Heavy Metals and Arsenic in Sediment and Muscle Tissues of African Sharptooth Catfish (*Clarias gariepinus)* from Lake Ngami

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Abstract

Concentrations of trace elements were determined in sediment and muscle tissues of African sharptooth catfish (*Clarias gariepinus*) from Lake Ngami in Botswana. Sediment and tissue samples were acid digested and analyzed using ICP-OES. Element concentrations followed the order iron>>manganese>>zinc>copper>chromium>nickel>lead>arsenic in sediment and iron>zinc>arsenic>chromium>copper>lead>manganese>molybdenum in fish. Levels in the sediment were higher than in fish muscles for all but molybdenum, which was below detection in sediment; and arsenic, which was 2.8 times more in fish muscles. The concentration of arsenic in muscle tissues was also 11 times higher than reported in previous studies of catfish from African waters. The cancer risk and target hazard quotient (THQ) for each element in four groups (two Okavango Delta communities, average persons in Botswana and Sub-Saharan Africa) were assessed following the US-EPA risk assessment method. The THQ for only arsenic and chromium were above 1, suggesting that the two elements pose an appreciable risk of deleterious non-carcinogenic effects to human consumers of the fish. The cancer risk from exposure to arsenic in fish exceeded the acceptable level of 10^{-4} and ranged from 0.0004 to 0.007. These results support the need for future research of arsenic and chromium bioaccumulation in benthivorous fish species, which may be posing health risks to the Okavango Delta communities who rely on these fish as a protein source.

Keywords: Okavango Delta; African sharptooth catfish; target hazard quotient; cancer risk; risk assessment.

Introduction

When David Livingstone visited Lake Ngami in 1849, he was impressed by the massive shimmering sheet of water he witnessed. While indeed magnificent, the water and its source — the Okavango Delta — contain high levels of arsenic and heavy metals, relative to the world's average in freshwater environments (Huntsman-Mapila *et al*., 2006 and Mogobe *et al*., 2019). Trace elements in the Okavango Delta and its environ are suggested to be of geological origin and derived from the weathering of rocks in the catchment area (Huntsman-Mapila *et al*., 2006 and Rango *et al*., 2010). Other known sources of trace elements in the environment are anthropogenic, and include the combustion of fossil fuels, mining, and industrial and agricultural activities. Aquatic systems serve in most of the cases as the ultimate depository of these contaminants, where living organisms are then exposed to them. These elements can be assimilated into animal tissues and passed on through the food web. Consequently, apex predators like fish and aquatic birds can accumulate the contaminants to levels that are several times higher than the concentrations in the water (Bowles *et al*., 2001; Tasneem *et al*., 2020 and Yi *et al*., 2011). Globally, for most people, food is the main

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route of exposure to trace elements (Hajeb *et al*., 2014). Therefore, people who consume contaminated food in large quantities may face chronic toxicity. Although elements such as iron and manganese are essential elements, they become toxic at high concentrations. Other elements, including cadmium, mercury, lead and arsenic, offer no known biological benefit and can be toxic even at low concentrations (Canfield *et al*., 2003; Datta *et al*., 2009; Karagas *et al*., 2012).

In aquatic systems, trace elements generally bind to sediment particles through chemical and physical processes such as adsorption and coagulation and settle to the bottom. This removal from the water column limits the pool of the toxic elements that actively cycle in the ecosystem and enter the aquatic biota. Benthic organisms, however, experience elevated exposure to these elements since they live and feed in the sediment. As such, benthivorous fish can end up accumulating trace elements to a greater extent than pelagic fish (Wei *et al*., 2014 and Yi *et al*., 2011). Secondly, biogeochemical processes in the sediment, primarily driven by changes in reduction-oxidation potential, impact chemical speciation of trace elements, their bonding with sedimentary mineral and organic components, and influence their bioavailability, toxicity, transport, and fate. For example, the microbial degradation of organic matter with iron oxyhydroxide as an electron acceptor, releases bound arsenic into the water (McArthur *et al*., 2004; Mukherjee and Bhattacharya 2001). Also, in anoxic sediments, during the degradation of organic matter by sulfate reducing bacteria, mercury is transformed into more toxic methylmercury (Compeau and Bartha 1985). Thirdly, sediment resuspension events can trigger the release of sediment-bound elements into the water column, making them more available to aquatic biota (Seelen *et al*., 2018; Liu et al., 2020). To preserve aquatic systems and protect human health, it is important to determine the levels of trace elements in water, sediment, and biota. In addition, it is crucial to evaluate the human health risks that these elements pose to people who regularly consume fish from these water bodies.

Lake Ngami is an important waterbody in the northwestern part of Botswana. It provides food and income to the local communities due to its high productivity and diverse fish species (Mosepele *et al*., 2018). One of the most important and dominant species in the lake is the African sharptooth catfish (*Clarias gariepinus*), which is popularly sold nationally and in Southern Africa (Mosepele and Kolawole 2017). *C. gariepinus* is native to Africa and can be found in many African fresh waters (Cambray 2003). It is also a resilient species which can withstand harsh conditions (Cambray 2003). In Lake Ngami, *C. gariepinus* is the dominant fish species in mass during extremely low water levels (Mosepele et al., 2018).

