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Accumulation of Heavy Metals in Water and Some Fish Samples from Onuimo River, Imo State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author CIN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CIE and CI managed the analyses of the study. Author CIE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Accumulation of some heavy metals (Cu, Zn, Cd and Cr) was determined in selected fish; Moon fish (*Citharinus citharus*), Tilapia fish (*Oreochronus niloticus*), Mud fish (*Clarias anguillaris*), Cat fish (*Clarias gariepinus*) and Carp fish (*Labeo coulbie*) and water samples from Onuimo River in Imo State in Nigeria. Accumulation order of heavy metals in fish samples comprised of Moon fish > Cling fish > Mud fish > Tilapia fish > Cat fish. Bioconcentration factor model used in the present study showed the following order; Moon fish = Carp fish > Cat fish > Mud fish > Tilapia fish and Moon fish = Carp fish > Tilapia >Mud fish > Cat fish for copper and zinc metals respectively. The concentration of cadmium and chromium in water samples were below detection limits of the Atomic Absorption Spectrophotometer (AAS) Machine. Concentrations of Cu, Cd, Zn and Cr were also below permissible limits of some international regulatory bodies.

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1. INTRODUCTION

In recent years, world consumption of fish has increased simultaneously with the growing concern of their nutritional and therapeutic benefits [1]. Fish not only serve as good sources of protein but are rich in unsaturated fatty acids basically as essential minerals and vitamins [1]. Excessive consumption of heavy metal contaminated fish can result in bioaccumulation of these toxic wastes in the body over time [2]. Fish which are relatively situated at the top of the aquatic food chain can accumulate heavy metals in their tissues either from food, water, and sediments [2-4]. Bioaccumulation of heavy metals in fish and their subsequent distribution to various organs of the fish are greatly interspecific.[2,5][Some factors that can influence metal uptake by fish include the following; metal type, fish species and tissue, sex, size, age feeding behaviour, swimming pattern, reproductive cycle and geographical location [2, 5-7]. Entry of heavy metals into the organs of fish mainly takes place by adsorption and absorption along kidney, liver, gut tract walls, muscular and gills surfaces [8]. Thus, the rate of accumulation becomes a function of uptake and depuration rates [8]. This makes fish a good indicator of heavy metal contamination in water [9-10].

Essential heavy metals like zinc, iron, manganese and copper are vital for biological systems like enzymatic activities but they can also be toxic at high concentration [11]. Non essential heavy metals like nickel, chromium, cadmium, mercury, lead, arsenic and silver have no known essential role in living organisms. They exhibit extreme toxicity at very low concentration and have been regarded as major threat to life of both organisms and humans. [12-14]. Toxicity occurs due to inability of excretory, metabolic, storage and detoxification mechanisms to counter metal uptake [15]. This eventually leads to histopathological and physiological changes [16-17]. Excessive heavy metals in fish tissues can invalidate their health benefits to humans who consume the fish. Several adverse effects of heavy metals in fish samples have been extensively studied [18]. Some of the adverse effects are cardiovascular, renal and peripheral vascular diseases, neurologic and non–behavioural disorders, cancer, proteinuria, liver and kidney failure, gastrointestinal toxicity, nephropathy, hematologic disorder, encephalopathy, pulmonary fibrosis, nasopharyngeal tumors, nephrotoxicity, tremor, nausea, and damage to fetus among others [19-21]. As a result of these effects, many I monitoring programs have been established in order to assess the quality of fish for human consumption and also to monitor the health of aquatic ecosystem [22-25].

