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Trace Elements in Marketed Rastreneobola Argentea and Clarias Werneri Caught from Selected Aquatic Ecosystems in Uganda

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Abstract - Samples of two fish species *Rastreneobola argentea* and *Clarias werneri* from Lake Victoria, Lake Kyoga and Nakivubo and Lubigi wetlands were analysed for Cu, Zn, Pb and Cd using an Atomic Absorption Spectrophotometer - Perkin-Elmer Model 2380. The results revealed that Zn concentrations were the highest in both species, followed by Cu; Pb and Cd being the lowest. The concentrations of Cu, Zn, Pb and Cd in *R. argentea* from Lake Victoria ranged between 20.5- 115 µg/Kg, 175 - 495 µg/kg, 15 - 77.5 µg/Kg and 2.5 - 4.45 µg/kg respectively, mean while their concentrations in the same fish species from Lake Kyoga ranged between 12.5 - 37.5 µg/kg (Cu), 35-100 µg/kg (Zn), 5-12.5 µg/kg (Pb), 0.5-1.5 µg/kg (Cd) dry weight. In *C.werneri*, heavy metals varied between 197-337.5 µg/kg (Cu), 235-670 µg/kg (Zn), 60-77.5 µg/kg (Pb) and 2.5-6.98 µg/kg (Cd) in Lubigi wetland and in Nakivubo wetland, concentrations of heavy metals ranged between 752-1320 µg/kg for Cu, 1230-1437 µg/kg for Zn, 85-97.5 µg/kg for Pb and 2.2-7.75 µg/kg for Cd. Heavy metal concentrations obtained in this study were below permissible limits issued by WHO/FAO. Therefore, the consumption of these fish species may not pose any threat to human health. However periodic monitoring of these metals in the fish caught from water bodies located in urban areas is highly essential to public health.

Keywords - *Rastreneobola argentea; Clarius werneri;* Lake Victoria; Lake Kyoga; Nakivubo; Lubigi wetland; Trace metals

1. Introduction

Uganda has realized rapid economic development in the last three decades due to the prevailing peace in the country [1], this has led to rapid growth in the industrial sector especially in the production of building and construction materials such as corrugated roofing sheets, reinforcing rods and paints. These heavy metal related industries have increased the input of trace elements into aquatic ecosystems that are simultaneously being exposed to increasing pollution loads resulting from rapid national population growth [2], rapid urbanization and industrialization [3]. This is the case in the region surrounding the major urban wetlands of Nakivubo and Lubigi and Lake Victoria, a lake that is regarded as the most important natural resources in East Africa, with fishing as the main economic activity [4].

Previous studies indicate that trace elements have become more wide-spread in Uganda over the recent past to such an extent that they have now reached detectable levels in a large number of biota including fish [5-10]. Mercury [4, 7] and arsenic [11] have been detected in fish from Lake Victoria. Similarly, trace elements were found in soils and plants grown in Lake Victoria catchment area [12]. In a recent study, zinc, copper, lead and cadmium were detected in fish from Lake Kyoga [7]. Substantial work has also been carried out on trace element contamination in urban wetlands, particularly in Nakivubo [5,6,12-15]. The presence of trace elements in various water bodies in the country suggests that Ugandan aquatic ecosystems receive considerable inputs of trace elements from various anthropogenic sources. The trace elements which leach into the aquatic ecosystems may cause a serious deterioration of their water quality [7, 11] and a probable poisoning of the aquatic life [8].

The identified immediate causes of trace element contamination are: enhanced effluent discharge; enhanced discharge of solids; runoff and storm water; and atmospheric deposition [10,13]. The pollution of aquatic environment by trace elements is a serious problem since they are not biodegradable, have potential toxic effects on organisms [16,17], and have the ability to bioaccumulate in aquatic ecosystems [18-21] and may further be transferred up to the top trophic levels [19, 22, 23]. Food chain contamination is one of the important pathways by which trace elements enter into the human body [23-25]. Several trace elements such as Co, Fe, Mn, Cu and Zn are essential to the metabolism of organisms. The development, growth and general health of the organisms are optimal if essential metals are present in sufficient amounts in their tissues [25], but are greatly toxic once the amount required for metabolic processes is exceeded [19]. The compounds of lead and cadmium appear to be dangerous contaminants even at low concentrations, having a direct toxic effect on the aquatic organisms and through the food chain process eventually threaten the health of humans [26].

