Trace metal concentrations in the abiotic and biotic components of River Rwizi ecosystem in Western Uganda, and the risks to human health

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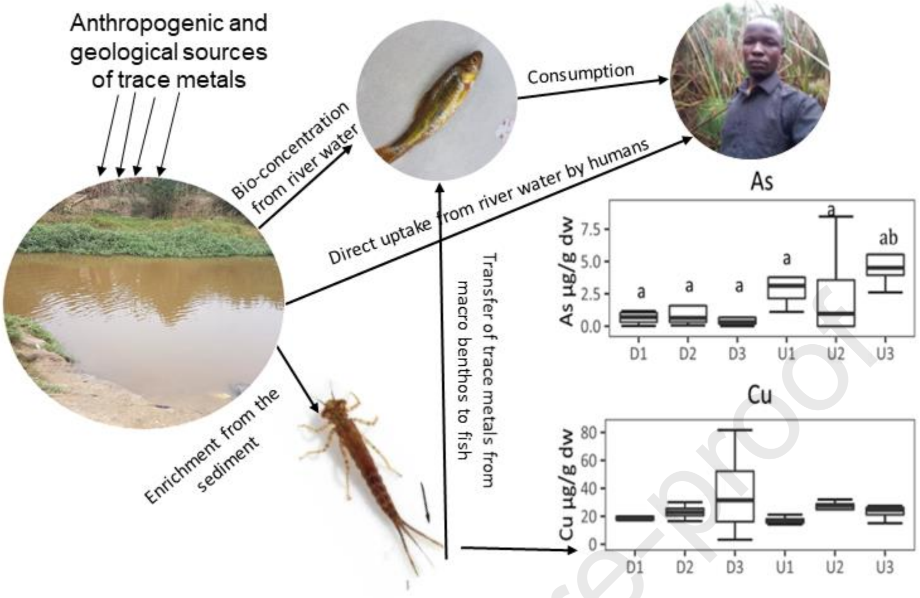
Anthropogenic and geological sources of trace metals
Bio-concentration from river water
Consumption
Direct uptake from river water by humans
Transfer of trace metals from macro benthos to fish
Enrichment from the sediment
As
As µg/g dw
7.5
5.0
2.5
0.0
a
a
a
a
a
ab
D1
D2
D3
U1
U2
U3
Cu
Cu µg/g dw
80
60
40
20
0
D1
D2
D3
U1
U2
U3

Trace metal concentrations in the abiotic and biotic components of River Rwizi ecosystem in Western Uganda, and the risks to human health.

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HIGHLIGHTS

- Surface waters at Katenga and Kayanja were contaminated with gold and mercury
- Concerning metals the surface water was generally safe for human consumption at most sites
- The sediment trace metal levels posed no ecological risks to the benthic biota ~~such~~
- Arsenic and mercury concentrations in *Brycinus sadleri* muscle posed a potential human health risk.

Abstract

The distribution of metals in the Rwizi River ecosystem was investigated and human health risks were assessed. Samples of water, sediment, damselfly larvae (*Ceriagrion glabrum*) and fish species (*Brycinus sadleri* and *Barbus altianalis),* were collected at six sites. In all samples the trace elements As, Al, Au, Cd, Co, Cu, Fe, Hg, Mn, Pb, Zn, were quantified. Sediment samples near the gold mine had significantly higher concentrations of Hg, Fe and Al although all the concentrations were below the probable effect concentrations (PEC). The dissolved concentrations of trace metals were within the European standards and WHO drinking water guidelines. However, Fe and Mn concentrations exceeded the standards at three sites. The damselfly larvae were good indicators of local metal pollution. The fish species accumulated metal levels in the order gills>liver>muscle for most metals except for Hg. Multiple regressions between accumulated metals in damselfly with environmental metal levels showed only for Au and Cd significant positive relationships. Relating environmental metal levels and physicochemical characteristics to the levels in the invertebrates, only for Cu and Pb significant relationships were found. With respect to the measured metals, the fish were safe for human consumption in most cases although *Brycinus sadleri* posed a potential health risk due to a As hazard quotient (HQ) of 2.2 that exceeded the critical value of 1. Similarly, the maximum edible risk-free quantity (Q) for As in *Brycinus sadleri* was 1.5 g (95 % CI), less than the minimum risk free quantity of 31.5 g. In conclusion, the river water was safe for drinking but the extraction of gold using Hg should be replaced with an environmentally friendly method or an effective wastewater treatment should be instituted. People should be cautioned from consuming *Brycinus sadler i* to avoid potential health hazards.

Keywords: Hazard quotient, trace metals, River Rwizi, *Ceriagrion glabrum, Barbus altianalis, Brycinus sadleri*

1 Introduction

Trace metal pollution in aquatic ecosystems has become a global threat (Onyari and Wandiga, 1989), caused by increased urbanization, industrialization, burning fossil fuels and mining activities releasing metals into the environment. For example, the global number of artisanal and small scale gold miners have increased from 6 million in 1993 to 40.5 million in 2017 (IGF, 2017), resulting in an augmented annual release of 1400 tons of mercury into the environment (IGF, 2017). Metals are increasingly used in industrial, domestic, technological, medical, and agricultural fields (Tchounwou et al., 2012; Rehman et al., 2018), thus, mining them is indispensable. Trace metals are naturally present in the aquatic environment in low soluble quantities (Duffus, 2002). However, the increase of metals in the environment is mainly anthropogenically influenced. If trace metals are deposited into aquatic ecosystems, they dissolve or adsorb to suspended particles or sediment (Mataba et al., 2016), rendering the benthic habitats a sink and source for secondary contamination in the ecosystem (Onyari and Wandiga, 1989). From the sediment or water, metals may be taken up by biota such as fish, plants or invertebrates (Goodyear and Mcneill, 1999; Nabulo et al., 2008), and potentially posing human health risks via the consumption of contaminated water and food (Rice et al., 2014).

Trace metals are mostly transferred by rivers from contamination points to other aquatic ecosystems such as seas, lakes, oceans, estuaries and wetlands. For example, the River Nyamwamba-Rukoki in Uganda which originates from the Rwenzori Mountain (Mwesigye and Tumwebaze 2017) deposits 30 tons of Cu and 13 kg of Cd annually into Lake George from Kilembe mines (Hartwig et al., 2005). Rwizi River in southwestern Uganda, which has been cited to be polluted with trace metals (Egor et al., 2014; Ojok et al., 2017; Semwanga et al., 2020), drains into lakes Mburo, Kachera, Nakivale, and Victoria.

In Africa, trace metal pollution in the environment has reached unprecedented levels (Yabe et al., 2010; Fasinu and Orisakwe, 2013) although most governments have put less effort to combat the menace and have prioritized malnutrition, infant and maternal mortalities, and communicable diseases (Fasinu and Orisakwe, 2013). In Uganda, presence of trace metals in sediment and invertebrates are usually not addressed, hence leaving a knowledge gap. Because of bioavailability and toxicity mechanisms of the trace metals in the aquatic ecosystem, measurements in the sediment, invertebrates, fish and surface water should be included for a complete understanding of the system status. Sediment inhabits biota and may act as a secondary source of nutrients as well as bring metals into the aquatic ecosystem through resuspension (Mataba et al., 2016). Further, most benthic invertebrate species are sedentary, and therefore appropriate biological tools to monitor bioavailability of local pollutants (Wakwabi et al., 2006).

Very little information is available on the distribution of metals in aquatic ecosystems in rivers in Uganda and information on metals in edible fish from Uganda is scarce (Omara et al. 2019) and non-existing for the study area.

Therefore the central aim of this study was to investigate the distribution of trace metals in the River Rwizi ecosystem in the vicinity of a gold mine. More specifically the objectives were (1) to evaluate the extent of metal pollution in water, sediment and biota in the catchment of River Rwizi, (2) to establish relationships between trace metal levels in the environment and the biota, taking into account water and sediment characteristics and (3) to assess the human health risks of drinking water and consuming fish contaminated with trace metals.

2 Materials and methods

2.1 Study area

The Rwizi River originates in the hills of Buhweju hills in Western Uganda and flows for 55 km through Lake Mburo, Nakivali, Kachera, Kijanebalola to Lake Victoria (Songa et al., 2015; Semwanga et al., 2020). Despite being shallow with a maximum depth of about 2 to 3 m, it is the principal source of water for domestic, agricultural and industrial activities in southwestern Uganda (NEMA, 2009). Ecologically, it supports a network of highly biodiverse ecosystems such as the Lake Mburo National Park and two Ramsar sites, namely Lake Mburo-Nakivali Wetland System (Songa et al., 2015) and the Sango Bay Wetland Forests (Ojok et al., 2017). The river catchment area of 2,282 km² contains natural resources such as gold, sand and fertile soil, which have attracted human exploitation (Mugonola, 2013; Mugonola et al., 2015). Herewith, the wetlands and river banks were reclaimed for cultivation, human settlement, gold and sand mining (NEMA, 2009; Mugonola, 2013).

Six study sites were selected and marked by GPS 12XL- Garmin (figure 1). Katenga (U1) was situated upstream of the river near the gold mining field followed by Kayanja (U2). Sheema (U3) was located downstream from the mining field and was only affected by farm inputs. Rwebikona (D1) received industrial wastes and Katete (D2) received municipal wastewater. Finally, Buleba (D3) was considered a control site with less human interference.

2.2 Water characteristics

At each site, water pH, conductivity and temperature were measured in quadruple in-situ at about 3 cm depth with a multi-probe Hanna meter (HI9828, USA).

2.3 Collection, laboratory preparation, and analysis of samples

2.3.1 Field sample collection and preservation

At each site, invertebrates, sediment and surface water samples were randomly collected, stored in polypropylene tubes and preserved in a cooler box in the field. Four replicate water samples were taken from each of the six sites (table 1) at a depth of ca. 5 cm using acid- washed 50 ml polypropylene (PP) tubes, which were closed airtight. Four replicate sediment samples were collected with a 2.5-inch diameter hand-held inox corer from the same locations where water samples were collected, and stored in 50 ml PP tubes (table 1) Upon arrival in the lab water samples were filtered over a cellulose acetate filter (0.2µm), acidified by adding 150 µl HNO_3 (69% ultrapure) and transferred to 14 ml PP tubes. All samples were frozen at -20°C before shipment to Antwerp University for trace metal analysis.

For sediment characterization from each site, a subsample of 5 g was taken from each replicate sample and pooled. Subsamples were obtained per site for the measurement of Total Organic Carbon (TOC) and clay content. For TOC analysis about 10 g per site was transferred to pre-weighed 50 ml PP tubes and dried at 60°C for 24 h to obtain the dry weight (DW_{60}). The content was transferred to crucibles and placed in a muffle oven that was slowly heated up to 550°C for one hour, hereafter this temperature was kept during another four hours. The samples were cooled and weighed again (DW_{550}). The TOC was calculated from the loss of ignition method (LOI) as described by Heiri et al.(

2001) according to the equation: TOC (%) = $((DW_{60}-DW_{550})/DW_{60}*100)/1.742$. With DW_{60} and DW_{550} the dry weight after heating at 60 and 550°C respectively. The correction factor 1.742 was applied since it is assumed that 57 % of the organic matter is carbon (Nelson & Sommers 1996) .

For the determination of the clay content another subsample of about 1 g was pre-treated with 40ml of H_2O_2 (33%) and 9ml of HCl (30%) to digest organic material and iron conglomerates. The percentage of clay (fraction < 2µm) in the sediment samples was measured by laser diffraction (Malvern Mastersizer 2000).

Following the USEPA (1995) and Barbour et al. (1998), invertebrates samples were randomly taken at every location using a 1-mm mesh sweep net and washed off through a 500-µm mesh benthic net. At each site four larvae of common waxtail damselfly (*Ceriagrion glabrum*) of comparable size were sorted out and preserved (table 1).

At each site an attempt was made to collect fish by a hook and line. Only at three out of the six sites fish could be collected (table 1). In total, nineteen individual fish were collected. Nine Rippon barbel (*Barbus altianalis*), of a total length (TL) of 8.9-25.9 cm were captured from the river urban area (Katete, D2), and five of TL =17.4-28.7 cm from the remote area (Rwebikona, D1) (table 1). Rippon barbel is an omnivorous freshwater cyprinid inhabiting the inland waters in preferably sand and gravel substrate of most water bodies in East Africa, and migrates upstream to spawn in marginal and vegetation covers (Lévêque, 1997; FishBase team RMCA & Geelhand, 2016). Its stocks are threatened by climate change and overfishing (Balirwa, 1979; Rutaisire et al. 2015). Five Sadler's robber *(Brycinus sadleri*) of TL=11.2-14.2 cm were captured at Kayanja (U2) which was near a gold mining site U1 (table 1). Sadler's robber is a freshwater characid, which mostly lives in open waters, wetlands, marginal vegetation covers, and migrates upstream to spawn (Ojuok 2008; Olowo et. al 2004; Fishbase 2016). Under drastic conditions such as severe pollution, over-fishing and habitat degradation it becomes a generalist, feeding on plants during day and insects at night (Wanink et al., 2007). Both species were selected because of their high abundance during the study period. Before dissection, fish were washed with deionized water. Gills, muscles and liver were collected and stored at -20^oC.

2.3.2 Laboratory preparation of samples.

Invertebrate, sediment and fish tissue samples were transferred into pre-weighed PP tubes and the total weights were assessed (Mettler Toledo AT261 Delta Range). Procedure blanks and standard reference material including mussel tissue (NIST-2976; National Institute of Standards and Technology, USA)) and estuarine sediment (BCR-277R) were included. The samples were freeze-dried for four days in a Thermo Scientific, Heto PowerDry LL3000 freeze dryer, which was connected to a Thermo Savant VL P80 Valu Vacuum Pump. The samples were removed and stored until digestion. They were grouped into two categories according to dry weight and appropriate acid volumes were added.

Samples were transferred to 10 ml glass tubes, and a mixture of HNO_3 (69%) and HCl (37%) (1:3; Aqua Regia) was added to keep mercury in a stable solution, hereafter they were left at room temperature for 24h. Subsequently, 200 µl of H_2O_2 was added to digest the fat tissue. The samples were digested in two steps at 120°c and 160°c respectively in a pressurized microwave system (CEM Discover SP-D; CEM Corporation, Matthews, NC 28106, USA). After digestion, the samples were diluted with Milli-Q water. For water samples, a mixture of HNO_3 and HCl (1:3) was added and stored at -20°C until analysis (Mataba et al., 2016).

