GIS-based source estimation of Cu pollution in Lake Itezhi-tezhi and metal accumulation profiles in *Oreochromis spp.* from both field and laboratory studies

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Abstract

The Copperbelt region, upstream of the Kafue River, including Lake Itezhi-tezhi (ITT), in Zambia has extensive copper (Cu) mines. In our field study, Geographic Information System (GIS) analysis in lake sediment indicated that the northern part of the lake, Copperbelt region, could be the Cu pollution source. Concentrations of Cu in stomach contents between fish species were not significantly different. However, *Oreochromis spp*. liver showed significantly higher Cu concentrations than those in other fish species. Log liver [Cu], standard length, and nitrogen stable isotope ratio were positively correlated only in *Oreochromis spp*. In the laboratory study, *O. niloticus* and *Oryzias latipes* were exposed to Cu for four days and recovery phases up to 28 days were examined. *O. niloticus* showed significantly higher concentrations of Cu compared with *O. latipes* at all sampling points. Significantly higher concentrations of Hg in *Schilbe intermedius* liver than for other fish species were observed, while *Oreochromis macrochir* showed significantly higher concentration of Cd. In conclusion, the northern part of the lake could be the source of Cu pollution in Lake ITT. Diet may not be the reason for high Cu accumulation in *Oreochromis spp*. Results from both field and laboratory studies imply that *Oreochromis spp.* contain high concentrations of Cu under normal physiological conditions.

Keywords: Lake Itezhi-tezhi; *Oreochromis spp*; metal accumulation profiles; stable isotope ratio

The Kafue River, including Lake Itezhi-tezhi (ITT), plays an important role in providing drinking water for humans, livestock, and wildlife in Zambia. The Copperbelt region, upstream of the Kafue River, is one of the core mining areas in Zambia. Previous studies have shown that water, sediment, and fish in the Kafue River, downstream of the Copperbelt region, contain higher concentrations of heavy metals, especially Cu, compared with samples collected upstream of the mining site (Choongo et al., 2005; Ikenaka et al., 2010; Mwase et al., 1998; Norrgren et al., 2000).

Recently, we reported that concentrations of Cu in the sediment, and liver of fish (*Oreochromis niloticus*) from Lake Itezhi-tezhi (ITT) in the Kafue National Park were very high, most likely due to the discharge of Cu wastes from the Copperbelt mining area, located approximately 450 km upstream (north) of the Kafue River (Nakayama et al., 2010). However, whether Cu waste from the Copperbelt region unequivocally reaches Lake ITT and affects the lake ecosystem is still unclear. Another interesting question from our previous study is why *Oreochromis spp.* accumulated higher concentrations of Cu compared with other fish species. This difference could be attributed to the particular feeding habits of various fish as it has been reported that *O. niloticus* can feed on detritus (Bowen, 1980) and that algae (*Oscillatoria sp*.) are an important component in the diet of *O. niloticus* (Getachew and Fernando, 1989). Interestingly, *Oscillatoria angustissima* are known to adsorb Cu (Mohapatra and Gupta, 2005). Given these facts, we hypothesized that diet is one of the reasons why *Oreochromis spp.* accumulate high concentrations of Cu.

In order to clarify the two observations above, namely the source of Cu pollution in the lake and relationships between high Cu accumulation and diet in *Oreochromis spp.*, we used Geographic Information System (GIS) and stable isotope

ratio (carbon and nitrogen) analysis. Since GIS analysis has been used as a powerful method for displaying pollution patterns (Nakayama et al., 2011), we used it to determine the source of pollution and predict the distribution of metals in lake sediments. Stable isotope ratio analysis has increasingly been used to assess the relationship between trophic levels and accumulation levels of environmental pollutants in an ecosystem (Revenga et al., 2011). Moreover, stable carbon isotope ratios ($\delta^{13}C$) have been used to evaluate dietary sources within a freshwater food web (Peterson and Fry, 1987), whereas nitrogen isotope ratios ($\delta^{15}N$) are reported to increase 2–4‰ (average: 3.4‰) with increasing trophic levels (Minagawa and Wada, 1984). In our field study, we focused on Cu as we noted above that the Lake ITT could be contaminated with Cu. Cd and Hg were also included on the assumption that these metals may be bio-accumulative in the trophic food web (Croteau et al., 2005; Mason et al., 1996).

In order to clarify the Cu accumulation profiles in *Oreochromis sp.*, we exposed Nile Tilapia (*O. niloticus*) to Cu under laboratory conditions. *O. niloticus* is frequently used in laboratory metal exposure studies (Daramola and Oladimeji, 1989) and is closely related to *O. andersonii* and *O. macrochir* that were included in our field study. We used Japanese medaka (*Oryzias latipes*) as a control species for the following reasons: (1) guidelines for conducting toxicity tests in medaka fish are available; (2) medaka has been used as a surrogate for many studies in environmental toxicology; and (3) medaka can be easily maintained in a laboratory with limited space (Chen et al., 2001).

Our objectives were to: (1) clarify the distribution of these metals in the lake sediment by using GIS analysis to predict the source of Cu pollution; (2) compare metal

levels among several fish species with different food habits to reveal if diet is related to Cu accumulation in fish; (3) determine the relationship between stable isotope ratio and metal levels (Cu, Cd, and Hg) in fish to reveal the bio-accumulation (or bio-dilution) profiles of these metals in the freshwater ecosystem; and (4) perform laboratory Cu exposure in order to clarify the species difference in Cu accumulation profiles between *O. niloticus* and *O. latipes*.

Materials and Methods

Study area and sampling in the field study

The study was conducted in Lake ITT, which is located in the Kafue River basin, during the dry season in August 2010 (Fig. S1). Lake ITT is an artificial lake (length: ~ 50 km, mean depth: ~ 19.5 m, surface area: ~ 370 km^2) that floods part of the Kafue National Park. The lake has several inflows, with the main inflow of the Kafue River in the northern part of the lake, and only one outflow (also Kafue River) in the middle (Fig. S1). We collected water (n = 54) and sediment (n = 54) from the lake. Information on lake water is summarized in Table S1. Surface water samples were collected and kept in plastic bottles. Surface lake sediment samples (0–5 cm) were collected using an Ekman-Birge grab sampler. The geographical coordinates of each location were recorded using Global Positioning System (GPS)**.** Each sediment sample was air-dried in the laboratory at room temperature and passed through a 2 mm sieve prior to analysis of metal concentration and stable isotope ratios. The water content of each sample was measured after 12 h of drying in an oven at 105 °C. To determine organic matter (OM) content in the sediment, the ignition loss of each sample was measured after 5 h in an oven at 600 °C.

