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Bioaccumulation of Trace Elements in Liver and Kidney of Fish Species from three Freshwater Lakes in the Ethiopian Rift Valley

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Abstract

The objective of the present work was to obtain scientific information on the ecological health of three freshwater lakes (Awassa, Koka and Ziway) situated in the Ethiopian Rift Valley by investigating possible trace element contamination accumulated in fish. Accordingly, fish liver and kidney samples were collected from three commercially important fish species (*Barbus intermedius*, *Clarias gariepinus*, and *Oreochromis niloticus*) in the lakes to determine the concentrations of chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), cadmium (Cd), and lead (Pb), using ICP-MS. Trace element concentrations were generally higher in *O. niloticus* compared with concentrations in *B. intermedius* and *C. gariepinus*. Compared to background values of most freshwater fish species, higher liver concentrations of Cu in *C. gariepinus* and *O. niloticus*, Mn in *O. niloticus*, Co in all except *B. intermedius*, and Zn in *C. gariepinus* from Lakes Ziway and Awassa were found. Cr, Co, Ni, Cd, and Pb were enriched in kidney, while Mn, Cu, Zn, As, and Se seems retained in the liver tissues. Assessment of transfer factors indicated that bioaccumulation from water and diet occurred, while uptake from sediments was low. Furthermore, the transfer factor values were generally higher for essential elements compared to the non-essential elements. Multivariate statistical analyses showed that the differences between the trace element levels were generally not significant among the lakes ($p = 0.672$), while significant differences were found between the fish species ($p = 0.042$), and between accumulation in kidney and liver ($p = 0.002$).

Key words: Nile Tilapia, African catfish, African big barb, bioaccumulation factor, trace elements, multivariate statistical analysis

Introduction

The three lakes that are considered in this study (Awassa, Koka, and Ziway) belong to the chain of lakes on the floor of the Ethiopian Rift Valley which provide water for domestic use and irrigation, and fish as a good protein source for the neighboring population, and means of income for local fishers. As these lakes are found in close proximity to both agricultural lands and fast growing cities mainly characterized by high population density and very poor waste management practices, they are often direct recipients of chemical pollutants from agricultural fields, urban runoff, domestic and small-scale industrial discharges. Inputs from natural sources such as chemical weathering of exposed rocks and soils caused by human activities such as deforestation and cattle grazing cannot be discounted (Zinabu and Elias 1989; Zinabu 1998). Therefore, it is important that the levels of chemical pollutants in these water bodies are monitored for the sake of the environment as well as the safety of the local population.

Trace elements such as aluminum (Al), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), cadmium (Cd), mercury (Hg), and lead (Pb), are present in all compartments in aquatic systems (water, sediment, and biota) **(Avigliano et al. 2019; Kumari 2018; Rajeshkumar et al. 2018)**. Transfer of trace elements to fish occurs either from water (uptake *via* gills or other respiratory surfaces), or *via* food (uptake *via* the digestive tract) **(Bjerregaard and Andersen 2007)**. Although skin has been considered as one of the possible pathways for metal uptake in fish, it is assumed that fish body surface does not play a significant role in metal uptake from the surrounding water **(Dallinger et al. 1987)**. Some of the trace elements are essential for biological systems (e.g. Cu and Zn), but should not exceed their optimum concentrations, while other elements (e.g. Hg, Cd and Pb) have no established biological function and may cause harmful effects to the biota when present even in very small concentrations **(Walker et al. 2006)**. Furthermore, trace metals can be transferred to humans via the food chain and thereby have the potential to cause health risk to humans **(Arumugam et al. 2020)**.

Trace element mobility, bioavailability, biological uptake and toxicity are influenced by the external environmental conditions (e.g. pH, redox conditions, water hardness, suspended solids, organic matter, etc.) as well as biological characteristics (e.g. biological species, sex, age, reproductive stage and feeding behavior) of organisms **(Chapman et al. 1996; Spry and Wiener 1991)**. Fish is often considered as good bio-indicator **(Agah et al. 2009; Gadzala-Kopciuch et al. 2004)** for the levels of trace elements and other contaminants in the environment. However, it is essential that focus is given to trace element levels in fish tissues known to be major sites of accumulation **(Ney and Van Hassel 1983)**. Most trace metals accumulate in the liver, kidney and gills **(Yang and Chen 1996)**, representing potential hazard for the fish species, while uptake in muscle tissues may represent hazards for man due to dietary intake in man **(Bradley and Morris 1986)**.

There have been a few studies of the lakes considered in this study focusing on trace metal concentrations in fish. In the first study **(Ataro et al. 2003)**, concentrations of 5 trace elements in muscle tissue from African catfish (*Clarias gariepinus*) (Burchell, 1822) and Nile tilapia (*Oreochromis niloticus*) (Linnaeus, 1758) collected in Lakes Awassa and Ziway were reported. Then, trace elements in muscle, bone, gill and liver of *O. niloticus* from the Lakes Awassa and Ziway were reported **(Kebede and Wondimu 2004)**. In addition, the Hg concentrations in muscle tissue have been investigated in fish species from Lake Awassa **(Desta et al. 2006, 2007, 2008)** and Lake Ziway **(Tadiso et al. 2011)**. In another study **(Dsikowitzky et al. 2013)**, the authors focused only on the determination of the concentrations of six trace elements (Cr , As, Se, Cd, Hg, and Pb,) in fish muscle, liver and gills; while the concentrations of trace elements in fish kidney were not analyzed. According to a recent study on Lake Awassa and neighboring Boicha stream **(Samuel et al. 2020)**, Cr, Cu, As and Hg concentrations determined in the muscle of *C. gariepinus* and *O. niloticus* have the potential to increase the health risk provided the two fish species are consumed regularly. The latest review on Lake Ziway **(Merga et al. 2020)** has concluded that "nutrients and trace metals, including $PO_4{}^{3-}$, $NO_3{}^{-}$, $NH_4{}^{+}$, Ca^{2+}, Ni and Cu in the lake have shown increasing temporal trends in concentration". A recent study on Lake Koka **(Tessema et al. 2020)** reported that Cr concentration in Nile tilapia, catfish, and common carp exceeded the WHO's permissible limits for human consumption. Thus, information on trace metals in fish muscles from the 3 selected lakes is available, while the retention of trace elements in vital organs such as liver and kidney seems missing.

Therefore, the main objectives of the present study were to: 1) Compare the levels of Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd, and Pb accumulated in kidney and liver collected from the African big barb *Barbus/Labeobarbus intermedius* (Rüppell, 1836), *C. gariepinus,* and *O. niloticus* in Lakes Awassa, Koka and Ziway; 2) Investigate the relationships between the trace element levels and fish length (as index of age/exposure time); and to 3) Investigate the relationship between the trace element levels in kidney and liver from each fish species. Thus, the present study is more comprehensive than previously performed, both with respect to the number of selected trace elements, number of fish species and number of organs investigated.

Materials and methods

Study area

The study area encompasses Lakes Awassa, Koka, and Ziway which are located in the Ethiopian Rift Valley Lakes Region (ERVLR) (Fig. 1). Information about the climate, main inflows, general water quality characteristics, and major activities around these lakes has previously been provided (Masresha et al. 2011). Therefore, only some basic characteristics of the lakes and the fish species in the study lakes are included in the present article.

Lake Awassa is a closed lake located at an elevation of 1680 m above sea level and has a maximum depth of 22 m, while the surface and catchment areas are 90 and 1250 km^2, respectively **(Kebede-Westhead et al. 1994)**. Lake Koka, also cited in some literature as the Koka Reservoir **(Mesfin et al. 1988; Zinabu and Pearce 2003)** is an artificial lake built over the River Awash primarily to generate electricity. The lake is located at an altitude of 1660 m above sea level (a. s. l) and has a surface area of 200 km^2 **(Kebede-Westhead et al. 1994)**. Lake Ziway, the largest fresh water resource in the Central Rift Valley, is located at an elevation of 1636 m (a. s. l). The maximum depth, surface and catchment areas of Lake Ziway are, respectively, 7 m, 442 and 7025 km^2 **(Kebede-Westhead et al. 1994)**.