Heavy metals are elements with a relative density greater than 5.0 g/cm^3 occurring naturally as components of the earth's crust. Metals enter the environment through anthropogenic processes like mining, extraction and refining, combustion and electro wining process, indiscriminate discharge of automobile and industrial wastes, fertilizer, pesticides and herbicides application in farm lands, river run off like hydrothermal vent [26] and volcanic eruptions [27]. Due to great benefits of fish consumption, there is an urgent need for humans to ascertain the levels of heavy metals in fish species that are consumed daily by both humans and animals to minimize health hazards associated with consumption of contaminated fishes. Some researchers have reported presence of toxic metals in various fish samples in water bodies such as Onuimo in Nigeria [24, 28-35]. Onuimo river plays an important role in water supply (domestic and industrial), flood control and fisheries and agricultural purposes to the rural communities. Presence of massive economic and agricultural activities around the river is believed to be the major sources of pollution through river run offs. Hence presence of contaminants like heavy metals and its bioaccumulation in water, soil, sediments, and aquatic organisms especially fishes in river could cause adverse health risk to people who consume products from the river [36-37]. The main objective of this work was to investigate the bioaccumulation of four heavy metals in selected fish samples from Onuimo River namely; Moon fish (*Citharinus citharus*), Tilapia fish (*Oreochronus niloticus*), Mud fish (*Clarias anguillaris*), Cat fish (*Clarias gariepinus*) and Carp fish (*Labeo coulbie*) as well as to assess the levels of Cu, Cd, Cr and Zn in the water phase of the same river.

2. MATERIALS AND METHODS

2.1. Site Description

Onuimo River is located in Umungwa Community in Obowo Local Government Area of Imo State,

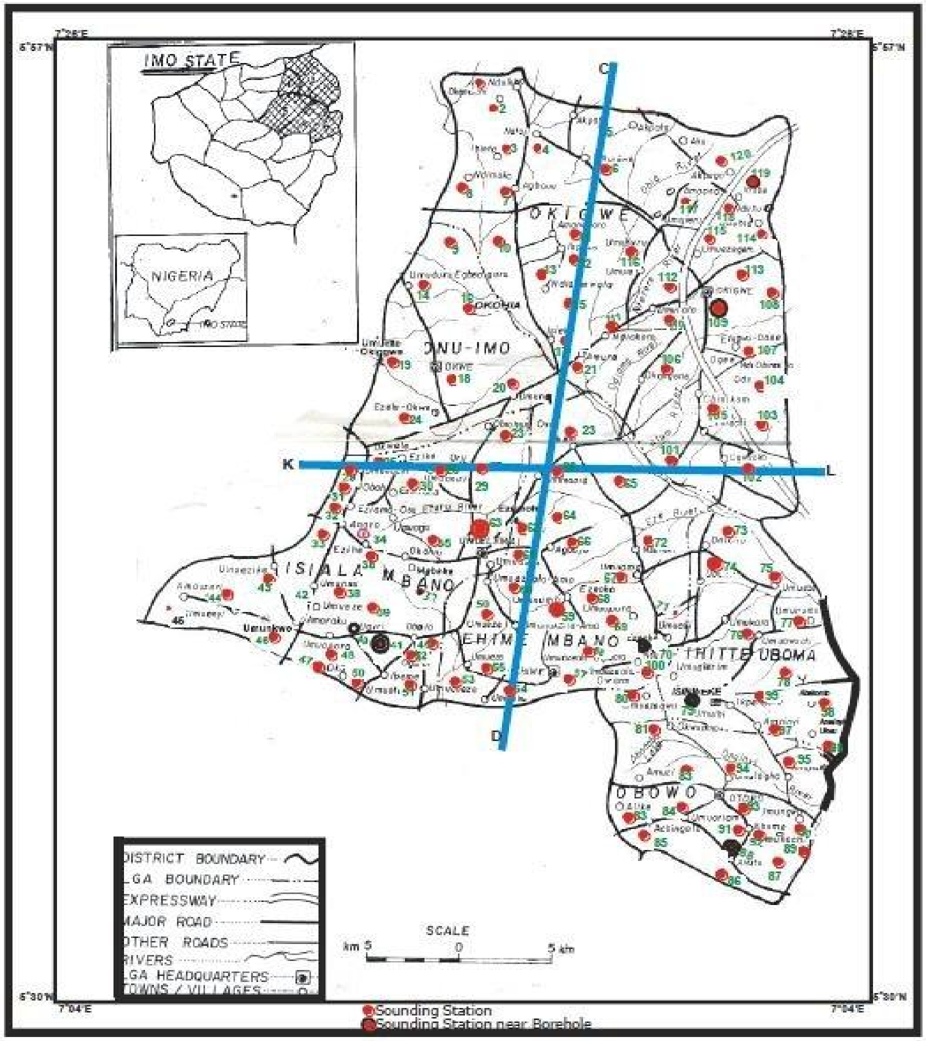

Fig. 1. Geological map showing Onuimo River

Nigeria and the river lies between Longitude 5°50 '56 N and latitude 7˚14 ' 20 W. The River is also linked to Umunachi River which is also in Obowo Local Government Area. The river is located close to Onuimo industrial market and has been seen to be a repository for waste generated in the market. The river runs approximately ten kilometers across Umuahia down to Obowo where it is located.