While few studies have assessed the concentrations of trace elements in the sediments, in surface and ground-waters of Lake Ngami (Huntsman-Mapila *et al*., 2006; Schaller *et al*., 2016), none has determined the concentrations in fish from the lake. Therefore, the overall aim of this study was to assess the concentrations of trace elements in the sediments and in *C. gariepinus* during low water levels. The study sought to answer the following research questions: i) what are the concentrations of selected trace elements in the sediment and muscle tissues of *C. gariepinus* from Lake Ngami? ii) what is the relationship between element concentration and organic matter content in the lake sediment? and iii) what are the potential human health impacts of trace element exposure from the consumption of *C. gariepinus* from Lake Ngami? Health impacts were evaluated as the risk of suffering carcinogenic and non-carcinogenic deleterious effects using the Integrated Risk Information System (IRIS) developed by US Environmental Protection Agency (US-EPA). This is a desktop risk assessment method. Future studies should consider using more rigorous methods of risk assessment.

Materials and Methods

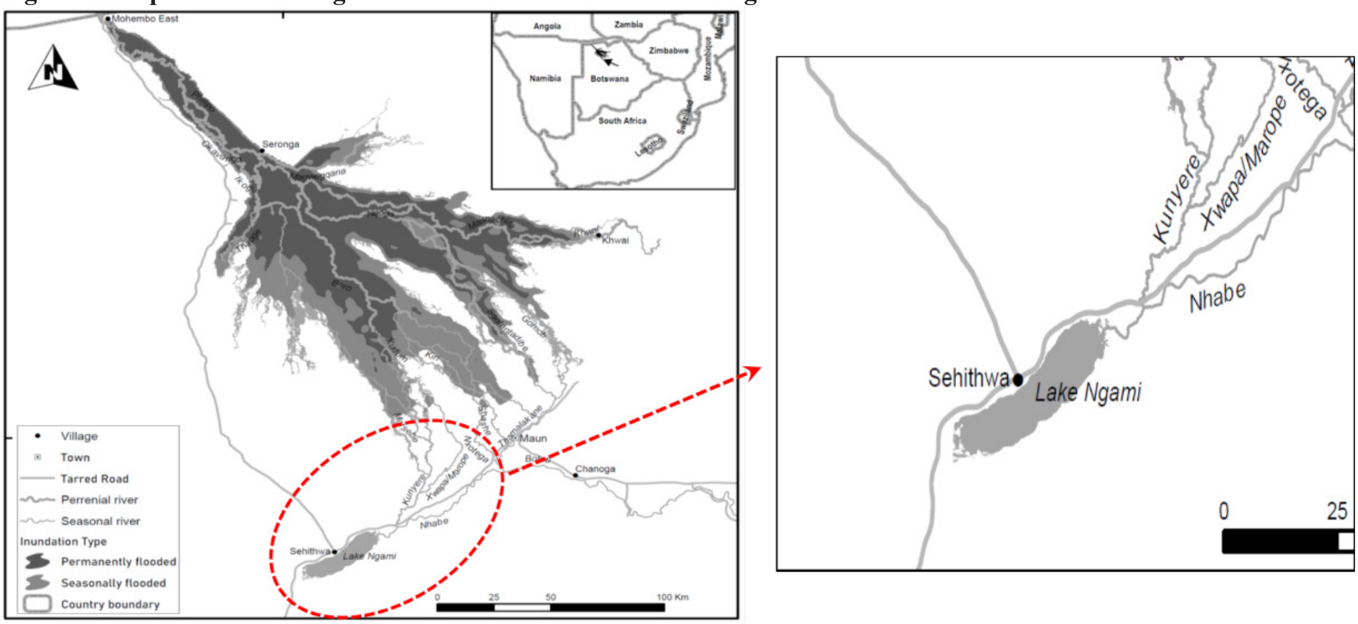
Study site

Lake Ngami is in the semi-arid region in the northwestern part of Botswana (Figure 1). It is an endorheic lake that receives 80% of its waters from floods that flow into the Okavango Delta from the highlands of

Angola (Shaw 1985). Floodwaters enter the Delta at Mohembo annually peaking between April and May and slowly traverse 250 km downstream reaching Maun three to four months later (Milzow *et al*., 2009). Outflow depends on water inflow via the Okavango River as well as local rainfall over the Delta and is only large enough to reach Lake Ngami during high flood periods (Gondwe *et al*., 2018; Wolski and Murray-Hudson 2006). Although agricultural activities are low in the river basin, water passes through villages and the Town of Maun on its journey to Lake Ngami. Most of the outflow from the Delta is through the Boro River which drains into Thamalakane River (Huntsman-Mapila *et al*., 2006). The Thamalakane bifurcates downstream to form the Boteti and Nhabe Rivers. Nhabe River joins the Kunyere River at Toteng and the combined flow spills into Lake Ngami.

Historically, the lake has desiccated on several occasions including the period from 1989 to 2004 (Kurugundla *et al*., 2019; Shaw 1985; Wolski and Murray-Hudson 2006). The flood extent of 2019 in the Okavango Delta was the lowest since 1974 at approximately 3500 km^2 (ORI MU 2021). Both the Kunyere and Thamalakane rivers, remained dry throughout the 2019 flood season. During our sampling expedition in October 2019, very little water remained in the lake. It eventually dried out completely for several months until the 2020 floods reached the lake.

Figure 1: Map of the Okavango Delta with the Position of Lake Ngami shown



Source: Map produced at UB-ORI GIS laboratory by D. Masego.

Specimens of African sharptooth catfish were bought on site from the local fishermen. As the lake was close to drying up, only 11 specimens were available for purchase during our sampling trip. Grab sediment samples were also collected around the lake. Three samples of the sediment (Sed 1-3) were scooped into separate Ziplock bags from a section of the lake that was mostly mud but with some water. Another three grab sediment samples (Sed 4-6) were scooped close by in recently (few weeks) dried up areas of the lakebed. All samples were immediately transported to the University of Botswana's Okavango Research Institute (ORI) environmental laboratory in a cooler box for processing and analysis.