The study area's geological characteristics have resulted from of volcano-tectonic activities and the subsequent sedimentation processes. Therefore, "most of the rift valley flat plains around lakes are covered with thick lacustrine deposits and volcano-clastic Quaternary sediments" **(Ayenew and Legesse 2007)**. The known fish species in Lake Awassa are *O. niloticus*, *C. gariepinus*, *B. intermedius*, *B. paludinosus*, *G. quadrimaculata*, and *A. antinorii* **(Dadebo 2000)**. The fish species dominating the fisheries in the Lake Koka are *O. niloticus*, *C. gariepinus*, *B. intermedius*, and *Cyprinus carpio* **(Vijverberg et al. 2012)**. The commercial fish species in Lake Ziway are *O. niloticus*, *C. gariepinus*, *Carassius carassius*, *Tillapia Zilli* and *B. intermedius* and three other species (*Barbus paludinosus*, *Barbus ethiopicus* and *Garra herticeps*) **(Tugie and Taye 2004)**. The diets of *B. intermedius*, *C. gariepinus* and *O. niloticus* in Lakes Awassa, Koka and Ziway are presented in Table 1.

Fish sampling and handling

Between July and September 2009, a total of fifty-nine (59) *O. niloticus*, sixty (60) *C. gariepinus*, and fifty-one (51) *B. intermedius* fresh fish, caught in Lakes Awassa, Koka and Ziway, were directly purchased from local fishermen at their respective landing sites. The fish specimens were transported on ice to a laboratory at Hawassa University in Ethiopia; afterwards, the fish dissection was carried out following the EMERGE protocol for live fish sampling (Rosseland et al. 2001). Kidney and liver obtained after dissection were kept in a deep freezer at -20 ^{0}C. Frozen samples were finally transported by airplane to the Norwegian University of Life Sciences (NMBU), Norway and stored at -20 ^{0}C until analysis.

Sample preparation and chemical analyses

Kidney and liver samples were freeze-dried and weighed in Teflon tubes and then digested using an UltraClave (Milestone Microwave Laboratory Systems, USA). To samples with masses between 0.1 and 0.3 g, 3.5 mL of ultrapure HNO_3 (69.0-70.0 %, Sigma-Aldrich) and 250 µL internal standard solution (4 mg/L of rhodium (Rh), tellurium (Te), indium (In) and thallium (Th)) were added. Also, to samples with masses below 0.1 g, 1.05 mL of ultrapure HNO_3 and 75 µL of the same internal standard solution were added. The digested samples were finally diluted with deionized water (Barnstead, >18 MΩ.cm^{-1}) to 50 and 15 mL so that the concentrations of HNO_3 and the internal standard (In) in the final solution would be 7 % and 20 µg/L, respectively. Reagent blanks and two types of certified reference materials (DOLT-4 (dogfish liver, National Research Council of Canada) and DORM-3 (dogfish muscle, National Research Council of Canada)) were used for determining the accuracy of the measurements. Replicate samples (n = 5) for both kidney and liver subsamples were taken from one fish to estimate the precision of the analytical method used. All measurements were carried out using Inductively Coupled Plasma-Mass spectrometer (ICP-MS, Perkin Elmer, ELAN 6000) calibrated using external calibration method. The concentrations of trace elements in kidney and liver tissues were calculated on a dry weight basis and reported as µg/g dry weight.

The uncertainty of the measurements for the different elements was determined for both kidney and liver samples by taking five sub-samples (n = 5) for all elements except Ni (n = 4) in both kidney and liver samples, as one value proved to be an outlier using Dixon's Q-test.

Bioaccumulation factors (BAF and BSAF) were used as indices of trace element uptake and retention in fish from the aquatic environment. According to the formula: $\mathbf{BAF} = {}^{\mathbf{C\ org}}/_{\mathbf{C\ water}}$ (unitless), where C_{org} is the trace element concentration in the specific organ of fish, and C_{water} is the trace element concentration in the water from which the fish is sampled. The availability and uptake of trace elements from lake sediment to fish was calculated as follows **(Nakayama et al. 2010; Rashed 2001)**: $\mathbf{BSAF} = {}^{\mathbf{C\ org}}/_{\mathbf{C\ sediment}}$ (unitless), where C_{org} is the trace element concentration in the specific organ of fish, and $C_{sediment}$ is the trace element concentration in the lake sediment from which the fish is sampled. Total concentrations of the trace elements in lake waters were taken from (Masresha et al. 2011), while element concentrations in sediments (average top 2-cm) were taken from Masresha **(2012)**.

Multivariate statistical analyses

The software CANOCO 4.5 and CanoDraw 4.14 were applied for the multivariate statistical analyses. Principal Component Analysis (PCA) and Redundancy Analysis (RDA) are both linear ordination methods, but the former is an unconstrained method while the latter is a constrained method. Hence, PCA extracts the maximum variation in the data, while RDA extracts the maximum variation in the data explained by a set of explanatory variables, i.e. the ordination axes are weighted sums of the explanatory variables.

Principal Component Analysis (PCA) and partial Redundancy Analysis (pRDA = RDA including co-variables in the analysis) were conducted in order to perform a hierarchical analysis of the fish data variation in terms of metal concentrations in kidney and liver samples obtained from three different species from each of the three lakes: Lakes Awassa, Koka and Ziway. In other words, this would enable us to know how much of the variation in the metal concentrations could be assigned to the spatial levels: lake, fish species and organ. A set of explanatory variables (factors) were made according to these hierarchical spatial levels by using dummy variables (1 and 0): Lake (n = 3), Fish species (n = 3 x 3 = 9) and Organ (n = 3 x 3 x 2 = 18).

To run the hierarchical analysis properly, we decided to randomly remove some of the samples in order to achieve a perfectly balanced design. Hence, 138 samples out of a total of 336 samples were removed. In order to see if this removal gave any significant changes in explained variation, a PCA was conducted on the total data set and on the balanced data set. The overall results from these two analyses were similar in respect to the percent explained variation, i.e. the first four axes in both analyses explained 86 % of the variation. Hence, the removal of samples had little or no effect on the overall results in terms of explained variation.

The dataset contained 198 samples obtained from kidney and liver samples from three different fish species from each of the three lakes (i.e. 198 = 3 lakes x 3 fish species x 2 organs x 11 replicates). The samples were analyzed for nine metals giving a data matrix with a total of 1782 single measurements. The concentrations of Ni, As and Pb were below the Limit of Detection (LOD) in some of the samples, 15 (7.6 %), 3 (1.5 %) and 74 (37.4 %) samples, respectively. According to Antweiler and Taylor **(2008)**, substituting left-censored data below LOD with LOD*0.5

is an adequate method if the amount of censored data is not too high. In our study, we decided to use a threshold value of 15 % for a specific variable (i.e. element). Hence, Ni and As concentrations below LOD were substituted with LOD*0.5. Lead did not fulfill this criterion and was only included in the analyses as a passive variable, i.e. not included in the statistical calculations, but displayed in the ordination plot for interpretation purposes. Prior to the analyses, the data was log(x+1) transformed to reduce the effects of extreme values, and in addition, centered and standardized (i.e. bringing their means to zero and their variance to one).

In order to perform the variance decomposition, we partitioned the variance into four sources according to the set-up provided in Table 2.The set-up is based on the procedure given by **(2003)**. To assess the effects of lake, fish species and organs upon the variation in trace element concentrations, the individual samples cannot be permuted at random. Hence, the groups representing the individual cases of the spatial levels immediately below the tested level were held together. This was obtained by using a split-plot design. The analyses were statistically tested using the Monte Carlo permutation test with $p < 0.05$ as criterion of significance (499 permutations and significance of canonical axes together were chosen).

Also, taking the complete data set, PCA and RDA were run on the liver data subset (n = 170) and kidney data subset (n = 166) separately in order to investigate the relationship between trace element concentrations between kidney/liver concentrations and fish size (length and weight). In this case also, the trace element data was log (x+1) transformed and centered and standardized. Pb was included as a passive variable due to many samples having values below LOD. Similarly, length and weight data were log (x+1) transformed. The statistical significance was tested by using Monte-Carlo permutation tests. No restrictions were made on the data, i.e. the samples were freely permuted (499 permutations).

The results are displayed in the ordination diagrams. The various trace elements are displayed as arrows in the way that each arrow points in the direction of steepest increase in concentrations for the corresponding trace element. The explanatory variables, represented by the various categorical variables (i.e. individual groups of samples) are displayed as centroids. The distance between the centroids approximates the average dissimilarity between the groups being compared, measured by their Euclidean distance.

