Human health aspects of the metals zinc and copper in tissue of the African sharptooth catfish, *Clarias gariepinus*, kept in treated sewage effluent and in the Krugersdrift Dam

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Abstract

Bioaccumulation and health risks of the metals zinc and copper were studied in the liver, kidney and muscle tissues of the African sharptooth catfish, *Clarias gariepinus*, kept in treated sewage effluent and in the Krugersdrift Dam, Bloemfontein, South Africa. Metal concentrations were also studied in the water and sediment of the mentioned localities. Marked differences in the bioconcentrations of Zn and Cu were measured in the different types of tissue. Concentrations of the selected trace elements were noticeably higher in the livers and kidneys than in the muscle tissue. However, no set seasonal patterns could be established regarding the incidence of these elements in both localities. The occurrence of Zn and Cu in the water of both localities was very low and therefore it would not be considered harmful or toxic to aquaculture. The Cu and Zn concentrations found in the muscles of *C. gariepinus*, kept in domestic effluent from a biofilter plant, are not considered to be a health hazard to consumers. Gutted fish only would be recommended for intake due to the Cu and Zn concentrations found in the kidney and liver tissue. A diet containing fish would contribute to the daily requirement of 4 to 10 mg for Zn and approximately 3 mg for Cu.

Introduction

The alarming growth rate of 2.3% per year for the South African population, will result in a total population of approximately 138 m. in less than 35 years (Council for Population Development, 1990). The Council estimated the natural water sources of the country to be adequate for a maximum of 80 m. people. It is clear, therefore, that the development and utilisation of water sources in the Republic of South Africa is of the utmost importance. Fourie (1989) furthermore warns that more food will have to be produced per unit area in South Africa in order to satisfy future domestic needs. General water shortages areas suitable for aquaculture in South Africa necessitate investigations into the use of effluent, such as treated sewage effluent from municipal purification plants for food production. These usually abundant and nutritious waters (Duffer, 1982), are normally discharged into natural water sources. Especially in South Africa, it must be endeavored to improve the quality of available water and to make use of effluent as much as possible.

The utilisation of human and animal waste in fish dams has been known for decades (Feacham et al., 1978), but little information regarding the human health aspects of fish living in treated domestic effluent is currently available. These waters may pose a potential health risk to handlers and consumers of such fish (Guelin, 1962; Janssen, 1970; Reichenback-Klinke, 1973; Feachem et al., 1978; Lawton and Morse, 1980). For this reason the use of domestic effluent for aquaculture has not yet been approved by health authorities in South Africa. Additional drawbacks of fish production in maturation ponds are public disapproval of sewage-related products as well as conditions resulting in stress to the fish (Wrigley et al., 1988). According to Sandbank and Nupen (1984), the most important problem regarding aquaculture in domestic effluent, is the accumulation of metals, pathogens and pesticides in the fish and, as a result, the possible transmission of diseases to man. The potential of fish being contaminated is very high where cultivation has taken place in effluent because of the possibility of contamination by bacteria, viruses and toxic chemicals (Hejkal et al., 1983). However, the health risks related to the consumption of fish kept under the controlled conditions of maturation ponds cannot be greater than in the case of fish kept under uncontrolled conditions (Hejkal et al., 1983). The authors suggest further that a sewage treatment system employed for aquacultural may be a potentially useful alternative to conventional sewage purification installations. Uncontrolled water sources on the contrary can be contaminated by pollutants at any time. The various discrepancies regarding health hazards to man consuming fish living in treated sewage effluent still have to be clarified.

Very little information is available on the effects of metals on local fish fauna (Bezuidenhout et al., 1990; Du Preez and Steyn, 1992; Du Preez et al., 1993; Van der Merwe et al., 1993; Wepener et al., 1992) as well as accompanying health risks for the consumer thereof. For this reason the primary goal of this study was to determine whether treated sewage effluent could be suitable for the culture of fish for table purposes. Generally known for its hardiness, the African sharptooth catfish (*Clarias gariepinus*) was chosen as experimental species, as research conducted by Prinsloo et al. (1989) on *C. gariepinus* in maturation ponds in Lebowa, had indicated that this species of fish thrived on the high organic content of the ponds. A secondary goal of this study was to compare the pollution status of the Krugersdrift Dam, as a natural water source, and treated sewage effluent.

