



# HOKKAIDO UNIVERSITY

|                  |                                                                                                                                                                                                                   |
|------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Title            | Heavy Metal Accumulation in Lake Sediments, Fish ( <i>Oreochromis niloticus</i> and <i>Serranochromis thumbergi</i> ), and Crayfish ( <i>Cherax quadricarinatus</i> ) in Lake Itzhi-tezhi and Lake Kariba, Zambia |
| Author(s)        | Nakayama, Shouta M. M.; Ikenaka, Yoshinori; Muzandu, Kaampwe et al.                                                                                                                                               |
| Citation         | Archives of Environmental Contamination and Toxicology, 59(2), 291-300<br><a href="https://doi.org/10.1007/s00244-010-9483-8">https://doi.org/10.1007/s00244-010-9483-8</a>                                       |
| Issue Date       | 2010-08                                                                                                                                                                                                           |
| Doc URL          | <a href="https://hdl.handle.net/2115/43285">https://hdl.handle.net/2115/43285</a>                                                                                                                                 |
| Rights           | The original publication is available at <a href="http://www.springerlink.com">http://www.springerlink.com</a>                                                                                                    |
| Type             | journal article                                                                                                                                                                                                   |
| File Information | AECT-59_291-300.pdf                                                                                                                                                                                               |



Heavy metal accumulation in lake sediments, fish (*Oreochromis niloticus* and *Serranochromis thumbergi*) and crayfish (*Cherax quadricarinatus*) in Lake Itzhi-tezhi and Lake Kariba, Zambia

<sup>a</sup>Shouta M.M. NAKAYAMA\*, <sup>a</sup>Yoshinori IKENAKA\*, <sup>b</sup>Kaampwe MUZANDU, <sup>b</sup>Kennedy CHOONGO, <sup>a</sup>Balazs OROSZLANY, <sup>c</sup>Hiroki TERAOKA, <sup>c</sup>Naoharu MIZUNO, and <sup>a</sup>Mayumi ISHIZUKA

<sup>a</sup>Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan

<sup>b</sup>Department of Biomedical studies, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia

<sup>c</sup>Department of Toxicology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan

Corresponding author

Mayumi ISHIZUKA

Email address: [ishizum@vetmed.hokudai.ac.jp](mailto:ishizum@vetmed.hokudai.ac.jp)

## Abstract

We measured the level of heavy metal accumulation in lake sediments, herbivorous (*Oreochromis niloticus*) and carnivorous (*Serranochromis thumbergi*) fish, and crayfish (*Cherax quadricarinatus*) from Lake Itzhi-tezhi (ITT) and Lake Kariba. We used atomic absorption spectrophotometry (AAS) to quantify the levels of 7 heavy metals (Cr, Co, Cu, Zn, Cd, Pb, and Ni). The sediment and the herbivorous fish *O. niloticus* accumulated a very high concentration of Cu in Lake ITT, most likely due to the discharge of Cu waste from a mining area 450 km upstream. The aquatic species we sampled in Lake Kariba had higher concentrations of Cr, Ni, and Pb relative to those in Lake ITT. This is most likely due to anthropogenic activities, such as the use of leaded petrol and anti-fouling agents in marine paints. Interestingly, we observed a negative correlation between the coefficient of condition (*K*) and Ni concentration in the crayfish hepatopancreas. Both *O. niloticus* and the crayfish had much higher biota-sediment accumulation factors (BSAF) for Cu, Zn, and Cd relative to Cr, Co, Pb, and Ni. The rank of BSAF values for *O. niloticus* (Cu > Cd > Zn) and *C. quadricarinatus* (Zn > Cd > Cu) differed from the expected ranks based on the general order of affinity of metals (Cd >> Zn > Cu).

Keywords: heavy metals, copper, Zambia, *Oreochromis niloticus*, *Serranochromis thumbergi*, *Cherax quadricarinatus*, sediment

## Introduction

The African continent has experienced rapid economic development during the last decade. Unfortunately, this has also led to an increase in environmental pollution (Akiwumi and Butler 2008). In many African countries, heavy metal contaminants are now a significant health issue. Humans and wildlife are exposed to heavy metals in drinking water, air, and soil due to contamination from activities such as mining and metal smelting. In many instances, mining operations have no containment measures. African aquatic environments are experiencing increasingly heavy metal pollution as a result of the growth in agricultural and industrial development (Lwanga *et al.* 2003, Berg *et al.* 1995), as well as increases in the use or spillage of leaded gasoline from watercraft (Muohi *et al.* 2003).

The Republic of Zambia is rich in mineral resources such as copper (Cu), cobalt (Co), zinc (Zn), and lead (Pb). Thus, mining is one of the key industries in Zambia. In 1997, 3% of the world's annual production of copper (Cu) and 20% of the annual production of cobalt (Co) was mined in Zambia. Furthermore, the majority of these metals were smelted within the country (Stockwell *et al.* 2001). As a result, heavy metal pollution is the biggest environmental issue in Zambia, with serious implications for the health of humans and animals. Despite this, little is known about the effects of heavy metal pollution on aquatic systems in Zambia (Berg *et al.* 1995). The Kafue River (including Lake Itzhi-tezhi (ITT)) and the Zambezi River (including Lake Kariba) are the largest rivers in Zambia. Both systems play an important role in providing drinking water for humans, livestock, and wildlife. The Copperbelt region, in the Kafue River basin, is one of the core mining areas in Zambia. Previous studies have shown that water, sediment, and fish in the Kafue River near the Copperbelt region contain higher concentrations of heavy metals compared with samples collected upstream of the mining site (Choongo *et al.* 2005, Norrgren *et al.* 2000, Mwase *et al.*

1998). The Zambia Consolidated Copper Mines Limited (ZCCM, 1982) also documented Cu poisoning in cattle caused by drinking water from the Kafue River. In Lake Kariba, there has been a dramatic increase in commercial fishing during the last few decades (Madamombe 2002). The commercial fleet relies heavily on leaded gasoline and is also a large consumer of anti-fouling marine paints which contain heavy metals (Diagomanolin *et al.* 2004, Muohi *et al.* 2003). Given these factors, there is increased risk to both the human population and wildlife from consuming fish from contaminated waterways.

Our objective was to measure the level of heavy metal accumulation in lake sediments, fish, and crayfish that were collected from Lake ITT and Lake Kariba. We used this data to evaluate spatial and species differences in heavy metal accumulation in the aquatic systems of Zambia. Lake sediments are often sampled to assess the level of environmental pollution (Ikenaka *et al.* 2005a). In polluted aquatic systems sediments are increasingly recognized as the most important sink for contaminants and a future source of pollutants (Ikenaka *et al.* 2005b). We sampled both herbivorous (*Oreochromis niloticus*: Nile tilapia) and carnivorous (*Serranochromis thumbergi*: Brownspot largemouth) fish species. Both species are relatively common and are widely distributed throughout Africa (Schwanck 1995). Furthermore, a number of researchers have already measured heavy metal accumulation in *O. niloticus* in other systems (Birungi *et al.* 2007, Coğun *et al.* 2003). We also measured heavy metal levels in *Cherax quadricarinatus* (Australian red claw crayfish), which is an alien species, but is widely distributed in Zambia. Crayfish live in contact with the sediment where metals accumulate and are known to concentrate trace metals in their body tissues (Anderson *et al.* 1978, Rincon-Leon *et al.* 1988). Thus, crayfish are an ideal biological indicator of heavy metal pollution in the aquatic environment.

