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Physicochemical Quality of Water and Health Risks Associated with the Consumption of the Extant African Lung Fish (*Protopterus annectens*) from Nyabarongo and Nyabugogo Rivers, Rwanda

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Abstract

Water is an indispensable natural resource that is often prodigiously threatened by anthropomorphic activities. This study evaluated the physicochemical properties of water and selected heavy metals in edible muscles of a piscivorous fish (*Protopterus annectens*) from Nyabarongo and Nyabugogo rivers of Rwanda. Edibility health risk was evaluated using the target hazard quotient method. Water samples were taken in triplicate from Ruliba station and Kirinda bridge on Nyabarongo river and Giticyinyoni on Nyabugogo river. Fish samples were obtained from the sampling stations on Nyabarongo river. All samples were analyzed following standard methods and analytical results indicated that the average temperature, pH, total dissolved solids and electrolytic conductivity of water from the rivers were within WHO acceptable limits. The statistical mean concentrations of the ionic components of the water samples were 1.61 ± 0.03, 0.53 ± 0.002, 0.24 ± 0.02 and 0.051 ± 0.01 mg/L for Fe, Mn, Cu and Pb respectively at Ruliba station and 0.63 ± 0.02, 0.02 ± 0.002, 0.09 ± 0.01, 0.06 ± 0.002 and 0.75 ± 0.02 mg/L for Fe, Mn, Zn, Cr and Pb respectively at Kirinda bridge. Water from Giticyinyoni had 1.57 ± 0.02, 0.49 ± 0.03, 0.29 ± 0.058, 0.43 ± 0.058, 0.15 ± 0.00 and 0.59 ± 0.058 mg/L of Fe, Mn, Cu, Zn, Cr and Pb respectively. Zinc, Cu, Cr and Cd were below detection limits in samples from Ruliba station and Kirinda bridge (Nyabarongo river). Edible muscles of *P. annectens* from Nyabarongo river contained 272.8 ± 0.36, 292.2 ± 0.25, 8.8 ± 0.36, 135.2 ± 0.15, 148.0 ± 0.21 and 432. 0 ± 0.50 mgkg^{-1} for Fe, Mn, Cu, Zn, Cr and Pb at Ruliba station and 336.0 ± 0.70, 302.6 ± 1.22, 6.4 ± 0.26, 44.7 ± 0.20, 138.2 ± 0.17 and 302.4 ± 1.50 mgkg^{-1} for Fe, Mn, Cu, Zn, Cr and Pb respectively at Kirinda bridge. Health risk assessment indicated that consumption of the edible muscles of *P. annectens* may lead to deleterious health effects as reflected by values of target hazard quotients being greater than one. Therefore, the Rwandese government should lay strategies to reduce pollution of the rivers. Further research should evaluate the heavy metal content of metabolically active organs of *P. annectens* from Nyabarongo river as well as the microbiological profile of water from the rivers.

Keywords: *Bioconcentration Factor; Estimated Daily Intake; Nyabarongo river; Protopterus annectens; Rwanda; Target Hazard Quotient.*

1.0 Introduction

Global deterioration of water quality and heavy metal pollution of aquatic ecosystems due to natural and anthropogenic activities such as industrialization, climate variability, poor waste management and high population growth rates have evoked concerns from international bodies as well as researchers [1-3]. World population registered a sharp increase to about 6.08 billion in 2000 following the industrial revolution and is expected to reach 9.7 billion by 2050 [4]. The current population of Rwanda stands at 12 million (population density of 470.6 people per km^2) and is expected to double by 2050 [5]. This implies that the country is densely populated in comparison to other East African countries [6, 7]. Several industries have sprung up and are causing pollution of water bodies due to discharge of untreated industrial wastes as well as sewages into surrounding rivers [8]. Thus, conservation and efficient utilization of water to meet the ever-growing requirement by the population and industries is necessary and this can only be achieved through constant monitoring of these water resources [9].

Environmental studies have reported pollution of major Rwandese rivers such as Mpazi, Nyabarongo, Rusine and Nyabugogo [8, 10-13]. Nyabugogo river, a tributary of Nyabarongo river (a tributary of Akagera transboundary river) pour its water into Lake Victoria, the life artery of East African countries [14]. The pollution of Lake Victoria due to anthropogenic activities is rated among the top ten worldwide [15] and the increasing load of contaminants has greatly deteriorated the quality of water and fish from this freshwater lake [16, 17]. The presence of toxic heavy metals in fish poses health threats such as development of cancer, renal failure, liver damage, cardiovascular diseases and eventually death [18-20]. Recently, heavy metals (manganese, zinc, copper, iron, nickel, lead and cadmium) were reported in the edible parts of *Colocasia esculenta, Amaranthus spinosus*, *Ipomoea batata* and soils from industrially active parts of Kigali, Rwanda [21, 22].

As a contribution to environmental monitoring and public safety, the current study investigated the physicochemical profile of water, heavy metal content of edible muscles of African lung fish (*Protopterus annectens*) and estimated the health risks associated with the consumption of this fish from Nyabarongo and Nyabugogo rivers of the Republic of Rwanda.

2.0 Materials and Methods

2.1 Description of the Study Area

Rwanda is a landlocked East African country covering an expanse of 26,338 km^2 on the Eastern shoulder of the Kivu-Tanganyika rift in Africa. It lies between 1°4' and 2°51' South latitude and 28°53' and 30°53' East longitude [6]. It shares common frontiers with Uganda to the North, Burundi to the South, Tanzania to the East and the Democratic Republic of the Congo (DRC) to the West. Like other East African countries, Rwanda is relatively rich in water resources (**Figure 1**) [5]. The current study was done in Nyabarongo (Nyawarungu) and Nyabugogo rivers which form part of the Rwandese drainage system. Nyabarongo river flows over 300 km from its source in western Rwanda southwards to its outlet into Lake Rweru in South Eastern Rwanda along the border with Burundi. Its main tributary is Akanyaru river that flows from the highlands of Nyungwe national park on the Congo-Nile divide in former Ruhengeri province along the Rwanda-Burundi frontier. The river then flow Eastwards through swampy valleys and small lakes in the lowlands of Bugesera-Gisaka in South Eastern Rwanda. Nyabugogo river traverses Kigali city through Nyarugenge district due to its numerous tributaries such as the Mwange, Rusine and Marenge rivers on its upstream portion. It is later fed by other rivers from the urbanized parts of Kigali such as Rwanzekuma, Ruganwa, Mpazi and Yanze. Floriculture, sugar processing at Kabuye works sugar industry, sugar cane plantations, agrarian and mining activities along this river are the major sources of anthropogenic pollution [8].