Fish is one of the most important part of human diet due to its high protein content, low saturated fats, omega fatty acids and several kinds of minerals [23, 25, 27-30]. It is also an important source of income to many people in developing countries [31]. In Uganda, some 24.7 million people depend wholly or partly on fishery for their livelihood [32, 33]. Recently, the consumption and demand for small fish as a cheap source of protein increased in Uganda due to the emergency of export markets for especially Nile perch and Nile tilapia [11]. The decline in Nile perch and Nile tilapia has increased the demand for non-export small fish species on the domestic market. Silver fish, *Rastreneobola argentea* and Cat fish, C*larius werneri* are the most abundant non-export small fish species found in Lakes and wetlands respectively. *R. argentea* is predominantly found in Lake Kyoga and to a lesser extent in Lake Victoria [7], they are caught by light attraction, where Kerosene pressure lamps are floated on water attracting fishes to light and are pulled out of the water in their thousands with scoop-nets [34]. They are then sun-dried directly on the beaches. *C. werneri* on the other hand live in papyrus swamps of Lake Victoria, Lake Kyoga and Lake Edward [35], and in major affluent rivers [36] and are common in Nakivubo and Lubigi papyrus swamps. *C. werneri* are caught in basket like structures by local fishermen, placed on long sticks and preserved by smoking, the traditional and low cost procedure commonly used in Uganda.

C. werneri and *R. argentea* are frequently and largely eaten in Uganda, so their toxic metal content should be of some concern to human health. Although these fish species are a major part of the human diet because of their nutritive value, there is a growing concern that metals accumulated in fish muscle tissues may represent a health risk, especially for populations with high fish consumption rates. Consequently, there was need to initiate systematic studies to evaluate the levels of the more likely heavy metal pollutants: Zn, Cu Pb and Cd in sample fish from the selected aquatic ecosystems in an effort to establish the full extent of the problem. The results from the study are considered to be an indirect but confirmatory indicator of pollution in the waterways. In this paper, the levels of copper, zinc, lead and cadmium in samples of *C. werneri* and *R. argentea* are reported and compared with the WHO/FAO guidelines for the safe consumption limits of fish. Monitoring two fish species from different geographical locations will not only help in understanding the extent of trace element contamination but also in elucidating the spatial variation in contamination pattern. Furthermore, the determination of trace element concentrations in different fish species helps in estimating the human dietary exposure to these chemicals.

2. Materials and Methods

2.1 Study area

The study area comprised of, seven landing sites, four on Lake Victoria and three on Lake Kyoga. In addition, parts of Nakivubo and Lubigi wetlands were also included in the study. Lake Victoria is located along the equator at 0^o 30' N, 3^o 00' E and 31^o 39' N, 34^o53' E, while Lake Kyoga is situated between 32^o 10' 34^o 20' E and 1^o 00' 2^o 00' N. Nakivubo wetland is located in Kampala District at 0^o 18' 13.32"N and 32^o 37' 39.36"E whereas Lubigi is located in Wakiso District Central Region, Uganda at 00^o 19' 56"N and 32^o 31' 34"E. The four sampling sites selected in Lake Victoria were Gaba, Port Bell, Kasenyi and Kigungu whereas Namasale, Biko and Kayago were the three sites selected in Lake Kyoga. The various sampling sites on Lakes Victoria and Kyoga are shown in Figure 1. Sampling sites in Nakivubo wetland were selected at Gaba and Bugolobi (Figure 2); this portion of the wetland is still intact with its natural vegetation, *Cyperus papyrus* and is the only part of the wetland where *C.werneri* are found. This part of the wetland receives most of the waste water carried by the channel before it joins Lake Victoria[4, 37]. The selected section of Lubigi wetland was between Bombo and Mityana roads where the wetland is still intact. One sampling station was set along the main stream at Namungona, along Hoima road; this part of the wetland receives municipal wastes and other wastes from Bwaise and Kawempe slums. Other sampling sites were set at points where Sentema and Mityana roads cut-across the wetland (Figure 3).

