass varying between 680 and 3 580 g. The fish varied in total length between 46 and 77 cm. Seven females and four males were used. Individual analysis showed no correlation of concentration of the metals with sex, age or mass. Mean values for the two metals for all fish were therefore obtained for each organ analysed (Table 2).

There was a considerable fluctuation in the concentration of zinc between the different organs with the highest concentration of this metal occurring in the brain (335 μg.g^{-1} ± 265,2), heart (196 μg.g^{-1} ± 66,2), gills (177 μg.g^{-1} ± 36,3), spleen (163 μg.g^{-1} ± 16,2) and kidneys (143 μg.g^{-1} ± 32,6), in that order.

The muscles (59 μg.g^{-1} ± 31,7) and body fat (50 μg.g^{-1} ± 41,5) contained the lowest concentration of zinc. The bioaccumulation for zinc of the various organs compared to the mean concentration for this metal in the sediments varied between a highest of 30% in the brain to a lowest of 4% in the body fat. The mean metal bioconcentration for all the organs investigated was 13% for all organs analysed expressed as a percentage of mean concentration of zinc in the sediments (Table 1). The highest fluctuations for zinc accumulated by the organs of *C. gariepinus* occurred in the body fat (C.V. = 84) and brain (C.V. = 79), while the lowest fluctuation occurred in the vertebrae (C.V. = 17), intestine (C.V. = 17) and liver (C.V. = 17).

Copper bioconcentration in the various organs followed an almost similar pattern as that encountered for zinc with the highest bioconcentration occurring in the brain (100 μg.g^{-1} ± 57,2) followed by heart muscle (41 μg.g^{-1} ± 9,6) and gills (45 μg.g^{-1} ± 16,7). Concentrations of copper in the liver (36 μg.g^{-1} ± 15,0), kidney (41 μg.g^{-1} ± 19,8) and spleen (38 μg.g^{-1} ± 16,4) were also amongst the highest values obtained for the various organs analysed. The concentration factor for the copper in the brain, compared with values obtained for the sediment, was much higher than for zinc, being 46%, with a lowest value of 4% in the muscle. Considering the concentrations of the two metals in the lake sediment, copper appeared to be accumulated at higher concentrations in the organs than was the case for zinc, being 17% for all organs analysed compared with 13% for zinc. In the case of copper, the highest variation in bioconcentrations recorded, occurred in the body fat (C.V. = 54), brains (C.V. = 57) and kidneys (C.V. = 48).

A statistical evaluation of the bioconcentration values in the various organs for zinc and copper, showed that although marked differences exist in the values obtained, no significant difference could be found for zinc bioconcentration between any of the organs. In contrast there was a significantly higher bioconcentration at the 95% level of confidence for copper between the brain and heart (P: 0,0200), brain and gills (P: 0,0084) and brain and spleen (P:0,0037).

Discussion

The uptake and accumulation of zinc and copper in the various

TABLE 2
MEAN VALUES OBTAINED FOR ZINC AND COPPER FROM THE VARIOUS ORGANS OF SPECIMENS OF THE AFRICAN SHARPTOOTH CATFISH CAUGHT IN GERMISTON LAKE DURING THE SUMMER OF 1988-89

	Zinc		Copper	
Organs	$\overline{X}$ ± S.D.	C.V.	$\overline{X}$ ± SD.	C.V.
Intestine	143 ± 23,6	17	26 ± 8,3	32
Spleen	163 ± 46,2	28	38 ± 16,4	43
Liver	143 ± 24,2	17	36 ± 15,0	42
Kidney	143 ± 32,6	23	41 ± 19,8	48
Body Fat	50 ± 41,5	84	13 ± 7,0	54
Gonads	126 ± 22,3	18	15 ± 2,8	19
Heart	196 ± 66,2	34	41 ± 9,6	23
Brain	335 ± 265,2	79	100 ± 57,2	57
Muscle	59 ± 31,7	54	9 ± 2,6	28
Vertebrae	75 ± 12,6	17	12 ± 4,8	40
Gill	177 ± 36,3	20	45 ± 16,7	37
Sediment	1104 ± 524,4	47	216 ± 151,8	70

$\overline{X}$: Mean values
S.D.: Standard deviation
C.V.: Coefficient of variability

organs of the sharptooth catfish have been clearly demonstrated in this investigation. The question arises via which pathways these metals may have entered the fish. According to the literature, there are four possible routes for substances such as metals to enter a fish. One way would be by means of the food items ingested (Waldichuck, 1974; Baudin, 1987). Another can be by means of absorption of the metal in its ionic form by the fish through the gills (Matthiessen and Brafield, 1977; Heath, 1987). This can occur through the gills, probably by simple diffusion and possibly through the pores in the gills (Bryan, 1979). Indications are that metal uptake in the gill tissues may be correlated with mass specific rates, with small fish accumulating metals more rapidly than larger ones (Anderson and Spear, 1980). A third possible route may be through drinking water (Eddy, 1981), whilst a fourth may be through absorption via the skin.