Results and discussion

Data quality

The limits of detection (LOD, in µg/g,) determined as 3 times the standard deviation of more than ten blanks were 0.3 (Cr), 0.2 (Mn), 0.002 (Co), 0.04 (Ni), 0.1 (Cu), 0.4 (Zn), 0.07 (As), 0.1 (Se), 0.01 (Cd) and 0.05 (Pb). Analytical precision of the method was within 10 % for both kidney and liver samples, and for all trace elements except Cr. Relative standard deviation (RSD) for Cr was 25 % in liver and 14 % in kidney, possibly due to low concentrations as Cr was < LOD in most of the samples.

Comparison of measured values with certified values for DOLT-4 and DORM-3 showed that our results were fairly accurate (Table 3). For DOLT-4, measured values were generally within 10 % of certified (given value for Cr and Co) values, except for Ni (19 %). No certified value was given for Cr, Mn and Co. For DORM-3, measured values were also within 10 % of average certified values for the trace elements Cu, Zn, As, Cd and Pb. No certified value was given for Co, Mn and Se.

Fish length and weight data

For each species of fish, the mean values of total length (L_T) and total weight (W_T) including the ranges are shown in Table 4. Based on mean length values, the smallest fish in each lake was found to be *O. niloticus* (Table 4). Length and weight of fish were positively correlated (Fig. 3 and 4).

Differences in trace element levels among lakes, fish species and organs

The geometric mean concentrations of the trace elements (µg/g dry weight) and their 95 % confidence intervals are presented in Tables 5 and 6. The summary of results from multivariate statistical data treatment of the fish from Lakes Awassa, Koka and Ziway is presented in Table 7 and Fig. 2**.** Results of pRDA indicated that lake differences explained very little of the total data variability and were also not significant ($p > 0.05$). However, the trace element concentrations differed significantly between the different fish species and specific organs (Table 7). The different lakes, fish species and organs are categorical explanatory variables and presented as centroids in Fig. 2. The response variables (trace element concentrations) are shown as vectors, originating from the origin. The distance between the centroids approximates the average dissimilarity between the groups being compared, measured by their Euclidean distance. Based on these techniques, it is clearly demonstrated that sample centroids for the same fish species and same organ (kidney/liver) collected in each lake appeared closer, indicating that significant differences exist between different species and their specific organs.

Trends in trace element concentrations within each fish species

The geometric mean trace element concentrations in kidney and liver for all samples, respectively, were in the ranges: < LOD–0.72 and < LOD–0.32 for Cr, 2.99–11.3 and 5.5–39.4 for Mn, 0.67–9.65 and 0.1–10.7 for Co, 0.06–4.2 and 0.03–0.93 for Ni, 5.5–20.0 and 37.0–1090 for Cu, 82–108 and 77–213 for Zn, 0.09–1.04 and 0.12–0.96 for As, 3.9–8.4 and 5–29 for Se, 0.32–3.95 and 0.09–0.89 for Cd, and 0.03–0.80 and 0.03–0.63 for Pb (Tables 5 and 6). Based on average values calculated from the geometric mean values obtained for each of the fish species, trace element concentrations in kidney were found to be in the order: Zn > Cu > Se > Mn > Cd > Co > Ni > Pb > Cr > As for *B. intermedius*, Zn > Cu > Se > Mn > Co > Cd > As > Cr > Ni > Pb for *C. gariepinus* and Zn > Cu > Mn > Se > Co > Ni > Cd > As > Cr > Pb for *O. niloticus.* Similarly, the concentrations of the trace elements in liver tissue were found to be in the order: Zn > Cu >Mn > Se > Cd > Pb >As > Cr > Co> Ni for *B. intermedius*, Cu > Zn > Se > Mn > Co > Cd > As > Cr > Pb > Ni for *C. gariepinus,* Cu > Zn > Mn > Se > Co > Cd Ni > As > Cr > Pb >for *O. niloticus.*

Comparison of trace element concentrations among species

As shown in Tables 5 and 6, the highest concentrations of many of the investigated trace elements were in kidney as well as in liver from *O. niloticus*. This is confirmed by the ordination plots presented in Fig. 3 and 4. However, Zn did not follow this pattern. Overall, the trace element concentrations in both kidney (p = 0.002) and liver (p = 0.002) tissues were significantly different when the different fish species were compared (Fig 3 and 4). Denny et al. (1995) also observed higher concentrations of Co and Cu in tilapia species compared to a carnivorous Protopterous *sp.* and explained the difference by indicating that Tilapia fish feed on metal enriched phytoplankton. In another study which was conducted in Lake Phewa in Nepal, it was found that Cu and Zn were among the highest trace metals in fish liver which the authors have associated with uptake from fish diet (Rosseland et al. 2017). The authors also indicated that *O. niloticus* tended to have higher concentrations of trace metals in fish liver as compared to other fish species of the lake. The main food components for *O. niloticus* could be detritus **(Bowen 1980)**, benthic and attached organisms such as blue greens and benthic diatoms early from the juvenile stage **(Tudorancea et al. 1988)**, and algae **(Getachew and Fernando 1989)** with surfaces of which trace element sorption may occur **(Mohapatra and Gupta 2005)**. On the other hand, Zn concentrations were the highest in the liver tissue from *C. gariepinus* which also could be attributed to enriched feed **(Dallinger et al. 1987; Moriarty et al. 1984)**.

The results obtained for Ni and Co in the present study were in agreement with the findings by Uzairu et al. **(2009)** and Lwanga et al. **(2003)**, respectively. However, in contrast to our finding, Uzairu et al. **(2009)** reported lower Zn levels in *C. gariepinus* compared to *O. niloticus*. Contrasting results were also reported for Cu. Cu in liver tissue from *O. niloticus* was higher than found in *C. gariepinus* from River Kubanni in Northern Nigeria. Similarly, Cu in liver of *O. niloticus* was significantly higher than found in the carnivorous *S. thumbergi* in Lake Itezhi-tezhi in Zambia **(Nakayama et al. 2010)**. However, Lwanga et al. **(2003)** found that concentration of Cu in liver was higher in *C. gariepinus* than *O. niloticus* in Lake George, Uganda. This indicates that some variations may exist depending on the specific context and conditions of the lakes that were studied.

Compared with results from a previous study (Kebede and Wondimu 2004), however, higher levels were found only for Cu in fish liver from Lake Ziway (1090 µg/g dry weight) and Mn from Lakes Ziway and Awassa. Again, comparing our results with background levels (µg/g dry weight) for most freshwater fish species, i.e., 0.01–1.0 for Cr, 0.5–15 for Mn, 0.02–0.25 for Co, 0.1–50 for Cu and 2–100 for Zn **(Denny et al. 1995)**, we found that geometric mean values of Cu in liver in both *C. gariepinus* and *O. niloticus*, Mn in *O. niloticus*, and Co in all except *B. intermedius* liver were much higher than the guideline value in all lakes. The Cr values were, however, in the range of background values. On the other hand, comparison of Cu and Mn levels in *O. niloticus* with observations in other water systems **(Allinson et al. 2009)** showed that the present values were much lower, and yet Allinson et al (2009) concluded that *O. niloticus* was uncontaminated by metals. Actually, it is known that excess concentration of Cu in fish could adversely affect growth, survival and reproduction **(Buckley et al. 1982; McKim and Benoit 1971)**. It is also possible that high levels of Cu in fish liver could adversely affect other animals in the food chain **(Nakayama et al. 2010)**. According to Geta **(2010)**, it was documented that the bird Abyssinia ground hornbill (*Bucorvus abyssinicus*), a common predator of *O. niloticus*, contained about 8 fold higher concentrations of Cu in its muscle compared to the concentrations in the prey *O. niloticus* collected from Lake Ziway. Given that there is no known Cu point source (e.g. mining tails) in the Lake Ziway area, and that the potential bioavailability is low (Masresha et al. 2011), particulate Cu in runoff from developed agricultural lands such as horticultural fields and flower farms or the use of Cu-containing fungicides could represent potential sources in the region.