This is the first report to show heavy metal accumulation in the aquatic life of Zambia.

## Materials and Methods

### Study area

The study was conducted in Lake ITT and Lake Kariba (Fig. 1). Lake ITT is located in the Kafue River basin, and Lake Kariba is located in the Zambezi River basin. Lake ITT is an artificial lake (length: 50 km, mean depth: 15 m, surface area:  $\sim 370 \text{ km}^2$ ) which has flooded part of the Kafue National Park. Lake Kariba is another large, artificial lake located on the Zambezi River. Lake Kariba is 320 km long, has a surface area of almost  $5,400 \text{ km}^2$ , and a mean depth of 31 m.

### Sampling

We collected samples of lake sediment, fish, and crayfish during the dry season from July 2008 to September 2008. The lake sediment samples were collected from a number of sites in Lake Itezhi-tezhi (ITT) (n=9) and Lake Kariba (n=12) (Fig. 1). Surface sediment samples were collected using an Ekman grab. Each sediment sample was air-dried in the laboratory at room temperature and passed through a 2 mm sieve prior to extraction. The dry weight of each sample was measured after 12 h of drying in an oven at  $105^\circ\text{C}$ .

We collected the fish and crayfish samples from the sites identified above. In Lake ITT, we collected *O. niloticus* (n=18), *C. quadricarinatus* (n=19), and *S. thumbergi* (n=6). *S. thumbergi* is not found in Lake Kariba (Skelton 1993), therefore, we only collected *O. niloticus* (n=16) and *C. quadricarinatus* (n=10) from this lake. We measured the body length and weight of each individual in the field. We also removed samples of liver (or hepatopancreas) and muscle tissue from each animal. The samples were stored separately at

-20°C for heavy metal analysis or water content measurement. For measurement of water content, the tissue samples were dried in an oven at 105°C for 24 h. The extraction and analysis methods are outlined in the following sections.

#### Extraction and analysis of heavy metals

The sediment samples were extracted using a method modified from Charlesworth and Lees (1999). Briefly, 1 g of each sample was placed into a 200 ml flask. We then added 0.2 ml sulfuric acid (poisonous metal analysis grade, Kanto Chemical Corp., Tokyo, Japan), 1 ml nitric acid (atomic absorption spectrometry grade, 60%, Kanto Chemical Corp.), and 5 ml perchloric acid (atomic absorption spectrometry grade, 60%, Kanto Chemical Corp.). The mixture was heated at 180°C for 3 h on a hotplate. After the mixture cooled, we added 1 g ammonium chloride (Wako Pure Chemical Industries Ltd., Osaka, Japan) and 20 ml 0.5 N HCl (special grade, 36%, Wako). The samples were then reheated at 180°C for 1 h and evaporated to a volume of ~10 ml. After the samples cooled, they were filtered into plastic bottles through ash-less filter paper 5B (Advantec, Tokyo, Japan). We then added 1 ml lanthanum chloride (atomic absorption spectrometry grade, 100g-La/L solution, Wako). The sample volume was standardized to 100 ml using 2% HNO<sub>3</sub>. A reagent blank was produced following the same process.

The liver and muscle samples were extracted by digestion using a method modified from Seymore *et al.* (1996). Briefly, 1 g of fresh tissue was placed into a 200 ml flask. We then added 20 ml nitric acid and 5 ml perchloric acid. The samples were heated for 12 h at 225°C (increased gradually) on a hotplate and evaporated to ~5 ml. When the samples formed a clear liquid, we added 0.2 ml lanthanum chloride (100 g La/L solution). The volume was then made up to 20 ml with 2% HNO<sub>3</sub>. A reagent blank was produced using the same

procedure.

We measured the concentrations of 7 elements (Cr, Co, Cu, Zn, Cd, Pb, and Ni) in the lake sediment, and fish and crayfish tissues using an atomic absorption spectrophotometer (AAS) AAnalyst 800 (Perkin Elmer Instruments, USA) with an acetylene flame (Cu and Zn) or an argon non-flame (Cr, Co, Cd, Pb and Ni), after preparation of the calibration standard. The overall recovery rates (Mean $\pm$ S.D.) for Cr, Co, Cu, Zn, Cd, Pb, and Ni were  $91 \pm 3.0$ ,  $92 \pm 3.4$ ,  $89 \pm 5.6$ ,  $91 \pm 2.3$ ,  $111 \pm 8.3$ ,  $90 \pm 3.5$ , and  $92 \pm 4.2\%$ , respectively. The detection limit for Cr, Co, Cu, Zn, Cd, Pb, and Ni was 0.5, 0.5, 1.0, 0.1, 0.2, 1.0, and 0.5  $\mu\text{g/kg}$ , respectively. Each heavy metal concentration was converted from mg/kg wet-weight to mg/kg dry-wt using the water content of each tissue. The total contaminant load was calculated by summing the 7 heavy metal concentrations ( $\sum_7$ ). We compared these levels to the recommended dietary intake levels published by the World Health Organization (Demirzen and Uruc 2006, WHO 1993).

#### Coefficient of condition ( $K$ )

The coefficient of condition ( $K$ ) in fish and crayfish was calculated for each sample using the formula  $K=W \times 10^5/L^3$ , where,  $K$ =coefficient of condition,  $W$ =weight in grams, and  $L$ =body length in millimeters (Choongo *et al.* 2005).

#### Biota-sediment accumulation factor (BSAF)

The biota-sediment accumulation factor (BSAF) is the ratio between the metal concentration in the liver (hepatopancreas) and that in the sediment (Abdallah and Abdallah 2008). The formula is:  $\text{BSAF}=\text{Ct}/\text{Cs}$ , where  $\text{Ct}$ =heavy metal concentration in the organism



and Cs=heavy metal concentration in sediment.

## Statistics

We analyzed for differences among the groups using a Mann-Whitney U test or a Tukey test after each datum was normalized by transforming to a base 10 logarithm. Statistical analyses were performed using JMP 7.0.1 (SAS Institute, Cary, NC, USA).

## Results

### *Concentration of heavy metals in lake sediments*

There was no significant difference between  $\sum_7$  heavy metal concentrations in Lake ITT and Lake Kariba (Table 1). However, the concentrations of Cu in Lake ITT (Cu:  $82 \pm 35$  mg/kg dry-wt) were significantly higher than in Lake Kariba (Cu:  $30 \pm 17$  mg/kg dry-wt) (Table 1, U test,  $p < 0.05$ ).

### *Coefficient of condition (K)*

The coefficient of condition ( $K$ ) in *O. niloticus* was  $3.2 \pm 0.4$  (Mean $\pm$ S.D) (Lake ITT) and  $3.2 \pm 0.9$  (Lake Kariba) (Table 2). The coefficient of condition ( $K$ ) in *C. quadricarinatus* was  $2.2 \pm 0.3$  (Lake ITT) and  $2.4 \pm 0.4$  (Lake Kariba). The coefficient of condition ( $K$ ) in *S. thumbergi* was  $2.5 \pm 0.3$ . There was no significant difference in  $K$  among individuals of the same species between the two lakes.