2.3.3 Trace metal analysis

Ten trace metals: aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), mercury (Hg), manganese (Mn), zinc (Zn) and the metalloid arsenic (As) were analysed using a High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS; Element XR, Thermo Scientific, Bremen, Germany). The instrument detection limits varied from 0.001 µg/L for Cd, Au, Pb, Co, and As to 0.01µg/L for Hg, Al, Mn, Fe, Cu, and Zn. The data was recorded in µg/L, and the recoveries for the metals ranged from 80 to 120% except for Hg which was 150% and a correction factor of x/1.5 was applied on Hg concentrations except for water samples (Thompson et al., 1996). For sediment and biota the concentrations were expressed as µg/g dry weight (µg/g dw) except for the risk for human consumption where concentration in muscle tissues were expressed on a wet weight basis (µg/g ww).

2.3.4 Heavy metal evaluation index (HEI) for surface water

The heavy metal evaluation index (HEI) by Edet & Offiong (2002) was used to evaluate the pollution status of Rwizi River surface water (equation 1)

$$\mathbf{HEI} = \sum_{n=1}^{n} \frac{\mathrm{Hc}}{\mathrm{Hmac}} \quad (1)$$

With Hc: mean concentration of each metal in the surface water at each site, and Hmac: maximum permissible levels in potable water by UNBS (2014). HEI values were categorized according to the mean value of the HEI for the overall study area (Prasanna et al., 2012; Biswas et al., 2017). The pollution status for each site was accordingly categorized as low (HEI: < 400), medium (HEI: 400-800), and high (HEI: > 800) (Edet & Offiong, 2002).

2.4 Human health risk assessment: maximum edible risk-free quantity of fish consumed without posing deleterious health risks to a person of 70kg average body weight

If an individual is exposed to trace metals through consuming contaminated fish meat, a quantity at which no health risks are posed can be calculated. For Hg it is assumed that 80-100 % of the total mercury in the fish muscle is present as methylmercury (EFSA, 2012), and thus a conversion factor of 1 was applied (EFSA, 2012). The maximum edible risk-free quantity (Q) was determined following Mataba et al. (2016) (equation 2):

$$Q=\frac{(W\times M\times 1000)}{C} \quad (2)$$

Where Q: Maximum quantity (g) of edible fish meat to be consumed without posing health risks to an individual of 70kg average body weight;

W: Weight (kg) of an average person;

M: Minimum Risk Levels (mg/kg/day) for the trace metals as determined by ATSDR (2018); and

C: the 50th and 95th percentile concentration (μg/g ww) in the fish meat for the two species.

A Hazard Quotient (HQ) was determined as a ratio of the daily fish meat consumed per person in Uganda (31.5 g, as noted by Kasozi et al. (2017)) to the maximum risk-free quantity (Q). A HQ >1 signified potential human health risks posed with consumption of contaminated fish meat with a particular metal and HQ< 1 showed no potential health risks.

2.5 Statistical analysis

Data analysis was performed in R 4.0.2 (R Core Team, 2020). Shapiro-Wilk and Levene's test were performed to test for data normality and homogeneity of variances respectively before parametric tests were conducted. Kruskal Wallis or One-way ANOVA was used to test for differences in the trace metal concentrations in water, sediment ,invertebrates and fish among the different sites. Tukey (HSD) or Pair-wise Wilcoxon tests were used as post hoc tests. Kruskal-Wallis was used to compare variations between trace metal levels in fish gills and muscle. Spearman ranks correlation test was performed to determine the relationship between the trace metal levels in water, sediment and invertebrates. Multiple regressions were performed to determine the relationship between the metal levels in damselfly with environmental trace metal concentrations taking into account the physicochemical characteristics (pH, conductivity, clay content, and TOC). No statistical tests were performed if 50% of the measurements were below the limit of detection (Custer et al., 2000). Outliers were examined by applying the Grubbs test (http://www.graphpad.com/quickcalcs/) and removed if found statistically significant.

3 Results

3.1 Water and sediment characteristics

Temperature, pH and conductivity increased from upstream (U) to downstream (D) of the river (Supplementary Information, SI table S1). Conductivity ranged from 28 to 100 µS/cm with the highest value recorded at D3 and the lowest at U1 and this was significantly different among sites ($\chi^2_{(1, 5)}$ =21.7, *p*= 0.0005). Temperature ranged from 16.1 to 22.7°C, with the lowest measured at D2 and the highest at D1 (table 2). However, the water temperature was not exactly measured at the same moment of the day at all sites and thus the differences could not be statistically tested. For pH, there was a significant difference between upstream and downstream ($\chi^2_{(1,)}$ =156.4, *p* = 0.0001); however, there were no statistically significant differences among sampled sites (p>0.05).

Table S2 show the mean % composition of clay content and Total Organic Content (TOC) in the sediment. High clay content percentage was recorded at U3 (6.27±1.26), followed by U2 (5.64±2.4), and least at D2 (1.24±0.00). The sites did not significantly differ in the % clay content ($\chi^2_{(1,\ 5)}$ = 4, *p* = 0.42). High percentage TOC was recorded at U2 (0.51±0.19), followed by D3 (0.28±0.00), and lowest at D1 and U3 (0.01±0.00). Similar to clay content, the sites did not significantly differ in their TOC concentration In the sediment ($\chi^2_{(1,\ 5)}$ = 5, *p* = 0.42).

3.2 Environmental trace metal levels in the sampled sites of Rwizi River (surface water and sediment)

Trace metals concentrations measured in surface water for the six sampled sites from Rwizi River are presented in table 2. Except for As, the metal concentrations were significantly different among sampled sites. Gold, As and Hg were below the detection limit at most sampled sites except at U1 (gold mining site) where Au was above detection limit. For other metals significant differences were recorded at particular sites. For example, at U1 the levels of Al, Hg and Pb were significantly different from other sites (table 2). Zinc, Mn and Co were significantly higher at U3 (Sheema) and comparable levels were measured for Cd between U2 and U3. Similarly, Cu concentrations were comparable between D3 and U3, and for Fe, significantly low levels were measured between U2 and U3.

In Figure 3 and table S3 of the supplementary information, the trace metal concentrations in the sediments are presented. Only Pb, Cd, Co, Cu and Hg levels did not differ between the six sampled sites ($p>0.05$). Gold, Al, As, Fe, Mn and Zn concentrations were significantly different among sites (Figure 3). Pair-wise Wilcoxon comparisons among sites showed that Al and Au were highest at U1, As was lowest at D1, Mn was highest at U2 while Fe and Zn were lowest at U2. Trace metal ranges in the sediment samples were determined for comparison with the standard sediment quality guidelines. Only trace metals with known probable effect concentrations and severe effect concentrations were evaluated.

Correlations between concentrations in water and sediment resulted in significant R-values ($p< 0.001$) for Cd, Hg and Zn only with R=0.99; 0.98 and 0.92 respectively.

3.3 Surface water pollution status of River Rwizi using Heavy metal evaluation index (HEI)

Table S4 presents the results of the Heavy metal Evaluation Index HEI for the six sites. Two outliers were found in the concentrations of Fe at U1 and removed before calculating the HEI index. HEI values decreased from upstream (U) to downstream (D) and ranged from 1.3 to 8.7. The downstream sites values ranged from 1.3 to 1.8, and the upstream from 2.4 - 8.7 with highest HEI at Sheema. The average HEI value for all sites was 3.3 and HEI was categorized as low (0-3), medium (3 to 6) and high (>6) (Table S4).

3.4 Trace element concentrations in damselfly larvae

Trace metal concentrations in damselfly larvae are presented in Figure 4 and table S5. All trace elements in the larvae were above the detection limits in all the sampled sites. Only the concentrations of Fe, Cu, Mn and Zn in the damselfly larvae did not statistically differ among the sampled sites (Figure 3). Multiple pair-wise Wilcoxon comparisons shows highest Au and Pb levels were observed at U1 while As, Cd, and Co concentrations differed at U3 compared to other sites. Higher Al levels were recorded at U3 and lower at U2.

3.5 Trace metal concentrations in *Barbus altianalis* and *Brycinus sadleri*

Metal concentrations in gills from *Barbus altianalis* at D1 (Rwebikona) and D2 (Katete) and from *Brycinus sadleri* at U2 (Kayanja) are presented in figure 4 and table S6. Only Mn and As concentrations did not differ among the sampled sites. However, *Brycinus sadleri* had significantly higher levels of Al Co, Cd, Hg and Zn compared to *Barbus altianalis.* The levels of Pb were significantly lower at D1 compared to U2 and D2.

Concentrations in muscle from the same species at the three sites are presented in figure 5 and table S6. Except for Cd and Cu at U2, for none of the measured trace metals significant differences were found.

Liver could only be collected from *Barbus altianalis* from Rwebikona (D1) and Katete (D2). No significant difference between the sites could be found for none of the measured metals (table S6).

For *Barbus altianalis* the order of magnitude of metal concentration in tissues was liver>gill>muscle for Au, Cd, Co, Cu, Fe and Zn and gill>liver>muscle for Al, Mn and lead. For As no significant differences were found among the tissues and for Hg concentrations in muscle equalled the ones in liver but were higher than in gills. For *Brycinus sadleri* we could only compare between gills and muscle. For Cd, Co, Cu, Fe and Mn ($p < 0.05$) significant higher concentrations were measured in gills. No significant differences were found for Al, As, Co, Hg, Pb and Zn ($p > 0.05$).

3.6 Relationship between environmental and accumulated concentrations

Since fish could not be captured at the same sites as where the sediments and the surface water were sampled, we could only relate the environmental concentrations to the concentrations in the invertebrates. Significant correlations ($p < 0.01$) between water and sediment concentrations were only found for Cd and Zn (R-values of respectively 0.99 and 0.92). For Hg correlation could not be investigated since only at one site the dissolved concentrations was above the detection limit. Multiple regressions were constructed to relate concentrations in the environment with accumulated concentrations in invertebrates, taking into account water (pH, conductivity) or sediment (TOC, clay content) characteristics. Only for Au, Cd, Cu, Fe, Hg and Pb significant relationships were found between concentrations in water or sediment and invertebrates. In a few cases, water or sediment characteristics contributed significantly to the described variation in accumulated metals. This was the case for TOC with Au, conductivity for Cd, pH for Fe and TOC for Hg and Pb (Table 3). Also, multiple regressions with both concentrations in the water and sediment with invertebrates showed that significant relationships were only observed for Au ($R^2=0.99$, $p<0.001$), Cd ($R^2=0.958$, $p <0.05$) and Pb ($R^2=0.85$, $p<0.01$) (Table 3).

3.7 Human health risks assessment

The maximum edible risk-free quantity (Q) consumed without potential health risks and the hazard quotients (HQ) were calculated (table 4). The Qs for the two fish species at both median and upper limit was greater than the daily consumption rate per person (31.5 g) in Uganda except for As (14 g) in *B. sadleri* at upper limit bound (95% CI). Similarly, the HQs for the assessed metals in both fish species were <1. However, the HQ for As in *B. sadleri* was 2.2 at the 95th percentile (table.6). The HQs for Fe, Mn and Au were not determined since no minimum risk levels (MRL) were available.

4 Discussion

4.1 Physicochemical characterisation of water and sediment

The surface water electrical conductivity (EC) significantly increased downstream of the Rwizi River. However, pH and EC were within the Uganda natural water quality standards (UNBS, 2014). Electrical conductivity is governed by natural river geology and hydrology but the high levels in the urban sites could be due to influx of ions from the catchment and transportation from the upstream sites of the river (Van Butsel et al., 2017).

Surface water pH of the downstream sites was significantly lower compared to the upstream sites. Earlier studies conducted on the Rwizi River in Uganda had similar pH ranges from 6 to 7 in the downstream areas (Egor et al., 2014; Semwanga et al., 2020). The low pH upstream could be attributed to leaching of hydrogen ions from the acidic soils of the riverbed and catchment (Banga, 2014). Studies on Sondu-Miriu Rivers in Kenya and Okpokiri River in Nigeria showed the variations in water pH were attributed to demineralisation from the riverbed (Vuai et al., 2012; Evbuomwan & Obinuchi, 2018). The pH range from 5.7 to 7.1 in the downstream part was perhaps influenced by influx of wastewaters from the urban catchment. The pH ranges were similar to Egor et al., (2014) and Semwanga et al. (2020) although sampling were conducted in different sites.

In the present study, the TOC and clay content were not significantly different among sampled sites. However, both Katenga (U1) and Katete (D2), which received high silt and organic matter from the goldmines and urban areas, respectively, had relatively high clay and TOC levels in the sediment which were higher than the levels in Thigithe River in Tanzania (Mataba et al., 2016). The sediment properties such as organic content and clay or silt content may have an important effect on the concentration of trace metals in the sediment (Mason, 2013). Total organic carbon increases binding capacity of some trace metals in the sediment and determines their release into the water or bioavailability to the biota such as invertebrates and fish (Allen, 1993). For example, metal bioavailability to chironomids decreased with the increase in TOC in the sediment (Bervoets et al., 1997, 1998). Further, the clay content will determine the rate of ion exchange over the sediment surface (Allen, 1993). For example, clay types such as smectitte and vermiculite are natural cation agents in the soils and sediment (Allen, 1993).

4.2 Trace metal concentrations in the sediment and water

In the present study, Au, As, Al, Mn, Zn and Fe were significantly different in the sediment among the sampled sites. The level of Au, was significantly higher at the goldmine which implied an anthropogenic enrichment due to wastewater deposal. In the surface water, Hg was only above the detection limit at Katenga and Kayanja, sites within and near the goldmining area respectively. The insignificant levels of Hg in sediment samples among sites but significantly higher concentrations in water at Katenga could perhaps be attributed to low sedimentation rate. Mataba et al. (2016) also observed significantly higher Hg levels near the goldmine in the sediment of Thigithe River in Tanzania. The significantly higher concentration of Al at Rwebikona (D1), and As at Katete (D2) were possibly due to enrichment from the catchment since these sites were located in the urban areas. Similar studies on trace metal levels in the sediment, for example, on lakes Kwania, Nakuwa, Opeta and Kyoga in Uganda measured 0-50 µg/g dw of Cu, 0-133 µg/g dw Zn and 10140-98930 µg/g dw Fe in sediments (Ocaya, 2010). These exceeded the levels of Cu and Fe measured in River Rwizi in the present study although the lakes were not affected by anthropogenic pollution sources (Ocaya

2010). Thus, trace metal enrichment in the sediment could be determined by natural geological sources (Jagus et al., 2013; Machowski et al., 2019). Bugenyi (1982) identified an influx of trace metals from the Kilembe copper mine into lakes George and Edward in Uganda. In the present study, a similar trend was observed for Hg, Al and Fe concentrations at U1 which was in close proximity to the Katenga gold mine. Previous studies which measured trace metals in River Rwizi ecosystem did not quantify the enrichment in sediment and hence the present study can be used a baseline for the river.