Trace elements accumulated in kidney and liver

Based on the ratio of geometric mean values of concentrations in kidney to liver (Table 8), trace elements which were more enriched in kidney were Cd, Co, and Ni in *B. intermedius* and C. *gariepinus*, as well as Cr, Ni, Cd, and Pb in *O. niloticus*. Based on the same criterion, trace elements which were found in relatively higher concentrations in the liver were Mn, Cu, As, Se and Zn in *B. intermedius* and *C. gariepinus*, Mn, Co, Cu, and Se in *O. niloticus* (Table 8). The results from multivariate statistical analyses also support this inference.. Other studies have also shown that Cr **(Palaniappan and Karthikeyan 2009)**, Co **(Mukherjee and Kaviraj 2009)**, Ni **(Pane et al. 2005)** and Cd **(Chowdhury et al. 2005)** preferentially accumulated more in kidney than in liver tissue; while Mn **(Crafford and Avenant-Oldewage 2011)**, Cu **(Cousins 1985)**, Zn **(Hogstrand and Haux 1996)** and Se **(Sato et al. 1980)** accumulated more in liver tissues. In another study **(Hazrat et al. 2020)**, the relative bioaccumulation magnitude of trace metals in fish liver and kidney also varied between the fish species studied. These results suggest that specific organs should be selected to obtain useful information about changes in the levels of trace elements in aquatic organisms, and thus the aquatic environment.

Relationship between trace element concentrations and fish size

The relationship between concentrations of trace elements in kidney and fish size (length and weight) is presented in Fig. 3. The RDA revealed that 23% of the variation could be attributed to the size (p = 0.0005). Accordingly, Se and Cd were positively correlated with fish length and weight, while Mn, Ni, As, and Pb were slightly negatively correlated with fish length and weight. Co, Cu, and Zn concentrations appeared uncorrelated to both length and weight. Likewise, the relationship between concentrations of trace elements in liver and fish size (length and weight) is presented in Fig. 4. The RDA revealed that 36% of the variation could be attributed to size (p = 0.0005). Accordingly, the Zn, Se and Cd concentrations correlated positively with fish length and weight; but Co and Cu seemed uncorrelated to fish length and weight. As observed for the kidney samples, Mn, Ni, As, and Pb tended to show a slightly negative correlation with fish length and weight.

The above results indicate that only few elements were positively correlated with fish size. The concentrations of the other trace elements were either slightly negatively correlated or showed no correlation at all. According to a review by Chapman et al. **(1996)**, the concentrations of trace metals, especially essential metals, do not increase with fish age as freshwater fish are known to maintain constant levels of trace metal concentrations. But, in the present study, Zn and Se tended to be positively correlated with fish size. Other studies have documented that an inverse relationship could occur between trace metal concentrations and fish size/age **(Allen-Gil and Martynov 1995; Ney and Van Hassel 1983)**. Different explanations have been given for such a behavior: 1) kidney and liver tissues grew at a faster rate than the rate of accumulation, leading to bio-dilution (e.g. “growth dilution” effect **(Desta et al. 2007; Ikemoto et al. 2008)**); 2) trace elements were excreted at a faster rate than the rate of accumulation which is element and species-specific behavioral differences during early development (e.g. habitat selection), 3) different fish species could be differentially exposed to different trace elements in a given environment, and 4) trace element like bone-seeking Pb would tend to accumulate more predominantly in the bone than in the kidney or other organs of the fish as the fish grows **(Mager et al. 2010)**.

Bioaccumulation and biota-sediment accumulation factor (BAF and BSAF)

In this study, BAF has been used instead of bioconcentration factor (BCF) since trace elements determined in the fish are accumulated from water and from natural diet from the lakes. The values obtained for BAF and BSAF are shown in Tables 9 and 10. BAF values for each trace element were mostly much higher than BSAF values. Fish species with the highest concentrations of the respective trace elements also had the highest BAF and BSAF values. The average BAF values for the combined kidney and liver values of each lake were in the order: Cd > Se > Cu > Zn > Co > As > Pb > Ni > Mn > Cr for Lake Koka, Cu > Cd > Zn > Se > Co > Ni > As > Cr > Mn > Pb for Lake Ziway, and Zn > Cu > Cd > Co > Se > Ni > Pb > Cr > Mn > As for Lake Awassa. Likewise, the BSAF values were in the order: Se > Cd > Cu > Zn > Co > As > Pb > Ni > Mn > Cr for Lake Koka, Se > Cu > Cd > Zn > Co > As > Ni > Mn > Cr > Pb for Lake Ziway, and Cu > Se > Cd > Zn > Co > As > Ni > Cr > Mn > Pb for Lake Awassa. Except for Cd, the results indicated that the retention of essential elements was relatively higher than of the non-essential elements.

Assessment of transfer factor (BAF and BSAF) values for trace elements showed that BAF values were all higher than unity, indicating that bioaccumulation from water and fish diet occurred. However, the typically very low BSAF values indicated that bioavailability and uptake of the trace elements in fish from sediments was quite low in these water systems. It was also observed that there was a large variation in both BAF and BSAF values for the same element in different lakes. It has also been documented that bioconcentration factor for trace elements can vary according to external concentrations **(Chapman et al. 1996; DeForest et al. 2007; McGeer et al. 2003)**. In the present study, generally higher BAF and BSAF values were obtained for essential elements compared to the non-essential elements, probably due to the fact that essential trace elements can be actively taken up by organisms as they are needed for life-supporting biological processes, being subject to internal regulation and kept within certain limits. Similarly, the concentrations of essential elements in fish liver **(Anandkumar et al. 2018; Mehmood et al. 2019)** and fish muscle **(Anandkumar et al. 2017; Gbogbo et al. 2018)** were found to be higher than the more toxic trace metals, indicating a corresponding higher BAF values for essential trace metals. Therefore, high BAF values obtained for these elements did not necessarily reflect toxicity, as toxicity could occur when the regulatory capacity of the organism is exceeded **(DeForest et al. 2007)**. BAFs for metals in most fish species were typically in the range 1000–500,000, exceeding laboratory derived BCF values (100–1000); rather, high BAF values frequently reflect natural conditions where external concentrations are low, including in diets and in water. When concentrations in water are low, the food chain transfer of metals may be the primary route of exposure (Clements, 1991). This argument applies well to the water systems studied since our previous study has shown that potential mobility and bioavailability of the studied metals/metalloids were rather low (Masresha et al. 2011). Still, BAF values greater than unity do, indeed, reflect that bioaccumulation of the trace elements has occurred due to uptake from water and/or from natural fish diet **(Rashed 2001)**. Among the non-essential trace elements, uptake of Cd is competing with essential trace elements both from water and sediments, showing that this metal has a relatively high bioavailability (Table 9).

Conclusions

In this study, selected trace elements have been determined in kidney and liver from three different common fish species in Lakes Awassa, Koka, and Ziway. The trace element levels determined in the three lakes was comparable, while the trace element concentrations were significantly different among fish species and also organ/tissues. Significant differences in levels of trace elements among fish species could be attributed to differences in feeding habits and habitat choices. Comparison of the results in the present study with previous studies has shown that no significant increase in trace element concentrations in fish tissues has occurred, indicating that the values obtained are probably local background values. However, the generally high concentrations of Cu in *O. niloticus* and *C. gariepinus* species, and the relatively higher than previous reported levels of Cu in liver tissue of *O. niloticus* from Lake Ziway indicated a possible anthropogenic influence, for instance, from agricultural activities. Similarly, higher levels of Mn reported in this study indicated that anthropogenic influences such as use of fertilizers could be the cause. As trace element tolerance limits for kidney and liver in the investigated fish species in these water systems have not been established, it is difficult to assess potential biological impact. Therefore, this work along with previously reported data could be used as baseline information for future studies aiming at monitoring future trends of trace elements in these Rift Valley lakes. In addition, it is recommended that environmental impact assessment is performed under prevailing conditions for at least Cu and Zn , and that trophic transfer of these metals from fish to fish-eating birds (e.g. white pelicans) residing on the shore of these lakes is addressed.