### *Comparisons of heavy metal concentrations in fish (O. niloticus) liver and muscle between Lake ITT and Lake Kariba*

The liver of *O. niloticus* from Lake ITT had a significantly higher concentration of Cu ( $1345 \pm 930$  mg/kg dry-wt) than in Lake Kariba ( $587 \pm 320$  mg/kg dry-wt) (Table 2, Figure 2) (U test, \*  $p < 0.05$ ). Conversely, the concentrations of Co, Zn, Cd and Pb were significantly higher in the fish from Lake Kariba than from Lake ITT (Table 2, Figure 2) (U test, \*  $p < 0.05$ ).

*O. niloticus* muscle had significantly higher levels of Cu in Lake ITT than in Lake Kariba (U test, \*  $p < 0.05$ ). In contrast, the concentrations of Cr and Ni were higher in the muscle of *O. niloticus* from Lake Kariba than in Lake ITT (Table 2, Figure 2) (U test, \*  $p < 0.05$ ).

#### *Comparisons of heavy metal concentrations in crayfish (C. quadricarinatus) hepatopancreas and muscle between Lake ITT and Lake Kariba*

There was no significant difference in the concentration of Cu in the hepatopancreas of crayfish (*C. quadricarinatus*) caught in Lake ITT and Lake Kariba. However, the concentration of Cd was significantly higher in the hepatopancreas of individuals collected in Lake ITT than in Lake Kariba (Table 2, Figure 3) (U test, \*  $p < 0.05$ ). The concentrations of Ni, Pb, and Co in Lake Kariba were significantly higher than in Lake ITT (Table 2, Figure 3) (U test, \*  $p < 0.05$ ). The muscle tissue of *C. quadricarinatus* from Lake Kariba contained significantly higher levels of Cr, Ni, Pb, Zn, and Cd than in the animals from Lake ITT (Table 2, Figure 3) (U test, \*  $p < 0.05$ ).

#### *Comparison of heavy metal concentration in liver and hepatopancreas of the three species*

The liver of *O. niloticus* contained the highest concentrations of Cu ( $1345 \pm 930$  mg/kg dry-wt), followed by the hepatopancreas of *C. quadricarinatus* ( $279 \pm 169$  mg/kg dry-wt), and the liver of *S. thumbergi* ( $48 \pm 43$  mg/kg dry-wt) (Tukey test,  $p < 0.05$ ) (Figure 4). The hepatopancreas of *C. quadricarinatus* contained significantly higher levels of Zn, Ni, and Co relative to *O. niloticus* and *S. thumbergi* (Tukey test,  $p < 0.05$ ) (Table 2, Figure 4). *S. thumbergi* had the lowest levels of Cu, Co, Cd, and Ni (Tukey test,  $p < 0.05$ ) (Table 2, Figure

4). There was no difference in Pb accumulation among the three species.

#### *Biota-sediment accumulation factor (BSAF)*

The biota-sediment accumulation factor (BSAF) was significantly higher for Cu, Zn, and Cd relative to Cr, Co, Pb, and Ni for both *O. niloticus* and *C. quadricarinatus*. Among these two species, the BSAF values for Cr and Cu were significantly higher for *O. niloticus* (U test,  $p < 0.05$ ). In contrast, the values for Zn and Ni were significantly higher in *C. quadricarinatus* (U test,  $p < 0.05$ ). Among the two lakes, the BSAF values for Co, Ni, and Pb were significantly higher in *O. niloticus* in Lake Kariba. There was no significant difference in the BSAF value for Cu among the two lakes. The BSAF values for Cu, Ni, and Pb were significantly higher in *C. quadricarinatus* from Lake Kariba relative to those from Lake ITT. The inverse was true for Cd.

#### *Correlation coefficient ( $R^2$ ) for the heavy metals in fish and crayfish*

We observed a positive correlation between Cu-Zn, Cu-Cd, Co-Zn, and Zn-Cd in the liver of *O. niloticus* from Lake ITT (Table 4). Similarly, there was a positive correlation between Cr-Ni, Cr-Pb, and Zn-Co in the liver of *O. niloticus* from Lake Kariba. There was also a positive correlation between Cu and Zn in the muscle of *O. niloticus* from Lake ITT and Cr and Ni in Lake Kariba. In the hepatopancreas of *C. quadricarinatus*, we observed a positive correlation between Cu-Zn, Cu-Cd, and Zn-Cd in the animals from Lake ITT, and between Cu-Zn, Cu-Cd, Cu-Co, and Zn-Co in the animals from Lake Kariba. The concentration of Ni in the hepatopancreas of *C. quadricarinatus* was negatively correlated with the coefficient of condition ( $K$ ) in those crayfish sampled from Lake ITT. However, there

was no such correlation in *S. thumbergi*.

## Discussion

### *Spatial variation in heavy metal concentrations in lake sediments*

We found high concentrations of Cu in Lake ITT sediments suggesting that the Cu waste discharged into the Kafue River was transported to the lake, more than 450 km downstream from the Copperbelt region. Pettersson and Ingri (2001) also noted that dissolved Cu concentrations in the Kafue River, 100 km downstream from the mining areas in the Copperbelt, remained elevated ( $143 \pm 61 \mu\text{g/L}$ ) compared with the background concentrations ( $2.54 \pm 3.81 \mu\text{g/L}$ ).

The pattern of heavy metal contaminant accumulation in Lake Kariba sediments was somewhat different than in Lake ITT, particularly for Cr and Ni. Berg *et al.* (1995) measured much lower levels of Cr (average:  $15.8 \pm 3.8 \text{ mg/kg dry-wt}$ ) and Ni ( $23.2 \pm 2.9 \text{ mg/kg dry-wt}$ ) in sediments collected from Lake Kariba (Kassesse Bay, in 1990 and 1991) than in our study ( $64 \pm 36$  and  $53 \pm 32 \text{ mg/kg dry-wt}$ , respectively). Taken together, these data suggest that the concentrations of heavy metals in Lake Kariba have increased significantly during the past 18 years.

### *Tissue differences in heavy metal accumulation in fish and crayfish*

The liver or hepatopancreas is the primary site for storage and detoxification of heavy metals (Bagatto and Alikhan 1987). The liver of *O. niloticus* and the crayfish hepatopancreas contained higher concentrations of heavy metals (except Cr) relative to the muscle tissue (Table 2, Figures 2 and 3). In addition, the concentrations of several of these metals were positively correlated in both the livers and the hepatopancreas (Table 4). We

hypothesize that the correlation among the metals is a function of their capacity to induce metal-binding proteins (e.g. metallothionein). Cu, Zn, and Cd bind readily to metallothionein (Klaassen *et al.* 1999). Thus, it is more likely that these metals would be positively correlated in the tissues.