2.2 Sample Collection

Sampling was conducted between March 2012 and 2013. Thoroughly dried *R. argentea* of length between 30-50 mm from mouth to tail were bought from the selected sites on Lake Victoria (V1-V4) and Lake Kyoga (K1-K3). The mean total lengths of fish were 45.38 mm in Lake Victoria and 33.35 mm in Lake Kyoga. Equal amounts of the fish were bought from four different people at each landing site and pooled to obtain a representative sample. The samples were carefully labeled, wrapped in aluminium foil, placed in air tight bags before being transported in dry boxes to the laboratory for analysis.

C.werneri of length between 13 and 20 cm (mouth to tail) were collected from sites (N1-N3) in the Nakivubo wetland and (L1-L3) in the Lubigi wetland by fishermen in basket like structures, dragged to the shores, killed and smoked. The mean total lengths of fish were (15.83 cm)

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in Nakivubo wetland and 16.40 cm in Lubigi wetland. Because *C. werneri* are consumed smoked by the local population, it was considered necessary to first smoke them. Samples were taken in triplicate per sampling site, wrapped in aluminium foil, placed in air tight bags and labeled. They were then kept in cold boxes containing ice during transportation to the laboratory where they were kept at -18°C until they were extracted for trace elements.

Antimonyl tartarate solution (0.0082 *M*, 5.0 mL), ammonium molybdate solution (0.032 *M*, 15.0 mL) and ascorbic acid solution (0.1 *M*, 30.0 mL) while shaking on each addition.

2.3 Apparatus

All glassware for the analyses of lead, copper, zinc and cadmium was acid cleaned and rinsed thoroughly with deionised distilled water and oven dried before use.

2.4 Chemicals and standards

All reagents used were of analytical reagent grade (BDH Chemicals Ltd, Poole, England). Standard stock solutions of lead, cadmium, zinc and copper were prepared by diluting concentrated solutions to obtain solutions of 1000 mg/L. The working solutions were freshly prepared by diluting an appropriate aliquot of the stock solutions through intermediate solutions using 5% HNO_3.

2.5 Sample preparation and digestion

The sample was prepared and digested according to the method described by AOAC [35]. Approximately 10 g of fish tissue was weighed into a nickel crucible and subsequently placed in an oven at 105°C for 24 h to remove moisture content. The dried sample was ground to powdery form using a pestle and mortar. The powder was sieved through a 2 mm nylon mesh. 2 ± 0.01 g of the sample was weighed into a 250 mL porcelain crucible, 10 mL of concentrated nitric acid, HNO_3 was added, the mixture was boiled gently for 45 min to oxidize all easily oxidisable matter. After cooling 5 mL of 70% perchloric acid, $HClO_4$ was added and the mixture boiled gently until dense white fumes appeared. After cooling, 20 mL of distilled water was added and the mixture boiled further to release more fumes. The solution was cooled, filtered and transferred to a 50 mL volumetric flask and made up to the mark with deionised distilled water. For each run, a duplicate sample, spiked samples, and two blanks were carried through the whole procedure.

2.6 Quality assurance

In this study, recoveries of each trace element were determined by the standard addition method [10], at three different spiking levels of 0.5, 1.0 and 2.0 µg/mL standard stock solutions of Cu^{2+},Zn^{2+}, Pb^{2+} and Cd^{2+}. The strengthened sample solutions were analysed according to the analytical procedures described below and recoveries were calculated for each metal. The recovery of copper ranged between 86.4 - 96.05%, zinc varied between 87.43 - 109.85%, lead 75.5 - 87.62% and cadmium 89.28- 97.33%. The precision of the analytical procedures, expressed as coefficient of variation (CV) varied between 2.0 to 9.5% for all the metals.

2.7 Trace element analysis

Trace element analysis was done using an Atomic Absorption Spectrophotometer-Perkin Elmer Model 2380. Standard solutions of concentrations 0.05, 0.10, 0.50, 1.00, 1.50 mg/L of all the metals (Cu, Zn, Pb and Cd) were analysed in tripricates and the data was used to prepare calibration curves. The instrument was set to zero concentration for all types of samples, using a reagent blank. Copper, zinc, lead and cadmium in the digested sample were determined by direct aspiration of the sample solutions into the air/acetylene frame and the metal concentration determined from the calibration curves. Each determination was based on the mean values of triplicate samples. Total trace element (Zn, Cu, Pb or Cd) was determined using the AAS technique based on the following equation:

$$\frac{1000 \times Absorbance_{sample} \times Concentration\ of\ Standard \times Volume\ of\ sample\ solution}{Absorbance_{standard} \times Weight\ extracted}$$

2.8 Statistical analysis

Statistical analysis of data was carried out using Graph pad Instat package program. Differences between and within species among sampling sites were investigated using a one-way factor analysis of variance (ANOVA). Correlations between different parameters were investigated using linear regression on the concentration data.