I. Tables (10)

Table 1. Diet composition of the different fish species from lakes Awassa, Koka, and Ziway

Lakes	Species	Diet	References
Awassa	*B. intermedius*	molluscs, aquatic insects, fish prey, macrophytes, fish eggs, and detritus	**(Desta et al. 2006)**
	C. gariepinus	*O. niloticus*	**(Dadebo 2000)**
	O. niloticus	algae	**(Getachew and Fernando 1989)**
Koka	*B. intermedius*	aquatic insects, fish, detritus and macrophytes	**(Deribe et al. 2011)**
	C. gariepinus	aquatic insects, fish and fish eggs	**(Deribe et al. 2011)**
	O. niloticus	algae, zooplankton	**(Deribe et al. 2011)**
Ziway	*B. intermedius*	–	–
	C. gariepinus	*O. niloticus*, *B. paludinosus*, insects and crustaceans	**(Tugie and Taye 2004)**
	O. niloticus	Zooplankton and blue green algae	(Kebede and Wondimu 2004)

Table 2. The hierarchical design needed to partition the total variation in the fish data in terms of trace element concentrations in kidney and liver samples obtained from three different species from each of the three lakes, Lake Awassa, Lake Koka and Lake Ziway

Variance component	Explanatory variables	Co-variables	Permuting in blocks	Whole-plots represent
Lake	Lake	None	No	Species
Species	Species	Lake	Lake	Organ
Organ	Organ	Species	Species	None
Residual	None (PCA)	None	Not applicable	Not applicable
Total	None (PCA)	None	Not applicable	Not applicable

Table 3. Comparison of measured values (µg/g dry weight) with certified values of reference materials (DOLT-4 and DORM-3)

Trace element	DOLT-4 (n = 12)		DORM-3 (n = 4)	
	Measured values (Mean ± S.D)	Certified values (Mean ± S.D)	Measured values (Mean ± S.D)	Certified values (Mean ± S.D)
Cr	1.4 ± 0.2	1.4*	2.27 ± 0.49	1.89 ± 0.17
Mn	9.5 ± 0.5	N/A	2.9 ± 0.1	4.6*
Co	0.23 ± 0.01	0.25*	0.26 ± 0.01	N/A
Ni	1.2 ± 0.4	0.97 ± 0.11	1.5 ± 0.24	1.28 ± 0.24
Cu	31 ± 1	31 ± 1	15 ± 1	15.5 ± 0.63
Zn	124 ± 4	116 ± 6	52 ± 1	51.3 ± 3.1
As	9.4 ± 0.4	9.7 ± 0.6	6.9 ± 0.1	6.9 ± 0.3
Se	9 ± 1	8.3 ± 1.3	4 ± 1	3.3*
Cd	24.4 ± 0.7	24.3 ± 0.8	0.31 ± 0.01	0.29 ± 0.02
Pb	0.16 ± 0.08	0.16 ± 0.04	0.41 ± 0.02	0.39 ± 0.05

N/A = Not Available; * = given value but not certified

Table 4. Mean length (L_T, cm) and weight (W_T, g) of the fish species collected from Lakes Awassa, Koka, and Ziway and the corresponding ranges are given in parentheses

Lakes	Species	Sample size (n)	Total length (L_T) (cm)	Total weight (W_T) (g)
L. Awassa	*B. intermedius*	20	25.7 (14.6–34.3)	187 (33.2–393)
	C. gariepinus	20	48.0 (27.8–67.0)	1063 (154–2500)
	O. niloticus	20	22.8 (16.9–33.0)	233 (86.7–700)
L. Koka	*B. intermedius*	20	34.1 (30.5–36.8)	392 (292–398)
	C. gariepinus	20	53.0 (35.0–58.2)	874 (700–3100)
	O. niloticus	20	25.8 (20.2–32.0)	305 (137–501)
L. Ziway	*B. intermedius*	11	33.3 (28.1– 44.4)	399 (202–900)
	C. gariepinus	20	42.0 (23.5–72.0)	647 (107–3000)
	O. niloticus	19	22.1 (12.7–29.1)	248 (35.6–525)

Table 5. Geometric mean concentrations (µg/g dry weight) and 95 % confidence intervals for Cr, Mn, Co, Ni and Cu in kidney and liver from the different fish species of the lakes Awassa, Koka and Ziway

Lakes	Species	Tissues	Cr	Mn	Co	Ni	Cu
Awassa	*B. intermedius*	Kidney	< LOD	2.99	1.15	0.07	5.5
			NA	(2.48–3.60)	(0.85–1.55)	(0.06–0.10)	(4.6–6.5)
		Liver	< LOD	6.35	0.10	0.05	52
			NA	(4.34–9.30)	(0.08–0.11)	(0.03–0.07)	(36–75)
	C. gariepinus	Kidney	< LOD	5.31	0.96	0.06	10.6
			NA	(4.61–6.10)	(0.74–1.23)	(0.05–0.07)	(10.0–11.2)
		Liver	< LOD	8.62	0.22	0.03	149
			NA	(7.05–10.6)	(0.15–0.30)	(0.02–0.04)	(110–201)
	O. niloticus	Kidney	0.49^{a}	11.3	0.75	2.55	18.7
			(0.39–0.62)	(7.8–16.3)	(0.61–0.93)	(1.77–3.68)	(13.3–26.4)
		Liver	0.32	36.0	2.47	0.93	454
			(0.18–0.56)	(19.6–66.2)	(1.94–3.15)	(0.78–1.11)	(348–592)
Koka	*B. intermedius*	Kidney	< LOD	15.3–24.5	0.81	14.1–17.8	6.9
			NA	6.2	(0.69–0.95)	1.99	(6.4–7.4)
		Liver	< LOD	(5.6–6.9)	0.21	(1.55–2.56)	41
			NA	7.3	(0.18–0.25)	0.43^{G}	(33–51)
	C. gariepinus	Kidney	< LOD	(6.2–8.4)	4.38	(0.28–0.66)	13.3
			NA	8.4	(3.37–5.70)	0.37^{i}	(11.3–15.5)
		Liver	< LOD	(7.5–9.4)	1.30	(0.29–0.48)	94
			NA	7.0	(1.00–1.70)	0.07^{H}	(69–128)
	O. niloticus	Kidney	0.72	(5.7–8.4)	9.65	(0.04–0.11)	20.0
			(0.63–0.84)	8.5	(8.37–11.13)	3.41^{h}	(13.9–28.9)
		Liver	< LOD	(6.1–11.7)	10.71	(2.87–4.04)	797
			NA	28.5	(9.00–12.73)	0.77^{G}	(653–973)
Ziway	*B. intermedius*	Kidney	< LOD	(18.7–43.3)	0.67	(0.58–1.00)	6.4
			NA	3.5	(0.50–0.91)	0.21	(5.8–7.1)
		Liver	< LOD	(3.1–3.9)	0.10	(0.13–0.33)	37
			NA	5.5	(0.08–0.14)	0.07	(23–58)
	C. gariepinus	Kidney	< LOD	(4.0–7.5)	1.09	(0.05–0.11)	16.1
			NA	5.7	(0.74–1.61)	0.15	(13.4–19.3)
		Liver	< LOD	(5.1–6.3)	0.37	(0.12–0.19)	396
			NA	6.9	(0.28–0.48)	0.06	(283–554)
	O. niloticus	Kidney	0.48	(5.9–8.0)	5.3	(0.05–0.09)	16.5
			(0.34–0.68)	13.2	(4.35–6.49)	4.2	(13.3–20.5)
		Liver	< LOD	(9.0–19.4)	5.44	(3.4–5.2)	1090
			NA	39.4	(3.94–7.52)	0.50	(805–1475)
		Liver*	–	(27.1–57.4)	11.0–11.8	(0.37–0.68)	757–797
				8.8–21.5		17.6–21.3	

* Data obtained from (Kebede and Wondimu 2004). For individual trace element concentrations <LOD, 0.5*LOD values were substituted to calculate the geometric mean

Table 6. Geometric mean concentrations (µg/g dry weight) and 95 % confidence intervals for Zn, As, Se, Cd and Pb in kidney and liver from the different species of Lakes Awassa, Koka and Ziway