Sample processing

At the laboratory, the fish were thoroughly washed with tap water on the outside after which the length was measured. A muscle tissue was then dissected from the dorsal side and the sex of the fish determined through examination of gonads. Tissue samples were rinsed with deionised water and stored in new Ziplock bags in the freezer. Sediment samples in Ziplock bags were also stored in the freezer at -4˚C. Frozen sediment and fish samples were freeze-dried then homogenised separately, to avoid inter-sample contamination, using a mortar and pestle. The mortar and pestle were wiped with a Kim wipe in between similar samples (i.e., fish to fish or sediment to sediment) but were thoroughly washed with soap and water and then acid cleaned between sediment and fish samples.

About 2 grams and 1 gram of dried and homogenised sediment and fish samples respectively, were digested in 16 mL of aqua regia (3 HCl:1 HNO_3) in covered glass tubes in a two-stage process (Tighe *et al*., 2004). Samples were first digested for 12 hours at 25°C and then a digestion block (AIM 600) set at 80˚C for 4 hours was used. Three preparation blanks and two duplicates each for sediment and fish samples were also digested. Digestates were then filtered into 100 mL volumetric flasks using acid rinsed Whatman 541 filter papers. The filtrates were diluted to 100 mL with 0.1N HNO_3 and stored in the fridge until analysis. All glassware used during the digestion and filtration process had been soaked in 10% HNO_3 acid for at least 24 hours then rinsed thoroughly with deionised water.

Sample analysis

Fish and sediment samples were analysed for 10 trace elements: iron, manganese, zinc, molybdenum, nickel, copper, cadmium, chromium, lead, and arsenic, using the ICP-OES (Perkin Elmer, Optima 2100DV). Elements were analysed in four batches to minimize interferences as recommended by US-EPA method 6010D. Multielement calibration standards for the four batches were prepared from 1000ppm stock solutions of individual elements (Merck, Fluka and Sigma Aldrich). All glassware used in preparation of standards were soaked in 10% HNO_3 for at least 24 hours followed by thorough rinsing with deionised water. A working standard (25ppm) containing all the elements in a particular batch was first prepared from the single element stock solutions. Three to six calibration standards were then prepared from the working standard. All the calibration curves had a high correlation coefficient ($R^2 > 0.99$). Concentrations of the elements in all samples were within the calibration curve except for iron in sediment which required further dilution. For quality control, analytical blanks and standard checks were included periodically during the run. The average recovery of the standards was 106% with the lowest and highest recovery recorded at 74% and 128% for nickel and iron, respectively.

Organic matter content as % Loss on Ignition (LOI) was determined on dried and homogenised sediment samples baked at 450°C for 5 h (Heiri *et al*., 2001). Soil pH was measured using a pH meter (WTW Inolab pH7110, Weilheim, Germany) after mixing 20g of freeze-dried sediment with 50 mL of deionised water (Hendershot *et al*., 2007). Sediment conductivity was measured two hours later, on the same sediment-water mixture using a WTW Inolab Cond7110 meter, Weilheim, Germany (Miller and Curtin 2007).

Health risk assessment

To determine if the concentrations of the elements in the fish pose a health hazard to human consumers, we calculated the estimated weekly intake (EWI) for each element (µg $kg\text{-}bw^{-1}$ wk^{-1}) and used it to evaluate the risk of suffering from deleterious non-cancer effects and from cancer as per the US-EPA Integrated Risk Assessment method. The EWI was calculated as:

$$EWI = \frac{C \times IR_{week}}{bw} \qquad \text{equation 1}$$

Where C is the concentration of the element in fish in µg/g wet weight, bw is body weight, and IR_{week} is the ingestion rate of fish per week. IR_{week} in the Okavango Delta is 680 g for low consumers (Delta-L), and 1400 g for high consumers (Delta-H) (Black *et al*., 2011). For the average person in Botswana and sub-Saharan Africa who consume much lower quantities of fish, their IR_{week} is 71 g and 171 g, respectively (FAO 2021a; 2021b).

Average body weight was assumed to be 60.7 kg for sub-Saharan Africans and 64.0 kg for Batswana (Walpole *et al*., 2012, Republic of Botswana and WHO 2015). Batswana in the Okavango Delta occupy rural land and likely have a body weight lower than that of an average Motswana (Letamo 2011). The actual EWI for Delta-L and Delta-H would thus be higher than what was calculated in this study. We also make no distinction between the weight of a man versus a woman. In Botswana, women are on average heavier than men, but the difference is slight at 1% (Republic of Botswana and WHO 2015). The gender specific EWI in each group would thus deviate slightly from what is reported here.

Non-carcinogenic risk of fish consumption

The deleterious non-carcinogenic risk posed by trace elements in the catfish of Lake Ngami was evaluated by calculating the target hazard quotient (THQ).

$$\text{THQ} = \frac{EWI}{RfD \times 7} \qquad \text{equation 2}$$

Where *RfD* is the oral reference dose of a contaminant in µg element kg-bw^{-1} day^{-1}. The *RfD* is the allowable maximum amount of a contaminant that a person can ingest on a regular basis and suffer negligible risk of deleterious health effects. The *RfD* and a similar metric known as the Provisional Tolerable Weekly Intake (PTWI) are published by US-EPA and WHO (US-EPA 2020; WHO 2020). The *RfD*/PTWI for arsenic and lead have been withdrawn and are currently being re-assessed as recent studies have shown that the previous values were not health protective (WHO 2011). Here, we have used the latest published *RfD*/PTWI for arsenic and lead, acknowledging that this may inaccurately represent the risk. Updated guidelines, once published, should be considered by future readers of this study.