In comparison with numerical sediment quality guidelines (MacDonald et al. 2000), the assessed metals were all below the Severe Effect Levels (SEL). However, both Zn and Hg levels exceeded the Probable Effect Concentrations (PEC) (Zn: 315 µg/g and Hg: 0.486 µg/g). Thus, Zn and Hg are potentially toxic for the benthic biota such as the damselfly. Mataba et al. (2016) reported similar findings on Thigithe River although severe effects levels were exceeded for As in the sediment. The present study findings were not comparable with Sekabira et al. (2010) on the metal loads in the sediment from highly polluted urban streams due to industrial discharge. For example, Zn levels of 177-442 µg/g dw and 341-1968 µg/g dw were measured in Nakivubo stream and industrial waste sediment in Kampala, Uganda. These levels exceeded both the SEL (820 µg/g) and PEC (315 µg/g) (MacDonald et al., 2000). Therefore, River Rwizi sediment can be considered as moderately polluted by trace metals from anthropogenic sources.

The trace metal levels in the surface water were similar to findings by Semwanga et al. (2020) but contrasted Egor et al. (2014) on Rwizi River although in all studies sampling was conducted in different sites. Egor et al. (2014) sampled in the downstream part of the river, and therefore less impact of gold mine wastewaters was expected. Since Hg and Au were not detected in the downstream sites but only in the sites (U1 and U2) near the goldmining site, it can be concluded that surface water was polluted anthropogenically from Katenga gold mines. Also, significantly higher Fe and Al concentrations at Katenga (U1) could suggest a possible influx from the mine wastes.

The higher Pb levels at Katete (D2) could perhaps be attributed to contamination from the wastewaters from both domestic and industrial activities (Tchounwou et al., 2012). Egor et al. (2014) reported similar trends of Pb levels increase in the urban sites although the levels of 0.75 ug/L were higher than in the present study. Further, Katete (D2) was near a road bridge and local people washed motorcycles in the river causing direct dumping of oil and fuel wastes into the water which were potentially the sources of Pb contamination at the site. At sites U2, U3 and D3 the maximal measured As concentrations exceeded the European water quality standards (European Commission 2013) whereas this was only the case for Co and Zn at site U3 (table 2). This may pose a potential risk to the aquatic communities.

At Buleba (D3), the significantly higher levels of Cu and Zn perhaps showed natural enrichment since there were less human activities. However, Egor et al. (2014) reported significantly higher levels of Zn 2.54 ug/L in 2011 and 2.53 ug/L in 2010 in the urban sites which was attributed to influx of runoff from zinc corrugated iron sheets from the urban area and perhaps it was carried downstream. The present study contrasted the findings of Mataba et al. (2016) on Thigithe River where most metals in surface water were below the detection limit. However, Omara et al. (2019) observed similar Hg concentrations. For example, 0.15 µg/L was measured near the gold mine in Namukombe stream in Uganda compared to 0.175 µg/L in Rwizi River near the Katenga gold mine. Similar trends of Hg levels decreasing downstream from the gold mine have been observed in Mataba et al. (2016). This trend suggested that the goldmines were the major source of the Hg in surface water. Mercury is mostly used in amalgamating Au from the mud slurry, however, contamination into the environment was attributed to inadequate treatment of wastewater.

In terms of human drinking water assessment, the metal loads in the surface water were within the Uganda national standards for potable natural water (WHO 2017) except for Fe and Mn mean levels at U1 and U2 where standards were exceeded (table 2). Similar findings on the same river were reported by Semwanga et al. (2020) and Egor et al. (2014). However, although the surface water was safe for humans to drink at most of the sites, this was based on the measured metals only at that particular time of sampling. Therefore, it was not exclusive for other contaminants or pollutants such as total coliforms, and turbidity, smell, taste and other compounds such as total nitrogen, phosphorus, soluble reactive silica and organic micro pollutants.

4.3 Surface water trace metal status using trace metal evaluation index

In the present study, Katenga (U1) had trace metal hotspots for iron based on the outliers which were detected at that site. However, the surface water had generally low trace metal loads as shown by the mean HEI values. Rwebikona (D1), Katete (D2), Buleba (D3) and Katenga (U1) had low surface water metal pollution. However, Kayanja (U2) and Sheema (U3) had medium and high metal pollution, respectively. However, the index was highly sensitive to outliers because the mean values were used to compute the index (Edet and Offiong, 2002). The outliers of Fe concentration at Katenga were attributed to the replicates obtained from stagnant water that possibly created a trace metal hotspot. Possibly, the high metal pollution at Sheema could be associated with natural elevated metal concentrations and to a lesser extent from point sources such as the influx from the nearby farms. Probably, the low metal pollution in the river was caused by a low influx from the catchment since the sampling was conducted in a dry season. Although Egor et al. (2014) and Semwanga et al. (2020) conducted the sampling in the wet seasons, the metal concentrations were not significantly different from the present study. Incidentally, the samples were collected from different sites but the studies indicated that natural enrichment was an important factor that determined the metal concentrations of the Rwizi River.

4.4 Trace metals in the biota: damselfly and fish.

In the present study, Au and Pb were significantly higher in the damselfly larvae from Katenga (U1) and Kayanja (U2). High accumulation of Au was observed at Katenga (U1), which was in close proximity to the goldmines. A study of Bervoets et al. (1997) on the impact of mining activities on Bolivian rivers, showed that chironomid larvae accumulated higher levels of Zn, Pb, Cu and Cd compared to measured concentrations in the damselfly from Rwizi River. Chironomid larvae are highly pollution tolerant (Bazzanti, 2000) because they possess a red blood pigment for extraction of oxygen at low levels (Sriariyanuwath et al., 2015). Simon et al. (2017)'s study on Tisza and Szamos rivers in Turkey affected by mining activities showed that *Gomphus flavipes* larvae accumulated Al, Zn, Mn, Fe, Pb and Cu concentrations similar to damselfly larvae from Rwizi River. In the Olifant River Basin (ORB), Verhaert et al. (2019) measured total Hg concentrations of 0.06 to 0.29 µg/g dw in the snail *Tarebia graniferia* and 0.08 to 0.69 µg/g dw in odonata larvae, which were comparable to the 0.05 to 0.27 µg/g dw in the damselfly larvae in the present study. The trend of trace metal accumulation in the damselfly larvae was similar to findings of Erasmus et al. (2020), which was higher in the macroinvertebrate families near or within platinum mining sites. In contrast, the annelids, molluscs, *Mugil sp., Solea sp.*, and *Tilapia sp.* from Lake Qarun in Egypt accumulated less Zn, Mn, Cu, Pb, Cd, Co and Fe (Mohamed and Mohamed, 2005), yet the lake was polluted by industrial and agricultural waste (Hussein et al., 2008). Similar to other studies, our study has indicated that macroinvertebrates significantly accumulated higher trace metal levels near mining sites and urban areas, indicating they are good bio-indicators of environmental pollution.

For most trace metals measured in *Barbus altianalis* gills and liver accumulated higher levels than muscle. A similar trace metal accumulation pattern was reported by Bervoets et al. (2001), Bervoets & Blust, (2003); Szarek-Gwiazda & Amirowicz (2006); Mataba et al. (2016); Semwanga et al. (2020). However, these studies have been conducted on different waterbodies and fish species. Copper, Fe, Zn, Pb, Cd and Mn concentrations in the liver and muscle of African catfish were lower than in the present study except for Al that was higher with 545 µg/g dw in the liver and with 69.2 µg/g dw in the muscle (Semwanga et al., 2020). This was probably due to the higher Al levels in water (225 µg/L) in addition to species specific differences (Van Ael et al., 2017). Similar to Teunen et al. (2017), Mataba et al. (2016), Gilbert et al. (2017) the order of Hg concentration was muscle>liver>gills. In *Oreochromis niloticus* captured 30 m downstream of the goldmine in Namukombe stream in Uganda, no Hg could be detected in the muscle (Omara et al., 2019). However, Hg levels of 0.11 µg/g dw were measured in muscle of *O. niloticus* captured within the goldmine (Omara et al., 2019). Similarly, low Hg levels

(0.08 µg/g dw) were detected in *Brycinus sadleri* muscle captured 200 m downstream of Katenga gold mine in the present study.

Physiologically the gills are used for gaseous exchange and thus exposed continuously to the polluted river water. Definitely, this makes them the main entry of dissolved metal ions and the target organ of metal toxicity (Olsson et al., 1998). Structurally, gills have a thin epithelium which provides a large surface area for uptake and storage of the metals. Similar to gills, the liver is constantly exposed to trace metals during detoxification and metabolism (Olsson et al., 1998). After detoxification, the metals are usually stored in the liver in a non-toxic form (Olsson et al., 1998). The edible muscle are neither in direct contact with polluted water nor physiologically active such as liver and gills, but they accumulated higher Hg levels. Mercury was not detected in water in the downstream sites but detected in the fish organs, which was a clear indication that the main route of Hg exposure was through food rather than water. The general low edible muscle metal loads might be attributed to the even distribution of metals over a larger mass compared to other organs (Vinodhini & Narayanan, 2008). The non-significant differences in the accumulated concentrations in fish tissues of most metals among sites suggests similar pollution levels exist along the river.

4.5 Relationship between environmental and biotic trace metals concentrations

The concentrations of trace metals in water, sediment and their physiochemical characteristics such as conductivity, pH for water and TOC, clay content and grain size may affect the bioavailability of trace metals to biota such as macroinvertebrates (Luoma, 1989; Allen, 1993; Mason, 2013). The invertebrates are vital in the food chain as they are food for fish, which may be consumed by humans, and thus understanding the metal accumulation in macroinvertebrates in aquatic ecosystem is necessary. Both studied species feed on aquatic invertebrates (fishbase.org). In the present study, weak correlations between metal levels in water and sediment were found except for Cd, Zn, and Hg. Further, only Au, Cd, Cu, Fe, Pb and Hg had significant relationships between concentrations in the invertebrates and concentrations in water or sediment. For Au, Hg and Pb TOC contributed significantly to the relationship with the concentration in the sediment. As expected and also found by Bervoets et al. (1997), for Pb, TOC contributed negatively to the relationship. For Au and Hg, however, higher TOC were related to higher accumulated levels in the damselfly larvae. This might be an indication that these metals are rather taken up via the food with higher exposure due to metals bound to the organic matter.

Clay content did not contribute to the relationships between metals in sediment and in biota. This is probably due to the relative low concentration of clay at all sites. With higher clay concentrations one would expect a significant negative effect of clay content on the relationship given the high adsorption capacity of fine material (Allen, 1993; Luoma ,1989).

When related to the water concentration, conductivity and pH contributed significantly negative to the accumulated concentrations for respectively Cd and Fe. Higher water conductivity is determined by higher amount of free ions and higher pH with lower amount of hydrogen ions (Canli and Canli, 2015) which will affect the bioavailability of metals to biota (e.g. Bervoets et al., 1996 and Bervoets & Blust, 2000).

Only for three metals significant relationships were found between concentrations in water and sediment. This is probably due to the differences in sediment characteristics between the sites (Bervoets & Blust, 2000).

4.6 Human health risk assessment

Except for As in *Brycinus sadleri* at the upper limit bound, the measured trace metals posed no human health risk through fish consumption because Q values were higher than the average fish consumption in Uganda. Also, the hazard quotient (HQ) was lower than 1 (critical value). Mataba et al. (2016) found a Q of 45 g and a HQ of 0.38 for As in Ningu from Thigithe River at upper limit bound. However, no immediate health risk were expected since the safe consumption of 45 g was higher than the national Tanzanian daily consumption of 17g (Mataba et al., 2016). Van Ael et al. (2017) reported similar potential human health risks due to As levels in muscle of fish collected in the Scheldt estuary.

Also, Hg accumulated in *Brycinus sadleri* and the Q value at upper limit bound was 85 g comparable to 65 g reported by Mataba et al. (2016) in *Labeo victorianus* from Thigithe River. About 80-100 % of total Hg in fish muscle is present as methylmercury (EFSA, 2012) which is highly neurotoxic in humans (Li et al., 2010; Pandey et al., 2012). Since the fish were continuously exposed to Hg loaded wastewater from the goldmine, health risks are prevalent. Similar studies by Ezemonye et al. (2019) on *Brycinus longipinus* from Benin River in Nigeria reported a Pb hazard quotient of 1.9. In Uganda, Omara et al. (2019) reported the HQ of 0.94 for Hg in *Oreochromis niloticus* suggesting potential toxicity to humans. The present study is cognisant of the differences in the size, age and location of the fish species assessed. Therefore, the study findings of *Brycinus sadleri* were mostly applicable for sites near the gold mine and could not be generalised along the river, and similarly, the *Brycinus altianalis* assessment was applicable for the urban catchment and nearby areas. However, both species indicated the general river nature in terms of health risk assessment.

5 Conclusions

From the present study it was shown that for most measured variables in the water no risk for human consumption is expected. Concerning the environmental quality possible risks may occur at sites U2 and U3. In addition the river sediment was anthropogenically polluted near the goldmining site although natural metal loading may affect the concentration in the sediment. As a consequence more research is needed to assess possible effects on the aquatic communities. Concerning the risk for human consumption, only in the case of fish captured near the gold mine, arsenic in muscle posed a possible risk for human consumption. However, more edible species should be measured in the future to assess possible health risks.

Because no trace metal concentrations have been measured in the invertebrates and sediment from the study river before, the present study can be used as a baseline.

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7 References

Allen, H.E., 1993. The significance of trace metal speciation for water, sediment and soil quality criteria and standards. Sci. Total Environ. 134, suppl 1. 23–45. https://doi.org/10.1016/S0048-9697(05)80004-X.

ATSDR, 2018. Agency for toxic substances and disease registry minimum risk levels (MRLs). 1–14.

Balirwa, J.S., 1979. A contribution to the study of the food of 6 cyprinid fishes in 3 areas of the Lake Victoria Basin, East Africa. Hydrobiologia 66, 65–72. https://doi.org/10.1007/BF00019141.