#### *Spatial variation in the accumulation of heavy metals in fish and crayfish*

We found very high concentrations of Cu in the liver of *O. niloticus* from Lake ITT compared with those in Lake Kariba (Table 2, Figure 2). Shaw and Handy (2006) experimentally exposed *O. niloticus* to Cu (2000 mg Cu/kg dry weight feed, 42 d) and found that these fish contained a high Cu concentration (liver: 727-1272 mg/kg) compared with the non-exposed controls (liver: 418 mg/kg). The authors also noted that there was an increase in the lipid content of the hepatocytes and a concomitant loss of sinusoidal space. The BSAF value for Cu was similar in *O. niloticus* that were collected in both lakes (Table 3). Thus, the *O. niloticus* in Lake ITT must have accumulated Cu from the lake environment itself, for example, through lake water, sediment, and diet. A study by Norrgren *et al.* (2000) demonstrated that caged three-spot tilapia (*Oreochromis andersonii*) exposed to Kafue River water accumulated higher Cu concentrations (liver:  $9700 \pm 810$  mg/kg dry-wt) downstream of the mining area compared with a locality upstream (Cu:  $4700 \pm 840$  mg/kg dry-wt). The WHO maximum recommended intake level for Cu is 0.9-30 mg/day. Based on this value, the consumption of 0.7-22 g/day of liver from *O. niloticus* caught in Lake ITT would exceed the WHO recommended level (Demirzen and Uruc 2006, WHO 1993). Given this, we believe that the high levels of Cu in these fish are likely to affect other animals in the food chain. Our results also suggest that the accumulation of Cu downstream of the mining areas along the Kafue River may also affect wildlife in the national parks.

The aquatic biota in Lake Kariba had higher concentrations of Cr, Ni, and Pb than in Lake ITT. We also observed a positive correlation between Cr and Ni in fish and crayfish from Lake Kariba. Cr and Ni compounds are commonly used as anti-fouling agents in marine paints (Diagomanolin *et al.* 2004) and Pb is often used in gasoline (Muohi *et al.* 2003, Caliceti *et al.* 2002). Given the increase in boat traffic on the lake and the recent increases in Cr and Ni in Lake Kariba sediments we hypothesize that these heavy metals are derived from commercial fishing around, and on, Lake Kariba. Indeed, the number of boats fishing in the Kapenta (*Limnothrissa miodon*) fishery, the largest fishery on the lake, increased from 175 in 1994 to 313 in 1999 (Madamombe, 2002). In addition, the catch of Kapenta also increased from 488 tons in 1974 to 17,974 tons in 1999 (Madamombe, 2002).

#### *Species difference of heavy metal accumulation in fish and crayfish in Lake ITT*

Of the three species we sampled from Lake ITT, the liver of *O. niloticus* contained the highest concentrations of Cu (Figure 4). Bowen (1980) reported that *O. niloticus* feeds on detritus in Lake Valencia, Venezuela. Moreover, Getachew and Fernando (1989) noted that algae (*Oscillatoria* sp.) are an important component in the diet of *O. niloticus*. Interestingly, *Oscillatoria angustissima* are known to adsorb Cu (Mohapatra and Gupta, 2005).

The carnivorous *S. thumbergi* had significantly lower concentrations of Cu, Co, Cd, and Ni (Table 2, Figure 4). This is consistent with the other reports that heavy metal concentrations are generally inversely correlated with the trophic level (Andres *et al.* 2000, Amundsen *et al.* 1997). *S. thumbergi* typically preys on insects and smaller fish, such as the Streep-rower (*Brycinus lateralis*) (Choongo *et al.* 2005; Utsugi and Mazingaliwa 2002, Skelton 1993), which have a lower total body load of Cu (whole animal: 4.0 mg/kg, Syakalima *et al.* 2001). Taken together, these results suggest that the feeding habits of *O.*



*niloticus* resulted in greater exposure to Cu in Lake ITT, relative to *C. quadricarinatus* and *S. thumbergi*.

The crayfish hepatopancreas had very high concentrations of Zn (Table 2, Figure 4). Moreover, the crayfish hepatopancreas contained much higher levels of Zn, Cu, Ni, and Co relative to Cd, Pb, and Cr (Table 2, Figure 3). Cu is an essential element in the respiratory pigment (hemocyanin) of crayfish. Similarly, Zn is used in metalloenzymes and as an activator of other enzyme systems (e.g. carbonic anhydrase) (Alcorlo *et al.* 2006). In some species, hemocyanins not only contain Cu, but also bind Zn (Bryan 1964, Bryan 1967). Studies have also shown that crayfish (*Orconectes hylas*) accumulate higher concentrations of Ni due to the increased bioavailability in surface and pore waters (Allert *et al.* 2008, Besser *et al.* 2008). These authors also observed a significant positive correlation between the concentrations of Ni, Co, and Zn in crayfish (*O. hylas*) and in their food (detritus, micro-invertebrates, and stonerollers). Thus, the accumulation of Zn and Cu in hemocyanin and the feeding ecology of crayfish may explain the higher concentrations of these heavy metals.

We observed a negative correlation between Ni and the coefficient of condition (*K*) in hepatopancreas of crayfish from Lake ITT (Table 4). This negative correlation is considered to be an indicator of sublethal/chronic toxic effects of Ni on *C. quadricarinatus*. Allert *et al.* (2008) observed an inverse correlation between survival in crayfish (*O. hylas*) and Ni concentrations in surface water, detritus, macro-invertebrates, and stonerollers during a 28 d *in situ* exposure. Interestingly, Mackevičienė (2002) reported that the exoskeleton of crayfish (*Astacus astacus* L.) contained substantial amounts of Ni relative to the hepatopancreas. The author also noted that the exoskeleton is involved in the absorption and excretion of Ni. Given this, we hypothesize that larger crayfish accumulate a greater amount of Ni in the exoskeletons than in the hepatopancreas. As the exoskeleton is shed on a regular basis this

would explain the negative correlation between Ni concentrations and the coefficient of condition.

#### *Biota-sediment accumulation factor (BSAF)*

The BSAF values for Cu, Cd, and Zn were above 1.0 in both *O. niloticus* and *C. quadricarinatus*. This indicates that these metals are readily bio-accumulated. Falusi and Olanipekun (2007) also reported that BSAF values for Cu, Cd, and Zn were higher than those for Ni and Pb in the tropical crab (*Carcinus* sp). Cadmium is known to be mobile in soils and available to plants (Alloway 1990). Thus, the herbivorous *O. niloticus* is more likely to accumulate higher concentrations of Cd relative to the lake sediment. Alcorlo *et al.* (2006) suggested that crayfish (*Procambarus clarkii*) could be used as bioindicator for Cd contamination based on their dose- and time-dependent accumulation of Cd. Taken together with our results, this suggests that the hepatopancreas of crayfish in Lake ITT and Lake Kariba may be used as an indicator of Cd contamination.

Generally, the affinity of metals for various soils is consistent with the value of the first hydrolysis constant of the cations: Cu (pK=7.7) >> Zn (pK=9.0) > Cd (pK=10.1) (Morera *et al.* 2001, Basta and Tabatabai 1992). Furthermore, the BSAF values may be ranked in the following order in the earthworm: Cd >> Zn > Cu (Dai *et al.* 2004). However, we noted a different order of accumulation (Cu > Cd > Zn) in *O. niloticus* and (Zn > Cd > Cu) *C. quadricarinatus*. The feeding ecology of these two species likely explains these differences. Both *O. niloticus* and *C. quadricarinatus* from Lake Kariba had significantly higher BSAF values for Ni and Pb than those from Lake ITT. This indicates that anthropogenic sources (e.g. leaded petrol or anti-fouling paint) are the likely cause of heavy metal contamination in Lake Kariba.