As equation 2 shows, THQ is simply the ratio of element intake by an individual to the maximum allowable intake. THQ greater than 1 indicates an appreciable risk of an individual suffering deleterious non-cancer effects due to chronic exposure to a contaminant (USEPA 2000).

Carcinogenic risk of fish consumption

The cancer risk is the probability of developing cancer in a lifetime from exposure to a carcinogen, in this case, lead and arsenic. It is calculated as follows:

$$CR = \frac{SF \times EWI \times 10^{-3}}{7} \qquad \text{equation 3}$$

Where, SF is the slope factor (0.0085 for lead and 1.5 for arsenic) (US-EPA 2000). The SF is defined as the increased cancer risk suffered in a lifetime due to chronic exposure to 1 mg element kg-bw^{-1} day^{-1} (USEPA 2000). A cancer risk of above 10^{-4} (i.e., 1 for every 10,000 people) from exposure to a contaminant is considered unacceptable (US-EPA 2018).

Results

The concentrations of all the trace elements in fish and sediment samples were above the method detection limit (MDL, determined as mean of analytical blank + 3 standard deviations) except for cadmium, nickel, and molybdenum. Cadmium levels were below detection for all sediment and fish samples. Molybdenum was below detection in all the sediment samples and 3 fish samples, and nickel was below detection in all but two fish samples. As such, concentrations of nickel in fish, molybdenum in sediment and cadmium in fish and sediment are not reported. The concentrations of copper, cadmium, and arsenic in the preparation blanks were also below detection. For all the other elements, sample concentration was corrected for the concentration in the preparation blank. In terms of reproducibility, the average relative percent difference (RPD) calculated for sediment and fish duplicate samples (weighed twice and digested separately) was 15% and 28%, respectively.

The concentrations of trace elements in the sediment are presented in Figure 2a. As can be seen, iron was found in the greatest amount followed by manganese, zinc, copper, chromium, nickel, lead, and arsenic in that order. Percent organic matter in the sediment, determined as % LOI, varied between 15% and 22%. Sed 1-3 had slightly more organic matter and moisture content and a higher pH and electrical conductivity than Sed 4-6 (Table 1).

Figure 2: Concentrations of trace elements in sediment (a) and in muscle tissues of *C. gariepinus* (b) from Lake Ngami. The concentrations of iron and manganese in the sediment and iron and zinc in the muscles are shown on the left axis of a and b, respectively.

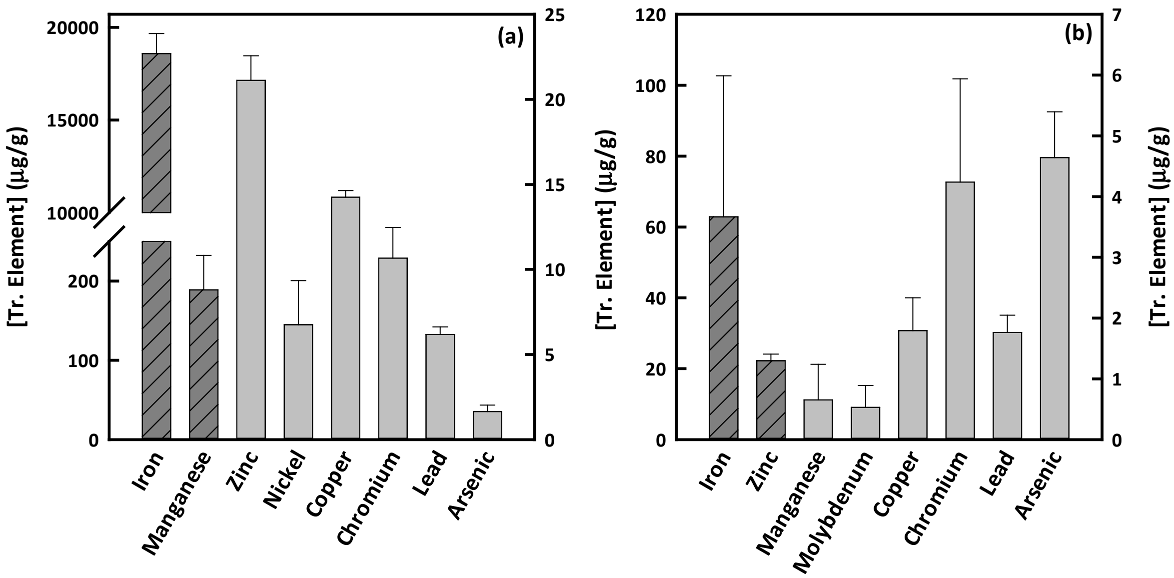


Table 1: Moisture, organic matter (%LOI) content, pH and electrical conductivity (EC) of sediments from Lake Ngami.

Sediment	Moisture (%)	LOI (%)	pH	EC (µS cm^{-1})
Sed 1	61	20	7.3	1151
Sed 2	65	22	7.4	1262
Sed 3	63	22	7.5	1265
Sed 4	52	16	6.4	560
Sed 5	50	17	6.7	608
Sed 6	46	15	6.0	435

Multivariate correlation analysis using SPSS statistical software (version 27) revealed that the organic matter content was significantly positively related to zinc, manganese, and arsenic, but negatively related to lead and chromium in the sediment ($p < 0.05$, Table 2). Though not significant at the 95%

confidence interval, organic matter was also positively related to nickel and iron and negatively related to copper. Concentrations of iron, manganese, zinc, nickel, and arsenic in the sediment were also positively related to each other though not all were significant at $p < 0.05$. On the other hand, lead, chromium, and copper were positively related to each other but the relation between lead and chromium with copper was not significant ($p > 0.05$). In essence, the elements formed two groups. Group A consisting of iron, manganese, zinc, nickel, and arsenic and Group B containing copper, chromium, and lead. Elements within a group were positively related to each other and negatively related to elements in the other group.