Additive and synergistic relationships among pairs of metals are concentration-dependent as is the case with Cu and Zn (Doudoroff and Katz, 1953; Lloyd, 1961; Sprague and Ramsay, 1965; Birge and Black, 1979). This was demonstrated by Sorensen (1991) with studies on the hatchability of catfish, goldfish and bass embryos with a 1:1 Cu:Zn solution. Due to their elemental interactions in the same habitat, Zn and Cu were selected for a first paper in a series on metals and related trace elements in treated effluent, sediment and tissue of catfish kept at the Bloemspruit Sewage Works and the Krugersdrift Dam near Bloemfontein, South Africa.

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Materials and methods

Water sampling, preparation and analysis

Water samples were collected monthly from January to December 1991 according to the method of Watling (1981). Sampling bottles were washed and left to stand in a 5% Decon 75 non-metallic detergent solution for 48 h. After rinsing with distilled water, the bottles were soaked in 50% HCl for 24 h. Finally, the items were washed with distilled water and stored in 1% HNO_3 prior to their use.

Pre-cleaned high-density polyethylene bottles with a 2 ℓ capacity were used for sampling. Samples were acidified on site with 10 mℓ concentrated nitric acid (SAARCHEM) (AR) per litre sample and the bottles sealed with high density polyethylene screw-tops. Samples were transported to the laboratory in a sample case at 15°C and stored in high density polyethylene bottles until they were analysed. The collected samples were divided into 3 subsamples and then filtered through an 0.45 µm membrane filter into a 335 mℓ high-density polyethylene bottle with a polyethylene top. The first 20 mℓ of the filtrate were discarded, and a separate subsample was collected in a test tube for analysis. The samples were analysed according to the method of Kempster (1986).

The Cu and Zn concentrations of water samples collected from each locality were determined by means of an ARL inductively coupled plasma (ICP) emission spectrometer (Model 3410).

Collection, preparation and analysis of sediment samples

For the collection of uncompacted sediment samples, a polyvinyl chloride pipe of 50 mm dia. was pushed manually into the sediment to a depth of 300 mm whereafter the top end was covered with a thick plastic bag. The pipe was then removed from the water and the rod-shaped samples placed into plastic bags. Each sample was divided into 3 lengths of 100 mm, sealed in polyethylene bags and transported to the laboratory in cool-bags filled with ice (Watling, 1981). Before drying, the samples were exposed to a temperature of -20 °C for 24 h (Bruwer et al., 1985) and thereafter passed through a 1 mm nylon sieve. Finally they were placed on a filter-disk between sheets of filter-paper and left to dry at room temperature for 7 d (Watling, 1981).

Sample dissolution was carried out according to the method of Watling (1981) and chemically analysed by means of ICP spectroscopy (Kempster, 1986).

Collection and keeping of fish

Healthy adult catfish were netted from a natural pond in the Brandfort district (Orange Free State) near Bloemfontein, South Africa in December 1990. The fish were kept in a polyethylene pond with a capacity of 2 150 ℓ for 2 weeks to rid the tissue of any foreign substances such as bacteria. The pond system had a domestic tap-water throughflow of approximately 20 $ℓ{\cdot}h^{-1}$. During this phase of the study the fish were fed commercial