2.2 Fish and Water Sampling

Fifteen commercial fish samples of the Moon fish (*Citharinus citharus*), Tilapia fish (*Oreochronus niloticus*), Mud fish (*Clarias anguillaris*), Cat fish (*Clarias gariepinus*) and Carp fish (*Labeo coulbie*) were harvested with a locally made gill net in the Onuimo River. The harvested fish samples were first washed with clean water at the point of harvest, separated according to species and preserved in an ice chest. They were then taken to the laboratory and kept frozen at -20 °C in a refrigerator 24 hours prior to analyses. On the other hand, water samples were collected alongside the fishes. Five water samples were harvested with separate acid pre washed 250ml water plastic containers, labeled, enclosed in a chest and transported to the laboratory where they were further refrigerated for a maximum of 24 hours prior to analyses.

2.3 Quality Control

All glass wares used for the analysis were Pyrex, and the reagents used were of analytical grade. The glass wares were washed in 10% nitric acid, 0.5% potassium permanganate and thoroughly rinsed with deionized water and air dried prior to their various uses, were first washed with. Standard operating conditions were observed to ensure accurate and precise analysis.

2.4 Determination of Heavy Metal Contents in Fish and Water Samples

In the laboratory, the fish samples were dried in an electric oven at a temperature range of 70 – 80 ℃ for three days. After drying, fish samples s were crushed to fine particle size using acid pre-washed mortar and pestle. Exactly 1 g of the grounded fish sample was accurately weighed and transferred into a 250 mL conical flask. 10 mL of digestion mixture in the ratio of (1:2:2) of perchloric, nitric and sulphuric acids ware added to the sample and heated on a hot plate in a fume cupboard at about 200 ℃ for 30 minutes until white fumes disappeared indicating complete digestion. The sample was allowed to cool after which 20 mL of distilled water was added to bring the metals into solution. Sample was later filtered with Whatman filter paper under gravity into a 100 mL volumetric flask. The filtrate was made up with deionized water up to the graduated mark of the volumetric flask. The following metals contents were analyzed using atomic absorption spectrophotometer of model AA 500PG after selecting the various wavelengths at which the heavy metals Cu, Zn, Cd and Cr were determined. Also an analytical blank was prepared in a similar method which was used in the calibration of the AAS machine. The analyses were validated by diluting the salt solutions of the investigated heavy metals (Zn, Cu, Cr and Cd) in various concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 ppm to enable the spectrophotometer measure the concentrations of the investigated heavy metals from their sample solutions [30]. The same procedure was repeated for all the fish samples and all analyses were done in triplicate. Refrigerated water samples were brought out and allowed to defrost and were transferred into separate beakers which was followed by digestion and analysis of samples [30]. Water samples were brought of the refrigerator and analyzed using AAS machine to determine heavy metal (Cu, Cr, Zn and Pb) contents in each sample [38]. The analyses were done in triplicate.

Table 1. An overview of regulatory bioaccumulation assessment endpoint and criteria [39]

S/No	Regulatory agency	Bioaccumulation endpoint	Criteria (log values)	Programme
1	Environmental Canada	K_{ow}	≥ 100000 (5)	CEPA (1999)*
2	Environmental Canada	BCF	≥ 5000 (3.7)	CEPA (1999)
3	Environmental Canada	BAF	≥ 5000 (3.7)	CEPA (1999)
4	European Union ‘bioaccumulative’	BCF	≥ 2000 (3.3)	REACH⁺
5	European Union ‘very bioaccumulative’	BCF	≥ 5000 (3.7)	REACH
6	United States ‘bioaccumulative’	BCF	1000 – 5000 (3.7)	TSCA ǂ, TRI
7	United States ‘very bioaccumulative’	BCF	≥ 5000 (3.7)	TSCA, TRI
8	United Nations Environment Programme	K_{ow}	≥ 100000 (5)	Stockholm convection §
9	United Nations Environment Programme	BCF	≥ 5000 (3.7)	Stockholm convection