3. Results and Discussion

3.1 Contamination pattern in Ugandan fish

The concentrations of trace elements (Cu, Zn, Pb and Cd) in the two fish species, *R. argentea* and *C.werneri* collected from different water bodies in Uganda are given in Table 1. The four elements were detected in all the samples. The results revealed that Zn concentrations were the highest followed by Cu and Pb. Cd being the lowest in both fish species. A similar order has been found in *R.argentea* from Lake Kyoga and Lake Victoria [7]. This could be explained by the fact that Zn and Cu are essential elements in the bodies of living organisms and have an important role in different physiological processes [39]. The essential metals can also produce toxic effects at high concentrations, but their concentrations in aquatic organisms tend to be highly regulated compared to non-essential metals.

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Table 1: Concentrations of trace elements (μg/kg) in fish collected from different water bodies in Uganda

Sampling Sites	Zn	Cu	Pb	Cd
Lake Victoria landing sites				
Kasenyi	225.52±12.89	35.68±3.64	21.38±3.54	3.23±0.44
Port Bell	397.32±27.18	73.36±12.51	56.91±4.71	3.89±0.32
Gaba	411.75±38.11	88.38±18.62	63.05±14.14	3.78±0.67
Kigungu	218.55±37.93	45.32±7.11	28.03±7.51	3.45±0.55
Lake Kyoga landing sites				
Namasale	66.22±9.71	5.12±1.91	8.16±2.25	1.11±0.28
Biko	50.5±10.22	6.13±1.09	8.51±2.54	1.09±0.15
Kayabo	72.35±16.47	7.50±2.53	9.43±1.92	1.01±0.31
Wetlands				
Nakivubo	1342.08±72.14	1255.58±41.11	88.3±5.72	4.79±0.59
Lubigi	300.42±37.94	260.73±29.09	67.23±8.08	2.59±0.18
WHO/FAO	50,000	30,000	500	500

Results presented as mean values of $N = 9$ determinations ± standard deviations (**Source**: this work)

The results show that trace element concentrations in the two fish species varied widely between the water bodies. All the metals attained their maximum values in Nakivubo wetland, followed by Lubigi wetland, Lake Victoria ranked third, while Lake Kyoga was least polluted. The spatial variation in trace element concentrations in the different water bodies suggests that the activities of the people living or working in the immediate vicinity of these ecosystems contribute a lot to their contamination. Fish in particular, collected from Nakivubo wetland showed the highest concentrations of all metals, this suggests localized contamination of its habitat, possibly from industrial and sewage outfalls. The Nakivubo channel passes through relatively dense industrial and residential areas [13, 14] where wastes could be discharged directly or indirectly into the wetland. Generally, fish from Lubigi wetland contained slightly lower concentrations of all the trace elements compared to Nakivubo wetland. Zn, Cu and Pb varied significantly ($P < 0.05$) in *C.werneri* from Nakivubo and Lubigi wetlands. This indicates that human activities which lead to waste discharge into Lubigi wetland are less intense. Although the fish from Nakivubo wetland contained the highest concentrations of all trace elements, Lake Victoria into which the channel drains contained slightly lower levels. This is likely to be either due to the effect of the natural vegetation, *Cyperus papyrus* which acts as a purifier of those pollutants [2] or dilution effect since Lake Victoria has a larger volume of water or the fish species, since the species from the wetland was different from that obtained from the Lake. Fish species [39], feeding habits, habitants [40], and age/size [41] are major factors responsible for pollutant accumulation in fish. The major source of trace elements to Nakivubo and Lubigi wetlands and Lake Victoria are industrial wastes, domestic sewage and motor vehicle workshops.