2.2 Analytical Reagents and Apparatus

Standard solutions were purchased from Sigma Aldrich (Steinheim, Swiss). The other reagents, except the certified reference material, were obtained from Merck (Darmstadt, Germany). Water used as solvent was double distilled water. The volumetric ware used were soaked overnight in 10% (v/v) nitric acid solution, rinsed with deionized water and oven dried prior to analysis. Mettler PM200 digital analytical balance (Marshall scientific, USA) was used for all analytical weighing. Hanna 211 digital microprocessor-based bench top pH/mV/°C meter (Hanna instruments, Italy) calibrated using pH 4.01, 7.01, 10 buffers was used for all laboratory-based pH measurements.

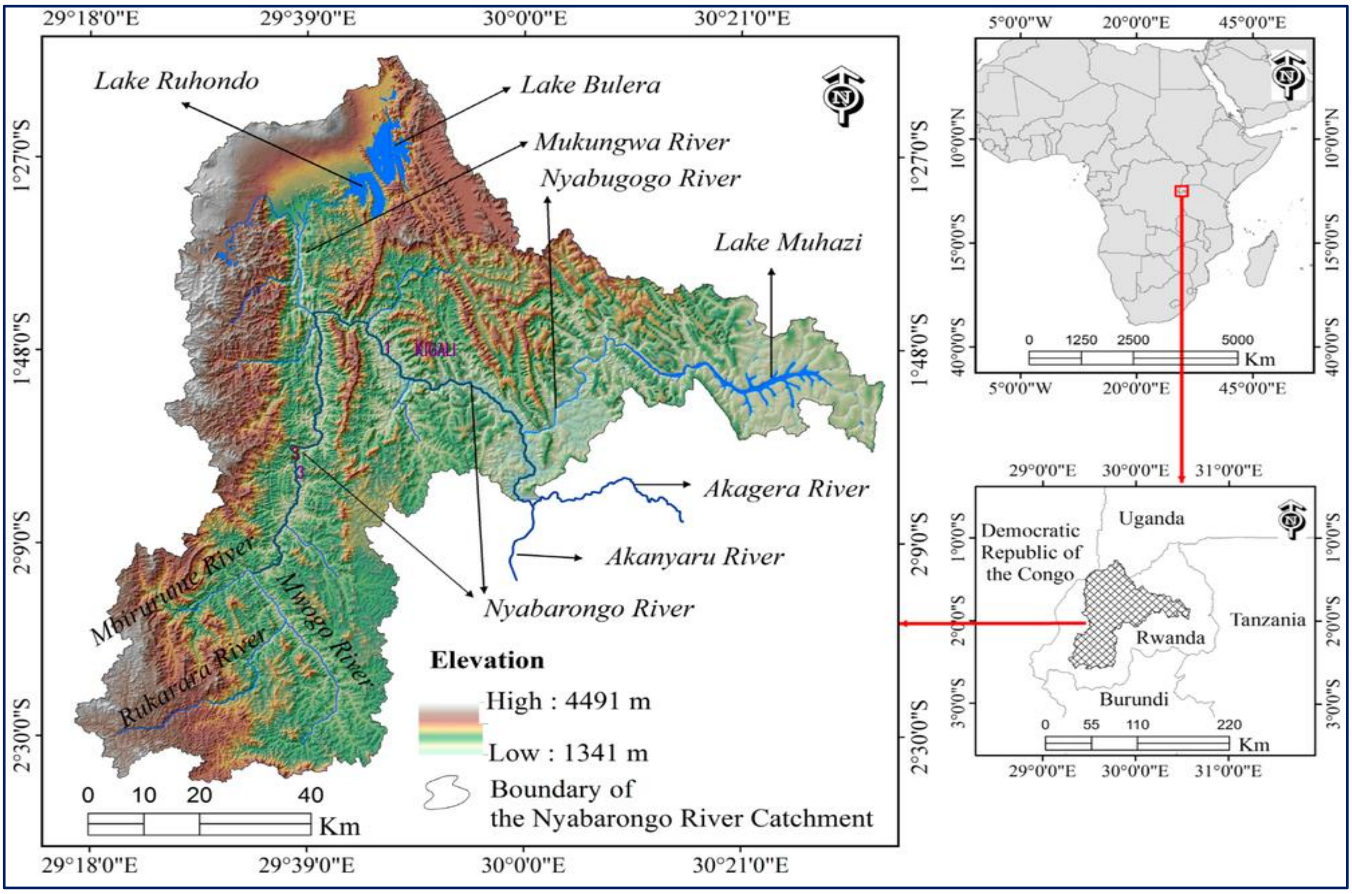

Figure 1. *Map of Rwanda showing the location of Nyabarongo and Nyabugogo rivers (Adapted from [23]).*

2.3 Ethical Approval

Approval to carry out this study was granted by the Department of Chemistry, College of Science and Technology, University of Rwanda, Rwanda for Papias Nteziyaremye (Approval No. **213000076**).

2.4 Sampling Procedure and Preparation of Samples

Three sampling points were purposively selected in this study after a prestudy survey which revealed that the selected points could be hot spots of anthropogenic pollution. For Nyabarongo river, two sampling sites were chosen. The first site (Ruliba station) was in Kigali city, Nyarugenge district, Ruliba cell in Nyabarongo wetland near Nyabarongo bridge at Kigali-Butare road (1^{0}58′37″S and 30^{0}0′50″E; **Figure 2**). The second sampling site (Kirinda bridge) was in Karongi district, Murambi sector, Shyembe cell at the bridge joint of Ruhango and Karongi districts (204 4″S and 290 20′46″E). On Nyabugogo river, samples were taken from Nyabugogo cell (Giticyinyoni) which lies at coordinates 10 55′22″ S and 300 2′52″ E.