Lakes	Species	Tissues	Zn	As	Se	Cd	Pb
Awassa	*B. intermedius*	Kidney	99	0.19	3.9	0.32	0.03
			(83–118)	(0.15–0.24)	(3.2–4.8)	(0.24–0.41)	(0.02–0.03)
		Liver	143	0.27	5	0.09	0.03
			(118–173)	(0.23–0.32)	(4.1–5.4)	(0.07–0.10)	(0.02–0.03)
	C. gariepinus	Kidney	86	0.19	4.8	0.49	0.03
			(81–90)	(0.15–0.26)	(4.1–5.7)	(0.30–0.80)	(0.03–0.04)
		Liver	213	0.36	19	0.32	0.06
			(191–238)	(0.24–0.53)	(14–26)	(0.19–0.52)	(0.04–0.08)
	O. niloticus	Kidney	85	0.79	5.1	1.00	0.30
			(79–92)	(0.66–0.95)	(4.7–5.6)	(0.83–1.21)	(0.25–0.37)
		Liver	86	0.96	5	0.80	0.20
			(72–103)	(0.78–1.19)	(4–7)	(0.62–1.03)	(0.15–0.27)
		Liver *	97.0–114	0.09	–	3.95	2.20–3.03
Koka	*B. intermedius*	Kidney	108	(0.08–0.11)	4.8	(3.16–4.94)	0.80
			(102–114)	0.12	(4.5–5.2)	0.69	(0.53–1.22)
		Liver	130	(0.09–0.18)	5	(0.53–0.91)	0.63
			(117–144)	0.17	(4.9–5.6)	2.20	(0.37–1.09)
	C. gariepinus	Kidney	92	(0.15–0.20)	8.0	(1.77–2.75)	0.14
			(84–101)	0.17	(7.3–8.6)	0.75	(0.06–0.36)
		Liver	161	(5.01–6.92)	26	(0.60–0.94)	0.10
			(142–183)	0.42	(20–34)	1.72	(0.06–0.16)
	O. niloticus	Kidney	82	(0.38–0.47)	6.4	(1.34–2.21)	0.19
			(79–85)	0.30	(5.8–7.1)	0.89	(0.15–0.24)
		Liver	94	(0.26–0.35)	13	(0.69–1.14)	0.15
			(81–110)	0.14	(10–16)	1.08	(0.11–0.21)
Ziway	*B. intermedius*	Kidney	105	(0.11–0.18)	5.7	(0.80–1.48)	0.03
			(99–111)	0.19	(5.3–6.1)	0.31	(0.02–0.03)
		Liver	130	(0.13–0.27)	6	(0.21–0.46)	0.03
			(88–191)	0.23	(5–8)	0.93	(0.02–0.04)
	C. gariepinus	Kidney	92	(0.19–0.28)	7.1	(0.61–1.43)	0.03
			(89–95)	0.26	(6.4–7.8)	0.47	(0.02–0.03)
		Liver	202	(0.20–0.34)	29	(0.33–0.67)	0.04
			(178–230)	1.04	(24.9–34.4)	1.21	(0.03–0.07)
	O. niloticus	Kidney	89	(0.78–1.40)	8.4	(0.97–1.50)	0.23
			(81–98)	0.87	(7.4–9.5)	0.69	(0.19–0.28)
		Liver	77	(0.65–1.15)	16	(0.54–0.87)	0.11
			(66–91)	–	(12–20)	1.08–1.75	(0.09–0.15)
		Liver*	85.6–116		–		2.70–3.37

* Data obtained from (Kebede and Wondimu 2004)

Table 7. Statistical parameters of the output from multivariate data analyses

Component	Explained variability (%)	DF	Mean square value	p-value
Lake	8.7	2	4.35	0.672
Species	36.6	6	6.1	0.042
Organ	28.3	9	3.14	0.002
Residual	26.4	180	0.147	n.a.
Total (PCA)	100	197	0.508	n.a.

Table 8. Kidney to liver ratio of the different trace elements studied in each fish species from the lakes Awassa, Koka and Ziway

Species	Elements									
	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Pb
B. intermedius	1.0	0.7	6.4	12.6	0.1	0.8	0.7	0.9	4.9	1.2
C. gariepinus	1.0	0.9	3.4	2.3	0.06	0.5	0.7	0.3	2.4	1.0
O. niloticus	2.8	0.3	0.8	4.7	0.02	1.0	1.1	0.6	1.7	1.6

Table 9. BAF and BSAF values for Cr, Mn, Co, Ni, and Cu in the lakes Awassa, Koka and Ziway. Superscripts K and L in the “parameters” column stand for kidney and liver, respectively.

Lakes	Fish species	Parameters	Cr	Mn	Co	Ni	Cu
Awassa	*B. intermedius*	BAF$_K$	1000	249	16429	143	4231
		BAF$_L$	1000	529	1429	102	40000
	C. gariepinus	BAF$_K$	1000	443	13714	122	8154
		BAF$_L$	1000	718	3143	61	114615
	O. niloticus	BAF$_K$	1633	942	10714	5204	14385
		BAF$_L$	1067	3000	35286	1898	349231
	B. intermedius	BSAF$_K$	0.01	0.003	0.2	0.003	0.5
		BSAF$_L$	0.01	0.006	0.01	0.002	5.1
	C. gariepinus	BSAF$_K$	0.01	0.005	0.1	0.003	1.0
		BSAF$_L$	0.01	0.008	0.03	0.001	15
	O. niloticus	BSAF$_K$	0.02	0.01	0.1	0.1	1.9
		BSAF$_L$	0.01	0.03	0.4	0.04	45
Koka	*B. intermedius*	BAF$_K$	5.9	14.7	105	50.5	332
		BAF$_L$	5.9	17.3	27	10.9	1971
	C. gariepinus	BAF$_K$	5.9	19.9	569	9.4	639
		BAF$_L$	5.9	16.6	169	1.8	4519
	O. niloticus	BAF$_K$	14.1	20.1	1253	86.5	962
		BAF$_L$	5.9	67.5	1391	19.5	38317
	B. intermedius	BSAF$_K$	0.003	0.005	0.04	0.02	0.2
		BSAF$_L$	0.003	0.01	0.01	0.01	1.0
	C. gariepinus	BSAF$_K$	0.003	0.01	0.2	0.004	0.3
		BSAF$_L$	0.003	0.01	0.1	0.001	2.2
	O. niloticus	BSAF$_K$	0.01	0.01	0.5	0.04	0.5
		BSAF$_L$	0.003	0.02	0.6	0.01	19
Ziway	*B. intermedius*	BAF$_K$	115	26	957	68	1882
		BAF$_L$	115	41	143	23	10882
	C. gariepinus	BAF$_K$	115	43	1557	48	4735
		BAF$_L$	115	52	529	19	116471
	O. niloticus	BAF$_K$	185	99	7571	1355	4853
		BAF$_L$	115	296	7771	161	320588
	B. intermedius	BSAF$_K$	0.01	0.002	0.05	0.004	0.2
		BSAF$_L$	0.01	0.003	0.01	0.001	1.4
	C. gariepinus	BSAF$_K$	0.01	0.003	0.1	0.003	0.6
		BSAF$_L$	0.01	0.004	0.03	0.001	15
	O. niloticus	BSAF$_K$	0.01	0.008	0.4	0.09	0.6
		BSAF$_L$	0.01	0.02	0.4	0.01	41

Table 10. BAF and BSAF values for Zn, As, Se, Cd, and Pb in Lakes Awassa, Koka and Ziway. Superscripts K and L in the "parameters" column stand for kidney and liver, respectively.

Lakes	Fish Species	Parameters	Zn	As	Se	Cd	Pb
Awassa	*B. intermedius*	BAF$_K$	495,000	79	3900	22857	333
		BAF$_L$	715,000	113	5000	6429	333
	C. gariepinus	BAF$_K$	430,000	79	4800	35000	333
		BAF$_L$	1065,000	150	19000	22857	667
	O. niloticus	BAF$_K$	425,000	329	5100	71429	3333
		BAF$_L$	430,000	400	5000	57143	2222
	B. intermedius	BSAF$_K$	0.6	0.02	4	1	0.002
		BSAF$_L$	0.9	0.02	5	0.4	0.002
	C. gariepinus	BSAF$_K$	0.5	0.02	5	2	0.002
		BSAF$_L$	1.3	0.03	18	1	0.004
	O. niloticus	BSAF$_K$	0.5	0.1	5	4	0.02
		BSAF$_L$	0.5	0.1	5	3	0.01
Koka	*B. intermedius*	BAF$_K$	1,098	31.0	4000	65833	94.1
		BAF$_L$	1,321	41.4	4167	11500	74.1
	C. gariepinus	BAF$_K$	935	58.6	6667	36667	16.5
		BAF$_L$	1636	58.6	21667	12500	11.8
	O. niloticus	BAF$_K$	833	144.8	5333	28667	22.4
		BAF$_L$	955	103.4	10833	14833	17.6
	B. intermedius	BSAF$_K$	0.5	0.02	16	32	0.04
		BSAF$_L$	0.6	0.02	17	6	0.03
	C. gariepinus	BSAF$_K$	0.5	0.03	27	18	0.01
		BSAF$_L$	0.8	0.03	87	6	0.01
	O. niloticus	BSAF$_K$	0.4	0.07	21	14	0.01
		BSAF$_L$	0.5	0.05	43	7	0.01
Ziway	*B. intermedius*	BAF$_K$	15,000	78	4750	54000	25
		BAF$_L$	18,571	106	5000	15500	25
	C. gariepinus	BAF$_K$	13,143	128	5917	46500	25
		BAF$_L$	28,857	144	24167	23500	33
	O. niloticus	BAF$_K$	12,714	578	7000	60500	192
		BAF$_L$	11,000	483	13333	34500	92
	B. intermedius	BSAF$_K$	0.5	0.03	8	7	0.002
		BSAF$_L$	0.6	0.04	8	2	0.002
	C. gariepinus	BSAF$_K$	0.4	0.05	10	6	0.002
		BSAF$_L$	1.0	0.05	41	3	0.002
	O. niloticus	BSAF$_K$	0.4	0.2	12	8	0.01
		BSAF$_L$	0.4	0.2	23	5	0.01