Banga, H., 2014. Tracing the root of Buhweju. In (Treasure in a thousand & one Poverty Eradication Initiatives). New Vis. 1, 9.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999 rapid bioassessment Protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish. EPA 841-B-99-002, U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Bazzanti, M., 2000. Ecological requirements of chironomids (diptera: Chironomidae) on the soft bottom of the river arrone, central Italy. J. Freshw. Ecol. 15, 397–409. https://doi.org/10.1080/02705060.2000.9663758.

Bervoets, L., Blust, R., 2003. Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor. Environ. Pollut. 126, 9–19. https://doi.org/10.1016/S0269-7491(03)00173-8.

Bervoets, L., Blust, R., 2000. Effects of pH on cadmium and zinc uptake by the midge larvae *Chironomus riparius*. Aquat. Toxicol. 49, 145–157. https://doi.org/10.1016/S0166-445X(99)00066-1.

Bervoets, L., Blust, R., Verheyen, R., 2001. Accumulation of metals in the tissues of three spined stickelback (*Gasterosteus aculeatus*) from natural fresh waters. Ecotox. Environ. Safe. 48, 117–127. https://doi.org/10.1006/eesa.2000.2010.

Bervoets, L., Blust, R., Verheyen, R., 1996. Uptake of zinc by the midge larvae *Chironomus riparius* at different salinities: Role of speciation, acclimation, and calcium. Environ. Toxicol. Chem. 15, 1423–1428. https://doi.org/10.1002/etc.5620150823.

Bervoets, L., de Wit, M., Blust, R., Verheyen, R., 1997. Relationships between river sediment characteristics and trace metal concentrations in tubificid worms and chironomid larvae. Environ. Pollut. 95, 345–356. https://doi.org/10.1016/S0269-7491(96)00134-0.

Bervoets, L., Solis, D., Romero, A.M., Van Damme, P.A., Ollevier, F., 1998. Trace metals in chironomid larvae and sediments from a Bolivian river: impact of mining activities. Ecotox. Environ. Safe. 41, 275–283. https://doi.org/10.1006/eesa.1998.1707.

Biswas, P.K., Uddin, N., Alam, S., Tamjid-Us-Sakib, Sultana, S., Ahmed, T., 2017. Evaluation of heavy metal pollution indices in irrigation and drinking water systems of Barapukuria coal mine area, Bangladesh. Am. J. Wat. Resour. 5, 146–151. https://doi.org/10.12691/ajwr-5-5-2.

Bugenyi, F.W.B., 1982. Copper pollution studies in lakes George and Edward, Uganda - the distribution of Cu, Cd and Fe in the water and sediments. Environ. Pollut. 3, 129–138. https://doi.org/10.1016/0143-148X(82)90047-7.

Canli, E.G., Canli, M., 2015. Low water conductivity increases the effects of copper on the serum parameters in fish (*Oreochromis niloticus)*. Environ. Toxicol. Pharmacol. 39, 606–613. https://doi.org/10.1016/j.etap.2014.12.019

Custer, T.W., Custer, C.M., Hines, R.K., Sparks, D.W., 2000. Trace elements, organochlorines , polycyclic aromatic hydrocarbons , dioxins , and furans in lesser scaup wintering on the Indiana Harbor Canal. Environ. Pollut. 110, 469–482. https://do.org/10.1016/S0269-7491(99)00315-2.

Duffus, J.H., 2002. "Heavy metals" a meaningless term? (IUPAC Technical Report). Pure Appl. Chem. 74, 793–807.

Edet, A.E., Offiong, O.E., 2002. Evaluation of water quality pollution indices for heavy metal contamination monitoring. A study case from Akpabuyo-Odukpani area, Lower Cross River Basin (southeastern Nigeria). GeoJournal 57, 295–304. https://doi.org/10.1023/B:GEJO.0000007250.92458.de

EFSA (European Food Safety Authority), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food 1. EFSA J. 10, 1–241. https://doi.org/10.2903/j.efsa.2012.2985.

European Commission 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. www.ec.europa.eu

Egor, M., Mbabazi, J., Ntale, M., 2014. Heavy metal and nutrient loading of River Rwizi by effluents from Mbarara municipality, Western Uganda. Int. J. Chem. Mater. Res. 2, 36–47.

Erasmus, J.H., Malherbe, W., Zimmermann, S., Lorenz, A.W., Nachev, M., Wepener, V., Sures, B., Smit, N.J., 2020. Metal accumulation in riverine macroinvertebrates from a platinum mining region, Sci. Total Environ. 703, 13478. https://doi.org/10.1016/j.scitotenv.2019.134738.

Evbuomwan, B.O., Obinuchi, W.B., 2018. Distribution of heavy metals and dissolved oxygen concentration along Okpokiri River stretch (case study of Onne, Nigeria). Glob. Sci. Journals 6, 535–546.

Ezemonye, L.I., Adebayo, P.O., Enuneku, A.A., Tongo, I., Ogbomida, E., 2019. Potential health risk consequences of heavy metal concentrations in surface water, shrimp (*Macrobrachium macrobrachion*) and fish (*Brycinus longipinnis*) from Benin River, Nigeria. Toxicol. Reports 6, 1–9. https://doi.org/10.1016/j.toxrep.2018.11.010.

Fasinu, P.S., Orisakwe, O.E., 2013. Heavy metal pollution in sub-saharan Africa and possible implications in cancer epidemiology. Asian Pacific J. Cancer Prev. 14, 3393–3402. https://doi.org:10.7314/APJCP.2013.14.6.3393.

FishBase team RMCA & Geelhand, D., 2016. Brycinus sadleri. The IUCN Red List of Threatened Species 2016 [WWW Document]. https://doi.org/http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T60758A47214050.en.

Gilbert, B.M., Hussain, E., Jirsa, F., Avenant-Oldewage, A., 2017. Evaluation of trace element and metal accumulation and edibility risk associated with consumption of *Labeo umbratus* from the Vaal Dam, South Africa. Int. J. Environ. Res. Public Health 14, 678. https://doi:10.3390/ijerph14070678.

Goodyear, K.L., Mcneill, S., 1999. Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: a review. Sci. Total Environ. 229, 1-19. https://doi: 10.1016/S0048-9697(99)00051-0.

Hartwig, T., Owor, M., Muwanga, A., Zachmann, D., Pohl, W., 2005. Lake George as a sink for contaminants derived from the Kilembe copper mining area, western Uganda. Mine Water Environ. 24, 114–123.

Heiri, O., Lotter, A.F., Lemcke, G., 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments : reproducibility and comparability of results. J. Paleolimn. 25, 101–110. https://doi: 10.1023/A:1008119611481.

Hussein, H., Amer, R., Gaballah, A., Refaat, Y., Abdel-Wahab, A., 2008. Pollution monitoring for Lake Qarun. Adv. Environ. Biol. 2, 70–80.

IGF, 2017. Global Trends in Artisanal and Small-Scale Mining (ASM): A review of key numbers and issues. Winnipeg: IISD.

Jagus, A., Khak, V., Rzetala, M.A., Rzetala, M., 2013. Accumulation of heavy metals in the bottom sediments of the Irkutsk Reservoir. Int. J. Environ. Heal. 6, 350–362. https://doi.org/10.1504/IJENVH.2013.056976

Kasozi, N., Rutaisire, J., Nandi, S., Sundaray, J.K., 2017. A review of Uganda and Indias freshwater aquaculture: Key practices and experience from each country. J. Ecol. Nat. Environ. 9, 15–29. https://doi.org/10.5897/JENE2016.0615.

Lévêque, C., 1997. Biodiversity dynamics and conservation: the freshwater fish of tropical Africa., ©Universit. ed. Syndicate of University of Cambridge, London.

Li, P., Feng, X.B., Qiu, G.L., 2010. Methylmercury exposure and health effects from rice and fish consumption: A review. Int. J. Environ. Res. Public Health 7, 2666–2691. https://doi.org/10.3390/ijerph7062666.

Luoma, S.N., 1989. Can we determine the biological availability of sediment-bound trace- elements? Hydrobiologia 176/177, 379–396. https://doi.org/10.1007/BF00026572.

MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31. https://doi.org/10.1007/s002440010075

Machowski, R., Rzetala, M.A., Rzetala, M., Solarski, M., 2019. Anthropogenic enrichment of the chemical composition of bottom sediments of water bodies in the neighborhood of a non-ferrous metal smelter (Silesian Upland, Southern Poland). Sci. Rep. 9, 1–13. https://doi.org/10.1038/s41598-019-51027-w

Mason, R.P., 2013. Trace metals in aquatic systems, 1st ed. Blackwell Publishing Ltd, Oxford, UK.

Mataba, G.R., Verhaert, V., Blust, R., Bervoets, L., 2016. Distribution of trace elements in the aquatic ecosystem of the Thigithe river and the fish *Labeo victorianus* in Tanzania and possible risks for human consumption. Sci. Total Environ. 547, 48–59. http://dx.doi.org/10.1016/j.scitotenv.2015.12.123.

Mohamed, H.H.A., Mohamed, R.A.F., 2005. Accumulation of trace metals in some benthic invertebrate and fish species relevant to their concentration in water and sediment of lake Qarun , Egypt. Egypt. J. Aquat. Res. 31, 289–301.

Mugonola, B., 2013. Optimal management of farm-level resources in the Lake Victoria catchments: a case of upper Rwizi and Iguluibi micro-catchments, Uganda. Master thesis Katholieke Universiteit Leuven.

Mugonola, B., Mathijs, E., Poesen, J., Deckers, J., Wanyama, J., Isabirye, M., 2015. Soil and water conservation technologies in the upper Rwizi micro-catchment of Southwestern Uganda, in: Nicol, A.; Langan, S.; Victor, M.; Gonsalves, J. (Eds.) 2015. Water-smart agriculture in East Africa. Colombo, Sri Lanka: International Water Management Institute (IWMI). CGIAR Research Program on Water, Land and Ecosystems (WLE); Kampala, Uganda.

Mwesigye, R.A., Tumwebaze, B.S., 2017. Water contamination with heavy metals and trace elements from Kilembe copper mine and tailing sites in Western Uganda;

implications for domestic water quality. Chemosphere 169, 281–287. https://doi.org/10.1016/j.chemosphere.2016.11.077.

Nabulo, G., Origa, H.O., Nasinyama, G.W., Cole, D., 2008. Assessment of Zn , Cu , Pb and Ni contamination in wetland soils and plants in the Lake Victoria basin. Int. J. Environ. Sci. Technol. 5, 65–74. https://doi.org/10.1007/BF03325998.

Nelson, D.W., Sommers, L.E., 1996. Total Carbon , Organic Carbon , and Organic Matter, in: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (Eds.), Methods of Soil Analysis: Part 3 Chemical Methods, 5.3. Soil Science Society of America, Inc., American Society of Agronomy, p. 53711. https://doi.org/10.2136/sssabookser5.3.c34.

NEMA, 2009. "Uganda: Atlas of Our Changing Environment." Natl. Environ. Manag. Auth. 205 pp.

Ocaya, H., 2010. Selected trace metal concentration in bottom water and sediments of Kyoga basin lakes , and its potential to aquatic pollution. Jinja, Uganda, National Fisheries Resources Research Institute (NaFIRRI), 14pp

Ojok, W., Wasswa, J., Ntambi, E., 2017. Assessment of seasonal variation in water quality in river Rwizi using multivariate statistical techniques, Mbarara Municipality, Uganda. J. Water Resour. Prot. 9, 83–97.

Olowo, J.P., Chapman, L.J., Chapman, C.A., Ogutu-Ohwayo, R., 2004. The distribution and feeding ecology of the characid *Brycinus sadleri* in Lake Nabugabo, Uganda: Implications for persistence with Nile perch (*Lates niloticus)*. African J. Aquat. Sci. 29, 13–23. https://doi.org/https://doi.org/10.2989/16085910409503788.

Olsson, P.E., Kling, P., Hogstrand, C., 1998. Mechanics of heavy metal accumulation and toxicity in fish. Met. Metab. Aquat. Environ. 321–350.

Omara, T., Karungi, S., Kalukusu, R., Nakabuye, V., Kagoya, S., Musau, B., 2019. Mercuric pollution of surface water, superficial sediments, Nile tilapia (*Oreochromis nilotica* Linnaeus 1758 [Cichlidae]) and yams (*Dioscorea alata*) in auriferous areas of Namukombe stream, Syanyona, Busia, Uganda. PeerJ 7, e7919. https://doi.org/10.7717/peerj.7919.

Onyari, J.M., Wandiga, S.O., 1989. Distribution of Cr, Pb, Cd, Zn, Fe, and Mn in Lake Victoria sediments, East Africa. Bull. Environ. Contam. Toxicol. 42, 807–813. https://doi.org/10.1007/BF0170619.

Pandey, G., Madhuri, S., Shrivastav, A.B., 2012. Contamination of mercury in fish and its toxicity to both fish and humans: An overview. Int. Res. J. Pharm. 3, 44–47.

Prasanna, M.V., Praveena, S.M., Chidambaram, S., Nagarajan, R., Elayaraja, A., 2012. Evaluation of water quality pollution indices for heavy metal contamination monitoring : a case study from Curtin Lake , Miri City , East Malaysia. Environ. Earth Sci. 67, 1978-2001. https://doi.org/10.1007/s12665-012-1639-6.

R Core Team, 2020. R: A Language and Environment for Statistical Computing.

Rehman, K., Fatima, F., Waheed, I., Akash, M.S.H., 2018. Prevalence of exposure of heavy metals and their impact on health consequences. J. Cell. Biochem. 119, 157-184. htpps://doi.org/10.1002/jcb.26234.

Rice, K.M., Walker, E.M., Wu, M., Gillette, C., Blough, E.R., 2014. Environmental mercury and its toxic effects. J. Prev. Med. Public Health 47, 74–83. https://doi.org/10.3961/jpmph.2014.47.2.74

Rutaisire, J., Levavi-Sivan, B., Aruho, C., Ondhoro, C.C., 2015. Gonadal recrudescence and induced spawning in *Barbus altianalis*. Aquac. Res. 46, 669–678.

https://doi.org/10.111/are.12213.

Sekabira, K., Origa, H.O., Basamba, T.A., Mutumba, G., Kakudidi, E., 2010. Assessment of heavy metal pollution in the urban stream sediments and its tributaries. Int. J. Environ. Sci. Technol. 7, 435–446. https://doi.org/10.1007/BF03326153.

Semwanga, M., Prohasaka, T., Zitek, A., Odong, R., Kasozi, N.G., 2020. Trace metal levels in the surface water and African catfish, *Clarias gariepinus* from River Rwizi , Uganda. Manuscr. Submitt. Publ. 1–17.