Figure 2. *Sampling points on Nyabarongo river (a) Ruliba station (near Ruliba Clays Limited); (b) at Kirinda bridge.*

Water was selected to determine the concentration of heavy metals being released into the rivers and the deterioration of water quality due to anthropogenic activities whereas fish was chosen because it constitutes the diet of many communities both in rural and urban areas. All samples were obtained in triplicate between April 2019 and May 2019 (10:00 am to 11: 00 am, Central African Time).

Water samples were collected in 500 ml Teflon plastic bottles. The bottles, previously cleaned by washing in non-ionic detergent were rinsed with tap water, soaked in 10% nitric acid for 72 hours and finally rinsed with deionized water before use. Each bottle was rinsed 3 times with river water at each sampling point, filled 20-25 cm below the water surface and capped with airtight stoppers while still under water. The samples were preserved with 5 ml of concentrated nitric acid to achieve $pH < 2.0$ (to minimize precipitation and sorption losses to the bottle walls). The samples were placed in a cooler box with ice and transported to the laboratory where they were stored at 4℃ until commencement of analysis.

African lung fish samples (6.2 to 8.1 cm fork length; 700-903 g fresh weight) were caught using hooks fed with earthworms as baits with the help of local fishermen [24]. Fish samples were only obtained from Nyabarongo river because fish caught from Nyabugogo river were small sized and not of edible size thus would not give representative samples. The samples were taken in ice cool boxes and kept frozen at − 20 ℃ in the laboratory until further analysis. The samples were eviscerated within two days of collection.

Drying of whole edible parts of the fish samples was done in an electric oven at 105 °C for 48 hours. The edible muscles of the dried fish samples were separately pulverized using clean porcelain mortars and pestles to obtain fine fish powders. Aliquots (2.15 ± 0.01 g) of the homogenized fish powders were ashed at 550 °C for 3 hours and subsequently transferred into 250 ml volumetric flasks. Measured 15 ml of 6 M Nitric acid and 5 ml of 5 M concentrated hydrochloric acid were added to the flasks and the samples were digested for 15 minutes, filtered into 100 ml standard volumetric flasks and made to the marks with sterile double distilled water [24].

2.5 Physicochemical Analysis of Water Samples

Water samples were analyzed for physicochemical parameters: temperature, pH, electrical conductivity (EC), total nitrogen (TN), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammoniacal nitrogen (NH_4-N), total phosphorus (TP), phosphates (P), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), chromium (Cr), cadmium and lead (Pb).

Temperature and pH were determined on-site using a handheld Jenway 370 pH/mV/Temperature meter (Jenway Gransmore Green, England) precalibrated using pH 4.0, 7.0, 9.22 and 10.0 standard buffer solutions (manual override). The electrodes were thoroughly rinsed with distilled water between measurements. Conductivity was determined on-site using a precalibrated Jenway 4520 Conductivity/TDS meter (Keison Products, England) [25].

All the chemical parameters of water samples were determined in the laboratory following standard analytical methods for examination of water and wastewater [26]. Total nitrogen (persulfate digestion method), NO_3-N (cadmium reduction method), NO_2-N (diazotization method), NH_4-N (Nessler method), phosphates and sulphates were analyzed using HACH DR/2500 scanning spectrophotometer. Ionic components of water samples (Fe, Mn, Cu, Zn, Cr, Cd and Pb) were quantified using Varian AA240 atomic absorption spectrometer.

2.6 Spectroscopic Analysis of Fish Samples

Prepared fish samples were analysed for Fe, Mn, Cu, Zn, Cr, Cd and Pb using Atomic Absorption Spectrophotometer (Varian AA 240) after selecting the various wavelengths at which the heavy metals were determined. The results in mg/L from the instrument were converted to the standard unit for solid foods (mgkg^{-1}) for easy comparison with the set international compliance limits [24].

2.7 Human Health Risk Assessment

2.7.1 Estimated daily intake and Target Hazard Quotient

Estimated daily intake (EDI) for fish from Nyabarongo river was calculated to averagely estimate the daily loading of the heavy metals into the body system of a specified body weight of a consumer (adult/child) through consumption of contaminated fish. This provides the relative availability of heavy metals but does not take into cognizance the possibility of metabolic ejection of these metals. EDI in mgkg^{-1}day^{-1} was calculated using **Equation 1** used in preceding studies [14, 24].

$$\text{EDI} = \frac{E_f \times E_d \times F_{ir} \times C_f \times C_{hm}}{W_{ab} \times T_{aet}} \qquad (1)$$

Where E_f = exposure frequency (365 days/year), E_d = exposure duration, the average lifetime (67 years for an adult Rwandese) [27], F_{ir} is the fresh food ingestion rate (g/person/day) = 48 for fish, C_f is the conversion factor (0.208) for fresh weight (F_w) to dry weight (D_w) for fish, C_{hm} = heavy metal concentration in the fish muscles (mgkg^{-1} *Fw*), W_{ab} = average body weight (considered to be 15 kg for children and 60 kg for adults [14, 28], T_{aet} = average exposure time for non-carcinogens (given by the product of E_d and E_f) [29].

Health risk index, the total risk of a non-carcinogenic element through three exposure pathways was evaluated using Target hazard quotient in accordance with US EPA region III risk-based concentration table [30] used in preceding studies [14, 24]. According to the criterion, target hazard quotient less than unity (1.0) indicate that the exposure is very unlikely to have adverse effects while target hazard quotient greater than 1.0 represent a possibility of non-carcinogenic effects, with its probability increasing as the value of the target hazard quotient increases. Target hazard quotient was calculated as the ratio of EDI to the reference dose (R_fD) **(Equation 2)** [14, 24, 30].

$$\text{Target hazard quotient } = \frac{EDI}{R_f D} \qquad (2)$$

The R_fD of Fe, Mn, Cu, Zn, Cr, Cd and Pb are 0.7, 0.3, 0.04, 0.3, 1.5, 0.001 and 0.14 mgkg^{-1} respectively [30]. The R_fD is the maximum daily dose of a metal from a specific exposure pathway that is believed not to lead to an appreciable risk of deleterious effects to sensitive individuals during a life time [31]. If the EDI is lower than R_fD, the target hazard quotient is less than 1 and adverse health effects are unlikely to appear while EDI exceeding R_fD gives THQ greater than 1 and adverse health effects are likely to appear [32].