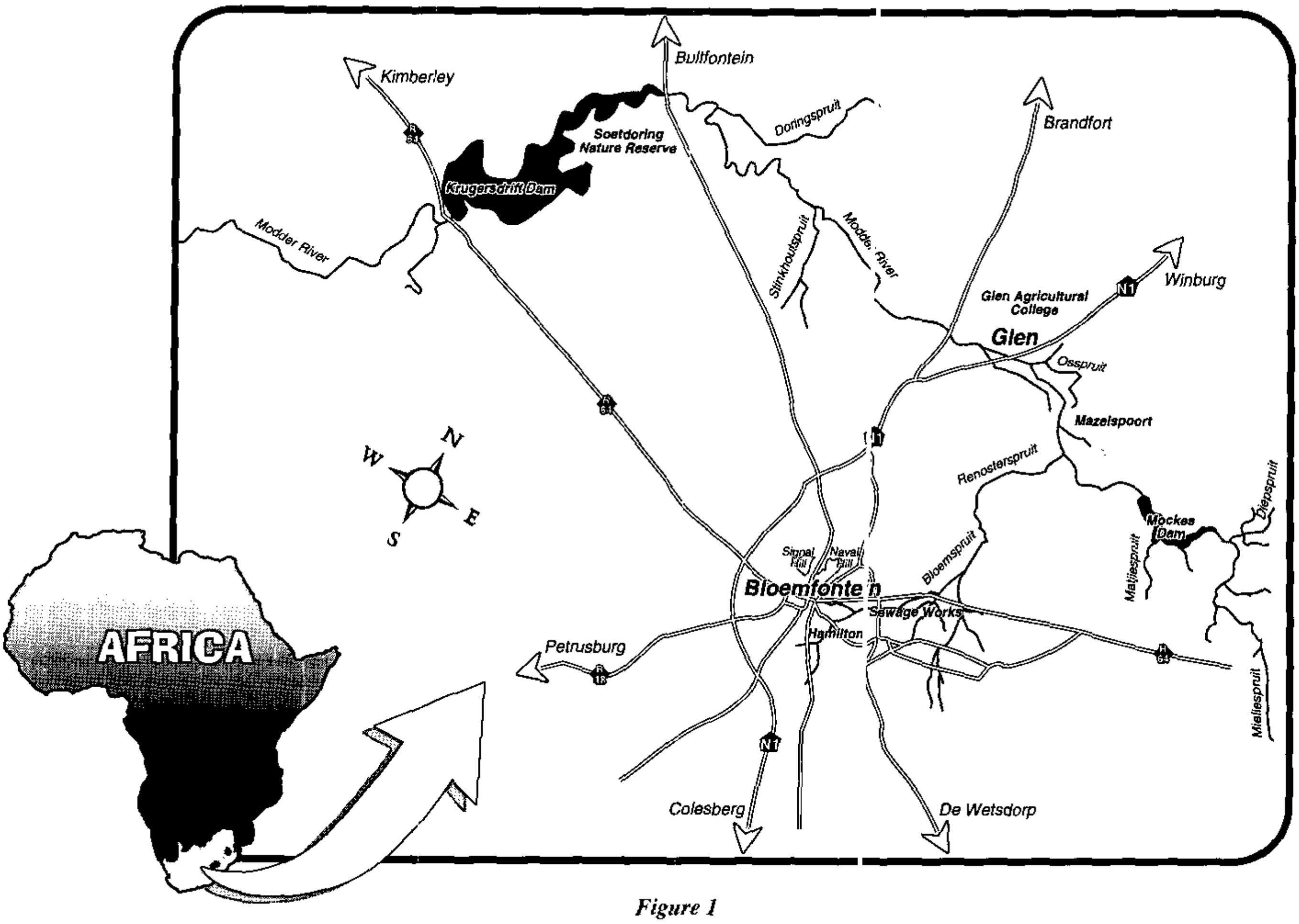

Figure 1
Map of the Modder River catchment area showing the geographical location of the sampling localities (Bloemspruit Sewage Works and Krugersdrift Dam)