Songa, P., Rumohr, J., Musota, R., 2015. A shared water risk assessment for a vulnerable river basin: River Rwizi in Uganda. WIT Trans. Ecol. Environ. 197, 213-224. https://doi.org/10.2495/RM150191.

Sriariyanuwath, E.O., Sangpradub, N., Hanjavanit, C., 2015. Diversity of chironomid larvae in relation to water quality in the Phong river, Thailand. AACL Bioflux 8, 933–945.

Szarek-Gwiazda, E., Amirowicz, A., 2006. Bioaccumulation of trace elements in roach, silver bream, rudd, and perch living in an inundated opencast sulphur mine. Aquat. Ecol. 40, 221–236. https://doi.org/10.1007/s10452-004-7341-3.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metals toxicity and the environment. EXS 101, 1–30. https://doi.org/10.1007/978-3-7643-8340-4_6.

Teunen, L., Belpaire, C., Blust, R., Bervoets, L., 2017. Mercury accumulation in two freshwater fish species in Flanders (Belgium). Internal distribution and effects of length , weight and sex ., in: 15th International Conference on Environmental Science and Technology Rhodes, Greece, 31 August to 2 September 2017. pp. 2–5.

Thompson, M., Elliosn, S.L.R., Fajgelj, A., Willetts, P., Wood, R., 1996. Harmonised guidelines for the use of recovery information in analytical measurement (Technical Report). Orlando, USA, 4-5 th. September. 1996.

UNBS, 2014. Potable water-Specification, UNBS. Uganda.

USEPA, 1995. Environmental monitoring and assessment program-Surface waters: Field operations and methods for measuring the ecological conditions of the wadeable streams.

Van Ael, E., Blust, R., Bervoets, L., 2017. Metals in the Scheldt estuary : From environmental concentrations to bioaccumulation. Environ. Pollut. 228, 82–91. https://doi.org/10.1016/j.envpol.2017.05.028

Van Butsel, J., Donoso, N., Gobeyn, S., De Troyer, N., Van Echelpoel, W., Lock, K., Bwambale, G., Muganzi, E., Muhangi, C., Nalumansi, N., Peeters, L., Goethals, P.L.M., 2017. Report: Ecological water quality assessment of the Mpanga catchment, Western Uganda. Ghent University, 37 pages.

Verhaert, V., Teuchies, J., Vlok, W., Wepener, V., Addo-Bediako, A., Jooste, A., Blust, R., Bervoets, L., 2019. Bioaccumulation and trophic transfer of total mercury in the subtropical Olifants River Basin, South Africa. Chemosphere 216, 832-843. https://doi.org/10.1016/j.chemosphere.2018.10.211

Vinodhini, R., Narayanan, M., 2008. Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). Int. J. Environ. Sci. Technol. 5, 179–182. htpss://doi.org/10.1007/BF03326011.

Vuai, S.A.H., Ibembe, J.D., Mungai, N., 2012. Spatial variation of nutrients in Sondu-Miriu and Simiyu-Duma rivers: Implication on sources and sactors influencing their transportation into the Lake Victoria. J. Earth Sci. Clim. Change 3, 2.

https://doi.org/10.4172/2157-7617.1000119.

Wakwabi, E.O., Balirwa, J., Ntiba, M.J., 2006. Aquatic biodiversity of Lake Victoria basin, in: Odada, E.O., Olago, D.O., Ochola, W.O. (Eds.), Environment for Development: An Ecosystems Assessment of Lake Victoria Basin. United Nations Environment Programme (UNEP), Pan African START Secretariat (PASS) Nairobi, Kenya, Nairobi, pp. 77–121.

Wanink, J.H., Joordens, J.C.A., 2007. Dietary shifts in *Brycinus sadleri* (Pisces: Characidae) from southern Lake Victoria. Aquat. Ecosyst. Heal. Manag. 10, 392–397. htpps://doi.org/10.1080/14634980701709352.

WHO (2017). Guidelines for drinking-water quality. 4th edition, incorporating the 1st addendum. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.

Yabe, J., Ishizuka, M., Umemura, T., 2010. Current levels of heavy metal pollution in Africa. J. Vet. Med. Sci. 72, 1257–1263. htpps://doi.org/10.1292/jvms.10-0058.

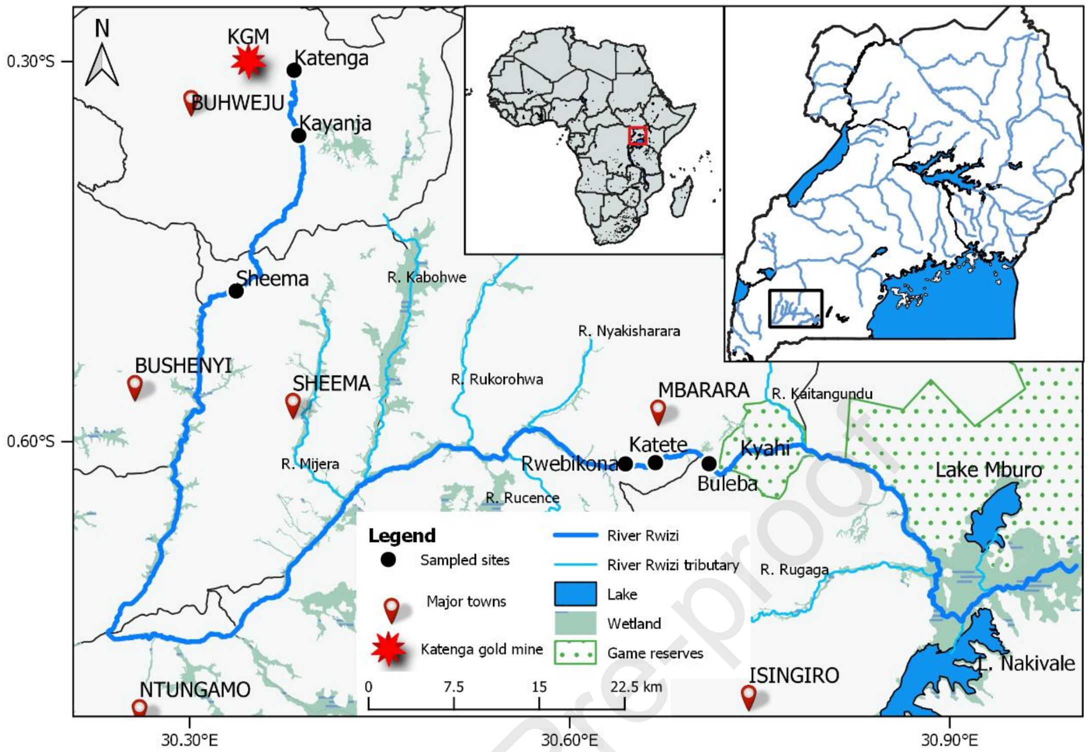


Figure 1. Study sites; upstream sites; Katenga (U1) near the gold mine; Kayanja (U2) and Sheema (U3); downstream sites; Rwebikona (D1); Katete (D2) and Buleba (D3)

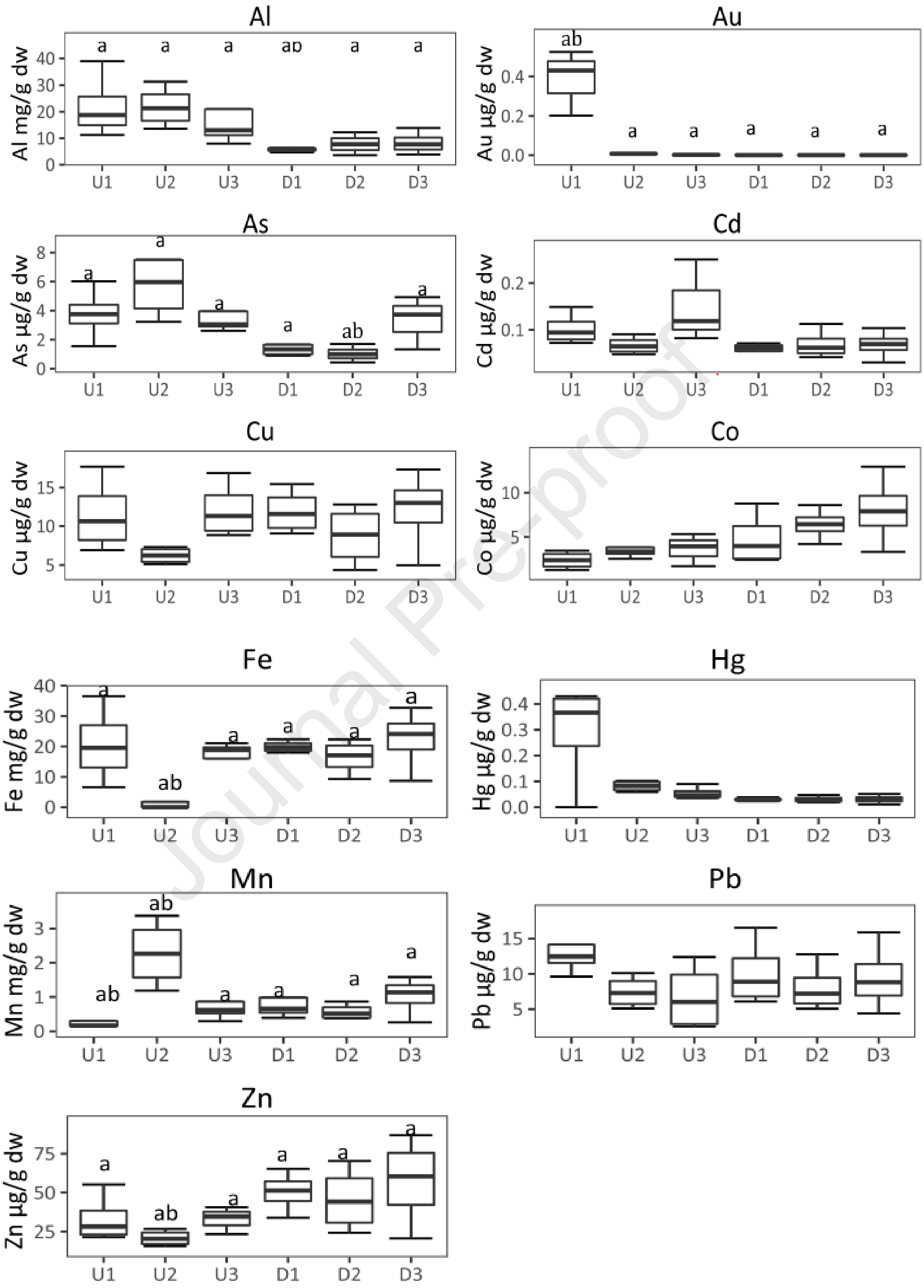


Figure 2: Trace metal concentrations in the sediment samples from the six sites in the Rwizi River. U1 to D3: sampled sites (table 1). Different letters indicate significant differences among sites, N=4

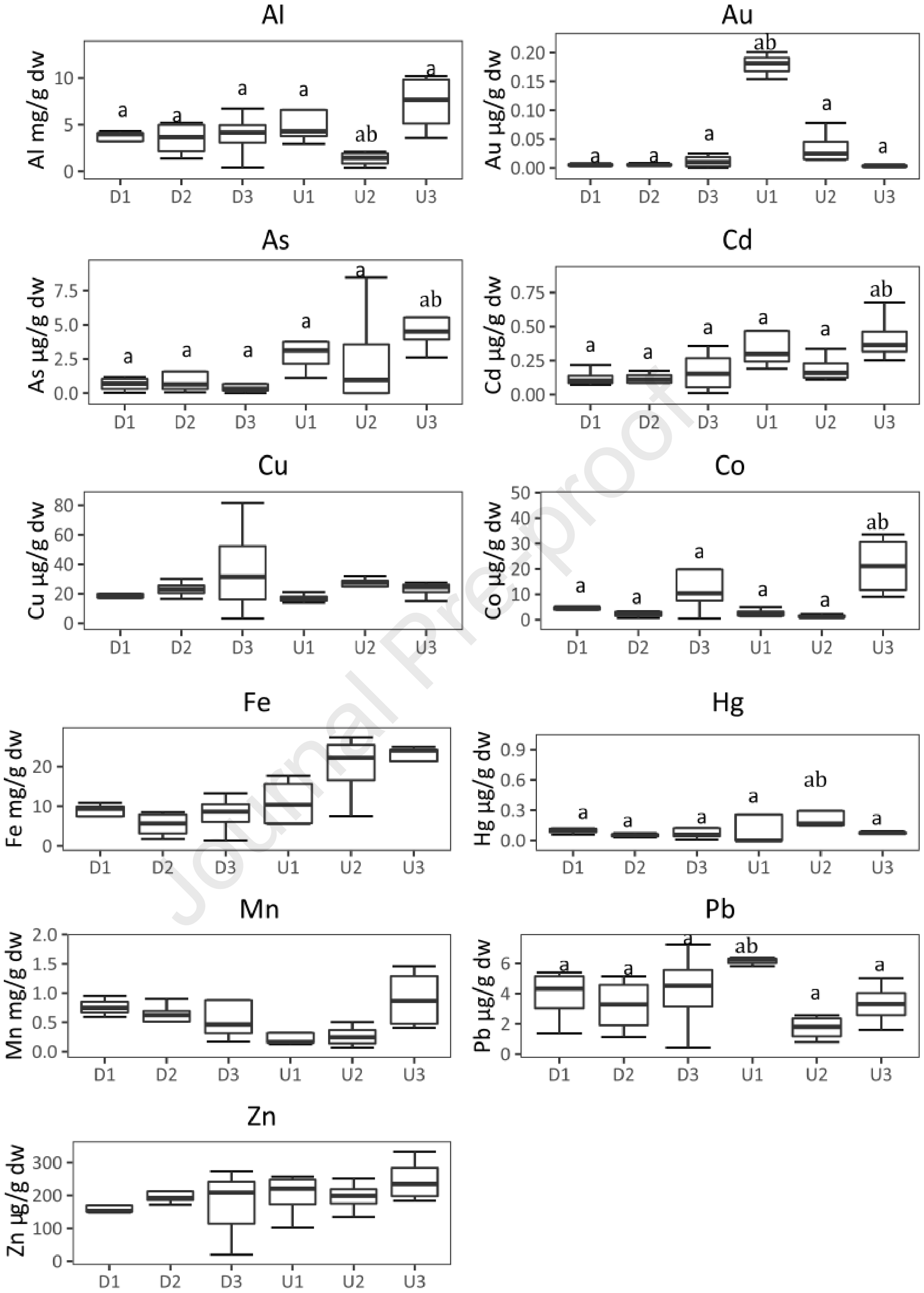
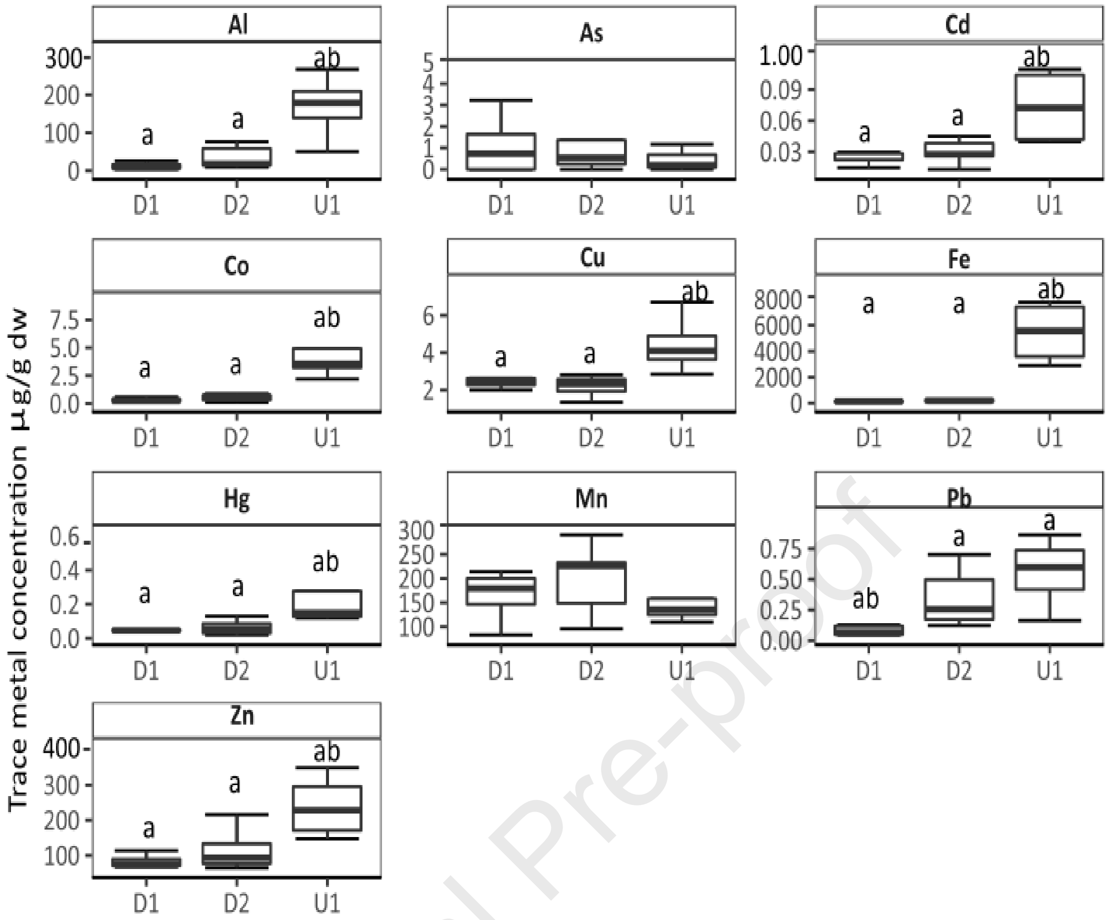