II. Figures (4)

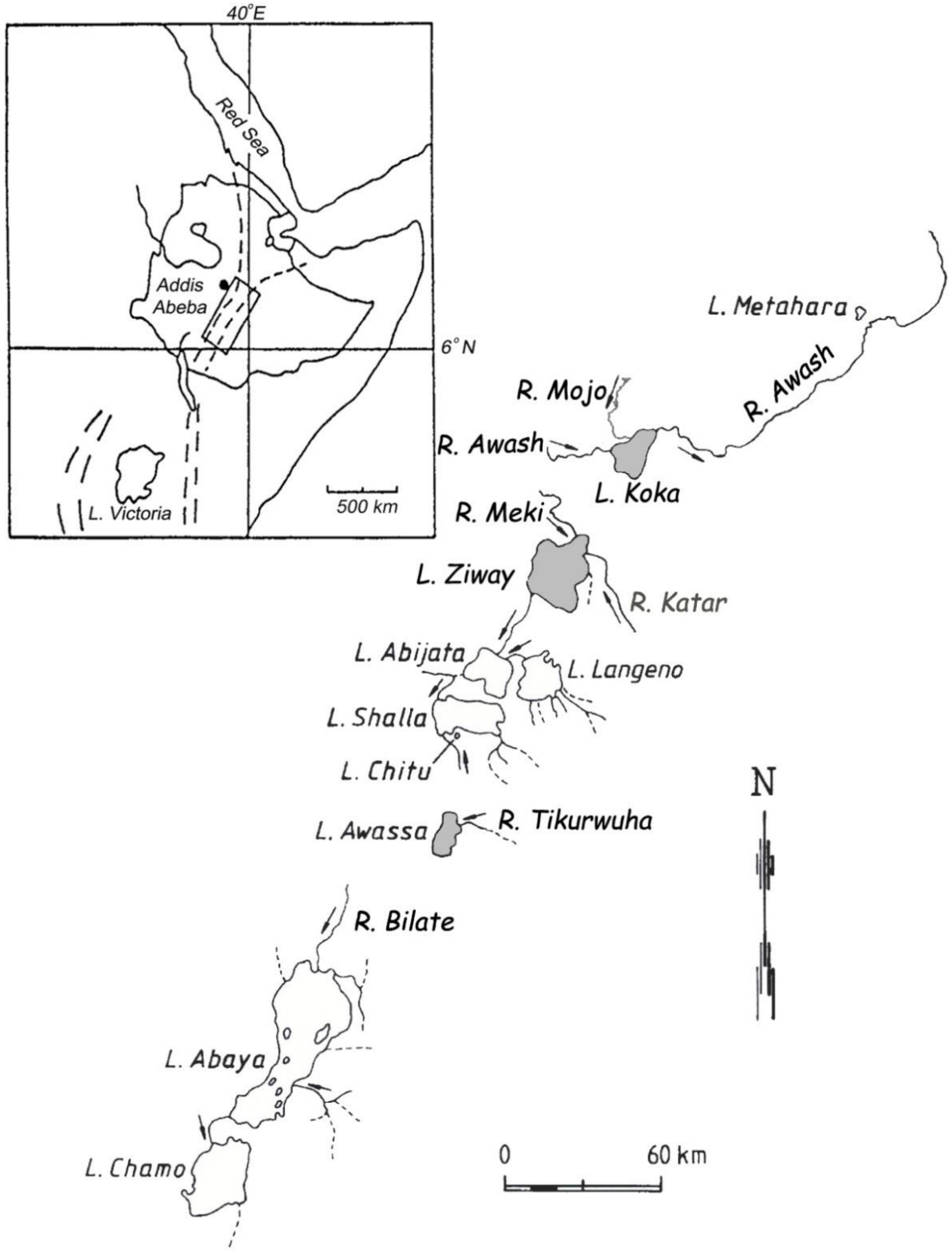

Fig. 1 Location and drainage pattern of the Ethiopian Rift Valley Lakes (Lake Awassa, Lake Koka and Lake Ziway highlighted) and their inflows modified from **(Kebede-Westhead et al. 1994)**

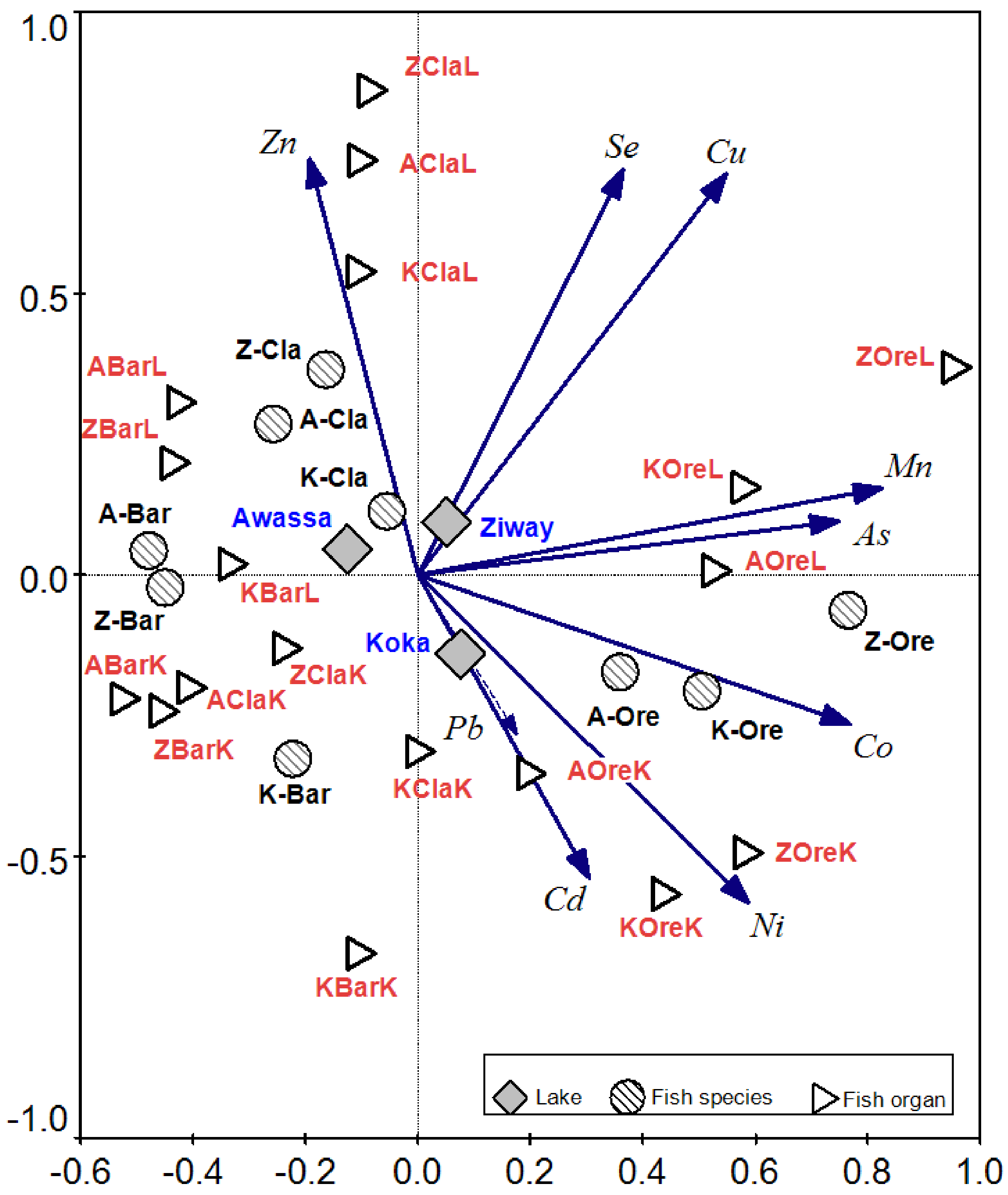

Fig. 2 RDA biplot showing fish species investigated and the relationship between the trace element levels in kidney and liver in the lakes Awassa, Koka and Ziway. In the figure, A, K and Z represent lakes Awassa, Koka and Ziway respectively, while, Bar, Cla, and Oreo represent *B. intermedius*, *C. gariepinus*, and *O. niloticus*, respectively. K and L stand for kidney and liver tissues, respectively