Fish samples of *C. gariepinus* bought from the local fishermen were composed of six adult females and five adult males ranging from 565–793mm in length (mean 693 ± 75mm). Average element concentrations in fish are given in Figure 2b above. Concentrations in fish decreased as follows: iron>zinc>arsenic>chromium>copper>lead>manganese>molybdenum. No relation was observed between the concentrations in muscles and sex or length. Generally, trace elements in the fish were found at a lower concentration than in the sediment. Arsenic, however, had a concentration 2.8 times higher in fish than in sediment. Molybdenum was also higher in fish at 0.5 µg/g dw and below detection in sediment. To illustrate the magnitude of enrichment in the fish muscles compared to the sediment, biota-sediment accumulation factors (BSAF) were calculated as the ratio of element concentration in fish to the concentration in sediment. BSAF for the elements were as follows: 0.003 (iron and manganese), 0.126 (copper), 0.285 (lead), 0.398 (chromium), 1.054 (zinc) and 2.818 (arsenic). The BSAF for molybdenum and nickel could not be calculated as their concentrations in sediment and fish, respectively, were below detection. The detection of molybdenum in fish and not in sediment suggests that the element has a relatively high bioaccumulation potential. The opposite is true for nickel.

Table 2: Correlation of trace elements and organic matter as %LOI in the sediment of Lake Ngami

	Iron	Manganese	Zinc	Nickel	Copper	Chromium	Lead	Arsenic
Manganese	0.78							
Zinc	0.78	**0.92**						
Nickel	0.71	0.75	**0.93**					
Copper	-0.21	-0.63	-0.30	-0.02				
Chromium	-0.46	-0.78	-0.60	-0.53	0.74			
Lead	-0.81	**-0.99**	**-0.85**	-0.69	0.67	**0.83**		
Arsenic	0.61	**0.86**	**0.92**	**0.93**	-0.33	-0.76	**-0.82**	
% LOI	0.69	**0.99**	**0.87**	0.69	-0.70	**-0.82**	**-0.98**	**0.85**

values in bold are statistically significant at $p<0.05$.

The risk of suffering negative health effects from the consumption of *C. gariepinus* from Lake Ngami was evaluated for an average adult in sub-Saharan Africa, Botswana, and the Okavango Delta, consuming 171g, 71g and 680/1400g of fish per week, respectively, as explained above. For each consumer group we calculated the EWI of each element and determined the corresponding THQ and cancer risk following the US-EPA risk assessment method (Table 3 and 4). As mentioned earlier, THQ is the ratio of estimated intake of element to the maximum allowable intake. THQ value of 1 indicates the maximum allowable exposure. Further declining values are associated with lowering risks of adverse health outcomes.

Table 3: The estimated weekly intake (EWI) of trace elements, for the average person in sub-Saharan Africa, Botswana and for low and high fish consumers in the Okavango Delta, consuming *C. gariepinus* from Lake Ngami. The reference dose and maximum allowable weekly intake are shown for each element.

Trace Element	SS Africa (µg kg-bw^{-1} wk^{-1})	Botswana (µg kg-bw^{-1} wk^{-1})	Delta-L (µg kg-bw^{-1} wk^{-1})	Delta-H (µg kg-bw^{-1} wk^{-1})	Ref. Dose (µg kg-bw^{-1} day^{-1})[a]	Max. Intake (µg kg-bw^{-1} wk^{-1})[c]
Iron	57	22	214	441	800[b]	5600
Manganese	1	0.2	2	5	140	980
Zinc	20	8	76	156	300	2100
Molybdenum	0.5	0.2	2	4	5.0	35
Copper	2	1	6	13	500[b]	3500
Chromium	4	2	14	30	3.0	21
Lead	2	1	6	12	3.6[b]	25
Arsenic	4	2	16	33	0.3	2.1

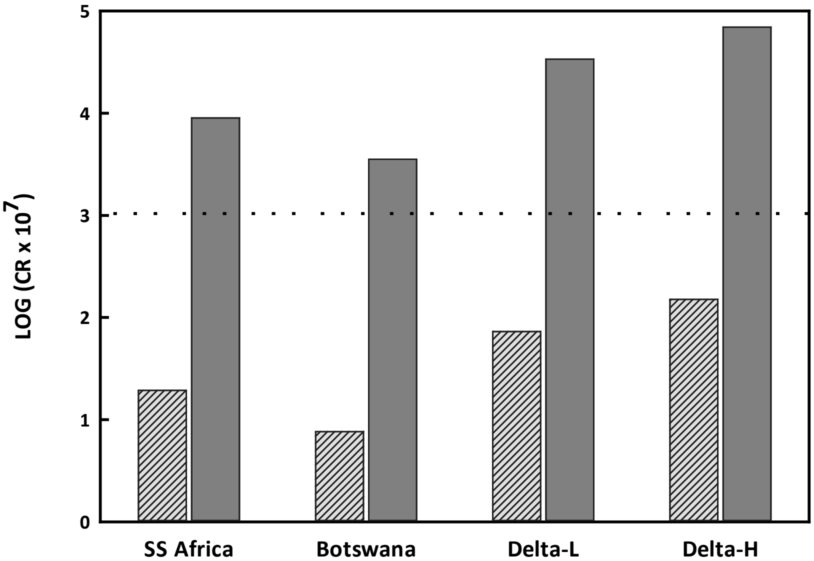
[a] Obtained from USEPA (2020) [b] Obtained from WHO (2020) [c] Calculated as Ref. Dose x 7

The THQs for all the measured elements in the four geographically classified groups of people were less than one, except for arsenic and chromium. The THQ for chromium was above 1 (at 1.4) for the Delta-H group only (Table 4). THQ for arsenic was above 1 in all groups except the Botswana group (2.0 for Sub-Saharan Africa, 7.5 for Delta-L and 15.5 for Delta-H). The risk of cancer was evaluated for the relevant carcinogenic elements arsenic and lead using equation 3. The cancer risk due to lead exposure for all consumer groups was less than the maximum acceptable risk level (ARL) of 0.0001. Exposure to arsenic resulted in a cancer risk above the maximum ARL and ranged from 0.0004 in the Botswana group to 0.007 in Delta-H (Figure 3).