We collected eight fish species (n = 103) with different diets, such as fish-eating or not (Table 2) and measured the standard length and weight of each individual in the field. We also removed samples of liver, muscle, and stomach contents from each fish. The samples were transferred into properly labeled and sealed plastic containers and transported to the Graduate School of Veterinary Medicine, Hokkaido

University, Japan for analysis. The samples were stored at −20 °C for water content measurement, analysis of heavy metals, and stable isotope ratio. For measurement of water content, each sample was dried in an oven at 105 °C for 24 h.

Coefficient of condition (K)

The coefficient of condition (*K*) in fish was calculated for each sample using the formula $K = (W/L^3) \times 10^2$, where, *K* = coefficient of condition, *W* = weight in grams, and *L* = standard length in centimeters (Choongo et al., 2005; Nakayama et al., 2010).

Extraction and analysis of heavy metals

Metals in sediment samples were extracted using a microwave digestion system according to the manufacture's instruction (Speedwave two, Berghof, Germany). Briefly, 1 g of each sediment sample was placed in a prewashed digestion bomb, and 10 mL of 60% nitric acid (Kanto Chemical Corporation, Tokyo, Japan) was added. The microwave unit was calibrated to a temperature of 200 ^{o}C and digestion was performed for 25 minutes. After the samples cooled, they were filtered into plastic bottles using ash-less filter paper 5B (Advantec, Tokyo, Japan). Next, 0.5 mL of Lanthanum chloride (atomic absorption spectrometry grade, 100 g/L solution; Wako Pure Chemical Industries Ltd., Osaka, Japan) was added. The sample volume was standardized to 50 mL using distilled, deionized water. A reagent blank was prepared using the same procedure.

Metals were extracted from the fish livers and stomach contents using a

microwave digestion system according to the manufacture's instruction (Berghof). Briefly, 0.5 g of each sample was placed in a prewashed digestion bomb, and 5 mL of 60% nitric acid (Kanto) and 1 mL of 30% hydrogen peroxide (Kanto) were added. Digestion conditions were as follows, 160 ^{o}C (5 min), 190 ^{o}C (10 min), and 75 ^{o}C (20 min). After the samples cooled, they were transferred into plastic tubes and 0.1 mL of lanthanum chloride (Wako) was added. The volume was then made up to 10 mL with distilled, deionized water. A reagent blank was prepared using the same procedure. Prior to metal analysis, 0.5 mL of HNO_3 was added to each 50 mL of water sample and kept for 24 h at room temperature to free the metals that could be bound to the tubes. The concentrations of Cu and Cd in lake water, sediment, and fish samples were measured using an atomic absorption spectrophotometer (AAS) (Z-2010, Hitachi High-Technologies Corporation, Tokyo, Japan) with either an acetylene flame or argon non-flame method, after preparation of the calibration standard. Analytical quality control was performed using three certified reference materials as follows; BCR-320R (channel sediment, Community Bureau of Reference of European Commission, Brussels, Belgium), SRM 1944 (New York/New Jersey Waterway Sediment, National Institute of Standards and Technology, New York, USA) and DOLT-4 (Dogfish liver, National Research Council of Canada, Ottawa, Canada). Recovery rates (%) of sediment using certified reference materials (BCR-320R and SRM 1944) were acceptable; Cu (88-95) and Cd (113-120). For liver tissue, recovery rates (%) of Cu and Cd were acceptable; Cu (88-90) and Cd (91-108). The detection limits (μg/kg) of Cu and Cd were 1.0 and 0.2, respectively. The detection limits (μg/L) of Cu and Cd in water samples were 0.5 and 0.2, respectively. Metal concentration in sediment and fish samples was converted from mg/kg wet-weight (wt) to mg/kg dry-wt using the

calculated water content, whereas those in water were expressed as μg/L.

Analysis of total mercury (Hg)

The concentration of total Hg in water, sediment, and fish liver samples was measured by thermal decomposition, gold amalgamation, and atomic absorption spectrophotometry (Mercury Analyzer, MA-3000, Nippon Instruments Corporation, Tokyo, Japan), after preparation of the calibration standard. Recovery rates of Hg for the three certified reference materials (BCR-320R, SRM 1944 and DOLT-4) ranged from 92 to 103%. The recovery rate (%) of Hg was 94.3 ± 4.2. The detection limit of Hg in soil and fish samples was 2.0 pg of total Hg. The detection limit (μg/L) of Hg in water samples was 0.01. Concentration of Hg was converted from mg/kg wet-wt to mg/kg dry-wt using the calculated water content, while those in water were expressed as μg/L.

Stable isotope ratio analysis

The sediment samples for $\delta^{13}C$ and $\delta^{15}N$ analyses were dried at 60 ^{o}C for 24 h, decarbonated by 1 M hydrochloric acid (Kanto) for 12 h, rinsed with distilled deionized water, and dried again (Usui et al., 2006). Fish muscle samples were ground into a homogeneous powder after drying at 45 ^{o}C for 48 h and treated with a 2:1 chloroform:methanol solution (Kanto) for 12 h to remove lipids (Logan and Lutcavage, 2008). After each sample was weighed out into a tin capsule, stable isotope ratio was determined using an isotope ratio mass spectrometer equipped with an elemental

analyzer (Fisons NA1500-Finnigan MAT 252). Stable isotope ratios were expressed in δ notation, as the deviation from standards in parts per thousand (‰), according to the following formula: $\delta X = [(R_{sample}/R_{standard} - 1)] \times 1000$, where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Minagawa and Wada, 1984). Data are presented as the values based on the international standard of v-PDB (Vienna Peedee Belemite) and atmospheric N_2 for C and N, respectively (Minagawa and Wada, 1984). The replicate error was within 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ analyses.