It has also been reported that exposure to two or more toxicants result in additive and/or interactive effects [33-36]. Thus, the total target hazard quotient (TTHQ) was treated as the arithmetic sum of the individual metal THQ values [2, 36, 37].

The assumptions made during the health risk calculations were that the ingested dose is equal to the dose absorbed into the body [38] and cooking have no effect on the trace metal content of the edible muscles of fish [36, 39].

2.7.2 Assessment of bioaccumulation factors

Metal accumulation and uptake by living organisms can be determined by use of bioconcentration factor (BCF). This is one of the multipliers (bioaccumulation factors) that is used to estimate concentrations of chemicals that accumulate in tissues through any route of exposure [40]. Thus, the bioconcentration factor of the different metals were calculated from the numerical ratio of the concentration of the respective metals in the whole edible muscles of *P. annectens* to the concentration of the same metal in water [14, 41].

2.8 Analytical quality assurance and quality control

All reagents used in this investigation were of analytical grade. Quality control was performed with spiked samples analyzed once for every 10 samples. Recovery percentages from the spiked samples ranged from 97.6% to 102.5%. Control samples were prepared from fish tissue homogenate (CRM IAEA-407, International Atomic Energy Agency, Vienna, Austria) and the recovery values for Fe, Mn, Cu, Zn, Cr, Cd and Pb ranged from 98.9 to 101.4%.

Method detection limits with reagent blanks were calculated and used as a verification tool as well. The detection limits were 1.50, 0.20, 0.60, 2.50, 0.50, 1.14, 1.20 and 0.37 mg/kg for Fe, Mn, Cu, Zn, Cr, Cd and Pb respectively. Bottle, analytical, equipment and filtration blanks were determined throughout the analyses and blank subtractions were used to correct the metal concentrations obtained. All samples were analyzed at least in triplicate to obtain a relative uncertainty of less than 5%. Thus, precision in the analyses were in good agreement, better than ± 5.0% relative standard deviation.

2.9. Statistical analysis of results

Analytical data were subjected to statistical evaluation to calculate means and standard deviations. Differences among means were determined using one-way analysis of variance (ANOVA). To identify significant differences between groups, Tukey test was done. Statistical analyses were performed at a 95% confidence interval using Minitab statistical software (v 17, Minitab Inc., USA).

3. Results and Discussion

3.1 Physicochemical profile of water from Nyabarongo and Nyabugogo rivers

The physicochemical properties of water from the studied rivers are given in **Table 1**.

Table 1. *Hydrochemical properties of water from Nyabarongo and Nyabugogo rivers.*

Parameter	Nyabarongo river		Nyabugogo river	WHO guidelines [42]
	Ruliba station	Kirinda bridge	Giticyinyoni	
Temperature (℃)	22.6 ± 0.30	23.0 ± 0.26	22.5 ± 0.40	25.0
pH	8.15 ± 0.07	7.96 ± 0.06	8.22 ± 0.03	6.5-8.5
Conductivity (µs/cm)	74.3 ± 3.84	20.0 ± 0.15	102.0 ± 1.0	1000
TDS (mg/L)	39.0 ± 2.0	10.0 ± 0.01	52.9 ± 0.36	2000
TN (mg/L)	1.62 ± 0.02	1.56 ± 0.001	**12.81 ± 9.51**	3.0
NO_2-N (mg/L)	0.01 ± 0.00	0.017 ± 0.02	0.014 ± 0.003	0.1
NO_3-N (mg/L)	0.40 ± 0.01	1.39 ± 0.01	1.07 ± 0.058	10
NH_4^+-N (mg/L)	0.153 ± 0.015	0.117 ± 0.01	0.12 ± 0.006	0.5
SO_4^{2-} (mg/L)	0.58 ± 0.017	0.270 ± 0.02	0.86 ± 0.03	1000
PO_4^{3-} (mg/L)	0.42 ± 0.01	0.21 ± 0.02	0.39 ± 0.058	5.0
Fe (mg/L)	**1.61 ± 0.03**	**0.63 ± 0.02**	**1.57 ± 0.02**	0.3
Mn (mg/L)	**0.53 ± 0.002**	0.02 ± 0.002	**0.49 ± 0.03**	0.1
Cu (mg/L)	0.24 ± 0.02	*BDL*	0.29 ± 0.058	0.5
Zn (mg/L)	*BDL*	0.09 ± 0.01	0.43 ± 0.058	3.0
Cr (mg/L)	*BDL*	0.06 ± 0.002	0.15 ± 0.00	0.05
Cd (mg/L)	0.106 ± 0.002	*BDL*	*BDL*	0.01
Pb (mg/L)	**0.051 ± 0.01**	**0.75 ± 0.02**	**0.59 ± 0.058**	0.01

BDL-Below method detection limit. Values in **bold** indicate exceedance of WHO drinking water standards [42].

3.1.1 Temperature and pH

Water temperatures from all the rivers were normal. Ruliba station and Kirinda bridge on Nyabarongo river recorded 22.6 ± 0.30 and 23.0 ± 0.26 ℃ respectively. Giticyinyoni sampling station on Nyabugogo river had 22.5 ± 0.40 ℃. Overall, the temperatures were below the maximum temperature of 25.0 ℃ recommended for drinking water [42]. Temperature impacts both the chemical and biological characteristics of water such as pH, density, oxidation reduction potential and conductivity. The solubility of oxygen and other gases are also known to decrease as temperature increases [43].