Table 4: Target hazard quotient (THQ) of trace elements estimated for the average person in sub-Saharan Africa, Botswana, and for low and high fish consumers in the Okavango Delta, consuming *C. gariepinus* from Lake Ngami.

Trace Element	SS Africa	Botswana	Delta-L	Delta-H	% THQ
Iron	0.0	0.0	0.0	0.1	0
Manganese	0.0	0.0	0.0	0.0	0
Zinc	0.0	0.0	0.0	0.1	0
Molybdenum	0.0	0.0	0.1	0.1	1
Copper	0.0	0.0	0.0	0.0	0
Chromium	0.2	0.1	0.7	**1.4**	8
Lead	0.1	0.0	0.2	0.5	3
Arsenic	**2.0**	0.8	**7.5**	**15.5**	88
Total	2.3	0.9	8.6	17.7	100

Figure 3: The risk of cancer from exposure to lead (lined) and arsenic (solid) to an average person in sub-Saharan Africa, Botswana and to low and high fish consumers in the Okavango Delta, consuming *C. gariepinus* from Lake Ngami. CR is the cancer risk factor calculated following the US-EPA risk assessment method with 0.0085 and 1.5 as the slope factors for lead and arsenic, respectively. Dotted line shows the maximum acceptable risk level.



Discussion

Trace elements and organic matter in sediment

The correlation of different parameters measured in the sediment is presented in Table 2 above. Results suggest that the elements formed two groups. Iron, manganese, zinc, nickel, and arsenic, which were positively related to each other and to organic matter, formed one group (Group A), while lead, chromium, and copper, which were positively related to each other but negatively related to organic matter, formed another group (Group B). These relationships imply that there may be two different sources of the elements at Lake Ngami. Previous studies have suggested that trace elements in the Okavango Delta originate from the weathering of mafic-ultramafic and proterozoic granitoids rocks in the catchment area (Huntsman-Mapila *et al*., 2006; Mogobe *et al*., 2019). Research also shows that regional dust from places such as the Makgadikgadi salt pan could be contributing up to 80% of fine-grained material in Okavango soils (Humphries *et al*., 2014 and 2020).

While both atmospheric deposition and the weathering of rocks likely contribute to the presence of elements in the Okavango Delta, one may be more important than the other for certain elements. Elements in Group A include the more abundant metals on the earth's crust i.e., iron and manganese; here too found at a much higher sediment concentration than the other elements. Thus, we hypothesise that Group A elements at Lake Ngami mostly originate from the weathering of rocks. These likely enter Lake Ngami via riverine inputs transported with organic matter, and hence their positive relation with organic matter. On the other hand, Group B elements are likely introduced into Lake Ngami via atmospheric deposition while associated with inorganic particles in dust. Areas with more dust deposited would have more B elements and more inorganic material than areas which receive less dust. Consequently, these areas would also have a lower fraction of organic matter content.

Group B elements entering Lake Ngami via atmospheric deposition could come from both natural and anthropogenic sources. Anthropogenic sources of the elements such as mining activities, including copper mining, happen in the vicinity of Lake Ngami and could be an important source of lead, chromium, and copper.

Comparison of trace elements in fish with previous studies

Concentrations of trace elements in the muscles of *C. gariepinus* from Lake Ngami are presented in Figure 2b above and compared to previous studies of catfish in African waters in Table 5 below. The study closest to the current study in location is that of Mogobe *et al*., (2015) which assessed zinc, manganese, copper and iron in the Silver catfish, *Schilbe intermedius*, from Chanoga Lagoon, Botswana (Figure 1). Levels of zinc, manganese, copper and iron in *S. intermedius* were found to be almost 6, 18, 3, and 2 times higher respectively, than those we found in the catfish from Lake Ngami (Mogobe *et al*., 2015). Like Lake Ngami, Chanoga Lagoon is located at the distal end of the Okavango Delta. Both systems receive their inflow from the Delta's outflow. The Delta's outflow through the Thamalakane River divides after Maun, to form the Boteti River (which flows to Chanoga) and the Nhabe River (which joins the Kunyere and carries water to Lake Ngami). As both Lake Ngami and Chanoga Lagoon receive their waters from the Delta's outflow after it had passed the Town of Maun, it is unlikely that differences in human activities along the split rivers would contribute significantly to differences in element concentrations in the two systems. Also, Lake Ngami is endorheic while Chanoga Lagoon is not. One would, therefore, expect the concentrations of the elements to be higher at Lake Ngami than at Chanoga. The higher concentrations of trace elements at Chanoga are thus likely due to a greater bioavailability of the elements or due to inter-species differences in uptake and assimilation.