Animals and acclimation condition of laboratory Cu exposure

Treatment of all animals was performed according to the policies of the Institutional Animal Care and Use Committee of Hokkaido University.

In the present study, *O. niloticus* (n = 77) were maintained in our laboratory and used for this experiment. *O. latipes* (n = 77) were purchased from a local fish farm in Sapporo, Japan. Both *O. niloticus* and *O. latipes* were maintained under the same conditions. Body weight (g) of the two fish species was not significantly different (*O. niloticus*; 0.24 ± 0.12, *O. latipes*; 0.22 ± 0.08, mean ± S.D.). Fish were kept in a dark room with a light:dark period of 14:10 h. Each fish species was separately acclimated in 60 L glass aquariums for 20 days before exposure. Each tank was filled with de-chlorinated and filtered well-water at 26 ^{o}C and continuously aerated. The concentrations of Cr, Co, Cu, Zn, Cd, Pb, Ni, and As in the filtered water were below the detection limit of AAS. Both fish species were fed the same commercial flakes (Tetra-KilliMin, Tetra Japan, Tokyo, Japan), which contained normal Cu levels (Cu: 8.3 ± 0.3 mg/kg), once per day. This low level was chosen to avoid contamination of the

aquarium water with Cu in feed. The feed contained a crude protein 48% (minimum), crude lipid 11% (minimum), crude fiber 2% (maximum), and ash 11% (maximum). Water pH was constant (8.09 ± 0.16, mean ± S.D.) during the acclimation and experimental period and was similar to levels in our field study.

Cu concentration used for exposure

Previous studies demonstrated that 0.964 mg/L Cu was the LC50 value for 96 h exposure to *O. niloticus* (Daramola and Oladimeji, 1989), while 2 mg/L Cu caused 40–60% mortality during six days of exposure to *Oreochromis mossambicus*, which is closely related to *O. niloticus* (Wu et al., 2008). In our preliminary experiment using *O. niloticus* and *O. latipes*, and a previous study using *O. mossambicus* (Wu et al., 2008), no mortality was found with exposure to 0.2 mg/L Cu for four days (96 h), and following 28 days recovery phase. Furthermore, saturation of Cu accumulation was observed with exposure to 0.2 mg/L Cu for 96 h (Wu et al., 2008). Therefore, 0.2 mg/L Cu waterborne exposure for 96 h was performed in the present study. The 0.2 mg/L Cu water was prepared using $CuSO_4 \cdot 5H_2O$ (Kanto) with distilled, deionized water and the actual concentration (0.2 mg/L) was confirmed by AAS.

Cu exposure to *O. niloticus* and *O. latipes*

In the current experiment, fish were starved for 24 h prior to sampling to allow all feed to be excreted. Before exposure (0 h), seven fish of each species were randomly collected for the ‘no exposure’ control. We exposed *O. niloticus* (n = 70) and *O. latipes*

(n = 70) to 0.2 mg/L Cu water for up to four days with aeration and feeding once a day. Cu concentration of the water was monitored and adjusted every 24 h to maintain accurate exposure. Seven fish were randomly removed from each tank at 24, 48, 72, and 96 h and the body surface of each fish was washed with Cu-free water in order to prevent surface contamination of Cu. After 96 h exposure, we transferred all remaining fish samples (n = 42 for each species) to Cu-free, de-chlorinated, aerated, and filtered well-water for up to 28 days in order to reveal differences between the species Cu excretion profiles. Seven fish were collected at 24, 48, 72 h, and 7, 14, and 28 days after fish were transferred to Cu-free water. Neither mortality nor weight change was observed during the exposure and depuration periods. To assess the Cu accumulation and elimination ability of whole fish, we used the whole body for Cu analysis. Each fish was individually dried in an oven at 50 ^{o}C for 24 h. Cu extraction and measurement was individually performed using the same procedures described above.

Statistical analysis and GIS-based spatial analysis

Statistical analyses were performed using JMP 9 (SAS Institute, Cary, NC, USA). Data were normalized by base 10 logarithm transformations. In the field study, we analyzed for differences in metal concentration and stable isotope ratio among the fish species using a Tukey test ($p < 0.05$). Pearson product-moment correlation (r) was used to analyze relationships between metal concentration and standard length of fish. Principal component analysis (PCA) was performed with each standard length, weight, nitrogen stable isotope ratio, and normalized metal concentration. In the laboratory Cu exposure experiment, differences in Cu concentration were analyzed by Student's *t*-test

(comparison between two fish species) or Dunnett's test (comparison with control in each species) ($p < 0.05$). Mapping of each water depth and metal concentration in sediment was performed by using ArcGIS 9.3 (ESRI, New York, USA). Spline was adopted for the interpolation of geographical data.

Results and Discussion

The Copperbelt mining area as a possible pollution source for Cu in Lake ITT

Metal concentrations in water and sediment are summarized in Table 1. Concentrations of Cd in lake water samples were below the detection limit. In contrast, Cu and Hg were detected in 83% and 67% of water samples, respectively, though these values were far below WHO guidelines for drinking water (World Health Organization, 2004). Approximately 40% of the sediments showed higher concentrations of Cu compared with the benchmark value, while Cd and Hg concentrations were below the benchmark value (Forstner, 1981; Yabe et al., 2010). Moreover, current results in Lake ITT sediments showed higher concentrations of Cu compared with the Cu levels in sediments from Lake Kariba in Zambia (6–56 mg/kg dry-wt, Student's *t*-test, (Nakayama et al., 2010)). According to GIS analysis, relatively higher concentrations of Cu were recorded in the sediment from the northern part of the lake, suggesting that Cu wastes actually reached Lake ITT (Fig. 1). In agreement with previous studies that reported high concentrations of Cu in water and sediment around mining areas in the Copperbelt region (Choongo et al., 2005; Ikenaka et al., 2010; Mwase et al., 1998; Norrgren et al., 2000), we observed that Cu pollution occurred in the northern part of

the lake, probably from the Copperbelt mining area, where Cu wastes passed through the Kafue River and reached Lake ITT located in the Kafue National Park. On the contrary, Cd and Hg distribution patterns were different from those of Cu. These metals were evenly distributed and relatively higher concentrations were detected from both northern and southern parts of the lake (Fig. 1).