The pH levels of water for all the sampled sites were not significantly different ($p < 0.05$). The highest pH (8.22 ± 0.03) was in water from Giticyinyoni (Nyabugogo river). Usanzineza *et al.* [12] reported that water in Lake Muhazi was slightly alkaline (mean pH 7.8) which is only slightly lower than 7.96 ± 0.06 reported at Kirinda bridge on Nyabarongo river in this study. The pH values recorded in this study agreed well with those recorded by previous reports. Nhapi *et al.* [8] reported a pH of 7.24 ± 0.18 at Rwesero, the first point after Nyabugogo river flows out of Lake Muhazi. Further, a more or less constant pH in Nyabugogo swamp was reported [11] and the author implicated this to be the effect of high pH of water in Rwanzekuma and Ruganwa rivers which receive alkaline reagents from Usine Textile Du Rwanda (UTEXRWA) textile industry and other factories in Kigali city. Another study [44] bewrayed that the pH increment of Mpazi river water was attributable to the effluent from Nyabugogo abattoir which had pH of 8.9 ± 0.2. Overall, the pH values obtained in this study were within the acceptable limits for drinking water recommended by WHO [42].

Medically, consumption of excessively acidic or alkaline water is harmful to the body. Drinking water should have a pH value in the range of 6.5 to 8.5 [42]. It should be emphasized that even within the acceptable pH range, slightly high or low pH values of water can be unpleasant for several reasons; high pH causes water to have a slippery feel, tasting like baking soda whereas low pH on the other hand may cause water to have a bitter or metallic taste and such water may cause corrosion of fixtures when used in industrial production [25].

3.1.2 Electrical Conductivity and Total Dissolved Solids

Conductivity ranged from 20.0 ± 0.15 to 102.0 ± 1.0 µS/cm which were lower than the maximum level of 1000 µS/cm. The electrical conductivity of water estimates the total amount of solids dissolved in the water (the water's TDS) [45, 46] and it depends on the water's temperature; the higher the temperature, the higher the electrical conductivity and vice versa. This ability is directly related to the concentration of ions in the water, and this is supported by the low levels of ions registered in this study [25].

Total dissolved solid is a measure of inorganic salts (principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides, and sulphates) and some small amounts of organic matter that are dissolved in water [47]. The TDS readings recorded in this study ranged from 10.0 ± 0.01 mg/L for samples from Kirinda bridge to 52.9 ± 0.36 mg/L for samples from Giticyinyoni. These readings are quite lower than those previously reported for water from Nyabugogo marshland, Nyabugogo, Rwanzekuma and Ruganwa rivers [12]. High TDS affects the aesthetic quality of water, interfere with washing operations and can be corrosive to plumbing fixtures [47].

3.1.3 Total nitrogen (TN)

Three forms of nitrogen commonly measured in water bodies include ammoniacal, nitrate and nitrite nitrogen. Total nitrogen is the sum of total Kjeldahl nitrogen (ammonia, organic and reduced nitrogen) and nitrate-nitrite. It can be obtained from monitoring organic nitrogen compounds, free-ammonia and nitrate-nitrite individually and adding the components. The TN levels recorded ranged from 1.56 ± 0.001 mg/L at Kirinda bridge (Nyabarongo river) to 12.81 ± 9.51 mg/L in water from Giticyinyoni (Nyabugogo river). The lower TN levels reported in Nyabarongo river are comparable to 2.06 mg/L and 1.55 mg/L reported in Tigris river (Turkey) and Xin'anjing river (China) [48, 49]. Only water from Giticyinyoni had TN higher than the maximum acceptable limit of 3.0 mg/L. Such high TN values (33.71 and 35.21 mg/L) have been reported in Awash and Walleme rivers of Ethiopia [50, 51]. Excess amount of TN in water may lead to low levels of dissolved oxygen and negatively alter various plant life and organisms. Sources of nitrogen include wastewater treatment plants, runoff from fertilized lawns and croplands, failing septic systems, runoff from animal manure and storage areas and industrial discharges that contain corrosion inhibitors.

3.1.4 Ammoniacal nitrogen (NH_4-N)

This is the sum of non-ionized ammonia and ionized ammonium ion species. The NH_4-N levels were 0.153 ± 0.015 and 0.117 ± 0.01 mg/L for Nyabarongo river (at Ruliba station and Kariba bridge respectively) and 0.12 ± 0.006 mg/L at Giticyinyoni of Nyabugogo river (**Table 2**). A previous study reported that there were high levels of NH_4-N downstream towards Kigali city due to release of domestic wastewater from Kigali urban areas [8]. The NH_4-N values reported in this study are comparable to 1.14 mg/L reported in contaminated wastewater in a Zimbabwean urban river [52]. Ammonia in the environment is reported to originate from metabolic, agricultural and industrial processes including disinfection operations using chloramine [42]. Natural ammonium levels in ground and surface water are usually below 0.2 mg/L and in the current study, all the levels were below WHO maximum permissible limit of 0.5 mg/L.

3.1.5 Sulphates and Phosphates

The sulphate levels recorded in water from the studied rivers were all below 1.0 mg/L which is less than 0.1% of the maximum permissible levels. This corroborates a previous report [8] which speculated that high levels of sulphates in some sites of Nyabugogo river could be due to pollution by wastes from UTEXRWA textile industry. Sulphate content of water is used for determination of the suitability of water for public and industrial use. Sulphate may also contribute to the corrosion of pipelines in the distribution system [53]. According to WHO, typical sulfate levels range from 0 to 630 mg/L in rivers [42]. High doses of sulphate (particularly magnesium sulphate) causes catharsis or purging of the bowels [54]. In drinking water, the taste threshold of sulphates are 200 - 500 mg/L for sodium, 250 mg/L for calcium and 400 - 600 mg/L for magnesium [55].

The highest concentration of phosphates (0.42 ± 0.01 mg/L) was in water from Ruliba station on Nyabarongo river (**Table 2**). In comparison to WHO standards, the values were relatively lower implying that there is little risk due to phosphate pollution. The values recorded in this study were lower than that reported in Rusine river (1.11 ± 1.76 mg/L) and Nyabugogo river [8]. Technically, elevated phosphate levels in a river are indicative of pollution from domestic sewage discharges [25].

3.1.6 Nitrite nitrogen (NO_2-N) and Nitrate nitrogen (NO_3-N)

The NO_2-N results were generally lower than 0.1 mg/L maximum permissible level. The values ranged from 0.010 ± 0.00 to 0.017 ± 0.02 mg/L. Insignificant differences in NO_2-N levels in water samples in Nyabugogo river stretch have been reported previously [8]. Presence of nitrite indicate that there is oxidation which itself is affected by various environmental factors such as natural re-aeration, photosynthesis and presence of ammonium which are usually fluctuating in an aquatic ecosystem.