It is suggested by Heath (1987) that in the case of freshwater fish, metal uptake through the gills may be due to solvent drag as a result of the osmotic inflow of water. According to Heath (1987) there is no apparent evidence for the active transport of metals in fish via the gills, although there may be cases of carrier mediation, in this case calcium (Bryan, 1979).

The most likely pathway through which *C. gariepinus* could have obtained the concentrations of copper and zinc would be through the uptake of food in the lake (Bryan, 1976; Moore and Ramamoorthy, 1984). *C. gariepinus* has omnivorous feeding habits (Groenewald, 1964; Schoonbee, 1969; Van der Waal, 1972; Gaigher, 1977; Willoughby and Tweddle, 1978; Bruton, 1979) taking small items such as zooplankton, bottom dwelling aquatic organisms such as Oligochaeta, chironomid larvae, Ephemeroptera and Hemiptera nymphs, aquatic weeds, detritus and fish. It is known from both unpublished data and the literature that these aquatic organisms are able to accumulate substantial quantities of metals, including zinc and copper, from their environments (Burrows and Whitton, 1983; Dixit and Witcomb, 1983; Yasuno *et al.*, 1985; Van der Merwe *et al.*, 1989).

According to the literature, zinc is mainly concentrated in the skin, bone, liver, gill and kidney in fish (Mount, 1964; Matthiessen and Brafield, 1977; Holcombe *et al.*, 1979). Our findings showed the bioconcentration of zinc in *C. gariepinus* to be the highest in the brain followed by the heart, gills, liver, spleen and kidneys with the vertebrae having the lowest concentration of zinc (Table 2).

Our results differ somewhat from results recorded for some other fish species mentioned in the literature when it comes to the bioconcentration of copper in the various organs. Felts and Heath (1984) showed that copper has a relatively low tendency to accumulate in the brain of the bluegill, *Lepomis macrochirus*. A similar tendency was found for the kidney of the brown bullhead, *Ictalurus nebulosis* (Brungs *et al.*, 1973), the bluegill, *Lepomis macrochirus* (Benoit, 1975) and for the coho salmon, *Oncorhynchus kisutch* (Buckley *et al.*, 1982). This is in contrast with our own findings for *C. gariepinus* in Germiston lake where the kidneys appeared to be amongst the more important organs in the bioconcentration of copper. Heath (1987) however cautions that the findings of Brungs *et al.* (1973), Benoit (1975) and Buckley *et al.* (1982) concerning the bioconcentration capacity of copper in the brains and kidneys of these fish need to be substantiated further as these investigations represented only three species of fish exposed to relatively low dosages of copper.

However, a factor which is not considered here and which may be important, would be the ability of *C. gariepinus* to excrete copper and zinc (Bryan, 1976) as this phenomenon has been well documented in the literature for a number of fish species (Cross *et al.*, 1973; Bryan, 1976). It has also been suggested that loss of metals in fish may occur via the skin and gills, probably involving the mucus layer covering these organs (Varanasi and Marky, 1978), a factor which should be investigated further for *C. gariepinus.*

Zinc is one of the essential trace elements in the human body (Förstner and Wittmann, 1981). It is an important constituent of cells with several enzymes depending upon it as a cofactor. This metal is classified as an intermediate element between hard and soft acceptors with ligands but seldomly interferes with sulphur and sulfhydryl groups in biological systems (Moore and Ramamoorthy, 1984). This metal is considered an essential element mediating a variety of metalloenzymes in the biosynthesis of nucleic acids. In the case where it may become toxic to human beings, which rarely occurs, it is from its synergistic and/or antagonistic interaction with other heavy metals, in particular cadmium. Judged by the concentrations of zinc in the muscle of the catfish, the meat is still suitable for human consumption and according to available information does not pose any serious threat to human health. This also applies to the copper concentrations found in the muscle of *C. gariepinus.* Copper has not found to be acutely toxic to humans (Moore and Ramamoorthy, 1984). There is also no indication that copper at concentrations recorded in *C. gariepinus* from Germiston lake could pose a carcinogenic or mutagenic threat to humans.

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