Stable isotope ratio, Cd and Hg levels in fish

Carbon and nitrogen stable isotope ratio in sediment and fish were summarized (Tables 1 and 2, Fig. S2). Sediment samples showed lower values of $\delta^{15}N$ compared to those of fish species. Significantly higher and lower values of $\delta^{13}C$ were observed in *Tilapia rendalli* (−15.01 ± 1.48‰) and *Synodontis sp.* (−26.77 ± 3.25‰), compared to other fish species. This result suggests that their carbon sources are different from those of the other fish species. Generally, C4 photosynthetic plant has higher value (about −12‰) of $\delta^{13}C$ compared to that of C3 photosynthetic plant (about −28‰) (Mbabazi et al., 2010). This large difference of $\delta^{13}C$ values could contribute to the variation of carbon isotope in fish. In addition, we found that $\delta^{13}C$ value in sediment was relatively similar to that in *Oreochromis spp.*, but different from in *T. rendalli*. According to literature (Skelton 1993), *Oreochromis spp*. prefer detritus whereas *T. rendalli* do not consume it. This diet difference is considered to be another reason why they showed different $\delta^{13}C$ values. Similar results of high $\delta^{13}C$ values in *T. rendalli* have been previously reported (Mbongwe et al., 2003).

In the present study, fish-eating species had higher values of $\delta^{15}N$ compared with other fish species, reflecting their food habits (Mbongwe et al., 2003; Skelton,

1993). The highest difference we found was 4.71‰ between *Serranochromis sp.* and *Labeo cylindricus*, indicating an increase in trophic level (Minagawa and Wada, 1984). According to the literature on stomach content analysis, *Oreochromis spp.* consumed algae, invertebrates, and small insects, but not fish (Mbongwe et al., 2003). That report is in accordance with the present results of relatively lower values of $\delta^{15}N$ in *Oreochromis spp*. In the present study, *T. rendalli* showed similar values of $\delta^{15}N$ to *Oreochromis spp.*, suggesting that this fish is also not a fish-eating species. It was reported that *T. rendalli* relied heavily on plant material and algae (Mbongwe et al., 2003).

Significantly higher concentrations of Hg in *S. intermedius* liver than for other fish species were observed, while *Oreochromis macrochir* showed significantly higher concentration of Cd (Table 2). Furthermore, fish species were classified into two groups on the basis of Hg and Cd concentrations (Fig. 2). One group mainly consisted of fish-eating species that accumulated higher concentrations of Hg than Cd (Fig. 2). On the contrary, another group included *T. rendalli* and *Oreochromis spp.*, which are not known to eat fish and accumulated higher concentrations of Cd than Hg. Results of PCA supported these accumulation profiles of Cd and Hg (Fig. 3). Results of component 1 suggested that levels of Hg and $\delta^{15}N$ were positively correlated, while $\delta^{15}N$ could negatively correlate with Cu and Cd levels in fish from the result of component 2. In the marine environment, similar Cd and Hg accumulation profiles were reported in seals (Watanabe et al., 2002). Seal species that accumulated Cd consumed invertebrates, whereas the preferred diet of Hg-accumulators was fish (Watanabe et al., 2002).

In all the fish species analyzed, there was no relationship between $\delta^{15}N$ and

liver Cd concentration (r^2 = 0.02, p = 0.16, data not shown), while $\delta^{15}N$ and liver Hg showed a weak positive correlation (r^2 = 0.11, p < 0.001, data not shown). Among metals, Hg (especially, methyl-mercury) is known to be the most biomagnified element (Mason et al., 1996).

Cu accumulation profiles in Oreochromis spp. from both field and laboratory studies

In our field study, as we expected, *Oreochromis spp*. accumulated significantly higher concentrations of Cu (164–7,205 mg/kg) in the liver than other fish species (Table 2). This result was in accordance with our previous study (Nakayama et al., 2010). It was reported that caged three-spot tilapia (*Oreochromis andersonii*), exposed to Kafue River water, accumulated higher Cu concentrations (liver: 9,700 ± 810 mg/kg dry weight) downstream of the mining area compared with a locality upstream (Cu: 4,700 ± 840 mg/kg dry weight) (Norrgren et al., 2000). Liver Cu concentrations in *Oreochromis spp.* in the current study were similar to those of exposed fish around the mining area.

To clarify the relationships of Cu accumulation between sediment and *Oreochromis spp.*, we analyzed spatial variations of Cu concentrations in *O. macrochir* livers from the northern, middle, and southern parts of the lake. In the current study, *O. macrochir* from the middle part accumulated significantly higher concentrations of Cu compared with those from the southern part (Fig. 4). This result directly reflected the water flow regime in the lake (Fig. S1) and the tendency of Cu distributions in the lake sediment (Fig. 1). Although standard fish lengths were different between sites, which could affect the Cu accumulation in the fish (Fig. 4), current results suggest the

possibility that Cu pollution in the sediment from the middle and northern parts of the lake caused higher Cu accumulation in *Oreochromis spp*. in these areas.

Interestingly, there was no difference in the concentration of Cu in stomach contents among the fish species (Table 2). This implies that diet may not be the reason for high accumulation of Cu in *Oreochromis spp*. and we believe that there could be some other mechanisms for higher accumulation of Cu in *Oreochromis spp*. In order to support this hypothesis, we analyzed relationships between standard length and log liver Cu levels in each fish species. Significant positive correlations were only observed in *Oreochromis spp.* (*O. andersonii* and *O. macrochir*), while other fish species did not show any significant correlations (Table 3). This result suggests that *Oreochromis spp.* can accumulate Cu in the liver as they grow.

In all the fish species in the present study, $\delta^{15}N$ and liver Cu concentration were negatively correlated, indicating a bio-dilution profile of Cu in the freshwater ecosystem (Fig. 5). As far as we are aware, this is the first report to show the bio-dilution profile of Cu using nitrogen stable isotope ratio in the freshwater ecosystem. Although Cu showed the bio-dilution profile in all the fish species, strong positive correlation between liver Cu level and muscle $\delta^{15}N$ value was observed only in *Oreochromis spp*. (Fig. 5). This result also suggests that *Oreochromis spp.* can accumulate Cu in the liver.