Nitrate nitrogen ranged from 0.40 ± 0.01 to 1.39 ± 0.01 mg/L in the water samples. The values were lower than those previously reported in water from Nyabugogo river [8] but is comparable to the results reported by Mvungi *et al.* [52] in Zimbabwe. Thus, there is no potential contamination of water from sewage discharges at the studied stations on Nyabarongo and Nyabugogo rivers.

3.1.7. Ionic Composition of the Water Samples

The chemical composition of natural water depends on factors such as the chemistry of atmospheric precipitation, mineralogy of the rocks encountered along the flow path, residence time of the water, topography and climate [56, 57]. As shown in **Table 2**, Fe and Pb concentrations in water from all the sampled stations were higher than their corresponding WHO recommended limits in drinking water. In addition, Mn levels were also exceeded in water from Ruliba station (Nyabarongo river) and Giticyinyoni (Nyabugogo river).

Iron is found in natural fresh water at levels ranging from 0.5 to 50 mg/L [42]. The high levels of Fe reported in this study agreed with previous studies. Usanzineza *et al.* [12] reported a mean value of 0.756 ± 0.734 mg/L for Fe at the Lake Muhazi outlet. Nhapi *et al.* [8] reported elevated Fe levels in water from Rusine river (8.76 ± 8.88 mg/L) and Marenge river (6.85 ± 5.92 mg/L). The study speculated that the pollution could have been due to the geological composition of red soils in the area. It was noted that inflows from Kigali city amplifies the concentration of Fe always recorded in Nyabugogo river. The levels of Fe recorded in this study is supported by a recent study [22] which reported that the concentration of iron was as high as 2,896 mg/kg in soils taken from Nyabugogo downstream. Earlier, iron leachate was reported in the range of 1023.1 to 2005.8 mg/kg in clay cooking pots in Rwanda [58]. It is reported that iron is a major inorganic water quality problem and is mainly transported in groundwater in the reduced form as Fe^{2+} [59]. Amadi [60], Eliku and Leta [51] reported lower Fe concentrations (0.011 to 2.897 and 1.11 to 4.12 mg/L) in water from Sosiani river (Kenya) and Awash river (Ethiopia) than is reported in Nyabarongo and Nyabugogo rivers in this study. Elevated levels of Fe in water (12.6 to 15.51 mg/L) comparable to that reported in this study were reported in Mara river of Tanzania by Kihampa and Wenaty [61]. The high levels of iron reported in this study could be due to iron, steel and household equipment made of iron that are dumped in the rivers. The dark brown color of water (Figure 2) is mainly attributed to the oxidation of iron in the ferrous form to ferric form and the formation of ferric hydroxide colloids and complexes [62].

The concentration of lead reported in this study were above WHO maximum limits. Nhapi *et al.* [8] reported that the high levels of lead in Mpazi river (Nyabugogo catchment) could be from Nyabugogo tannery which uses a lot of chemicals and has many car parks in the area. The high levels of Lead in Nyabugogo catchment was also reported by Usanzineza *et al.* [13] who hinted that the prevalence of Lead in this catchment warranted further investigations. Similar results have been reported in other African rivers; Mvungi *et al.* [52] reported 0.213 to 0.544 mg/L of lead in water from an urban river in Zimbabwe while Okonkwo and Mothiba [63] reported 0.010 to 0.012 mg/L of lead from three urban rivers in South Africa. The occurrence of lead in urban waters have been reported to be due to use of leaded petrol and thoughtless dumping of dead car lead acid accumulators [24]. Lead is a toxic non-essential trace metal even at meagre concentrations. Thus, the concentrations reported in this study is harmful to fish and humans when ingested or inhaled in high doses [64]. Lead is an antagonist that interferes with fundamental trace metals of comparable characteristics such as Calcium and Zinc. It is also associated with human kidney failure and liver degradation [65]. Lead retards interactive, survival, growth, development and metabolic processes in addition to increasing mucus synthesis and triggering nervous system disorders [66].

Manganese levels were high in water from Ruliba station and Giticyinyoni. A study conducted in Rwanda in the same catchment area recorded 10.28 ± 11.44 mg/L of Mn in water from Rwanzekuma river, 11.58 ± 11.46 mg/L in Ruganwa river, 28.85 ± 23.53 mg/L for Nyabarongo upstream and 25.56 ± 27.91 mg/L on Nyabarongo downstream water samples [8]. Nzeyimana [67] concluded that groundwater in Rwanda contained high levels of Mn and thus the high Mn levels recorded in this study could be due to the surrounding geological formation and disturbance of soils which causes discharge of Manganese-rich runoffs [8] .

Generally, physico-chemical parameters such as pH, redox potential and salinity affect the mobility of metals in water [68-70]. The water samples collected from all the stations showed low salinity with mean values between 0.01 and 0.05 mg/L of chloride ions. This could explain the high concentrations of Fe, Pb and Mn recorded in the water samples [71].

3.2 Heavy Metal Content of the Edible Muscles of Protopterus annectens

Although fish are migratory in an aquatic ecosystem, metal accumulation in fish gives evidence of exposure to polluted aquatic environment [72] and could be used to evaluate the health condition of the environment from which they are collected. Further, fish are good indicators of heavy metal contamination in aquatic systems because they occupy different trophic levels [73]. The heavy metal content of the edible muscles of *P. annectens* from Nyabarongo river are shown in **Table 3**.

Table 3. *Heavy Metal Concentrations in Edible Muscles of P. annectens from Nyabarongo River.*

Sampling point	Heavy metal concentration (mgkg^{-1})						
	Fe	Mn	Cu	Zn	Cr	Cd	Pb
Ruliba station	272.8 ± 0.36	292.2 ± 0.25	8.8 ± 0.36	135.2 ± 0.15	148.0 ± 0.21	*BDL*	432. 0 ± 0.50
Kirinda bridge	336.0 ± 0.70	302.6 ± 1.22	6.4 ± 0.26	44.7 ± 0.20	138.2 ± 0.17	*BDL*	302.4 ± 1.50
FAO/WHO [74]	30.0	1.0	30.0	40.0	10.0	0.5	0.5

BDL-below method detection limit.