Figure 3: Trace metal concentrations in the damselfly larvae from the six sites in the Rwizi River. Different letters indicate significant differences among sites. N= 4. U1 to D3: sampling sites (table 1).



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902 Figure 4. Trace metal concentration in the gill tissue of *Barbus altianalis* from Rwebikona
903 (D1, n=5) and Katete (D2, n=9) and *Brycinus sadleri* from Kayanja (U2, n=5). Different
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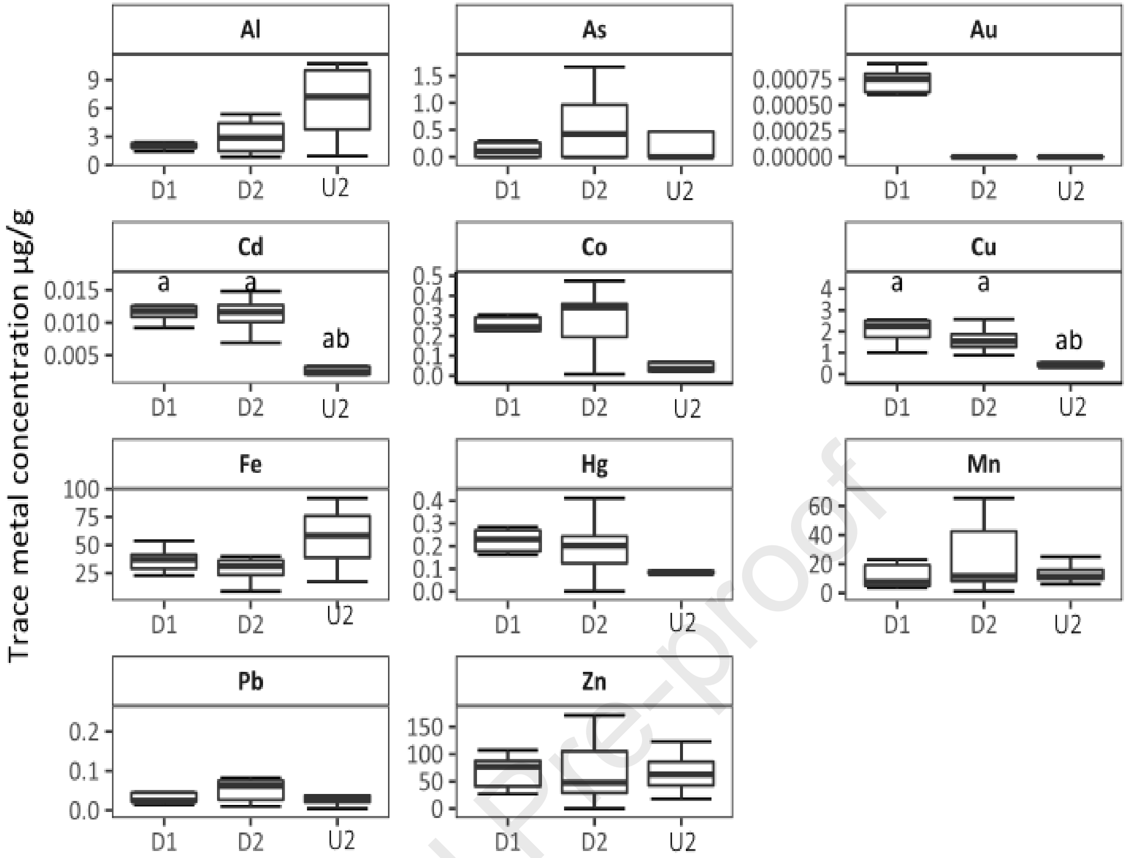

Figure 5. Trace metal concentrations in the muscle of *Barbus altianalis* collected at Katete (D2, n=9), Rwebikona (D1, n=5) and *Brycinus sadleri* from Kayanja (U2, n = 5). Different letters indicate significant differences among sites.

Table 1: Number of replicate samples collected for each sampled variable

	Replicate samples obtained							
	Water	Sediment	Invertebrates	*Barbus altianalis*			*Brycinus sadleri*	
				Liver	Gills	Muscle	Gills	Muscle
Katenga (U1)	4	4	4	0	0	0	0	0
Kayanja (U2)	4	4	4	0	0	0	5	5
Sheema (U3)	4	4	4	0	0	0	0	0
Rwebikona (D1)	4	4	4	5	5	5	0	0
Katete (D2)	4	4	4	9	9	9	0	0
Buleba (D3)	4	4	4	0	0	0	0	0

Table2: Range in trace metal concentrations in the surface water (µg/L) at the six locations in the Rwizi River.

Metals	U1	U2	U3	D1	D2	D3	Water quality standards	
							Drinking water	Environmental standards
Al	58.2 – 349^{ab}	7.9 - 15.4^{a}	4.3 - 13.4^{a}	6.2 - 9.2^{a}	4.65 – 110^{a}	3.2 - 11.2^{a}	-	-
As	BDL - 1.6	BDL - 5.72	0 - 5.43	BDL - 5.9	0 - 2.89	BDL - 6.2	10	3
Au	0.003 - 0.01	BDL	BDL	BDL	BDL	BDL	-	-
Cd	0.03 - 0.1^{a}	0.028 - 0.03^{ab}	0.19 - 0.25^{abc}	0.03 - 0.05^{a}	0.03 - 0.06^{a}	0.03 - 0.04^{a}	3	0.08-0.45
Co	0.2 - 8.1^{a}	0.27 - 0.3^{a}	9.7 - 10.8^{abc}	0.3 - 0.4^{a}	0.29 - 1.3^{a}	0.32 - 0.4^{a}	-	0.5
Cu	0.2- 0.9^{a}	BDL - 0.13^{a}	BDL - 0.23^{ab}	0.48 - 0.83^{a}	0.58 - 2.49^{a}	1.1 - 4.44^{ab}	2,000*	7.2
Fe	156 – 67267^{a}	424 – 535^{ab}	61 – 91^{ab}	110 – 160^{a}	89 – 433^{a}	79 – 107^{a}	50,000*	-
Hg	0.06 - 0.3	0 - 0.01	BDL	BDL	BDL	BDL	6.0*	0.05
Mn	36.6- 3751^{a}	87 – 629^{a}	740 – 831^{ab}	24.5 - 36.8^{a}	28.8 – 101^{a}	29.4 - 47.3^{a}	400*	-
Pb	0.06 - 0.22^{ab}	0.02 - 0.09^{a}	0.017 - 0.06^{a}	0.03 - 0.05^{a}	0.027 - 0.27^{a}	0.02 - 0.04^{a}	10*	7.2
Zn	3.14 - 11.9^{a}	3.48 - 10.2^{a}	89.25 – 102^{ab}	0.71 - 53.3^{a}	0.77 - 18.7^{a}	5.0 – 144^{a}	3,000*	20

BDL: Below Detection Limit. N=6.

U1-D3: Sampled sites along the River Rwizi (Table 1). Different superscript letters in a row indicate significant differences ($p < 0.05$) among sites for the trace metals; * drinking water standards WHO; environmental standards: EU 2013

Table 3. Results of the multiple regression analyses between metal(loid) concentrations in damselfly larvae (*Ceriagrion glabrum*) and in sediment or water.

Metals	**Sediment**	**Water**
Au	$Au_{invert} = 0.0022+0.267*Au_{sed}+0.049*TOC$ $R^2=0.99$, $p<0.001$	NS
	$Au_{invert} = 0.0034-986.9_{wat}+ 3.984_{sed}$, $p=0.00003$, $R^2= 0.99$, Significant	
Cd	NS	$Cd_{invert} = 0.471 + (1.39*Cd_{wat}) – 0.0043*Cond)$ $R^2=0.81$, $P=0.038$
	$Cu_{(inverts)} = 0.0687-9.4_{wat}+ 6.62_{sed}$, $p=0.009$, $R^2= 0.958$	
Cu	NS	$Cu_{invert} = 19.4 + (5.46*Cu_{wat})$, $R^2=0.49$, $p=0.05$
Fe	NS	$Fe_{invert} = 12.27 - (0.329*Fe_{wat}) – 17.5*pH)$ $R^2=0.89$, $p=0.016$
Hg	$Hg_{invert} = 0.029 +0.711*Hg_{sed}+0.479*TOC$, $R^2=0.97$, $P=0.015$	NS
Pb	$Pb_{invert} = -4.92 + (0.99*Pb_{sed})$ $R^2=0.79$, $p=0.011$ $Pb_{invert} = -4.21 + (1.02* Pb_{sed}) – (3.01*TOC)$ $R^2=0.92$, $P=0.011$	NS
	$Pb_{(inverts)} = -4.62 +8.953_{wat}+ 0.9_{sed}$, p=0.0.04, $R^2= 0.85$	

Invert=invertebrates, sed= sediment; wat = water; TOC= total organic carbon, NS= not significant

Table 4: Maximal daily amount (Q, g) of fish that could be eaten without health risk by a person of 70 kg average body weight and Hazard Quotient (HQ) for *Barbus altianalis* (*B.a*, n=14) and *Brycinus sadleri* (B.s, n=4) obtained from River Rwizi at 50 (median, mg/g ww) and 95 percentile (mg/g ww) trace metal levels

	Al	**As**	**Cd**	**Co**	**Cu**	**Hg**	**Pb**	**Zn**
MRL (mg/kg/day)	1	0.0003	0.00001	0.01	0.01	0.0003	0.00036	0.3
MRL for 70 kg person (mg/kg/day)	70	0.021	0.007	0.7	0.7	0.02	0.02	21
Concentration in *B.a* at 50 %	0.2	0.03	0.001	0.026	0.2	0.017	0.005	6.8
Concentration in *B.a* at 95 %	1.0	0.13	0.002	0.101	0.5	0.06	0.028	34
Q for *B.a* at 50 %	329739	820	5448	26788	4648	1271	4949	3076
Q for *B.a* at 95 %	69142	162	3189	6938	1340	350	911	614
HQ for *B.a* at 50 %	0.0	0.04	0.006	0.001	0.01	0.025	0.01	0.010
HQ for *B.a* at 95 %	0.0	0.19	0.010	0.005	0.02	0.09	0.04	0.051
Concentration in *B.s* at 50 %	9.7		0.003	0.037	0.4	0.08	0.03	62
Concentration in *B.s* at 95 %	121	1.5	0.01	0.17	0.8	0.2	0.1	116
Q for *B.s* at 50 %	7183		2755	19077	1821	259	857	337
Q for *B.s* at 95 %	577	14	729	4161	892	89	256	182
HQ for *B.s* at 50 %	0.0	0.00	0.01	0.002	0.02	0.1	0.04	0.09
HQ for *B.s* at 95 %	0.1	2.2	0.04	0.008	0.04	0.4	0.1	0.17

B.a: *Barbus altianalis*, B.s: *Brycinus sadleri*, HQ: Hazard Quotient, MRL: Minimum Risk Levels (ATSDR, 2018), Q: Maximum health risk free quantity

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Sheema (U3)	4	4	4	0	0	0	0	0
Rwebikona (D1)	4	4	4	5	5	5	0	0
Katete (D2)	4	4	4	9	9	9	0	0
Buleba (D3)	4	4	4	0	0	0	0	0

Table2: Range in trace metal concentrations in the surface water (µg/L) at the six locations in the Rwizi River.