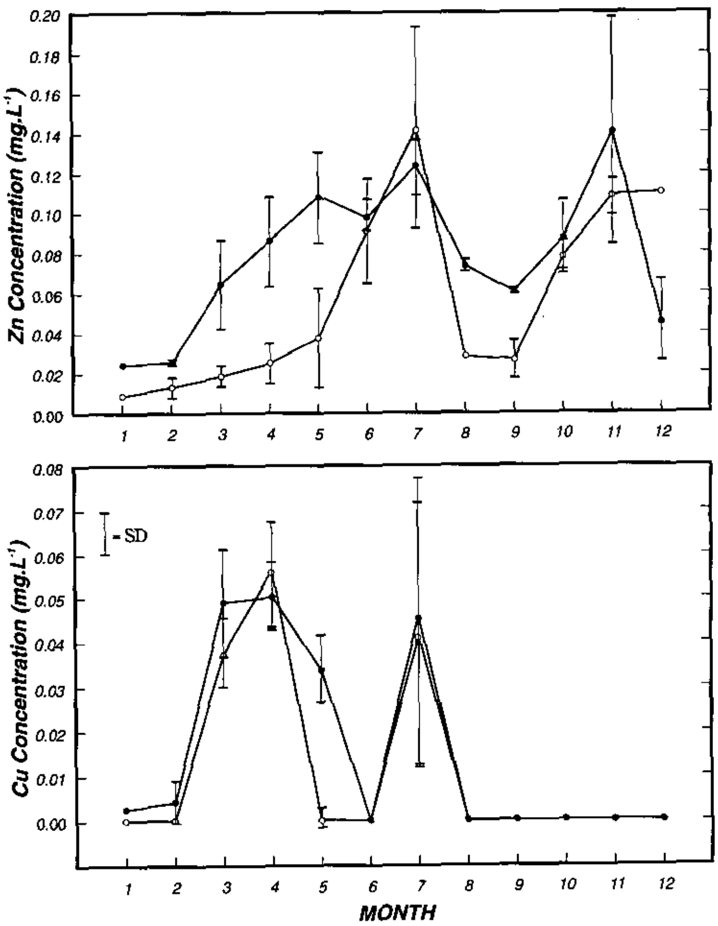

Figure 2
Concentrations and standard deviation from the mean of treated water from the Bloemspruit Sewage Works (•—•) and water from the intake of the Krugersdrift Dam (o—o) measured from January 1991 (month 1) to December 1991 (month 12)

breakfast food (Weet-bix) with a high protein content (0.116 $g \cdot g^{-1}$) every second day. Fish were fed to the ratio of 0.6 $g \cdot kg^{-1}$ body mass.

After the above freshening process, 30 fishes were transported to the first maturation pond of the Bloemspruit Sewage Works and the inlet of the Krugersdrift Dam respectively (Fig. 1). Here they were placed in 2 cube-shaped 10 x 50 mm galvanised mesh cages, each with a capacity of 8 m^3. The cages were firmly positioned on the bottom in such a way that the water could flow through freely. Approximately 20 cm of each cage protruded above the water allowing the fish to surface for air if necessary. The fish were not fed in the cages as sufficient food was found in the stomachs of individual specimens after periodic examination. A random sample of 2 fishes per month showed stomach fullness of 50%. The number of fish that died in captivity was negligibly small (3%).

Tissue sampling, preparation and analysis

In June 1991, 9 fishes were removed at random from each of the 2 cages with the aid of a scoop net and transported to the laboratory in 200 ℓ polyethylene drums filled with water from the 2 localities respectively. Both groups of 9 fishes were then subdivided into 3 groups of 3 each. The same procedure was followed in December 1991. All fishes were slaughtered for experimental purposes within one hour of being removed from the cages.

Liver, kidney and muscle tissue samples were collected from each fish and prepared for chemical analysis according to the method of Nupen (1983). Fish were scaled and dissected to obtain fillets, liver and the kidneys from each fish, and thoroughly washed in distilled water, whereafter portions of 2 g each were weighed accurately for analyses. As some specimens did not have sufficient liver and kidney tissue for a single analysis, tissue from each group of 3 fishes was pooled. Each pooled sample was again subdivided into 3 equal mass quantities. The collected samples were rinsed in running deionised water, sealed in airtight polyethylene bags and frozen to -20 °C. Tissue samples were digested and further prepared for chemical analysis according to the method of Watling (1981). Samples were dried at 95 °C for 24 h whereafter 20 mℓ concentrated HNO_3 was added to each sample. After 24 h, a 4:1 mixture of HNO_3 (SAARCHEM) (AR) and H_2O_2 (MERCK) (AR) was added and evaporated to dryness. Digestion was continued by adding 10 mℓ 10% HNO_3 with occasional shaking to suspend the residue in order to dissolve it. Each pooled tissue sample was analysed in triplicate for both metals by means of ICP spectroscopy.

A preliminary study was carried out at the start of the experiment to establish to what extent uniform concentrations of metals were present in tissue of the group.