Generally, high levels of heavy metals were recorded in edible muscles of *P. annectens* from Nyabarongo river. The chemical sequences were Pb > Mn > Fe > Cr > Zn > Cu > Cd for samples from Ruliba station and Fe > Mn > Pb > Cr > Zn > Cu > Cd for samples from Kirinda bridge. These observations agreed well with the conclusions of Watanabe *et al.* [39] and Masoud *et al.* [40] that absorbency of heavy metals in animal tissues varies from one trace metal to another. All the heavy metal concentrations recorded in this study except that of copper and cadmium were above the maximum permissible limit in fish recommended by FAO/WHO (**Table 3**).

Published data indicate that fish ingest heavy metals by direct uptake in aqueous solution or by epithelial absorption of heavy metal contaminated water that sluices through their gills, skin, oral cavity and digestive tract [73]. However, chronic intake of heavy metals by fish rest entirely on the metal concentration, volume of the ingested contaminated food or water, the heavy metal uptake speed, exposure duration, uptake route, ecological conditions external to the fish (including availability of water, temperature, pH) and innate factors such as fish age [14, 75], fish nutritional habits as well as the dynamic processes involved in the trace metal metabolism when ingested [76]. Thus, the high levels of heavy metals recorded in *P. annectens* in this study could be because it is a piscivorous species [14, 77, 78]. *Protopterus annectens* (popularly known as West African lung fish or Tana lungfish) feeds on small fish, molluscs, annelids, crustaceans and frogs [77, 78]. Further, the fish samples were taken during the dry season and this is known to influence the concentration of heavy metals recorded in aquatic organisms [75, 79, 80]. The results of this study are not in agreement with the report of Mohamed [81] in which *P. annectens* was reported to contain the least level of heavy metals (beryllium, boron and molybdenum) in both edible muscles and the head compared to the other species (*Barbus bynni, Labeo niloticus, Marcusenius cyprinoides, Mormyrus niloticus, Clarias lazera* and *Mormyrops anguilloides*). Further, the levels of heavy metals reported in *P. annectens* in this study are higher than that reported by preceding authors in other parts of Africa (**Table 4**). Only one study [82] reported a zinc concentration of 211.33 mgkg^{-1} which was higher than the concentration of zinc reported in this study.

Overall, the differences in the heavy metal concentrations in the fish samples can be attributed to the presence of metal contaminants in the rivers. Bioavailability of metals may be influenced by physiological activities of fish, pollutant loads, water chemistry and other environmental factors. Differences in temperature, salinity and water flow can also influence metal accumulation and bioavailability [72].

Table 4. *Comparison of Heavy Metal Concentrations in P. annectens from Nyabarongo river with Other Studies.*

Study area	Mean heavy metal concentration (mgkg^{-1})							Year	Authors
	Fe	Mn	Cu	Zn	Cr	Cd	Pb		
Nyabarongo river, Rwanda [a]	272.8 ± 0.36 (336.0 ± 0.70)	292.2 ± 0.25 (302.6 ± 1.22)	8.8 ± 0.36 (6.4 ± 0.26)	135.2 ± 0.15 (44.7 ± 0.20)	148.0 ± 0.21 (138.2 ± 0.17)	*BDL* *(BDL)*	432. 0 ± 0.50 (302.4 ± 1.50)	2019	**This study**
Lower River Benue, Nigeria	0.36 ± 0.02	ND	ND	ND	ND	ND	0.09 ± 0.01	2018	[83]
Oguta Lake, Nigeria	ND	ND	30.10	ND	3.75	0.41	18.10	2016	[84]
Nkisa River, Nigeria	174.66	11.81	4.92	211.33	1.03	0.79	0.98	2014	[82]
Benin City, Nigeria.	ND	ND	ND	ND	ND	0.32^{F}, 0.52^{D}	ND	2011	[85]
Anambra river, Nigeria	60.23 ± 0.37 [b] 54.60 ± 0.20 [c]	0.94 ± 0.06 1.00 ± 0.01	3.01 ± 0.40 2.86 ± 0.31	10.60 ± 0.08 11.40 ± 0.30	0.16 ± 0.03 0.17 ± 0.02	ND ND	0.01 ± 0.02 0.01 ± 0.02	2009	[79]

Notes: a-results in parenthesis are for fish from Kirinda bridge, b and c-results in these rows were obtained in wet and dry seasons respectively. BDL-below detection limit, ND-Not determined, F-Fresh sample, D-Dry sample. Years cited are the years the data were published, with most data collected in 1 month to 1 year prior to publication.

3.3 *Health* Risk Assessment From Consumption *of P. annectens*

Chronic low-level intake of priority trace metals has been implicated for deleterious human health effects which become apparent following years of persistent exposure [86-88]. Weber *et al.* [89] reported that aquatic organisms exposed to copious levels of waterborne trace metals bioconcentrate the metals upon absorption, ultimately transferring them to humans as they are inevitable in human nutrition. Thus, for the general population, dietary intake is the dominant exposure pathway to heavy metals [14]. Target hazard quotient method was used to assess the potential health risks of heavy metal accumulation through consumption of the edible muscles of *P. annectens*. The estimated daily intakes (EDIs) ranged from 0.011 $mgkg^{-1}day^{-1}$ for Cu at Kirinda bridge to 0.719 $mgkg^{-1}day^{-1}$ for Pb at Ruliba station for adults (**Table 5**).

Table 5. *Estimated Daily Intakes for P. annectens from Nyabarongo River Consumed by Adults and Children*

Consumer	Sampling point	Estimated Daily Intake ($mgkg^{-1}day^{-1}$)						
		Fe	Mn	Cu	Zn	Cr	Cd	Pb
Adults	Ruliba station	0.454	**0.486**	0.015	0.225	0.246	*ND*	**0.719**
	Kirinda bridge	0.559	**0.504**	0.011	0.074	0.230	*ND*	**0.503**
Children	Ruliba station	**1.816**	**1.945**	**0.059**	0. 900	0.985	*ND*	**2.875**
	Kirinda bridge	**2.236**	**2.014**	**0.043**	0.298	0.920	*ND*	**2.013**

ND-Not determined. Values in **bold** are higher than the reference oral doses of the heavy metals.