Metals	U1	U2	U3	D1	D2	D3	Water quality standards	
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As	BDL - 1.6	BDL - 5.72	0 - 5.43	BDL - 5.9	0 - 2.89	BDL - 6.2	10	3
Au	0.003 - 0.01	BDL	BDL	BDL	BDL	BDL	-	-
Cd	0.03 - 0.1^{a}	0.028 - 0.03^{ab}	0.19 - 0.25^{abc}	0.03 - 0.05^{a}	0.03 - 0.06^{a}	0.03 - 0.04^{a}	3	0.08-0.45
Co	0.2 - 8.1^{a}	0.27 - 0.3^{a}	9.7 - 10.8^{abc}	0.3 - 0.4^{a}	0.29 - 1.3^{a}	0.32 - 0.4^{a}	-	0.5
Cu	0.2- 0.9^{a}	BDL - 0.13^{a}	BDL - 0.23^{ab}	0.48 - 0.83^{a}	0.58 - 2.49^{a}	1.1 - 4.44^{ab}	2,000*	7.2
Fe	156 – 67267^{a}	424 – 535^{ab}	61 – 91^{ab}	110 – 160^{a}	89 – 433^{a}	79 – 107^{a}	50,000*	-
Hg	0.06 - 0.3	0 - 0.01	BDL	BDL	BDL	BDL	6.0*	0.05
Mn	36.6- 3751^{a}	87 – 629^{a}	740 – 831^{ab}	24.5 - 36.8^{a}	28.8 – 101^{a}	29.4 - 47.3^{a}	400*	-
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Katenga (U1)	4	4	4	0	0	0	0	0
Kayanja (U2)	4	4	4	0	0	0	5	5
Sheema (U3)	4	4	4	0	0	0	0	0
Rwebikona (D1)	4	4	4	5	5	5	0	0
Katete (D2)	4	4	4	9	9	9	0	0
Buleba (D3)	4	4	4	0	0	0	0	0

Table2: Range in trace metal concentrations in the surface water (µg/L) at the six locations in the Rwizi River.

Metals	U1	U2	U3	D1	D2	D3	Water quality standards	
							Drinking water	Environmental standards
Al	58.2 – 349^{ab}	7.9 - 15.4^{a}	4.3 - 13.4^{a}	6.2 - 9.2^{a}	4.65 – 110^{a}	3.2 - 11.2^{a}	-	-
As	BDL - 1.6	BDL - 5.72	0 - 5.43	BDL - 5.9	0 - 2.89	BDL - 6.2	10	3
Au	0.003 - 0.01	BDL	BDL	BDL	BDL	BDL	-	-
Cd	0.03 - 0.1^{a}	0.028 - 0.03^{ab}	0.19 - 0.25^{abc}	0.03 - 0.05^{a}	0.03 - 0.06^{a}	0.03 - 0.04^{a}	3	0.08-0.45
Co	0.2 - 8.1^{a}	0.27 - 0.3^{a}	9.7 - 10.8^{abc}	0.3 - 0.4^{a}	0.29 - 1.3^{a}	0.32 - 0.4^{a}	-	0.5
Cu	0.2- 0.9^{a}	BDL - 0.13^{a}	BDL - 0.23^{ab}	0.48 - 0.83^{a}	0.58 - 2.49^{a}	1.1 - 4.44^{ab}	2,000*	7.2
Fe	156 – 67267^{a}	424 – 535^{ab}	61 – 91^{ab}	110 – 160^{a}	89 – 433^{a}	79 – 107^{a}	50,000*	-
Hg	0.06 - 0.3	0 - 0.01	BDL	BDL	BDL	BDL	6.0*	0.05
Mn	36.6- 3751^{a}	87 – 629^{a}	740 – 831^{ab}	24.5 - 36.8^{a}	28.8 – 101^{a}	29.4 - 47.3^{a}	400*	-
Pb	0.06 - 0.22^{ab}	0.02 - 0.09^{a}	0.017 - 0.06^{a}	0.03 - 0.05^{a}	0.027 - 0.27^{a}	0.02 - 0.04^{a}	10*	7.2
Zn	3.14 - 11.9^{a}	3.48 - 10.2^{a}	89.25 – 102^{ab}	0.71 - 53.3^{a}	0.77 - 18.7^{a}	5.0 – 144^{a}	3,000*	20

BDL: Below Detection Limit. N=6.

U1-D3: Sampled sites along the River Rwizi (Table 1). Different superscript letters in a row indicate significant differences ($p < 0.05$) among ~~between~~ sites for the trace metals; * drinking water standards WHO; environmental standards: EU 2013

Table2: Range in trace metal concentrations in the surface water (µg/L) at the six locations in the Rwizi River.

Metals	U1	U2	U3	D1	D2	D3
Al	58.2 – 349^{ab}	7.9 - 15.4^{a}	4.3 - 13.4^{a}	6.2 - 9.2^{a}	4.65 – 110^{a}	3.2 - 11.2^{a}
As	BDL - 1.6	BDL - 5.72	0 - 5.43	BDL - 5.9	0 - 2.89	BDL - 6.2
Au	0.003 - 0.01	BDL	BDL	BDL	BDL	BDL
Cd	0.03 - 0.1^{a}	0.028 - 0.03^{ab}	0.19 - 0.25^{abc}	0.03 - 0.05^{a}	0.03 - 0.06^{a}	0.03 - 0.04^{a}
Co	0.2 - 8.1^{a}	0.27 - 0.3^{a}	9.7 - 10.8^{abc}	0.3 - 0.4^{a}	0.29 - 1.3^{a}	0.32 - 0.4^{a}
Cu	0.2- 0.9^{a}	BDL - 0.13^{a}	BDL - 0.23^{ab}	0.48 - 0.83^{a}	0.58 - 2.49^{a}	1.1 - 4.44^{ab}
Fe	156 – 67267^{a}	424 – 535^{ab}	61 – 91^{ab}	110 – 160^{a}	89 – 433^{a}	79 – 107^{a}
Hg	0.06 - 0.3	0 - 0.01	BDL	BDL	BDL	BDL
Mn	36.6- 3751^{a}	87 – 629^{a}	740 – 831^{ab}	24.5 - 36.8^{a}	28.8 – 101^{a}	29.4 - 47.3^{a}
Pb	0.06 - 0.22^{ab}	0.02 - 0.09^{a}	0.017 - 0.06^{a}	0.03 - 0.05^{a}	0.027 - 0.27^{a}	0.02 - 0.04^{a}
Zn	3.14 - 11.9^{a}	3.48 - 10.2^{a}	89.25 – 102^{ab}	0.71 - 53.3^{a}	0.77 - 18.7^{a}	5.0 – 144^{a}

BDL: Below Detection Limit. N=6.

U1-D3: Sampled sites along the River Rwizi (Table 1). Different superscript letters in a row indicate significant differences between sites for the trace metals.

Table 3. Results of the multiple regression analyses between metal(loid) concentrations in damselfly larvae (*Ceriagrion glabrum*) and in sediment or water.

Metals	Sediment	Water
Au	$Au_{invert}= 0.0022+0.267*Au_{sed}+0.049*TOC$ $R^2=0.99$, $p<0.001$	NS
	$Au_{invert} = 0.0034-986.9_{wat}+ 3.984_{sed}$, $p=0.00003$, $R^2= 0.99$, Significant	
Cd	NS	$Cd_{invert} = 0.471 + (1.39*Cd_{wat}) – 0.0043*Cond)$ $R^2=0.81$, $P=0.038$
	$Cu_{(inverts)} = 0.0687-9.4_{wat}+ 6.62_{sed}$, $p=0.009$, $R^2= 0.958$	
Cu	NS	$Cu_{invert} = 19.4 + (5.46*Cu_{wat})$, $R^2=0.49$, $p=0.05$
Fe	NS	$Fe_{invert} = 12.27 - (0.329*Fe_{wat}) – 17.5*pH)$ $R^2=0.89$, $p=0.016$
Hg	$Hg_{invert} = 0.029 +0.711*Hg_{sed}+0.479*TOC$, $R^2=0.97$, $P=0.015$	NS
Pb	$Pb_{invert} = -4.92 + (0.99*Pb_{sed})$ $R^2=0.79$, $p=0.011$ $Pb_{invert} = -4.21 + (1.02* Pb_{sed}) – (3.01*TOC)$ $R^2=0.92$, $P=0.011$	NS
	$Pb_{(inverts)} = -4.62 +8.953_{wat}+ 0.9_{sed}$, p=0.0.04, $R^2= 0.85$	

Invert=invertebrates, sed= sediment; wat = water; TOC= total organic carbon, NS= not significant

Table 4: Maximal daily amount (Q, g) of fish that could be eaten without health risk by a person of 70 kg average body weight and Hazard Quotient (HQ) for *Barbus altianalis* (*B.a*, n=14) and *Brycinus sadleri* (B.s, n=4) obtained from River Rwizi at 50 (median, mg/g ww) and 95 percentile (mg/g ww) trace metal levels

	Al	As	Cd	Co	Cu	Hg	Pb	Zn
MRL (mg/kg/day)	1	0.0003	0.00001	0.01	0.01	0.0003	0.00036	0.3
MRL for 70 kg person (mg/kg/day)	70	0.021	0.007	0.7	0.7	0.02	0.02	21
Concentration in *B.a* at 50 %	0.2	0.03	0.001	0.026	0.2	0.017	0.005	6.8
Concentration in *B.a* at 95 %	1.0	0.13	0.002	0.101	0.5	0.06	0.028	34
Q for *B.a* at 50 %	329739	820	5448	26788	4648	1271	4949	3076
Q for *B.a* at 95 %	69142	162	3189	6938	1340	350	911	614
HQ for *B.a* at 50 %	0.0	0.04	0.006	0.001	0.01	0.025	0.01	0.010
HQ for *B.a* at 95 %	0.0	0.19	0.010	0.005	0.02	0.09	0.04	0.051
Concentration in *B.s* at 50 %	9.7		0.003	0.037	0.4	0.08	0.03	62
Concentration in *B.s* at 95 %	121	1.5	0.01	0.17	0.8	0.2	0.1	116
Q for *B.s* at 50 %	7183		2755	19077	1821	259	857	337
Q for *B.s* at 95 %	577	14	729	4161	892	89	256	182
HQ for *B.s* at 50 %	0.0	0.00	0.01	0.002	0.02	0.1	0.04	0.09
HQ for *B.s* at 95 %	0.1	2.2	0.04	0.008	0.04	0.4	0.1	0.17

B.a: *Barbus altianalis*, B.s: *Brycinus sadleri*, HQ: Hazard Quotient, MRL: Minimum Risk Levels (ATSDR, 2018), Q: Maximum health risk free quantity

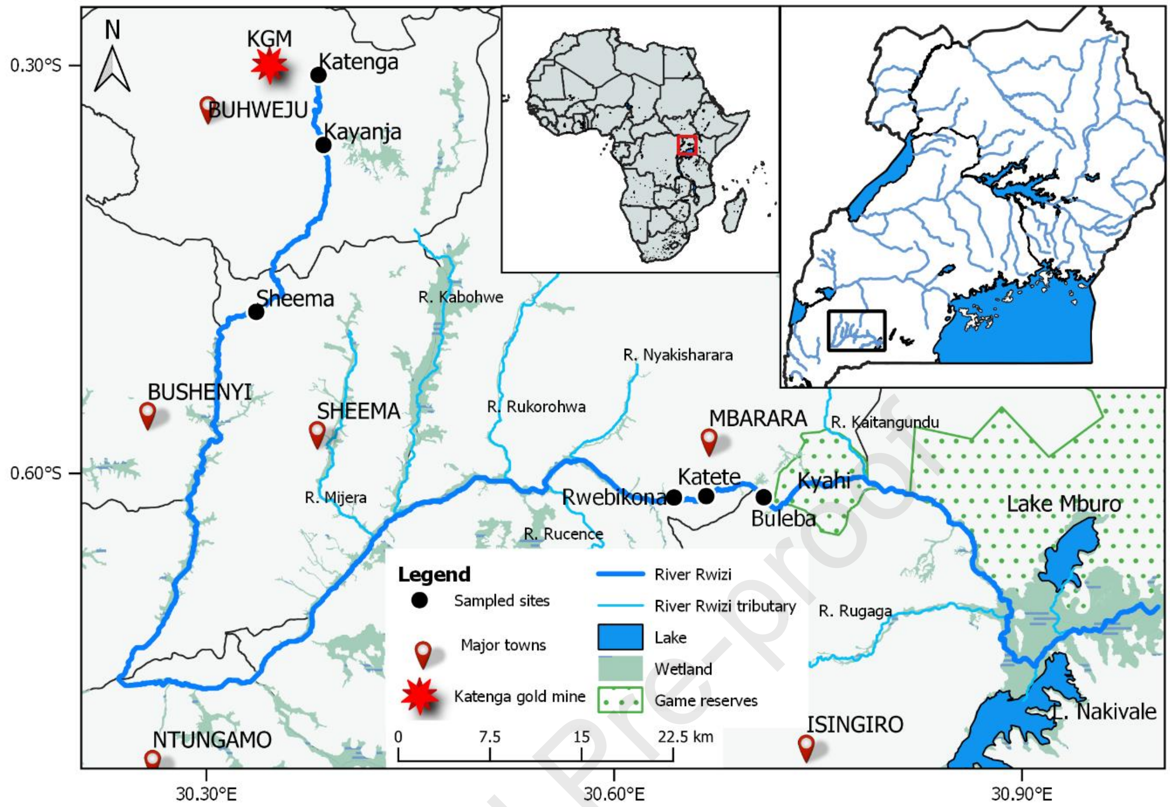

Figure 1. Study sites; upstream sites; Katenga (U1) near the gold mine; Kayanja (U2) and Sheema (U3); downstream sites; Rwebikona (D1); Katete (D2) and Buleba (D3)