In order to reveal the Cu accumulation profiles in *Oreochromis spp.* under laboratory conditions, both *Oreochromis niloticus* and Japanese medaka (*Oryzias latipes*) were exposed to Cu. In the exposure period, both species showed a significant increase in Cu concentrations after 24 h exposure (Dunnett's test, $p < 0.05$) (Fig. 6). In the recovery phase, Cu concentration in *O. niloticus* significantly decreased after 24 h

recovery, whereas medaka showed significant decrease after 48 h (Dunnett's test, $p < 0.05$) (Fig. 6). These results suggest that there are no distinctive differences in the Cu accumulation and excretion profiles between the two fish species. However, interestingly, *O. niloticus* showed significantly higher concentrations of Cu compared with medaka at all sampling points including 'no exposure' groups (0 h, Fig. 6) (Student's *t*-test, $p < 0.05$). This implies that *Oreochromis spp.* contains high concentrations of Cu under normal physiological conditions and is consistent with our results of high concentrations of Cu in *Oreochromis spp.* in the field study. Since the mechanisms involved in high Cu accumulation in *Oreochromis spp*. are still unclear, further laboratory study using a molecular biology approach is required.

Conclusions

In conclusion, GIS analysis in the lake sediment suggests that the northern part of the lake could be the source of Cu pollution in Lake ITT. Diet may not be the reason for high Cu accumulation in *Oreochromis spp*. Results from both field and laboratory studies imply that *Oreochromis spp*. contain high concentrations of Cu under normal physiological conditions.

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Table 1. Metal concentrations in water (μg/L) and sediment (mg/kg dry-wt), plus organic matter content (OM, %) and stable isotope ratio ($\delta^{13}C$ and $\delta^{15}N$, ‰) in sediment.

Water (n = 54)

	Cu (μg/L)	Cd (μg/L)	Hg (μg/L)
Median	1.2	ND	0.02
Range	ND-6.5	ND	ND-0.48
Benchmark [(a)]	2000	3	1

(a) WHO guidelines for drinking water (2004), ND: not detected

Sediment (n = 54)

	Cu (mg/Kg dry-wt)	Cd (mg/Kg dry-wt)	Hg (mg/Kg dry-wt)	OM (%)	$\delta^{13}C$ (‰) (Mean±S.D.)	$\delta^{15}N$ (‰) (Mean±S.D.)
Median	34	0.074	0.017	8	-24.14±1.78	3.83±0.73
Range	5-114	0.011-0.12	0.001-0.036	0.53-18		
Benchmark [(a)]	45	0.3	1	-	-	-

(a) Forstner 1981; Yabe et al., 2010

Table 2. Metal concentrations (mg/kg dry-wt), stable isotope ratio (carbon and nitrogen, ‰), and food habit in fish. Different letters indicate significant differences among fish species (Tukey test, $p < 0.05$)

Species, common name	Sample size	Code		Body weight (g)	Standard length (cm)	Coefficient of condition (K)	Cu (mg/kg dry-wt)		Cd (mg/kg dry-wt)	Hg (mg/kg dry-wt)	$\delta^{13}C$ (‰) (Mean±S.D.)	$\delta^{15}N$ (‰) (Mean±S.D.)	Food habit *
							Liver	Stomach content	Liver	Liver			
Serranochromis spp .	11	S	Median	383	26.5	1.9	21 ^{C}	12 ^{A}	0.04 ^{B}	0.16 ^{B}	-22.19±1.04 ^{CD}	11.00±0.86 ^{A}	Fish-eating
			Range	210-677	24.5-33.0	1.6-2.8	14-109	6-24	0.02-0.07	0.04-0.55			
Clarias ngamensis	12	C	Median	604	40.0	1.0	91 ^{C}	32 ^{A}	0.08 ^{B}	0.07 ^{B}	-22.10±1.18 ^{C}	9.70±0.99 ^{B}	Fish-eating
Blunttooth catfish			Range	461-2000	36.0-52.5	0.9-1.4	12-160	9-84	0.06-0.40	0.028-0.38			
Schilbe intermedius	6	I	Median	116	21.3	1.2	54 ^{C}	42 ^{A}	0.13 ^{B}	0.59 ^{A}	-24.76±1.16 ^{DE}	9.23±1.27 ^{B}	Fish-eating
Silver catfish			Range	88-286	20.0-28.0	1.1-1.3	38-84	10-73	0.10-0.20	0.33-3.2			
Synodontis sp.	14	Y	Median	62	13.8	2.3	212 ^{B}	28 ^{A}	0.17 ^{B}	0.19 ^{B}	-26.77±3.25 ^{E}	8.91±0.89 ^{B}	Fish-eating
Upside-down catfish			Range	43-115	12.0-16.0	1.9-3.5	47-520	17-90	0.08-0.36	0.05-0.55			
Tilapia rendalli	12	T	Median	231	19.0	3.4	268 ^{B}	11 ^{A}	0.06 ^{B}	0.01 ^{B}	-15.01±1.48 ^{A}	7.69±0.34 ^{C}	Fish-eating or not ?
Redbreast tilapia			Range	174-567	16.0-25.0	2.5-5.0	74-1040	6-20	0.003-0.09	0.004-0.05			
Oreochromis anderson	18	O or OA	Median	587	27.5	3.0	1555 ^{A}	19 ^{A}	0.41 ^{AB}	0.13 ^{B}	-22.02±0.94 ^{BC}	7.61±1.01 ^{C}	Not fish-eating
Threespot tilapia			Range	107-1908	15.0-43.0	2.2-3.6	245-7205	6-36	0.13-1.24	0.03-0.82			
Oreochromis macrochi	23	O or OM	Median	357	22.0	3.5	989 ^{A}	24 ^{A}	0.55 ^{A}	0.08 ^{B}	-21.08±1.34 ^{BC}	7.50±0.59 ^{C}	Not fish-eating
Greenhead tilapia			Range	131-910	15.5-29.0	3.1-4.5	164-4795	11-240	0.03-1.24	0.003-0.63			
Labeo cylindricus	7	L	Median	409	23.3	3.2	NA	NA	NA	NA	-19.54±1.15 ^{B}	6.29±0.37 ^{D}	Not fish-eating
Redeye labeo			Range	51-130	14.5-18.5	1.7-2.2	NA	NA	NA	NA			

Different letters indicate significant differences among fish species (Tukey test, $p < 0.05$), * Mbongwe et al. 2003, Skelton 1993

Table 3. Correlation coefficients (r^2) between metal concentrations in liver and standard length in fish. * indicates a significant correlation.