The EDIs ranged from 0.043 $mgkg^{-1}day^{-1}$ for Cu at Kirinda bridge to 2.875 $mgkg^{-1}day^{-1}$ for Pb at Kirinda bridge for fish consumed by children. Most EDIs exceeded the individual metal reference doses, implying that there are possible health risks from consumption of *P. annectens* from Nyabarongo river. Target hazard quotient of less one indicates the relative absence of health risks associated with intake of the metal through consumption of a heavy metal contaminated fish. As can be observed in **Table 6**, the individual target hazard quotients for Mn and Pb are above 1.0 for adults. In children, the risk is very high because only Zn at Kirinda bridge and Cr had THQ less than one. Thus, consumption of *P. annectens* from the studied parts of Nyabarongo river may have negative health impacts as reflected by the total target hazard quotients being greater than 1 in both adults and children. Clearly, children are more vulnerable to health risks associated with trace metal contamination than adults as the TTHQ for children were always higher than that of adults (**Table 6**). This risk is also high in pregnant women and those of childbearing age.

Table 6. Target hazard quotients for *P. annectens* from Nyabarongo river consumed by adults and children.

Consumer	Sampling Point	Heavy Metal Target Hazard Quotient							Total Target Hazard Quotient
		Fe	Mn	Cu	Zn	Cr	Cd	Pb	
Adults	Ruliba station	0.649	**1.620**	0.375	0.750	0.164	*ND*	**5.136**	**8.53**
	Kirinda bridge	0.799	**1.680**	0.275	0.247	0.153	*ND*	**3.593**	**6.75**
Children	Ruliba station	**2.657**	**6.483**	**1.475**	**3.000**	0.657	*ND*	**20.536**	**34.81**
	Kirinda bridge	**3.194**	**6.713**	**1.075**	0.993	0.613	*ND*	**14.379**	**25.89**

ND-Not Determined. Values in **bold** are greater than **1**.

3.4 Bioaccumulation factor

The bioconcentration factor computed for *P. annectens* in the sampled stations of Nyabarongo river are presented in **Table 7.**

Table 7. Bioconcentration Factor for *Protopterus annectens* in Sampled Stations of Nyabarongo River.

Location	Bioconcentration factor (L/kg)						
	Fe	Mn	Cu	Zn	Cr	Cd	Pb
Ruliba station	169.0	**551.3**	36.7	N/A	N/A	N/A	**8,640**
Kirinda bridge	**533.3**	**15,130**	N/A	496.7	**2,303.3**	N/A	**403.2**
Recommended limits [90]	200	500	200	1000	200	200	300

N/A-Not Applicable. Values in ***bold*** *are higher than the maximum permissible limits.*

The concentration of chemicals in aquatic organisms can be calculated by two factors; bioconcentration factor (BCF) and bioaccumulation factor (BAF). Both factors illustrate the partitioning of a chemical between water and aquatic organisms, often fish, at steady-state conditions [90]. BCF refers to chemical levels in organisms only due to uptake by the organism from the surrounding water while BAF includes uptake from food. In this study, Fe, Mn, Cr and Pb had BCF more than the recommended limits in edible tissues of freshwater fish especially at Kirinda bridge. Chromium had the highest BCF, about 11 times the recommended limit. Bioaccumulation is due to uptake and retention of elements in organisms and the process is usually complex to describe. The uptake of elements depends primarily on environmental conditions whereas the retention is dependent on biological features of the organisms [90]. The complexity of these processes may be one of the reasons why the range of the reported values for a given element can be very large [90]. Further, the BCF based on dry weight are reported to be 3 to 10 times higher than those based on wet weight, depending on the organism and sample preparation. Ash-weight based BAF values are 100 times higher than those based on wet weight [91].

Alinnor, Ayuk and Igbomezie [84] reported that the BCF of heavy metals in *P. annectens* followed the sequence Cu > Pb > Cd = Cr. In this study, *P. annectens* recorded the highest BCF of 130 for Cu and 27 for Pb. Such trace metal accumulation levels in fish as in this concerted study augment published data reported by preceding authors on different species of aquatic organisms [14, 37, 41, 92, 93]. Thus, this study suggests that *P. annectens* could be used as a sentinel organism for biomonitoring of aquatic ecosystems.

4.0 Conclusions and Recommendations

This study has shown that Nyabarongo river and Nyabugogo river water and fish are polluted with heavy metals (Fe, Mn Cu, Zn, Cr and Pb) with Fe, Pb and Mn being the major pollutants in concentrations exceeding maximum permissible WHO limits. Human health risk assessment indicates that the population consuming *P. annectens* are subjected to significant health hazard from ingestion of heavy metal contaminated fish. Nyabarongo and Nyabugogo rivers should therefore be protected from anthropogenic activities such as mining and agricultural activities carried out along their banks to mitigate water pollution with metals and agricultural chemicals. Further studies during different seasons of the year should be done to monitor the variation of the heavy metal content of the edible muscles of *P. annectens* in Nyabarongo river. Research should be done to evaluate the trace metal contents of metabolically active organs (gills, liver, kidney) of *P. annectens* from Nyabarongo river. The microbiological quality of water from the studied rivers should be assessed.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article (and its additional files).

Supplementary materials

Additional file 1. Physicochemical properties of water and the heavy metal content of the edible muscles of *Protopterus annectens* from Nyabarongo and Nyabugogo rivers.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' contributions

TO, PN & SA designed the study, PN performed the analytical experiments, TO, DWO, MDN, RO, SME, AJ & AM performed literature search, PN, LNK, BJK, SME & AJ analyzed the collected data. TO, PN, SA, DWO & RO wrote the first draft of the manuscript. All authors revised and approved the final manuscript.

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Abbreviations

BAF-Bioaccumulation factor
BCF-Bioconcentration factor
EDI-Estimated Daily Intake
R_fD-Reference dose
TDS-Total dissolved solids
THQ-Target hazard quotient
TTHQ-Total target hazard quotient

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