**CEPA , Canadian Environmental Protection Act, 1999 (Government of Canada 2000)*

⁺Registration, Evaluation and Authorization of Chemicals (REACH) Annex XII (European Commission 2001)

ǂCurrently being used by the US Environmental Protection Agency in its Toxic Substance Control Act (TSCA) and Toxic Release Inventory (TRI) Program (USEPA 1976)

§ Stockholm Convention on Persistent organic Pollutants (UNEP 2001)

2.5 Statistical Analysis

Statistical data analysis was done using SPSS 16.0 software. Descriptive statistics was conducted on the triplicate data to determine the mean, range and standard deviation.

2.6 Bioconcentration of Heavy Metals in Investigated Fish Samples

The competing uptake and elimination processes resulting in bioconcentration can be represented mathematically by an organism-water-two-compartment model where the organism is considered to be single compartment in which the chemical is homogeneously mixed [39].

$$\frac{dC_B}{dt} = (k_1 C_{WD}) - (k_2 + k_E + k_M + k_G)C_B \qquad \text{Eq. 1}$$

where C_B = chemical concentration in the organism ($g.kg^{-1}$),

t = unit time (d^{-1})

k_1 = chemical uptake rate constant from the water at the respiratory surface ($L.kg^{-1}.d^{-1}$)

C_{WD} = freely dissolved chemical concentration in the water ($g.L^{-1}$)

k_2, k_E, k_M, k_G = rate constants (d^{-1}) representing chemical elimination from the organism via the respiratory surface, fecal egestion, metabolic transformation and ngrowth dilution

when both C_B and C_{WD} no longer vary with exposure duration. That is, $\frac{dC_B}{dt} = 0$, the system has reached a steady state and equation (1) can be rearranged to calculate the BCF as;

$$BCF = \frac{C_B}{C_{WD}} = \frac{k_1}{(k_2 + k_E + k_M + k_G)} \qquad \text{Eq. 2}$$

Bioconcentration can also be calculated as the ratio of the chemical concentration in the organism to the chemical concentration in water at steady state [39].

$$BCF_{ss} = \frac{C_B}{C_{WD}} = \frac{Conc._{Biota}}{Conc._{Water}} \qquad \text{Eq. 3}$$

The steady state calculation also refered to as the "Plateau Method" is only valid if the staedy state actually occur (USEPA 1996a; OECD 1996) [40-41].

BCF can also be determined kinetically as the ratio of the chemical uptake rate constant from water and the total elimination or depuration rate constant k_T (d^{-1}),

$$BCF_k = \frac{k_1}{k_T}, \text{ where } k_T = k_2 + k_E + k_M + k_G \qquad \text{Eq. 4}$$

3. RESULTS AND DISCUSSION

Results of the study conducted showed that copper level in the investigated fish samples were in the range of; Moon fish (27.10 - 30.88) mg/kg; Cat fish (24.00- 30.36) mg/kg, Tilapia fish (13.02 - 33.56) mg/kg; Mud fish (24.20 - 25.12) mg/kg and Carp fish (27.10 - 30.77) mg/kg (Table 2). A trend of mean concentrations of copper (mg/kg) can be written as Tilapia (23.29) < Mud (24.66) < Cat (27.18) < Moon = Carp (28.99) mg/kg. These mean values were higher than permissible limits of some regulatory bodies like WHO (3.0 mg/kg), FEPA (1.3 mg/kg), EU (2008) (1.0 mg/kg) and those reported in *Cyprinus Carpio* and *Pelteobagrus Fluridraco* [23], *L. Coubie* and *M. Tapirus* [31], Indo-pacific king Mackerel and Tiger tooth Crocker [48]. Although copper is recognized as an essential element, excessive intake of it can lead to poisoning, nausea, nausea, diarrhea and fever, acute stomach pain and death [23].