Liver Cu-standard length		
Species	r^2	*p* value
Serranochromis sp.	0.01	0.98
Clarias ngamensis	0.16	0.19
Schilbe intermedius	0.30	0.25
Synodontis sp.	0.05	0.46
Tilapia rendalli	0.04	0.54
Oreochromis andersonii	0.85	< 0.0001 *
Oreochromis macrochir	0.30	< 0.001 *
Liver Cd-standard length		
Species	r^2	*p* value
Serranochromis sp.	0.00	0.99
Clarias ngamensis	0.00	0.9
Schilbe intermedius	0.01	0.85
Synodontis sp.	0.02	0.64
Tilapia rendalli	0.11	0.33
Oreochromis andersonii	0.70	< 0.0001 *
Oreochromis macrochir	0.34	< 0.001 *
Liver Hg-standard length		
Species	r^2	*p* value
Serranochromis sp.	0.32	0.07
Clarias ngamensis	0.76	< 0.001 *
Schilbe intermedius	0.39	0.18
Synodontis sp.	0.13	0.21
Tilapia rendalli	0.49	< 0.05 *
Oreochromis andersonii	0.53	< 0.001 *
Oreochromis macrochir	0.28	< 0.01 *

Figure legends

Fig. 1. Geographical maps of the water depth and concentrations of metals in lake sediment in Lake ITT.

Fig. 2. Plot of Cd and Hg concentrations (mg/kg dry-wt) in fish liver. Each letter indicates code for the name of fish species in Table 2. Red, blue and green letters in the figure indicate the fish species of fish-eating, not-fish eating and not known, respectively.

Fig. 3. Principal component analysis (PCA) of heavy metal concentrations (mg/kg dry-wt), nitrogen stable isotope ratio ($\delta^{15}N$), standard length (cm) and body weight (g) in fish. Each letter indicates code for the name of fish species in Table 2. Red, blue and green letters in the figure indicate the fish species of fish-eating, not-fish eating and not known, respectively.

Fig. 4. Spatial variation of liver Cu concentrations (mg/kg dry-wt) and standard length (cm) in *O. macrochir* from the northern (n = 5), middle (n = 8), and southern (n = 10) parts of the lake. Different letters indicate significant differences in Cu concentration between sites (Tukey test, $p < 0.05$).

Fig. 5. Relationships between log liver Cu concentration (mg/kg dry-wt) and nitrogen stable isotope ratio ($\delta^{15}N$) in all the fish species (left panel) and *Oreochromis spp.* only (right panel). Each letter indicates the code for the name of fish species in Table 2. Red,

blue and green letters in the figure indicate the fish species of fish-eating, not-fish eating and not known, respectively.

Fig. 6. Concentrations of Cu (mean S.D.) in *O. niloticus* and *O. latipes* during the four-day exposure and 28 days recovery phase. Blue and red in the figure indicate *O. niloticus* and *O. latipes*, respectively.* indicates a significant difference between two fish species at each sampling point (Student's *t*-test, $p < 0.05$). † indicates a significant difference compared to control group (0 h) in the same species during the exposure phase (Dunnett's test, $p < 0.05$). ‡ indicates a significant difference compared to 96 h exposure (0 h recovery) group in the same species during the recovery phase (Dunnett's test, $p < 0.05$).

Fig. 1.

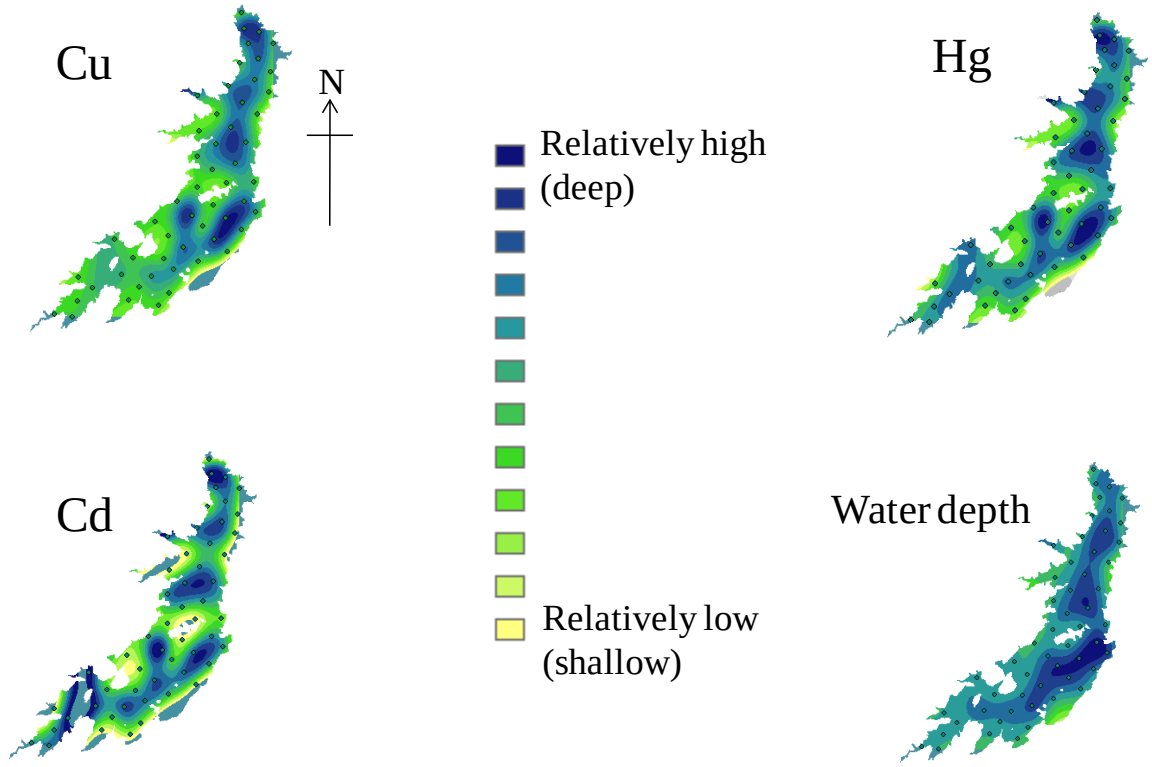

Cu
N
Hg
Relatively high
(deep)
Cd
Water depth
Relatively low
(shallow)

Fig. 2.