## Conclusion

We observed spatial variation in the accumulation of heavy metals between Lake ITT and Lake Kariba. The sediments and the herbivorous teleost *O. niloticus* accumulated very high concentrations of Cu in Lake ITT, located 450 km downstream of a mining area. This is most likely due to the discharge of Cu waste from the Copperbelt region. Conversely, the aquatic species that we sampled from Lake Kariba had higher concentrations of Cr, Ni, and Pb. We believe that the contaminants in this lake were derived from the use of leaded petrol and Cr-Ni compounds in anti-fouling marine paints. We also noted significant differences in the accumulation of heavy metals among the three species (*O. niloticus*, *S. thumbergi* and *C. quadricarinatus*) that are likely related to their feeding ecology, physiology, or metal sensitivity. The coefficient of condition (*K*) was negatively correlated with the Ni concentration in the hepatopancreas of the crayfish, suggesting that this species is sensitive to Ni toxicity. Interestingly, differences in BSAF rankings (Cu, Zn, and Cd) relative to the general order were observed in both *O. niloticus* and *C. quadricarinatus*.

## **Acknowledgments**

This study was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan awarded to M. Ishizuka (No. 19671001).

## References

- Abdallah MAM, Abdallah AMA (2008) Biomonitoring study of heavy metals in biota and sediments in the South Eastern coast of Mediterranean sea, Egypt. *Environ Monit Assess* 146:139-145
- Akiwumi FA, Butler DR (2008) Mining and environmental change in Sierra Leone, West Africa: a remote sensing and hydrogeomorphological study. *Environ Monit Assess* 142:309-318
- Alcorlo P, Otero M, Crehuet M, Baltanás A, Montes C (2006) The use of the red swamp crayfish (*Procambarus clarkii*, Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the River Guadiana (SW, Spain). *Sci Total Environ* 366:380-390
- Allert AL, Fairchild JF, DiStefano RJ, Schmitt CJ, Brumbaugh WG, Besser JM (2008) Ecological effects of lead mining on Ozark streams: In-situ toxicity to woodland crayfish (*Orconectes hylas*). *Ecotoxicol Environ Saf* 72:1207-1219
- Alloway BJ (1990) Cadmium. In: Alloway BJ (ed) *Heavy Metals in Soils*, Blackie, Glasgow. p 100-124
- Amundsen PA, Staldivik FJ, Lukin AA, Kashulin NA, Popova OA, Reshetnikov (1997) Heavy metal contamination in freshwater fish from the border region between Norway and Russia. *Sci Total Environ* 201:211-224
- Anderson RV, Vinikour WS, Brower JE (1978) The distribution of Cd, Cu, Pb, and Zn in the biota of two freshwater sites with different trace metal inputs. *Holarctic Ecol* 1:377-384
- Andres S, Ribeyre F, Tourencq JN, Boudou A (2000) Interspecific comparison of cadmium and zinc contamination in the organs of four fish species along a polymetallic pollution gradient (Lot River, France). *Sci Total Environ* 248:11-25

- Bagatto G, Alikhan MA (1987) Copper, cadmium, and nickel accumulation in crayfish populations near copper-nickel smelters at Sudbury, Ontario, Canada. *Bull Environ Contam Toxicol* 38:540-545
- Basta NT, Tabatabai MA (1992) Effect of cropping systems on adsorption of metals by soils: II: Effect of pH. *Soil Sci* 153:195-204
- Berg H, Kibus M, Kautsky N (1995) Heavy metals in tropical lake Kariba, Zimbabwe. *Water Air Soil Pollut* 83:237-252
- Besser JM, Brumbaugh WG, Allert AL, Poulton BC, Schmitt CJ, Ingersoll CG (2008) Ecological impacts of lead mining on Ozark streams: toxicity of sediment and pore water. *Ecotoxicol Environ Saf* 72:516-526
- Birungi Z, Masola B, Zaranyika MF, Naigaga I, Marshall B (2007) Active biomonitoring of trace heavy metals using fish (*Oreochromis niloticus*) as bioindicator species. The case of Nakivubo wetland along Lake Victoria. *Phys Chem Earth* 32:1350-1358
- Bowen SH (1980) Detrital amino acids are the key to rapid growth of Tilapia in Lake Valencia, Venezuela. *Science* 207: 1216-1218
- Bryan GW (1964) Zinc regulation in the lobster *Homarus vulgaris* I. Tissue zinc and copper concentrations. *J Mar Biol Assoc U.K.* 44:549-563
- Bryan GW (1967) Zinc Regulation in the Freshwater Crayfish (Including Some Comparative Copper Analyses). *J Exp Biol* 46:281-296
- Caliceti (2002) M, Argeese E, Sfriso A, Pavoni B (2002) Heavy metal contamination in the seaweeds of the Venice lagoon. *Chemosphere* 47:443-454
- Charlesworth SM, Lees JA (1999) The distribution of heavy metals in deposited urban dusts and sediments, Coventry, England. *Environ Geochem Health* 21:97-115
- Choongo CK, Syakalima SM, Mwase M (2005) Coefficient of condition in relation to copper levels in muscle of *serranochromis* fish and sediment from the Kafue river, Zambia. *Bull*

- Environ Contam Toxicol 75:645-651
- Coğun HY, Yüzereroğlu TA, Kargin F (2003) Accumulation of Copper and Cadmium in Small and Large Nile Tilapia *Oreochromis niloticus*. Bull Environ Contam Toxicol 71:1265-1271
- Dai J, Becquer T, Rouiller JH, Reversat G, Bernhard-Reversat F, Nahmani J, Lavelle P (2004) Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils. Soil Biol Biochem 36:91-98
- Demirzen D, Uruc K (2006) Comparative study of trace elements in certain fish, meat and meat products. Meat Sci 74:255-260
- Diagomanolin V, Farhang M, Ghazi-Khansari, Jafarzadeh N (2004) Heavy metals (Ni, Cr, Cu) in the Karoon waterway river, Iran. Toxicol Lett 151:63-68
- Falusi BA, Olanipekun EO (2007) Bioconcentration Factors of Heavy Metals in Tropical Crab (*Carcinus sp*) From River Aponwe, Ado-Ekiti, Nigeria. J Appl Sci Environ Manage 11:51-54
- Getachew T, Fernando CH (1989) The food habits of an herbivorous fish (*Oreochromis niloticus* Linn.) in Lake Awasa, Ethiopia. Hydrobiologia 174:195-200
- Ikenaka Y, Eun H, Watanabe E, Kumon F, Miyabara Y (2005a) Estimation of sources and inflow of dioxins and polycyclic aromatic hydrocarbons from the sediment core of Lake Suwa, Japan. Environ Pollut 138:530-538
- Ikenaka Y, Eun H, Watanabe E, Miyabara Y (2005b) Sources, distribution, and inflow pattern of dioxins in the bottom sediment of Lake Suwa, Japan. Bull Environ Contam Toxicol 75:915-921
- Klaassen CD, Liu J, Choudhuri S (1999) METALLOTHIONEIN: An Intracellular Protein to Protect Against Cadmium Toxicity. Annu Rev Pharmacol Toxicol 39:267-94
- Lwanga MS, Kansiime F, Denny P, Scullion J (2003) Heavy metals in Lake George, Uganda,