Results

Water analyses

The mean concentrations of Zn and Cu with the standard deviations from the means of treated water from the Bloemspruit Sewage Works and water from the intake of the Krugersdrift Dam measured from January 1991 to December 1991, are shown in Fig 2. Means and standard deviations were calculated for 9 samples collected at each locality during each month.

Concentrations of Zn in both localities exhibited an annual

TABLE 1
CONCENTRATIONS OF Zn AND Cu IN SEDIMENT SAMPLES COLLECTED MONTHLY OVER A 12-MONTH PERIOD DURING 1991 FROM THE FIRST MATURATION POND OF THE BLOEMSPRUIT SEWAGE WORKS AND THE KRUGERSDRIFT DAM. CONCENTRATIONS ARE EXPRESSED IN mg·g^{-1} DRY MASS.

	Maturation pond			Krugersdrift Dam		
Element	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD
Zn	1.20-1.78	1.49	0.23	2.93-3.44	3.38	0.74
Cu	1.30-1.96	1.73	0.30	1.78-2.11	1.89	0.43

$\bar{x}$: Mean concentration
SD : Standard deviation from the mean value

fluctuation of 0.010 to 0.150 mg·ℓ$^{-1}$ (Fig. 2). Although the Zn concentrations were, with the exception of samples taken in July and December 1991, higher for treated sewage effluent than for natural dam water, no significant difference ($p>0.05$) could be found between the average Zn values of the 2 localities (0.077 ± 0.039 and 0.059 ± 0.048 mg·ℓ$^{-1}$ for treated sewage effluent and natural dam water respectively).

Copper reached maximum concentrations of 0.05 mg·ℓ$^{-1}$ in treated sewage effluent and 0.056 mg·ℓ$^{-1}$ in natural dam water during 1991 (Fig. 2). Fluctuations in Cu concentrations exhibited more or less the same pattern in both localities throughout the year, except during May in the case of the Krugersdrift Dam. As for Zn, no significant difference ($p>0.05$) could be found between the average annual Cu concentrations for treated sewage effluent (0.013 ± 0.024 mg·ℓ$^{-1}$) and natural dam water (0.030 ± 0.121 mg·ℓ$^{-1}$).

Sediment analyses

The average Cu concentrations in the sediment of treated sewage effluent and natural dam water were found to be very similar. In contrast, the average Zn concentration for sediment samples of the Krugersdrift Dam was more than double that of treated sewage effluent (Table 1).

Zn and Cu concentrations in fish tissue

Zinc concentrations measured in the tissue of *C. gariepinus* are displayed in Fig. 3. No significant differences ($p>0.05$) could be found regarding the average annual Zn concentrations in the muscle tissue of the fish in the respective habitats (0.246 ± 0.217 mg·g^{-1} wet mass for sewage effluent fish and 0.213 ± 0.283 mg·g^{-1} wet mass for fish in natural dam water). In general, the Zn concentrations in the livers and kidneys found in this study, were considerably higher in December than in June 1991 (Fig. 3). The concentrations of Zn in the livers of fish kept in the Krugersdrift Dam were also significantly higher ($p<0.001$) in winter than those of fish kept in treated sewage effluent. The summer concentrations measured for Zn in liver tissue were again significantly higher ($p<0.0001$) for fish in treated sewage effluent than those kept in natural dam water. In December the average Zn concentration in the kidneys of fish kept in treated sewage effluent was much higher (7.117 ± 2.31 mg·g^{-1} wet mass) than for those kept in natural dam water (1.203 ± 0.24 mg·g^{-1} wet mass). Furthermore, the concentrations in the livers during the same period were 3.385 ± 0.851 mg·g^{-1} wet mass and 4.580 ± 1.396 mg·g^{-1} wet mass for fish in treated sewage effluent and natural dam water respectively ($p>0.05$).

Concentrations of Cu found in the liver of *C. gariepinus* were considerably higher in December and June compared to those in the kidney and muscle tissue (Fig. 3). No significant differences ($p>0.05$) could, however, be found between the Cu concentrations of fish collected from the 2 localities during winter and summer with regard to all 3 tissue types. The Cu concentration in the livers of fish in treated sewage effluent ranged from 0.455 to 0.891 mg·g^{-1} wet mass.