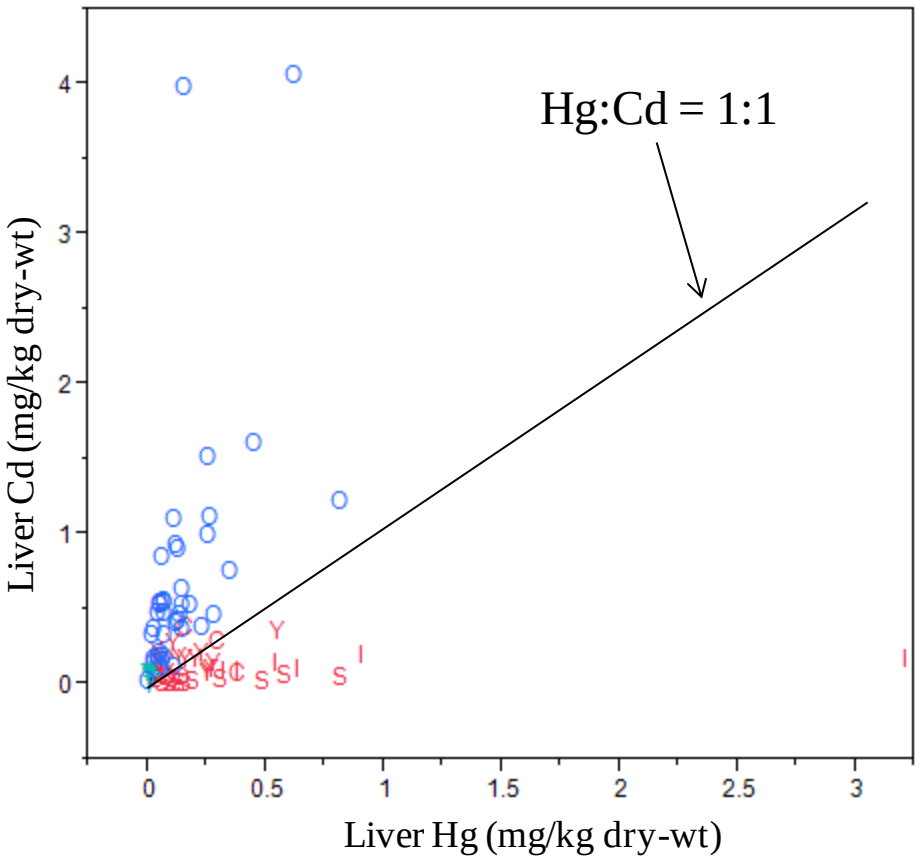

Hg:Cd = 1:1
Liver Cd (mg/kg dry-wt)
Liver Hg (mg/kg dry-wt)
0
1
2
3
4
0
0.5
1
1.5
2
2.5
3

Fig. 3.

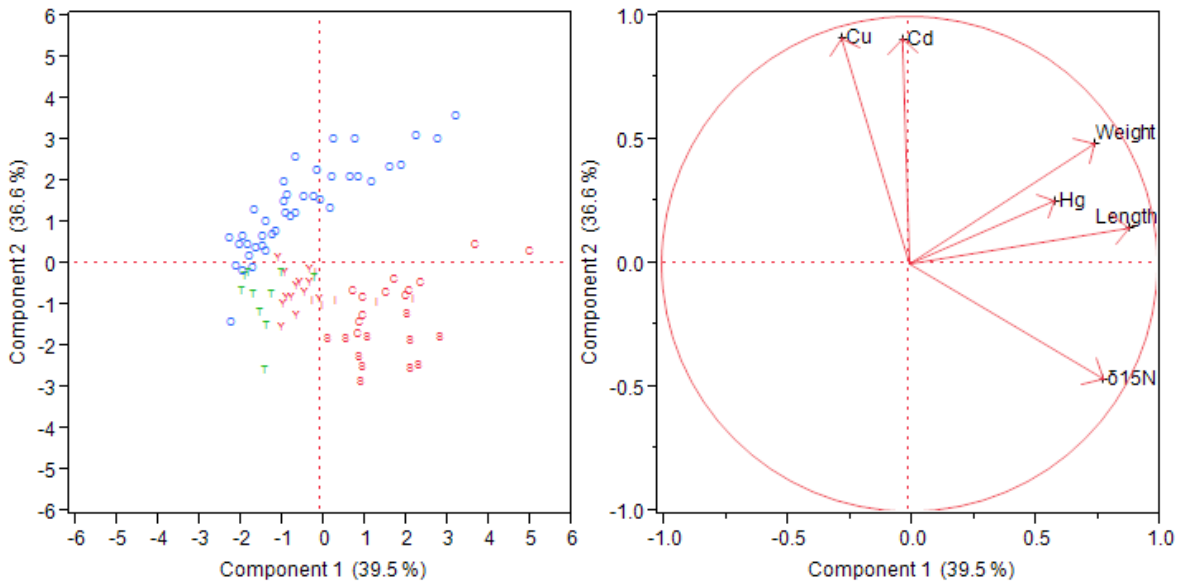


Fig. 4.