Cadmium is a highly toxic metal which has no biological function in both human system and aquatic organisms [48]. Cadmium can remain in human system for decades and cannot be efficiently metabolized. It can cause kidney damage, lung cancer, testicular tissue destruction, high blood pressure, proteinuria, red blood destruction and non-descended testes in young males [19,48-49]. Recent research show that exposure to cadmium at even low concentration can increase the risk of hormonal cancer [50]. Another research on Long Island also estimated that about 40% of breast cancer cases recorded in the United States might be associated with elevated cadmium levels [51]. Results of cadmium levels (Table 2) depict that cadmium recorded highest mean value of 1.88 mg/kg in Mud fish and the least value of 1.12 mg/kg in Carp fish. The various mean values as shown in Table 2 were also higher than permissible limits of EU (2008) but lower than that of FAO (1983).

Chromium showed an increasing trend in mg/kg as follows, Carp fish (0.32) $<$ Cat fish (0.88) $<$ Mud fish (1.27) $<$ Tilapia fish (1.74) $<$ Moon fish (2.61). Mean values of chromium in Carp and Cat fishes (Table 2) were observed to be lower than permissible limit of FAO (1983), EU (2008) and some literature values [52-53]. Chromium levels in the remaining fish samples were higher than the permissible limits above and those reported in *Balistoides Vridiscens* [19]. Bioaccumulation of chromium in humans can lead to; pulmonary fibrosis, lung cancer

Table 2. Levels of heavy metals in fish samples in river Onuimo compared to some regulatory standards

Sample	Cu	Cd	Cr	Zn
Damsel fish				
A	30.88	2.00	2.61	79.55
B	28.99	1.36	3.20	80.10
C	27.10	0.72	2.10	79.00
Range	27.10 – 30.88	0.72 – 2.00	2.02 – 3.20	79.00 – 80.10
$\bar{X} \pm$ SD	28.99 $\pm$ 1.89	1.36 $\pm$ 0.64	2.61 $\pm$ 0.59	79.5 $\pm$ 0.55
Tilapia fish				
A	33.56	2.10	1.74	100
B	23.29	1.14	1.40	61.37
C	13.02	0.18	2.08	22.64
Range	13.02 – 33.36	0.18 – 2.10	1.40 – 2.08	22.64 – 100
$\bar{X} \pm$ SD	23.29 $\pm$ 10.27	1.14 $\pm$ 0.96	1.74 $\pm$ 0.34	61.37 $\pm$ 38.73
Cat fish				
A	30.36	2.15	0.88	40.12
B	27.18	1.24	1.10	45.56
C	24.00	0.33	0.66	51.00
Range	24.00- 30.36	0.33 – 2.15	0.66 – 1.10	40.12 – 51.00
$\bar{X} \pm$ SD	27.18 $\pm$ 3.80	1.24 $\pm$ 0.91	0.88 $\pm$ 0.22	45.56 $\pm$ 5.44
Dat fish				
A	25.12	1.26	1.27	70.00
B	24.66	1.88	0.53	60.44
C	24.20	2.40	2.01	50.88
Range	24.20 – 25.12	1.36 – 2.40	0.53 – 2.01	50.88 – 70.00
$\bar{X} \pm$ SD	24.66 $\pm$ 0.46	1.88 $\pm$ 0.52	1.27 $\pm$ 0.74	60.44 $\pm$ 9.56
Cling fish				
A	30.77	0.21	0.41	79.35
B	29.10	1.12	0.32	80.20
C	27.10	2.03	0.23	79.10
Range	27.10 – 30.77	0.12 - 2.03	0.23 – 0.41	79.10 – 80.20
$\bar{X} \pm$ SD	28.99 $\pm$ 1.89	1.12 $\pm$ 0.91	0.32 $\pm$ 0.09	79.55 $\pm$ 0.55
[42] FEPA(2003)	1.3	-	0.15	-
[43] WHO (2006)	3.0	-	0.15	-
[44] EU (2001)	1.0	-	1.0	-
[45] EU (2008)	0.5 – 1.0	0.5 – 1.0	2.0	-
[46] Indonesia	80	-	-	200
[47] FAO (1983)	-	2.0	1.0	-

Table 3. Level of heavy metals in water, Bioconcentration Factor (BCF) of investigated fish samples