- with relation to metal concentrations in tissues of common fish species. *Hydrobiologia* 499:83-93
- Mackevičienė G (2002) Bioaccumulation of heavy metals in noble crayfish (*Astacus astacus* L.) tissues under aquaculture conditions. *Ekologija* 2:79-82
- Madamombe L (2002) The economic development of the Kapenta fishery lake Kariba (Zimbabwe/Zambia). Norwegian College of Fishery Science, University of Tromsø Thesis.
- Mohapatra H, Gupta R (2005) Concurrent sorption of Zn(II), Cu(II) and Co(II) by *Oscillatoria angustissima* as a function of pH in binary and ternary metal solutions. *Bioresour Technol* 96:1387-1398
- Morera MT, Echeverria JC, Mazkaran C, Garrido JJ (2001) Isotherms and sequential extraction procedures for evaluating sorption and distribution of heavy metals in soils. *Environ Pollut* 113:135–144
- Muohi AW, Onyari JM, Omondi JG, Mavuti KM (2003) Heavy metals in sediments from Makupa and Port–Reitz Creek systems: Kenyan Coast. *Environ Int* 28:619-647
- Mwase M, Viktor T, Norrgren L (1998) Effects on Tropical Fish of Soil Sediments from Kafue River, Zambia. *Bull Environ Contam Toxicol* 61:96-101
- Norrgren L, Pettersson U, Örn S, Bergqvist AP (2000) Environmental monitoring of the Kafue River, located in the Copperbelt, Zambia. *Arch Environ Contam Toxicol* 38:334-341
- Pettersson UT, Ingri J (2001) The geochemistry of Co and Cu in Kafue River as it drains the Copperbelt mining area, Zambia. *Chem Geol* 177:399-414
- Rincon-Leon F, Zurera-Cosano G, Pozo-Lora R (1988) Lead and cadmium concentrations in red crayfish (*Procambarus clarkii*, G.) in the Guadalquivir River Marshes (Spain). *Arch Environ Contam Toxicol* 17:251-256
- Schwanck EJ (1995) The introduced *Oreochromis niloticus* is spreading on the Kaufe floodplain, Zambia. *Hydrobiologia* 315:143-147



- Seymore H, du Preez HH, van Vuren JHJ (1996) Concentrations of zinc in *Barbus marequensis* from the lower Olifants River, Mpumalanga, South Africa. *Hydrobiologia* 332:141-150
- Shaw BJ, Handy RD (2006) Dietary copper exposure and recovery in Nile tilapia, *Oreochromis niloticus*. *Aquat Toxicol* 76:111-121
- Skelton P (1993) A complete guide to the freshwater fishes of Southern Africa. Southern Book Publishers. South Africa. pp 311-318
- Stockwell Hillier, Mills & White, (2001) World mineral statistics 1995-99, Keyworth, Nottingham. British Geological Survey 2001
- Syakalima M, Choongo K, Nakazato Y, Onuma M, Sugimoto C, Tsubota T, Fukushi H, Yoshida M, Itagaki T, Yasuda J (2001) An Investigation of Heavy Metal Exposure and Risks to Wildlife in the Kafue Flats of Zambia. *J Vet Med Sci* 63:315-318
- Utsugi K, Mazingaliwa K (2002) Field Guide to Zambian fishes, planktons and aquaculture. Japan International Co-operation Agency (JICA). pp 41, 81-82
- World Health Organization (WHO) (1993) Evaluation of certain food additives and contaminants. Technical Report Series, 1993, Number 837
- Zambia Consolidated Copper Mines Limited (ZCCM) (1982) A Review of Factors Relevant to the Reported Incidence of Cattle Losses on Farm Bordering the Mwambashi River. MITS/PA/30/82

## Figure Captions

Figure 1: Sampling sites (Lake ITT and Lake Kariba) for sediments, fish, and crayfish in Zambia. Each sampling point is shown as a black dot.

Figure 2: Concentration of heavy metals in *O. niloticus* liver and muscle in Lake ITT and Lake Kariba (U test, \*  $p < 0.05$ ).

Figure 3: Concentrations of heavy metals in *C. quadricarinatus* hepatopancreas and muscle in Lake ITT and Lake Kariba (U test, \*  $p < 0.05$ ).

Figure 4: Concentrations of heavy metals in fish liver and crayfish hepatopancreas in Lake ITT. Bars that share the same letter for a single heavy metal are not significantly different (Tukey test,  $p < 0.05$ ).

Table 1: Mean ( $\pm$  S.D.), and range of concentrations (mg/kg dry-wt) for heavy metals in the lake sediments. Values that have a different letter are significantly different (U test,  $p < 0.05$ ).

|                             | Cr (mg/kg)               | Co (mg/kg)               | Cu (mg/kg)               | Zn (mg/kg)               | Cd (mg/kg)                   | Pb (mg/kg)               | Ni (mg/kg)               | $\Sigma 7$                 |
|-----------------------------|--------------------------|--------------------------|--------------------------|--------------------------|------------------------------|--------------------------|--------------------------|----------------------------|
| <b>Lake ITT (n = 9)</b>     |                          |                          |                          |                          |                              |                          |                          |                            |
| AVERAGE                     | 54 $\pm$ 21 <sup>A</sup> | 26 $\pm$ 16 <sup>A</sup> | 82 $\pm$ 35 <sup>A</sup> | 39 $\pm$ 14 <sup>A</sup> | 0.06 $\pm$ 0.02 <sup>A</sup> | 13 $\pm$ 10 <sup>A</sup> | 39 $\pm$ 14 <sup>A</sup> | 254 $\pm$ 100 <sup>A</sup> |
| RANGE                       | 20 – 78                  | 1 – 42                   | 9 – 118                  | 12 – 58                  | 0.03 – 0.09                  | 3 – 30                   | 15 – 59                  | 60 – 369                   |
| <b>Lake KARIBA (n = 12)</b> |                          |                          |                          |                          |                              |                          |                          |                            |
| AVERAGE                     | 64 $\pm$ 36 <sup>A</sup> | 28 $\pm$ 19 <sup>A</sup> | 30 $\pm$ 17 <sup>B</sup> | 49 $\pm$ 30 <sup>A</sup> | 0.08 $\pm$ 0.03 <sup>A</sup> | 14 $\pm$ 8 <sup>A</sup>  | 53 $\pm$ 32 <sup>A</sup> | 237 $\pm$ 133 <sup>A</sup> |
| RANGE                       | 6 – 99                   | 1 – 46                   | 6 – 56                   | 5 – 92                   | 0.04 – 0.13                  | 3 – 25                   | 1 – 98                   | 29 – 383                   |

Table 2: Mean ( $\pm$  S.D.) body weight (g), body length (cm), coefficient of condition, and heavy metal concentration (mg/kg dry-wt) in the liver (hepatopancreas) and muscle of fish (*O. niloticus*, *S. thumbergi*) and crayfish (*C. quadricarinatus*).