Discussion

Experimental evidence indicates that a relationship exists between the functional role and incidence of metal ions in living systems and the availability and quantities thereof in nature (Williams, 1967; Vahrenkamp, 1973; Wood, 1975). Good summaries regarding the natural occurrence and toxic levels of metals in human and animal tissue are discussed in detail by Vahrenkamp (1973), Wood (1975), Overhoff and Forth (1978), Duffus (1983) and Moriarty (1991).

In Table 2 the average concentrations of Zn and Cu found in the water of the Krugersdrift Dam and of the first maturation pond of the Bloemspruit Sewage Works, were compared with values found for the same metals in other South African water sources. With the exception of Zn, the concentrations in the water of both localities were much lower than those of the Vaal Dam. The metal concentrations of the first maturation pond at the Bloemspruit Sewage Works listed in Table 2, can therefore, in comparison with other water sources, be reassuring.

In addition to the favourable comparison in Table 2, Zn concentrations for both treated sewage effluent and natural dam water were generally lower than the maximum concentration of 5 mg·ℓ$^{-1}$ prescribed by all authoritative organisations (USEPA, USPHS, WHO, SABS) dealing with water quality. According to WHO (1984), the mentioned maximum concentration was set because of aesthetic reasons due to higher concentrations in water resulting in an unpleasant taste and the forming of a greasy layer on the water when boiled. In the case of Cu, a maximum concentration of 1.0 mg·ℓ$^{-1}$ is recommended for drinking water (USEPA, 1976; WHO, 1984; SABS, 1984) and for treated waste water (South Africa, 1984). The highest average concentration measured during the course of this study was 0.056 mg·ℓ$^{-1}$ for natural dam water, and