								Bioconcentration factor				
Metal	**Conc. of water (mg/L)**	**WHO (2011) limits (mg/L) [54]**	**UNEP (2007) limits (mg/L) [55]**	**USEPA (mg/L) [56]**	**ECE (1998) (mg/L) [57]**	**FTP-CDW (mg/L) [58]**	**ADWG (mg/L) [59]**	**Moon**	**Tilapia**	**Cat**	**Mud**	**Carp**
Cu	0.065	2.000	2.000	1.300	2.000	1.000	2.000	446	358	418	379	446
Cd	BDL	0.003	0.003	0.005	0.005	0.005	0.002	-	-	-	-	-
Cr	BDL	0.050	0.050	0.010	0.050	0.005	0.050	-	-	-	-	-
Zn	0.112	3.000	3.000	0.500	-	50.00	3.000	710	548	407	540	710

[54] World Health Organization (WHO, 2011)
[55] United Nation Environmental Programme (2007)
[56] United States Environmental Protection Agency (USEPA, 2011)
[57] European Commission Environment (ECE, 1998)
[58] Federal-Provincial-Territorial Committee on drinking Water (CDW), Health Canada (FTP-CDW, 2010)
[59] Australian Drinking Water Guidelines (2011)

(inhalation), cardiovascular, renal, gastrointestinal, hematological and neurological effects [19].

Levels of zinc in the investigated fish recorded the least minimum value of 45.56 mg/kg in Cat fish and highest value of 79.55 mg/kg in both Moon and Carp fishes. A trend of decrease in mean values of zinc (mg/kg) in the analyzed fish samples can be seen as; Cat fish (45.56) $<$ Mud fish (60.44) $<$ Tilapia fish (61.37) $<$ Moon fish = Carp fish (79.55). These mean values are also higher than some permissible limits of Indonesia maximum limits of metals in food (Table 2) and some literature studies [30,31].

Results of heavy metals in water samples (Table 3) depict that level of copper (0.065 mg/L) was lower than standards of WHO, ECE, UNEP, ADWG (2.000 mg/L), USEPA (1.300 mg/L) and FTP-CDW (1.000 mg/L). Concentration of zinc in water sample (0.112 mg/L) was also lower than standards of WHO, UNEP, ADWG (3.000 mg/L) and USEPA (0.500 mg/L). Cadmium and chromium levels were observed to be below detection limit of the AAS machine. Results of Bioconcentration factor showed that copper have a BCF order of; Tilapia fish (358) < Mud fish (379) < Cat fish (418) < Moon = Carp (446). Also zinc has a decreasing BCF order of; Moon = Carp (710) > Tilapia (548) > Mud fish (540) > Cat fish (407). These values were found to be lower than the criteria (log value) of some regulatory agencies like CEPA { $\geq$ 5000 (3.7) }, REACH+ {$\geq$ 2000 (3.3)}, TSCA ǂ, TRI {1000 - 5000 (3.7)} and UNEP {$\geq$ 5000 (3.7)} as shown in Table 1. Bioconcentration factors of chromium and cadmium were not calculated because their levels in investigated water samples were below the detection limits of the AAS machine used. Levels of heavy metals in water samples as investigated showed that the mean concentration of copper (0.0065 mg/L) in water samples was lower than the permissible limits of some regulatory bodies like WHO (2.0 mg/L), USEPA (1.3 mg/L), ECE (2.0 mg/L) and FTP-CDW, 2010 (1.0 mg/L). Mean concentration of zinc (0.112 mg/L) was also lower than permissible limits of WHO (3.0 mg/L), USEPA (0.5 mg/L), FTP-CDW (50 mg/L) and UNEP (3.0 mg/L). However, concentrations of cadmium and chromium in investigated water were observed to be below detection limit of the AAS machine used.

4. CONCLUSION

The present study provides valuable information on levels of heavy metals in some selected fish and water samples from Onuimo River, Imo State. The rate of heavy metal sorption and accumulation in fish samples varied with species of fish and other specific factors like the feeding pattern, weight and age. From the data presented, it can be concluded that values of heavy metals in water and fish samples were lower than some permissible limits of some regulatory bodies. Bioconcentration factor model used showed that values of investigated heavy metal were lower than permissible limits of some regulatory bodies. Thus, the river can be said to be contaminated due to presence of heavy metals detected. Therefore, it is recommended that activities that release heavy metals and other contaminants in and within the river should be stop so as to prevent pollution of the river.

COMPETING INTEREST

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

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