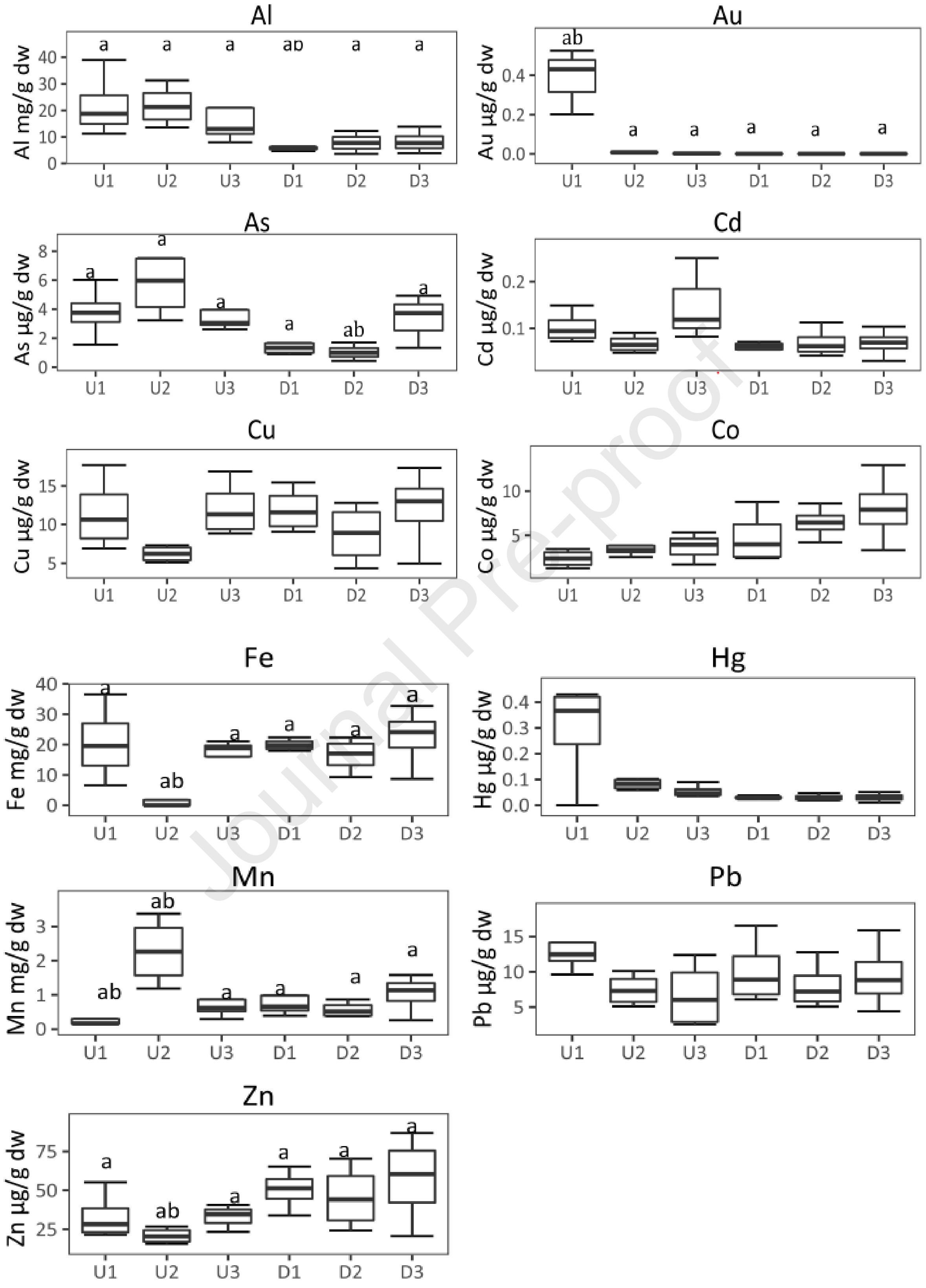

Figure 2: Trace metal concentrations in the sediment samples from the six sites in the Rwizi River. U1 to D3: sampled sites (table 1). Different letters indicate significant differences among sites, N=4

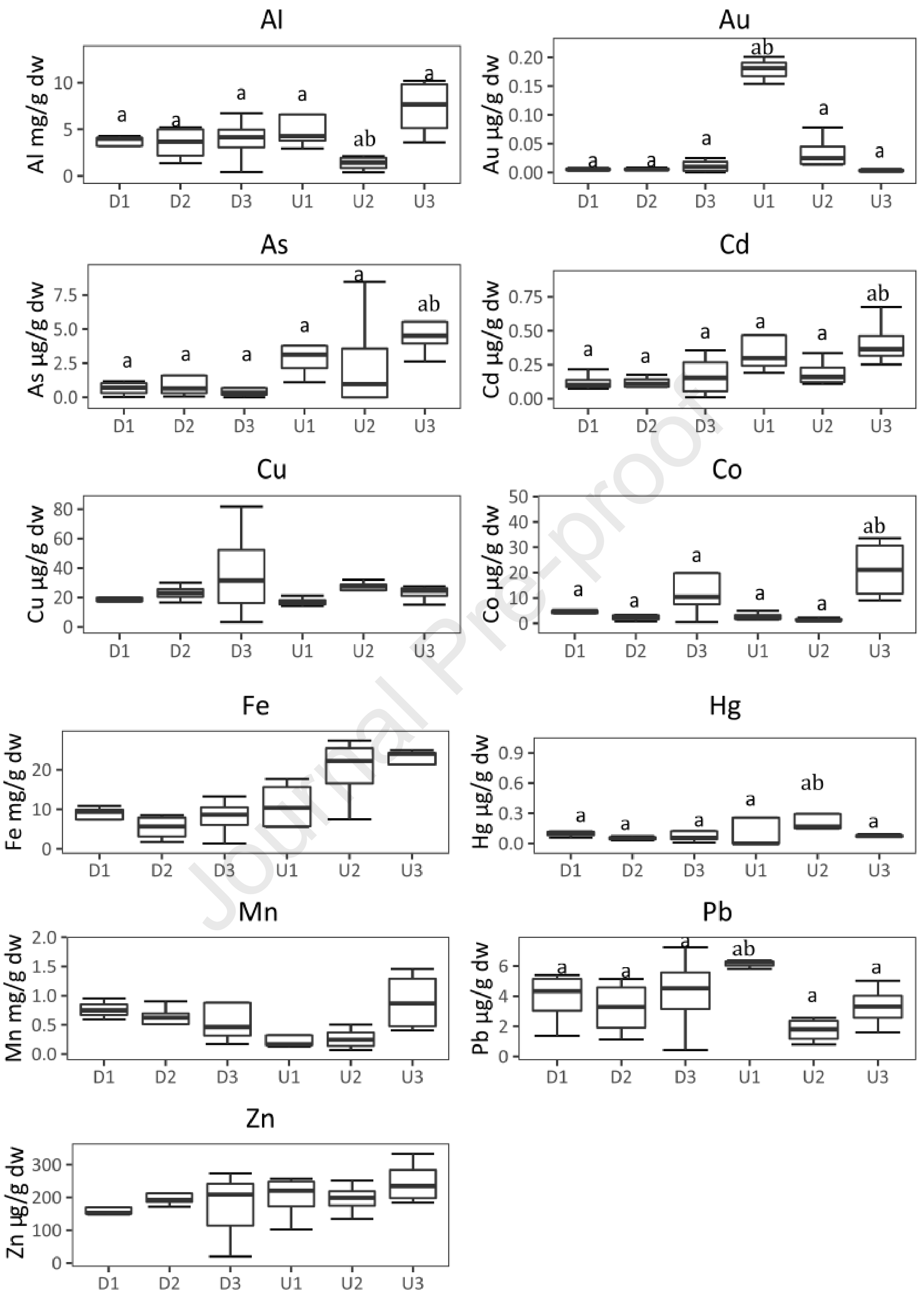

Figure 3: Trace metal concentrations in the damselfly larvae from the six sites in the Rwizi River. Different letters indicate significant differences among sites. N= 4. U1 to D3: sampling sites (table 1).

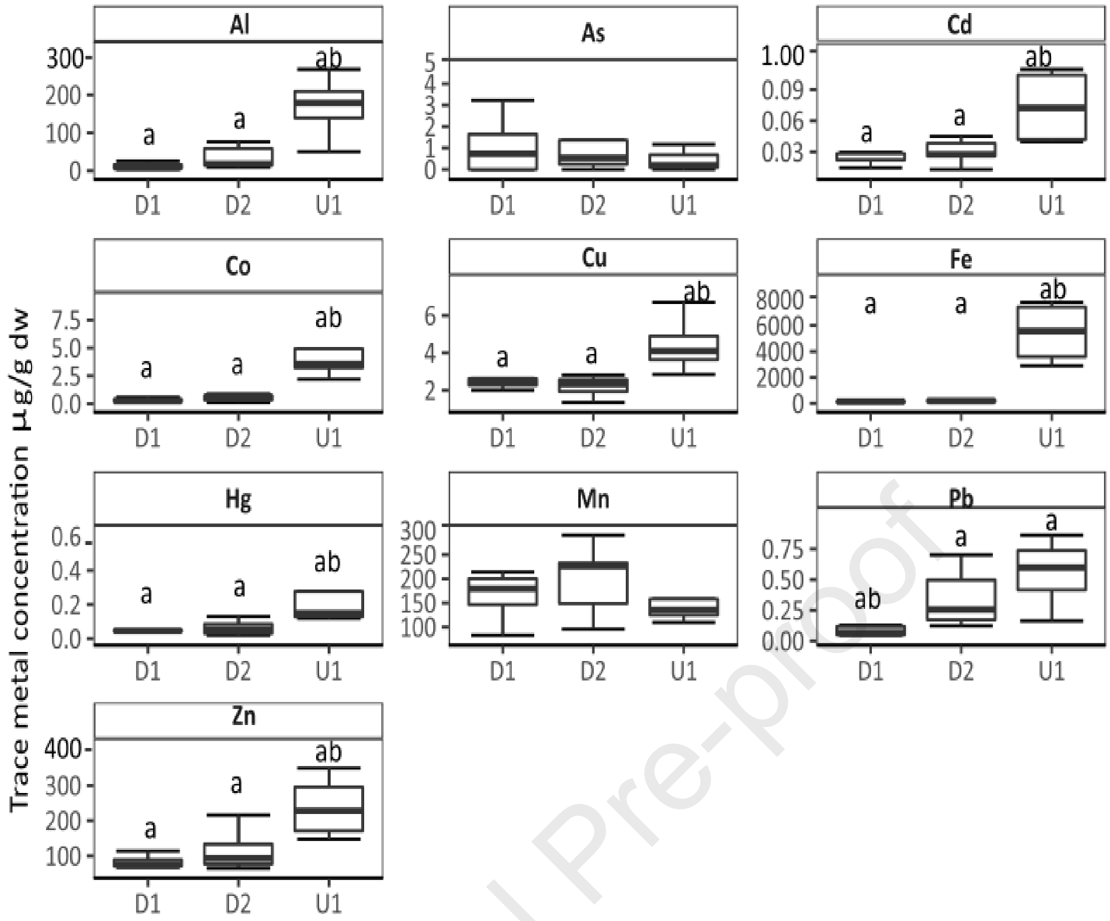

Figure 4. Trace metal concentration in the gill tissue of *Barbus altianalis* from Rwebikona (D1, n=5) and Katete (D2, n=9) and *Brycinus sadleri* from Kayanja (U2, n=5). Different letters indicate significant differences among sites.

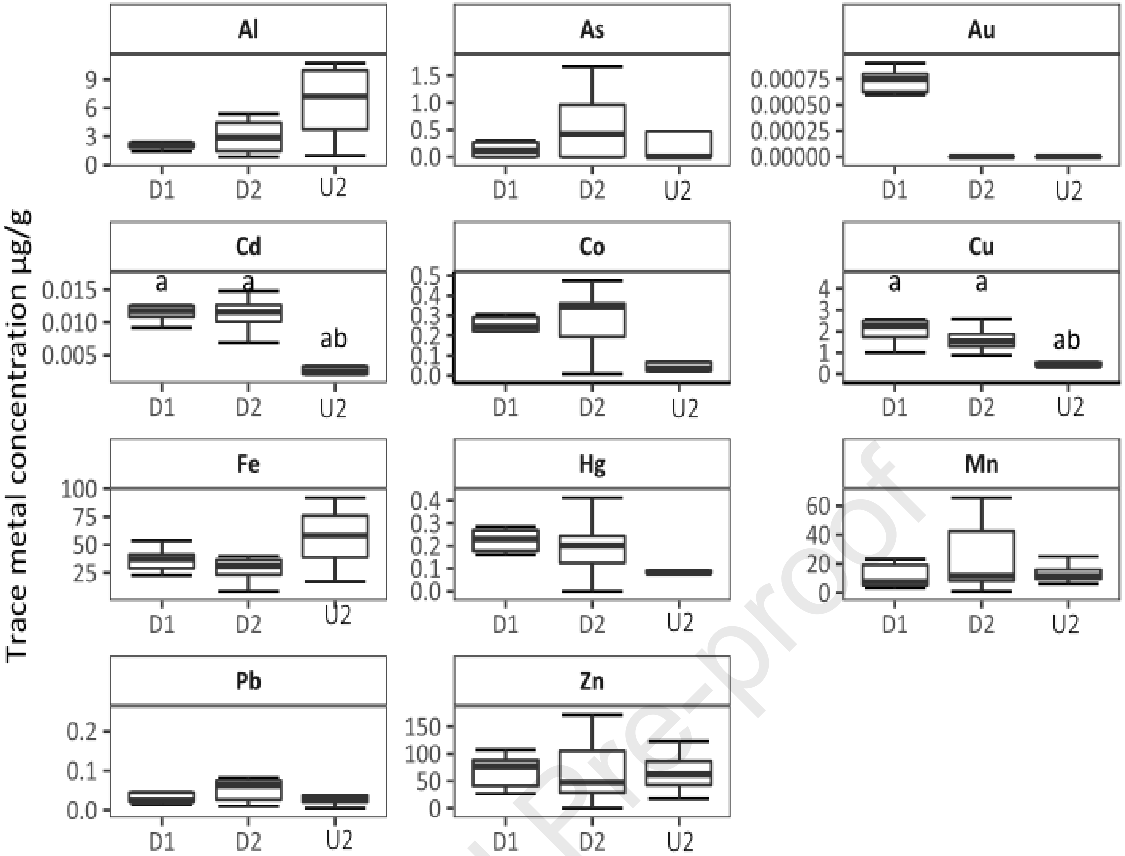

Figure 5. Trace metal concentrations in the muscle of *Barbus altianalis* collected at Katete (D2, n=9), Rwebikona (D2, n=5) and *Brycinus sadleri* from Kayanja (U2, n = 5). Different letters indicate significant differences among sites.

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