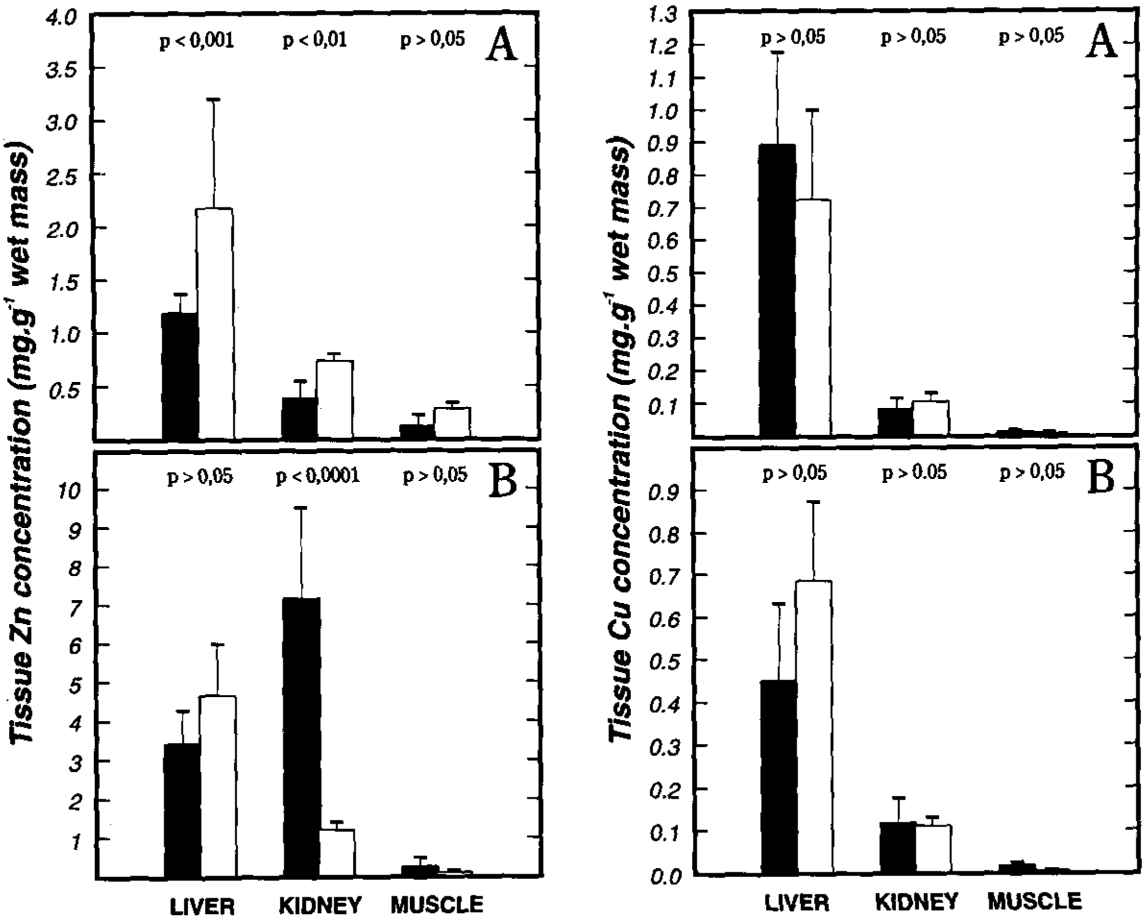

Figure 3

Concentrations measured in tissue samples of Clarias gariepinus *kept in treated sewage water (■) and natural dam water (□)*

A: Fish kept from January to June 1991 whereupon tissue analysis was carried out

B: Fish kept from January to December 1991 whereupon tissue analysis was carried out

TABLE 2
A COMPARISON BETWEEN CONCENTRATIONS OF Zn AND Cu ($mg \cdot \ell^{-1}$) MEASURED FOR SOME SOUTH AFRICAN RIVERS AND FOR WATER OF THE KRUGERSDRIFT DAM AND TREATED SEWAGE EFFLUENT OF THE BLOEMSPRUIT SEWAGE WORKS

River	Vaal River		12 Cape rivers	Transkei rivers	Maturation dam	Natural dam
Period	Oct 1981& Sept 1982		May 1975- Aug 1981	Sept 1980- Jan 1982	Jan 1991- Dec 1991	Jan 1991- Dec 1991
Source	Rand Water Board (1982)		Watling (1982a-f)	Du Preez (1985)		
Cu	0.040	0.040	0.0019	0.007	0.0137	0.0123
Zn	0.040	0.040	0.0044	0.011	0.077	0.0594

with a normal water intake of 2 ℓ·d^{-1}, Cu and Zn concentrations as measured in this study pose no health hazard. In comparison with the above-mentioned values, Zn concentrations measured by Massoud et al. (1981) at Lake Mariut in Egypt, varied less severely (0.009 to 0.014 mg·ℓ$^{-1}$) whilst Du Preez (1985) determined a noticeable lower average concentration of 0.011 mg·ℓ$^{-1}$ in rivers of the Transkei. The Cu concentrations found during this study were considerably higher than the average value of 0.007 mg·ℓ$^{-1}$ determined by Du Preez (1985) for Transkei Rivers.

Clarias gariepinus has an omnivorous feeding habit (Groenewald, 1964; Van der Waal, 1972; Gaigher, 1977; Bruton, 1979) and feeds mainly on zooplankton, benthos like Oligochaeta, Chironomidae larvae, Ephemeroptera and Hemiptera nymphs, Dutch grass (*Scirpus fluitans*), detritus and smaller fish species. As the above-mentioned organisms absorb large quantities of metals from the water and sediment (Burrows and Whitton, 1983; Dixit and Whitcomb, 1983; Yasuno et al., 1985; Van der Merwe et al., 1990), it could be expected that accumulation of metals in the tissue of catfish and other vertebrates should occur to a large extent. The relatively low concentrations of Zn and Cu found in the sediment of the 2 localities (Table 1), are in accordance with those found for water samples. It must, however, be emphasised that especially Zn occurred at a much lower concentration in sediment samples of treated sewage effluent compared to natural dam water.

Zinc(II), like Co(II), is also associated with enzymes (Förstner and Wittmann, 1983). Zinc apparently does not accumulate in food chains and is therefore considered to be one of the less dangerous elements to living organisms. According to Förstner and Wittmann (1983) high concentrations of Zn can, however, affect the metabolism of man, especially that of children and patients already experiencing irregular metabolism. The toxicity of this metal can also increase in the presence of As, Pb, Cd and Sb in the body (Duffus, 1983).