In South Africa, Jooste *et al*., (2015) reported on average six times more iron, five times more manganese and chromium, four times more zinc, and two times more copper and lead in *C. gariepinus* in the Olifants River than those we recorded at Lake Ngami. The concentration of arsenic, however, was six times lower than in our study (Jooste *et al*., 2015). When compared to *C. gariepinus* from River Nile, our study detected generally lower levels of all the elements except arsenic and molybdenum, which were not determined in the River Nile fish samples (Osman and Kloas 2010). In Lake Geriyo in Nigeria, Mudfish (*Clarias anguillaris*) when compared to *C. gariepinus* of Lake Ngami, had seven times more lead and two times more copper, but similar levels of zinc (Bawuro *et al*., 2018). In the lower Niger River, *C. gariepinus* had similar levels of lead and iron but six times more manganese than found in the catfish of Lake Ngami (Madu *et al*., 2017). *C. gariepinus* in central Ethiopia were also found to have five times more manganese, two times more lead, copper, and chromium, but similar levels of zinc and iron than those in Lake Ngami (Kassegne *et al*., 2019). Contrary to the studies above which mostly found similar or higher levels of trace elements than we recorded, Dsikowitzky *et al*., (2013), Nhiwatiwa *et al*., (2011), and Ouattara *et al*. (2020) reported lower levels of the elements (between 3-164 times) in *C. gariepinus* and Bagrid catfish (*Chrysichthys nigrodigitatus)* from rivers and lakes in Ethiopia, Zimbabwe, and Côte d'Ivoire, respectively.

Generally, the concentrations of trace elements in muscles tissues were on average lower at Lake Ngami than in the previous studies, for all elements except arsenic (Table 5). Arsenic in the Lake Ngami catfish was 11 times higher. All other elements (minus molybdenum - not reported in previous studies), were on average almost half or less than half of reported values in past studies (Table 5). A similar trend was observed when only studies of *C. gariepinus* were compared. Clearly, there is a substantial enhancement of arsenic in *C. gariepinus* of Lake Ngami. Although more studies are needed to confirm this trend, our results are in agreement with previous studies in the Okavango Delta and the Central Kalahari Game Reserve which have reported elevated arsenic concentrations of geological origin in the ground water, and higher than the world's average in surface waters (Huntsman-Mapila *et al*., 2006; Mladenov *et al*., 2014; Selebatso *et al*., 2018). High arsenic levels in the water would lead to greater uptake and accumulation in biota. While it has been known for years that the waters and sediments of the Okavango Delta region have high arsenic levels, this is the first study to report that the region also has elevated concentrations of arsenic in fish.

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Table 5: Range and average concentrations of trace elements (µg/g ww) in muscle tissues of catfish in previous studies

	Iron	**Manganese**	**Zinc**	**Copper**	**Chromium**	**Lead**	**Arsenic**
Current study[a,b]	4.67–49.38 (20.13 ± 12.7)	0.01–0.58 (0.22 ± 0.2)	6.08–8.26 (7.14 ± 0.6)	0.45–1.09 (0.58 ± 0.2)	0.51–2.05 (1.34 ± 0.5)	0.42–0.67 (0.58 ± 0.1)	1.15–1.92 (1.47 ± 0.3)
Mogobe at al. (2015)	37.3	3.9	39.5	1.7			
Jooste et al. (2015)[a]	22.5–236.9 (129.7)	0.8–1.5 (1.15)	6.6–49.8 (28.2)	1.0–1.4 (1.2)	3.4–9.6 (6.5)	0.4–1.7 (1.05)	0.2–0.3 (0.25)
Nhiwatiwa et al. (2011) [a]	1.06–2.88 (1.82)		2.00–2.23 (2.08)	trace–0.11 (0.06)	0.29–0.76 (0.48)	trace–0.08 (0.05)	
Dsikowitzy et al. (2013) [a]					0.029–0.232 (0.082)	0.004–0.054 (0.019)	0.006–0.017 (0.009)
Kassegne et al. (2019) [a,b]	42.30–89.55 (21.41)	1.32–7.22 (1.08)	4.88–38.77 (5.91)	ND–4.93 (1.14)	0.93–16.75 (2.44)	ND–5.32 (1.16)	
Osman and Klaos (2010) [a]	26.49–85.29 (54.2)	4.27–14.80 (8.5)	11.62–71.85 (46.2)	1.01–5.48 (3.7)	2.37–5.47 (4.3)	5.90–14.51 (9.3)	
Bawuro et al. (2018)			4.17–5.35 (4.76)	0.5–2.02 (1.26)		0.23–8.44 (4.34)	
Madu et al. (2017) [a,b]	11.98	1.32				0.83	
Ouattara et al. (2020)			1.04–1.28 (1.12)	0.04–0.16 (0.11)		0.08–0.12 (0.10)	0.12–0.20 (0.16)
Average concentration in previous studies	42.73 ± 46.5	3.18 ± 3.2	18.25 ± 19.2	1.31 ± 1.2	2.30 ± 2.7	2.11 ± 3.2	0.14 ± 0.1
Average concentration in previous studies of *C. gariepinus*	43.82 ± 51.9	3.00 ± 3.6	16.48 ± 20.6	1.21 ± 1.5	2.77 ± 2.7	2.07 ± 3.6	0.13 ± 0.2

[a] Concentrations of trace elements in *Clarias gariepinus*

[b] Converted to wet weight by multiplying reported concentrations in dry weight by 0.32 (Akpambang 2015)

Human health risk assessment

The risks of suffering from adverse health effects (cancerous and non-cancerous) were evaluated following the integrated risk assessment method developed by US-EPA. First, the Estimated Weekly Intake of each element was calculated for four consumer groups (Delta-H, Delta-L, Batswana, and sub-Saharan Africans), and the corresponding Target Hazard Quotients for the elements was calculated as the ratio of element intake to the maximum allowable intake (equation 2, Table 3 and 4). THQ > 1 indicates an appreciable risk of suffering non-cancerous deleterious effects. Our results show that except for arsenic in three groups (sub-Saharan Africa, Delta-H and Delta-L) and chromium in one group (Delta-H), the THQ was < 1. This implies that generally heavy metals in the catfish of Lake Ngami do not individually pose any potential risk of deleterious non-carcinogenic effect to human consumers.