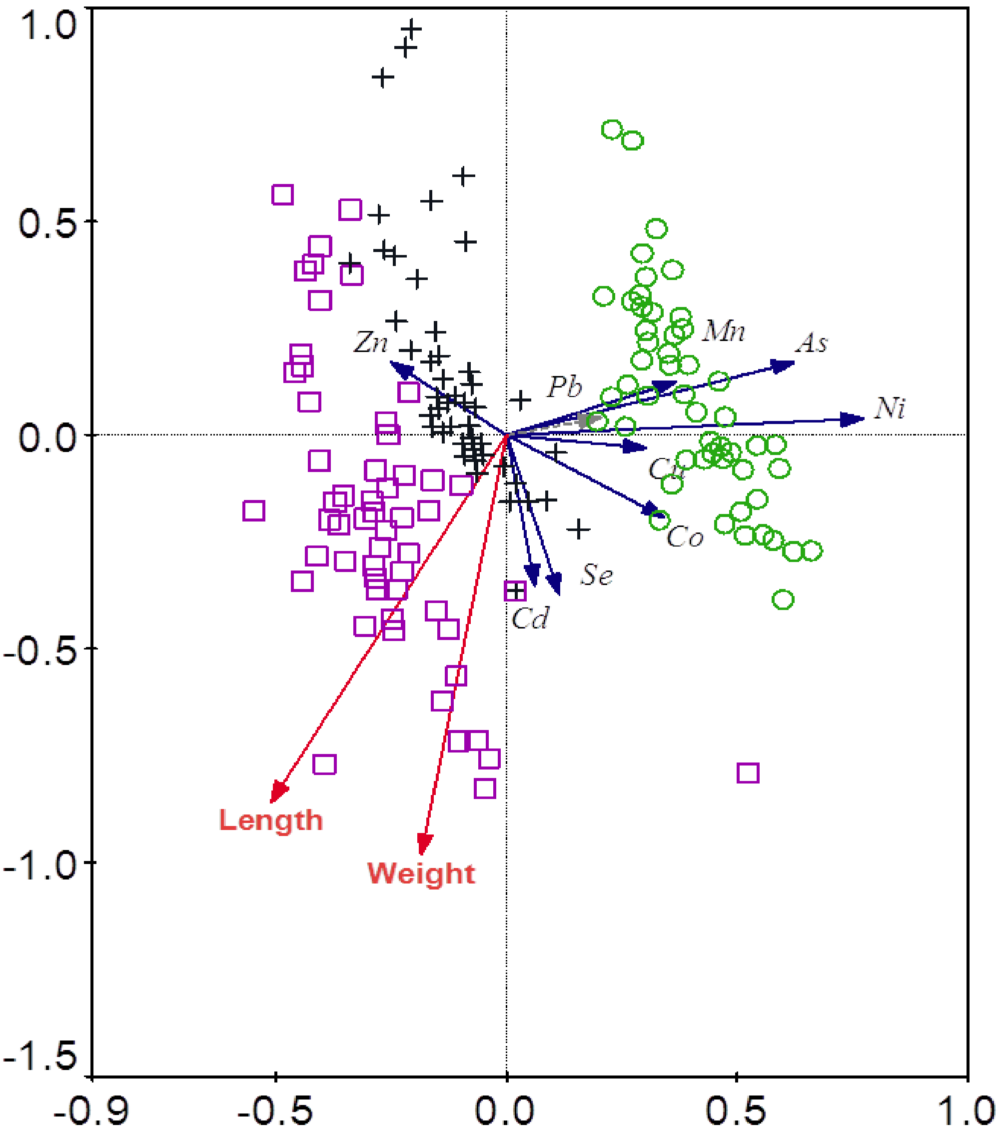

Fig. 3 RDA biplot showing trace elements in kidney from fish collected in all lakes as response variables (bold blue arrows), different fish species (+ = *B. intermedius*, □ = *C. gariepinus*, and ○ = *O. niloticus*) representing kidney samples, and weight and length of fish as explanatory variables (bold red arrows)

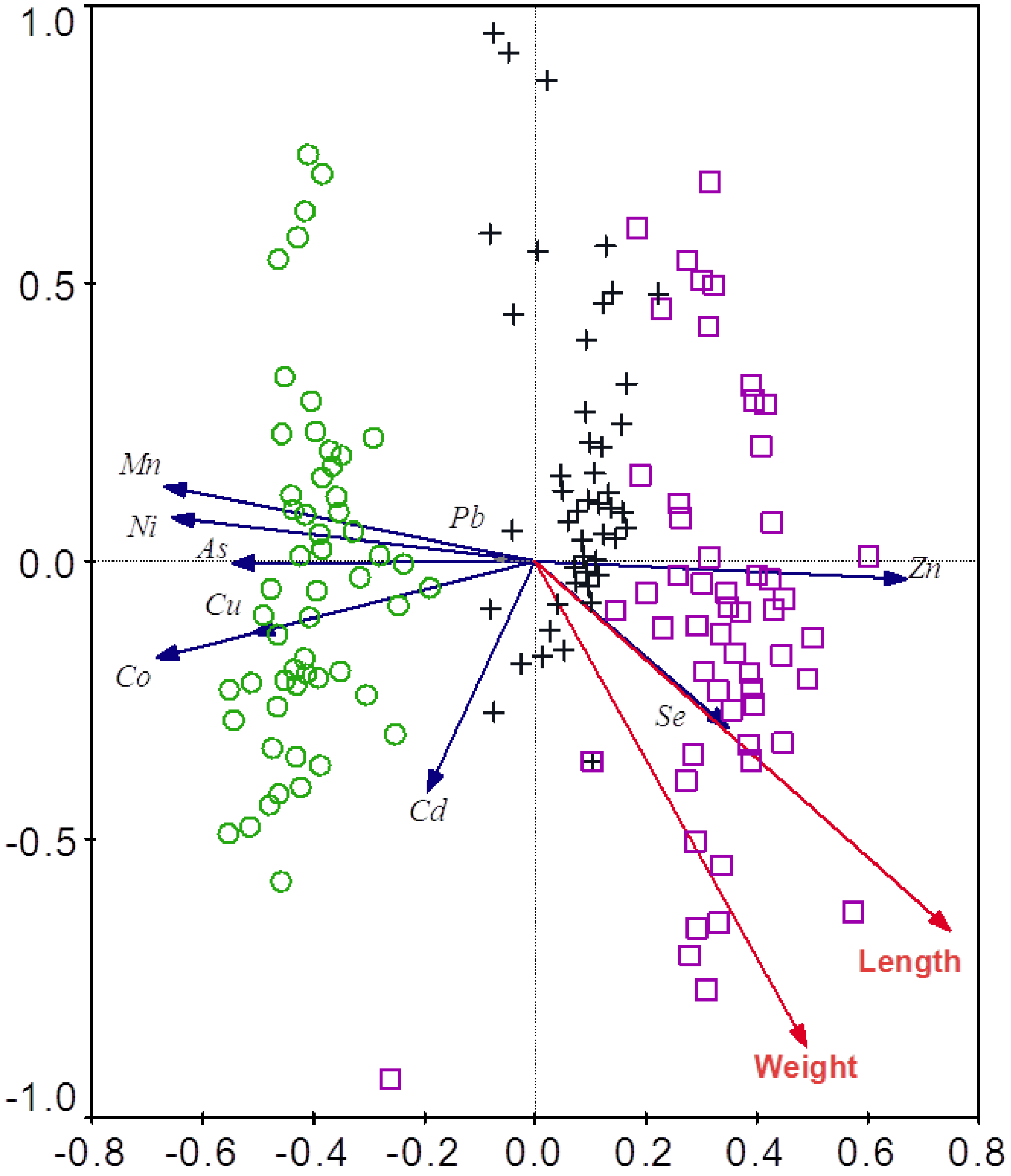

Fig. 4 RDA biplot showing trace elements determined in liver from fish collected in all lakes as response variables (bold blue arrows), different fish species (+ = *B. intermedius*, □= *C. gariepinus*, and ○ = *O. niloticus*) representing liver samples, and weight and length of fish as explanatory variables (bold red arrows)

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