|                               |        |    |                 |                 |               |                 |                   |                 |                 |                   |                 |                 |
|-------------------------------|--------|----|-----------------|-----------------|---------------|-----------------|-------------------|-----------------|-----------------|-------------------|-----------------|-----------------|
| <b>Liver (hepatopancreas)</b> |        |    |                 |                 |               |                 |                   |                 |                 |                   |                 |                 |
| species                       | lake   | n  | BW              | BL              | K             | Cr              | Co                | Cu              | Zn              | Cd                | Pb              | Ni              |
| <i>O. niloticus</i>           | ITT    | 18 | 1100 $\pm$ 409  | 32.5 $\pm$ 3.8  | 3.2 $\pm$ 0.4 | 0.60 $\pm$ 0.42 | 4.52 $\pm$ 2.93   | 1345 $\pm$ 930  | 67 $\pm$ 15     | 0.80 $\pm$ 0.73   | 0.19 $\pm$ 0.15 | 0.52 $\pm$ 0.28 |
|                               | Kariba | 16 | 849 $\pm$ 396   | 29.3 $\pm$ 4.2  | 3.2 $\pm$ 0.9 | 0.74 $\pm$ 0.58 | 11.1 $\pm$ 5.16   | 587 $\pm$ 320   | 87 $\pm$ 28     | 1.21 $\pm$ 0.88   | 0.50 $\pm$ 0.24 | 0.52 $\pm$ 0.56 |
| <i>C. quadricarinatus</i>     | ITT    | 19 | 103 $\pm$ 49.5  | 16.4 $\pm$ 2.0  | 2.2 $\pm$ 0.3 | 0.17 $\pm$ 0.25 | 8.37 $\pm$ 4.10   | 279 $\pm$ 169   | 484 $\pm$ 226   | 0.58 $\pm$ 0.36   | 0.18 $\pm$ 0.20 | 1.11 $\pm$ 0.79 |
|                               | Kariba | 10 | 98 $\pm$ 30     | 16.0 $\pm$ 2.1  | 2.4 $\pm$ 0.4 | 0.09 $\pm$ 0.03 | 12.6 $\pm$ 4.50   | 336 $\pm$ 207   | 493 $\pm$ 205   | 0.27 $\pm$ 0.19   | 0.34 $\pm$ 0.16 | 3.89 $\pm$ 2.17 |
| <i>S. thumbergi</i>           | ITT    | 6  | 362 $\pm$ 146   | 24.2 $\pm$ 4.2  | 2.5 $\pm$ 0.3 | 0.11 $\pm$ 0.10 | 0.47 $\pm$ 0.40   | 48 $\pm$ 43     | 50 $\pm$ 21     | 0.022 $\pm$ 0.016 | 0.35 $\pm$ 0.66 | 0.06 $\pm$ 0.11 |
| <b>muscle</b>                 |        |    |                 |                 |               |                 |                   |                 |                 |                   |                 |                 |
| species                       | lake   | n  | Cr              | Co              | Cu            | Zn              | Cd                | Pb              | Ni              |                   |                 |                 |
| <i>O. niloticus</i>           | ITT    | 18 | 1.53 $\pm$ 0.54 | ND              | 3 $\pm$ 1     | 21 $\pm$ 6      | 0.003 $\pm$ 0.005 | 0.12 $\pm$ 0.25 | 0.38 $\pm$ 0.29 |                   |                 |                 |
|                               | Kariba | 16 | 4.94 $\pm$ 4.20 | ND              | 2 $\pm$ 1     | 21 $\pm$ 5      | 0.002 $\pm$ 0.006 | 0.04 $\pm$ 0.06 | 0.82 $\pm$ 0.51 |                   |                 |                 |
| <i>C. quadricarinatus</i>     | ITT    | 19 | 1.15 $\pm$ 0.51 | 0.10 $\pm$ 0.16 | 33 $\pm$ 8    | 67 $\pm$ 12     | 0.003 $\pm$ 0.006 | 0.25 $\pm$ 0.39 | 0.28 $\pm$ 0.44 |                   |                 |                 |
|                               | Kariba | 10 | 1.86 $\pm$ 0.98 | ND              | 33 $\pm$ 8    | 78 $\pm$ 10     | 0.020 $\pm$ 0.015 | 1.36 $\pm$ 3.04 | 0.41 $\pm$ 0.12 |                   |                 |                 |

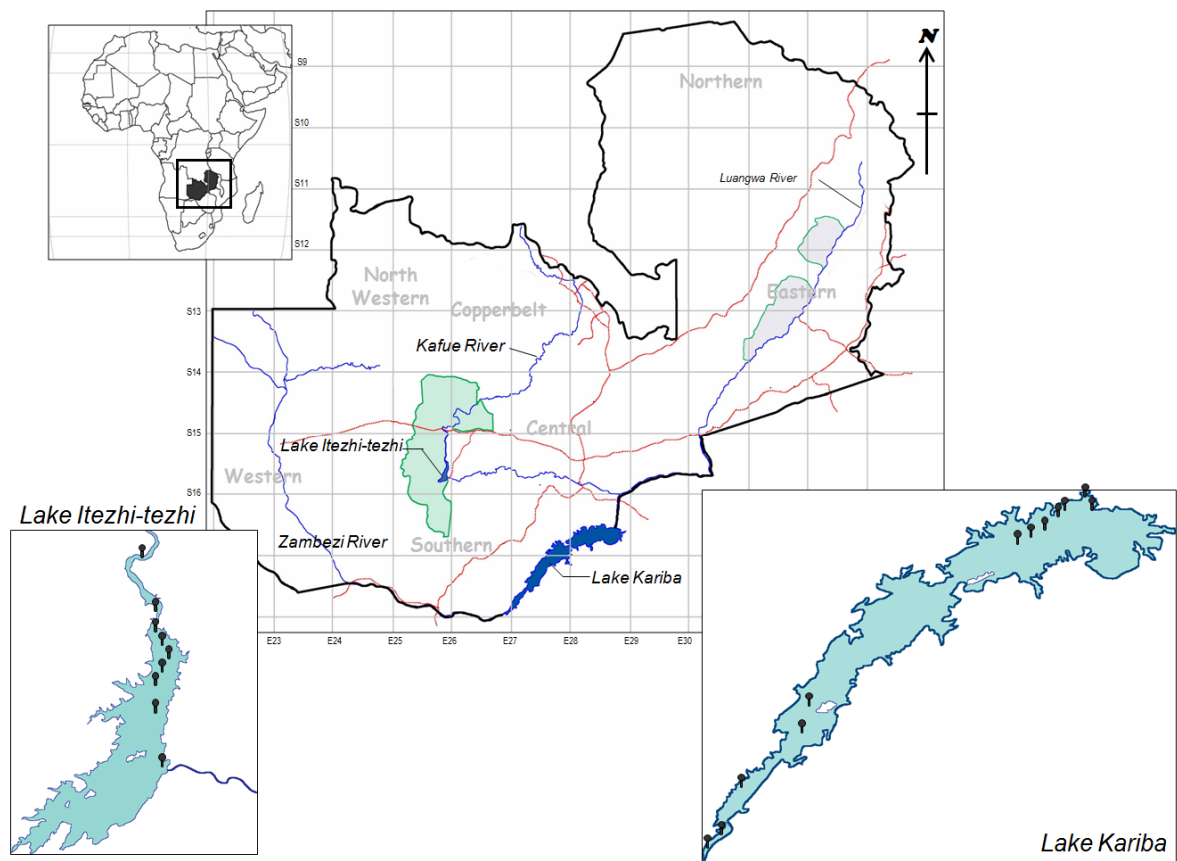
Body weight (BW), body length (BL), coefficient of condition (K), not detected (ND)

Table 3: Mean ( $\pm$  S.D.) biota-sediment accumulation factor values (BSAF) for *O. niloticus*, *S. thumbergi*, and *C. quadricarinatus*.