However, negative effects of trace elements can be additive or interactive thereby exacerbating the overall health effects of trace element exposure on a person (Yi *et al*., 2011). The sum of THQ values for all except the Botswana group was above one, suggesting increased risk from combined exposure. This is especially so for Delta-L and Delta-H with total THQs of 8.6 and 17.7, respectively (Table 4). The high total THQ was driven by arsenic, contributing 88% of the overall risk. Without arsenic the combined risk of deleterious effect in all the groups was significantly reduced. Apart from arsenic, levels of chromium in the catfish and to a lesser extent lead were also concerning. While all other metals contributed < 1% to the overall THQ, chromium and lead contributed 8% and 3% respectively. The THQ for lead was less than one in all groups but approached one at 0.5 in the Delta-H community. Chromium had a THQ above one in the Delta-H community and approaching one at 0.7 for Delta-L consumers (Table 4). Therefore, our results suggest that arsenic, chromium, and to some extent lead, pose a great risk of deleterious non-cancer effects to fish consumers in the Okavango Delta.

Our results also show that, the cancer risks evaluated for arsenic and lead using equation 3, were low from lead exposure but substantially high from arsenic exposure. In all consumer groups, the exposure to arsenic in the catfish resulted in a cancer risk above the ARL of 10^{-4} (Figure 3). This implies that more than one in 10,000 people would get cancer if they were to regularly consume *C. gariepinus* from Lake Ngami. The ARL for cancer ranges between 10^{-6}–10^{-4} (US-EPA, 2018). The cancer risk due to arsenic exposure in this study reached almost 10^{-2}. Specifically, cancer risk in the Delta-L and Delta-H groups, was 34 and 70 times higher than the ARL, respectively.

It is worth noting that the toxicity of arsenic varies with the speciation of the element (Taylor *et al*., 2017). In aquatic systems, arsenic exists as either inorganic arsenic (iAs) or organic arsenic (orgAs) classified broadly in to four groups: arsenobetaine, arsenosugars, arsenolipids and methylated arsenicals (Taylor *et al*., 2017). OrgAs is the form which mainly bioaccumulates up the food chain (Hasegawa *et al*., 2019; Taylor *et al*., 2017). In our risk assessment calculations, we used the reference dose for iAs as that for orgAs is yet to be established (Taylor *et al*., 2017). Since OrgAs is generally less toxic than iAs, our results overestimate the risk from arsenic exposure.

Nonetheless, recent studies have shown that some forms of orgAs could be cytotoxic (Meyer *et al*., 2014; 2015). Additionally, in the fruit fly *Drosophila melanogaster*, arsenolipids have been shown to cross the blood-brain barrier and accumulate in brain cells (Niehoff *et al*., 2016). Arsenolipids make up as much as 62% of total arsenic in oily fish (Lischka *et al*., 2013; Taleshi *et al*., 2010). Also, though the levels of iAs in fish are lower than orgAs, demersal fish accumulate increased levels of the more toxic iAs (Lorenzana *et al*., 2009). *C. gariepinus* is both a demersal and oily fish. It would thus contain more iAs and arsenolipids than other fish species with similar levels of total arsenic. Compared to other fish species, regular consumption of *C. gariepinus* from Lake Ngami, especially during periods of low floods, would therefore pose a greater health risk than assumed.

Conclusion

This study has provided important information on the levels of trace elements at Lake Ngami during low flood season. It is shown, based on Pearson analysis, that trace elements in the sediment formed two distinct groups. Iron, manganese, zinc, nickel, and arsenic formed one group (A) positively related to each other and to organic matter; and copper, chromium, and lead formed another group (B), positively related to each other and negatively related to organic matter. We hypothesise that group A elements at Lake Ngami possibly originate from the weathering of rocks while Group B elements come mostly from atmospheric deposition.

The study has also shown that, for all except arsenic, average concentrations of trace elements in muscles tissues of catfish from African waters in past studies, were higher than those from Lake Ngami. Arsenic, however, was 11 times higher in the Lake Ngami fish. The much higher concentration of arsenic in the fish at Lake Ngami relative to the previous studies, is attributed to higher uptake, possibly caused by a higher concentration of arsenic in the lake waters. Our results also show that to the average person in Botswana consuming *C. gariepinus* from Lake Ngami, the risk of non-cancerous deleterious effects is low. However, to the Okavango Delta communities with high fish consumption rates, the risk is higher and comes mostly from exposure to arsenic and chromium. In addition, exposure to arsenic in all the consumption groups resulted in a cancer risk greater than the acceptable risk level of 10^{-4}. Though most of the arsenic in the fish is likely the less toxic organic arsenic, the actual toxicity of organic arsenic is yet to be fully established.

As mentioned earlier, water levels at Lake Ngami fluctuate immensely in a year and between years from high water levels to complete desiccation. Here, we determined trace element concentrations at low water levels. During high-floods, concentrations of trace elements in water, and consequently in fish, would likely be lower. But since some trace elements enter through the river, the overall load of elements during high floods, would also increase. On the other hand, when the lake is completely dry, grazing animals roam the lakebed and deposit manure. The deposited manure would fuel biological productivity when water arrives, leading to high organic matter decomposition and changing redox conditions. This in turn would affect the cycling of elements in the different environmental compartments of Lake Ngami. Due to the changing dynamics of the lake, concentrations of trace elements in the fish and associated risks to humans would vary with the flood season.

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