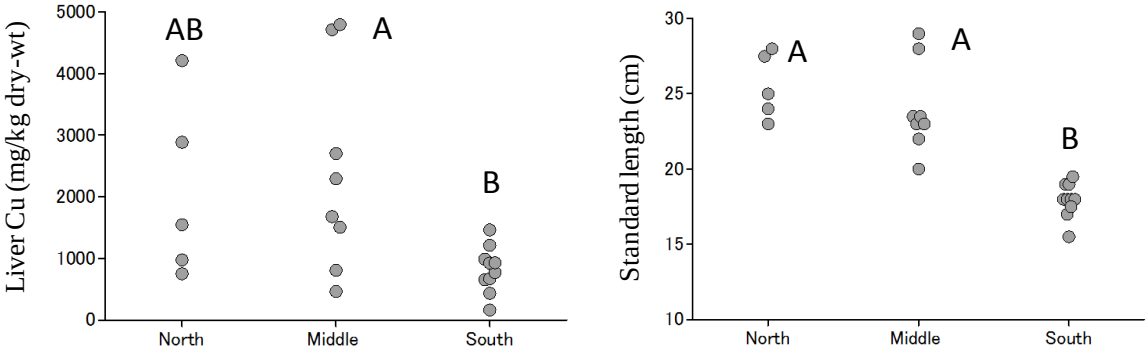

Liver Cu (mg/kg dry-wt)
5000
4000
3000
2000
1000
0
AB
A
B
North
Middle
South
Standard length (cm)
30
25
20
15
10
A
A
B
North
Middle
South

Fig. 5.

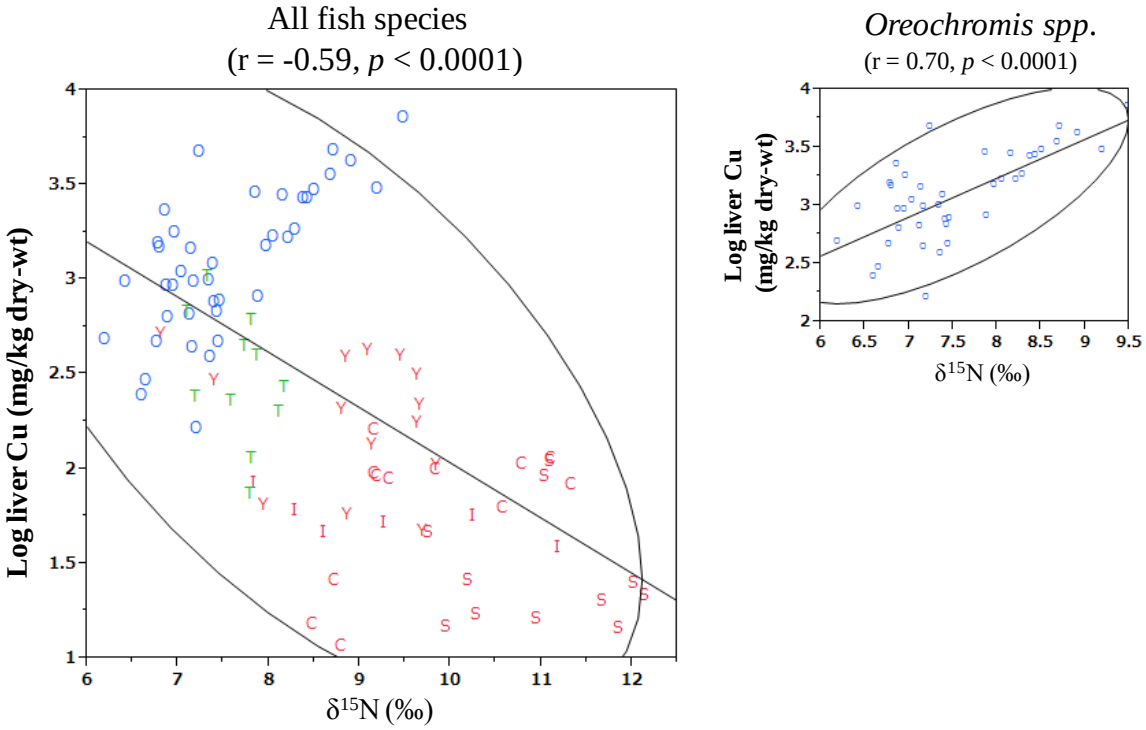
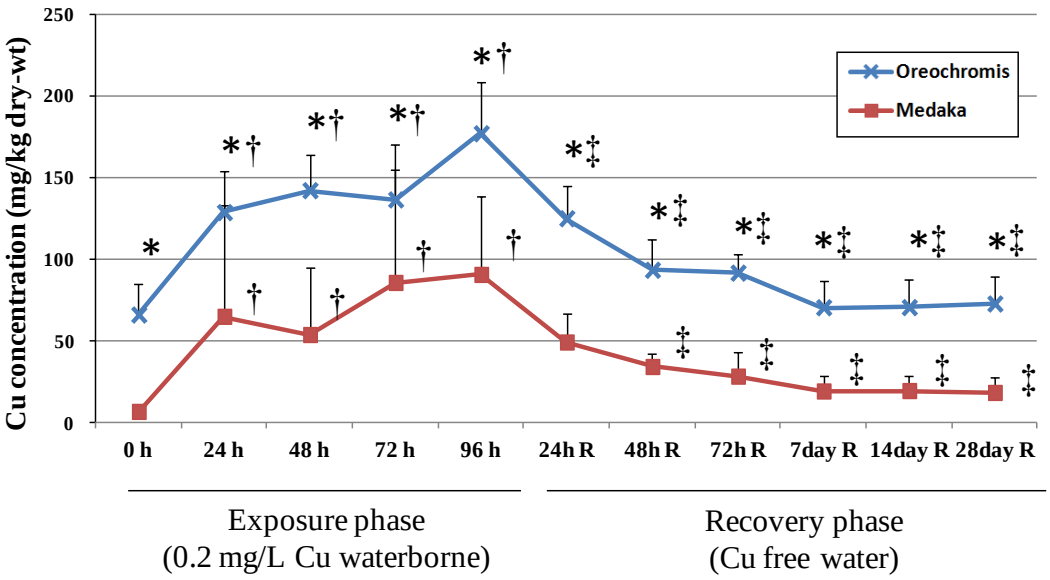

All fish species
(r = -0.59, p < 0.0001)
Log liver Cu (mg/kg dry-wt)
δ15N (‰)
Oreochromis spp.
(r = 0.70, p < 0.0001)
Log liver Cu (mg/kg dry-wt)
δ15N (‰)

Fig. 6.


Cu concentration (mg/kg dry-wt)
250
200
150
100
50
0
Oreochromis
Medaka
0 h
24 h
48 h
72 h
96 h
24h R
48h R
72h R
7day R
14day R
28day R
Exposure phase
(0.2 mg/L Cu waterborne)
Recovery phase
(Cu free water)