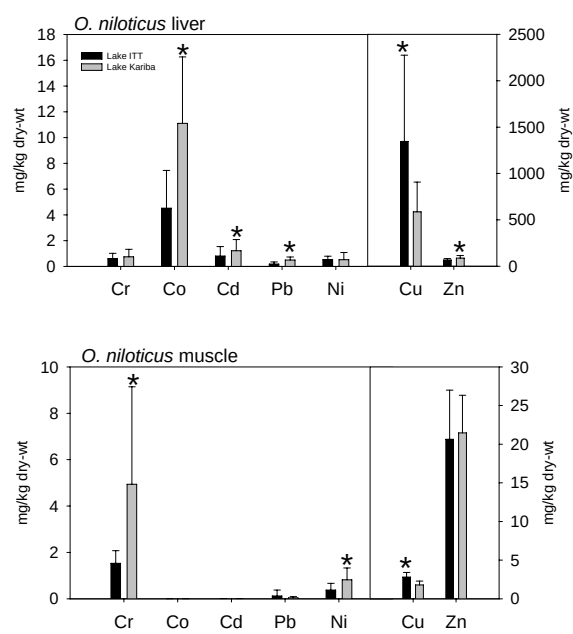
|                           |                   |                   |                 |                |                 |                   |                   |
|---------------------------|-------------------|-------------------|-----------------|----------------|-----------------|-------------------|-------------------|
| <i>O. niloticus</i>       | Cr                | Co                | Cu              | Zn             | Cd              | Pb                | Ni                |
| BSAF_ITT                  | 0.011 $\pm$ 0.009 | 0.17 $\pm$ 0.16   | 16.4 $\pm$ 13.3 | 1.7 $\pm$ 0.73 | 13.4 $\pm$ 13.0 | 0.015 $\pm$ 0.016 | 0.013 $\pm$ 0.009 |
| BSAF_Kariba               | 0.012 $\pm$ 0.011 | 0.40 $\pm$ 0.33   | 19.6 $\pm$ 15.4 | 1.8 $\pm$ 1.2  | 15.1 $\pm$ 12.3 | 0.036 $\pm$ 0.026 | 0.010 $\pm$ 0.012 |
| <i>C. quadricarinatus</i> | Cr                | Co                | Cu              | Zn             | Cd              | Pb                | Ni                |
| BSAF_ITT                  | 0.003 $\pm$ 0.005 | 0.32 $\pm$ 0.25   | 3.4 $\pm$ 2.5   | 12.4 $\pm$ 7.3 | 9.7 $\pm$ 6.8   | 0.014 $\pm$ 0.019 | 0.028 $\pm$ 0.023 |
| BSAF_Kariba               | 0.001 $\pm$ 0.001 | 0.45 $\pm$ 0.34   | 11.2 $\pm$ 9.4  | 10.1 $\pm$ 7.5 | 3.4 $\pm$ 2.6   | 0.024 $\pm$ 0.018 | 0.073 $\pm$ 0.06  |
| <i>S. thumbergi</i>       | Cr                | Co                | Cu              | Zn             | Cd              | Pb                | Ni                |
| BSAF_ITT                  | 0.002 $\pm$ 0.002 | 0.018 $\pm$ 0.019 | 0.59 $\pm$ 0.58 | 1.2 $\pm$ 0.7  | 0.36 $\pm$ 0.30 | 0.027 $\pm$ 0.055 | 0.002 $\pm$ 0.002 |

Table 4: Correlation coefficients ( $R^2$ ) for each heavy metal in fish (*O. niloticus*) and crayfish (*C. quadricarinatus*).

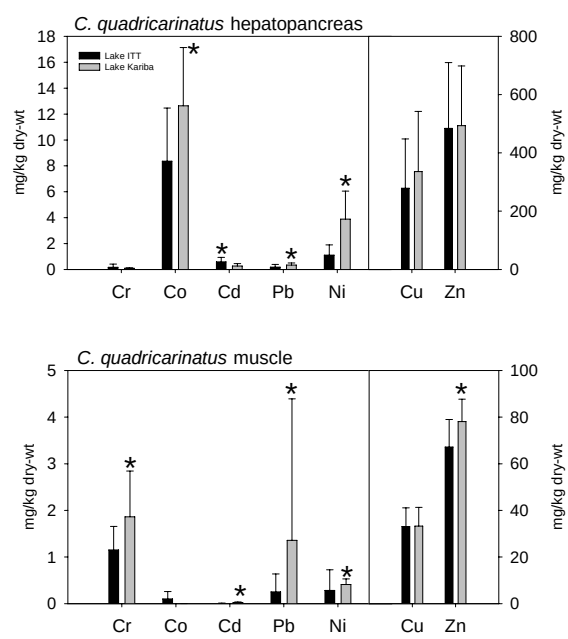
| species                                                               | tissue         | lake   | metal                       | R <sup>2</sup> | p value |
|-----------------------------------------------------------------------|----------------|--------|-----------------------------|----------------|---------|
| <i>O. niloticus</i>                                                   | liver          | ITT    | Cu-Zn                       | 0.54           | 0.0005  |
|                                                                       |                |        | Cu-Cd                       | 0.59           | 0.0002  |
|                                                                       |                |        | Co-Zn                       | 0.44           | 0.0028  |
|                                                                       |                |        | Zn-Cd                       | 0.41           | 0.0042  |
|                                                                       | liver          | Kariba | Cr-Ni                       | 0.54           | 0.0007  |
|                                                                       |                |        | Cr-Pb                       | 0.58           | 0.0006  |
|                                                                       |                |        | Zn-Co                       | 0.54           | 0.0012  |
|                                                                       | muscle         | ITT    | Cu-Zn                       | 0.41           | 0.0030  |
| muscle                                                                | Kariba         | Cr-Ni  | 0.41                        | 0.0079         |         |
| <i>C. quadricarinatus</i>                                             | hepatopancreas | ITT    | Cu-Zn                       | 0.60           | 0.0001  |
|                                                                       |                |        | Cu-Cd                       | 0.53           | 0.0004  |
|                                                                       |                |        | Zn-Cd                       | 0.58           | 0.0002  |
|                                                                       |                |        | Ni- <i>K</i> <sup>(a)</sup> | 0.51           | 0.0006  |
|                                                                       | hepatopancreas | Kariba | Cu-Zn                       | 0.50           | 0.0477  |
|                                                                       |                |        | Cu-Cd                       | 0.81           | 0.0028  |
|                                                                       |                |        | Cu-Co                       | 0.53           | 0.0375  |
|                                                                       |                |        | Zn-Co                       | 0.53           | 0.0361  |
|                                                                       |                |        |                             |                |         |
| Coefficient of condition ( <i>K</i> ), (a) means negative correlation |                |        |                             |                |         |



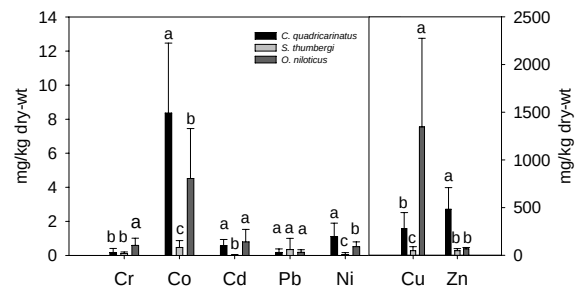
**Figure 1**



**Figure 2**



**Figure 3**



**Figure 4**