The daily Zn requirement needed by man through his diet is determined by age and sex and set between 4 and 10 mg (WHO, 1984). Pre-school children need approximately 0.3 mg Zn per kg body mass per day (USEPA, 1976). According to the National Academy of Sciences (NAS, 1980), American inhabitants have a notable Zn deficiency. Therefore Taylor et al. (1982) recommend a concentration of 1.1 mg·d^{-1} for babies, 2.2 mg·d^{-1} for children and adults, 2.8 mg·d^{-1} during puberty, 2.55 to 3.0 mg·d^{-1} during pregnancy and 5.45 mg·d^{-1} during lactation. The WHO (1984) states that long-term intake of Zn will have no adverse effect on man due to the human body's ability to readily excrete excess Zn in the faeces. Carson et al. (1991) state that the oral intake of Zn based on an LD_{50} range of 1 to 3 mg·kg^{-1} body mass for rats, is not toxic to man. The maximum concentration of 0.3 mg·g^{-1} muscle tissue found in *C. gariepinus* during the course of this study, therefore posed no danger to humans consuming this fish, provided that the fish is not consumed in excess daily. A portion of 150 g fish per day would be recommended in a normal diet. In addition, Zn mainly concentrates in the skin, bones, liver, kidneys and gills of fish (Mount, 1964; Mathiessen and Brafield, 1977; Holcombe et al., 1979) which are not normally part of man's diet. It is therefore recommended that the fish be gutted and the gills removed prior to cooking.

Before Cu was considered to be an "essential element" (Duffus, 1983; Förstner and Wittmann, 1983), it was found that it occurred in combination with blood protein in certain species of snails. Today it is known that Cu(I) is involved in the synthesis of enzymes. However, excessive intake of Cu leads to the accumulation thereof in liver tissue. The toxicity of Cu is usually increased by low intake of Mo, Zn and SO_4^{-2}.

Man's physiological body need for Cu varies between 2 and 3 mg·d^{-1} according to the NAS (1980). Sollman (1957) states that young children need approximately 0.1 mg of Cu·d^{-1} and adults approximately 2 mg·d^{-1}. The muscle tissue of *C. gariepinus* in both treated sewage effluent and natural dam water contained very low concentrations of this metal ion (6.6 and 3.8 µg·g^{-1} respectively) during the year and therefore poses no risk to humans consuming this fish. Actually the concentrations measured in this study are lower than those given for other fresh water species in the literature. Cross et al. (1973) and Bryan (1976) attributed this phenomenon to the exceptional ability of certain fish species to excrete Cu and Zn rapidly. The average Cu concentrations of 0.006 ± 0.006 mg·g^{-1} wet mass and 0.003 ± 0.003 mg·g^{-1} wet mass found in the muscle tissue of fish in treated sewage effluent and natural dam water, were also considerably lower than the value of 0.055 mg·g^{-1} dry mass reported for tilapia (Massoud et al., 1981). Available information indicates that there is no evidence that chronic exposure to Cu concentrations, like those found in *C. gariepinus*, poses a carcinogenic or mutagenic danger to man (Moore and Ramamoorthy, 1984); intake therefore can be unlimited.

Conclusions

The concentrations of Cu and Zn were noticeably higher in the livers and kidneys compared to the muscle tissue of catfish kept in treated sewage effluent and natural dam water. However, no set seasonal patterns could be established regarding the incidence of these elements in both localities. The occurrence of Zn and Cu in the water of both localities was very low and could therefore not be considered as contaminants due to bioaccumulation.

One of the most important findings of this study was that lower concentrations of Cu and Zn were recorded in the muscles of fish kept in treated sewage effluent compared to those kept in the Krugersdrift Dam. No toxic levels were recorded for either Cu or Zn in the muscle tissue of catfish kept in the 2 habitats. However, concentrations of Cu and Zn found in the livers and kidneys of *C. gariepinus* in both habitats may well pose a health risk when eaten. Human consumption of these organs is therefore not recommended. Fish must be gutted and gills removed before cooking and eating.

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