Comparison of the data concerning metal levels in fish from the two Lakes indicates that there were significant differences ($P < 0.05$) between Cu, Zn, Pb and Cd levels in *R. argentea* from Lake Victoria and Lake Kyoga. It can be said that Lake Kyoga is a relatively unpolluted environment, since almost no industry and only small settlements exist in the surrounding region. The metal concentrations in *R. argentea* from Lake Victoria varied widely and exhibited fluctuations between the sites (Table 1), but worthy to note were the slightly higher levels of all metals in fish samples from Gaba and Portbell. This is probably due to proximity of these sites to the Kampala city where there is a lot of wastes generated from the large settlements, garages, industries and heavy traffic. Significant differences ($P < 0.05$) among species (*R. argentea* and *C.werneri*) were also observed for all the metals except Cd ($P > 0.05$). These variations would either suggest that there were different contamination patterns in the different water bodies or the two fish species react differently to pollution, *C. werneri* being a bottom feeder accumulates high concentrations of trace elements due to its feeding habits and habitats [41]. However, sex [39], length, weight and age of the fish may play a part in explaining this wide range of variation [41]. Correlation analysis showed that, Zn concentrations in *C. werneri* were significantly correlated ($P < 0.05$), Cu was well correlated with Pb ($r^2 = 0.373$), and Zn ($r^2 = 0.361$) and a positive correlation ($r^2 = 0.392$) between Pb and Cd was observed. This indicates that there is a mutual relationship among the metals and hence the prediction of the level of any of the metal from the other is possible. It has been reported that Zn contamination affects the hepatic distribution of other trace elements in fish [42]. Zn, Cu and Mn compete for the same sites in animals [43]. This, no doubt, would affect tissue metal concentrations and certain physiological processes.

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Trace element concentrations in this study were lower than FAO/WHO [41] guideline values indicating that fish from Ugandan water bodies are safe for human consumption. However, people with high fish consumption rates may accumulate increased levels of these metals over time. Long-term exposure to small amounts of copper causes stomach upset, nausea, and diarrhea and can lead to tissue injury and disease. Cadmium and lead are dangerous contaminants even at low concentrations their effects being more pronounced in children than adults once exposed over the same period. In general, the concentrations of trace elements in fish samples observed in this study were lower than, and in some cases comparable to those reported in earlier studies as indicated in Table 2. The low levels of metals reported in this study could be attributed to the size of fish and their positions in the food web [44] since these factors are responsible for pollutant accumulation in fish.

Table 2: Trace element concentrations (μg/g) in fish collected from different parts of the world

Country water body	Fish species	Zinc μg/g	Copper μg/g	Lead μg/g	Cadmium μg/g	Reference
Uganda						
L. Victoria	*R. argentea*	2.71-9.62	0.79-7.94	0.09-0.24	0.37-0.69	[7]
L.Kyoga		0.34-1.32	ND-0.9	ND-0.02	ND-0.01	
L.Victoria	Nile perch			0.16-0.22		[12]
L.George	*O. leucostictus*			15.3-189		[42]
	B. docmas	78.2-187.5				
Tanzania						
L. Victoria	*Lates nilotus*		0.7±0.02	0.13±0.04	4.67±1.87	[43]
L. Victoria	*C.niloticus*			14.6-18.6	0.6-0.8	[44]
	R. argentea			13.4-15.8	0.9-1.8	
	L. niloticus			29.2-46.6	0.6-0.9	
Ethiopia						
L. Hashenge	Nile Tilapia	26610	404760	336360	66670	[23]
	Common Carp 49150		666670	375760	60920	
Turkey						
Black Sea	Demersal	1380-2714	1424-63290			[45]
	M. barbatus	8862-13277	3913-8952			
	M.euxinus	6234-57743	2610-4161			
	S.smaris	6601-35873	5496-9356			

4. Conclusions

The quantities of heavy metals in fish measured in this study provide information on concentrations and distribution of heavy metals in water bodies in Uganda. Spatial variation in heavy metal concentrations suggests that non-point sources lead to the contamination of the water bodies in Uganda. The results of this study indicate that heavy metal concentrations in the fish samples were below permissible limits issued by FAO/WHO. Therefore, the consumption of these fish species may not pose any threat to human health. However, periodic monitoring of these metals in the fish caught from water bodies located in urban centers is highly essential in order to prevent excessive build up of these toxic heavy metals in the human food chain.

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