

Bioaccumulation of Heavy Metals in water and some fish samples from Onuimo River, Imo State, Nigeria

ABSTRACT

Bioaccumulation of some heavy metals (Cu, Zn, Cd and Cr) was determined in selected fish samples from Onuimo River in Imo State. Concentrations of some heavy metals in water and fish samples were determined using atomic absorption spectrophotometer (AA 500 PG) and the results obtained varied significantly among the fish species. Bioaccumulation of heavy metals were found to follow the sequence as Damsel fish > Cling fish > Dat fish > Tilapia fish > Cat fish. Bioconcentration factor model implored in the study showed a decreasing order as follows; Damsel fish = Cling fish > Cat fish > Dat fish > Tilapia fish and Damsel fish = Cling fish > Tilapia > Dat fish > Cat fish for copper and zinc metals respectively. Those of Cadmium and chromium were not calculated due to the fact that there concentrations in water samples were below detection limits. Results of analyses of heavy metals in selected water samples revealed that concentration of cadmium and chromium was below detection limits of some international regulatory bodies whereas those of zinc and copper were also lower than these permissible limits.

Keywords: Atomic absorption spectrophotometer, bioaccumulation, bioconcentration, fish, heavy metals, Onuimo River, water analysis.

1. INTRODUCTION

Due to geometric increase in human population around the world, the rate at which fish is consumed globally had also increased owing to the fact that fish have both nutritional and therapeutic values. Fish not only serve as good sources of protein but are unsaturated fatty acids richly in various essential minerals and vitamins which nourishes the body when consumed [1]. These vital nutrients makes fish a perfect health food which when consumed adequately encourages optimal heart functioning. The American Heart Association (AHA) recommended the consumption of fish for at least twice per week in order to reach the daily intake of omega – 3 fatty acids [1].

However, excess consumption of heavy metal contaminated fish can result in bioaccumulation of these toxic wastes in the body over time. Fish which are relatively situated at the top of the aquatic food chain can accumulate heavy metals in their tissues either from food, water, sediments or all. [2-4]. Bioaccumulation of heavy metals in fish and their subsequent distribution at various organs of the fish are greatly interspecific. Some factors that can influence metal uptake by fish may include the following; metal type, fish species and tissue, sex, size, age feeding behaviour, swimming pattern, reproductive cycle and geographical location etc [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. Thus, the rate of accumulation becomes a function of uptake and depuration rates. This makes fish a good indicator of heavy metal contamination in water [8-10].

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, silver etc 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]. Toxic effect occurs due to inability of excretory,

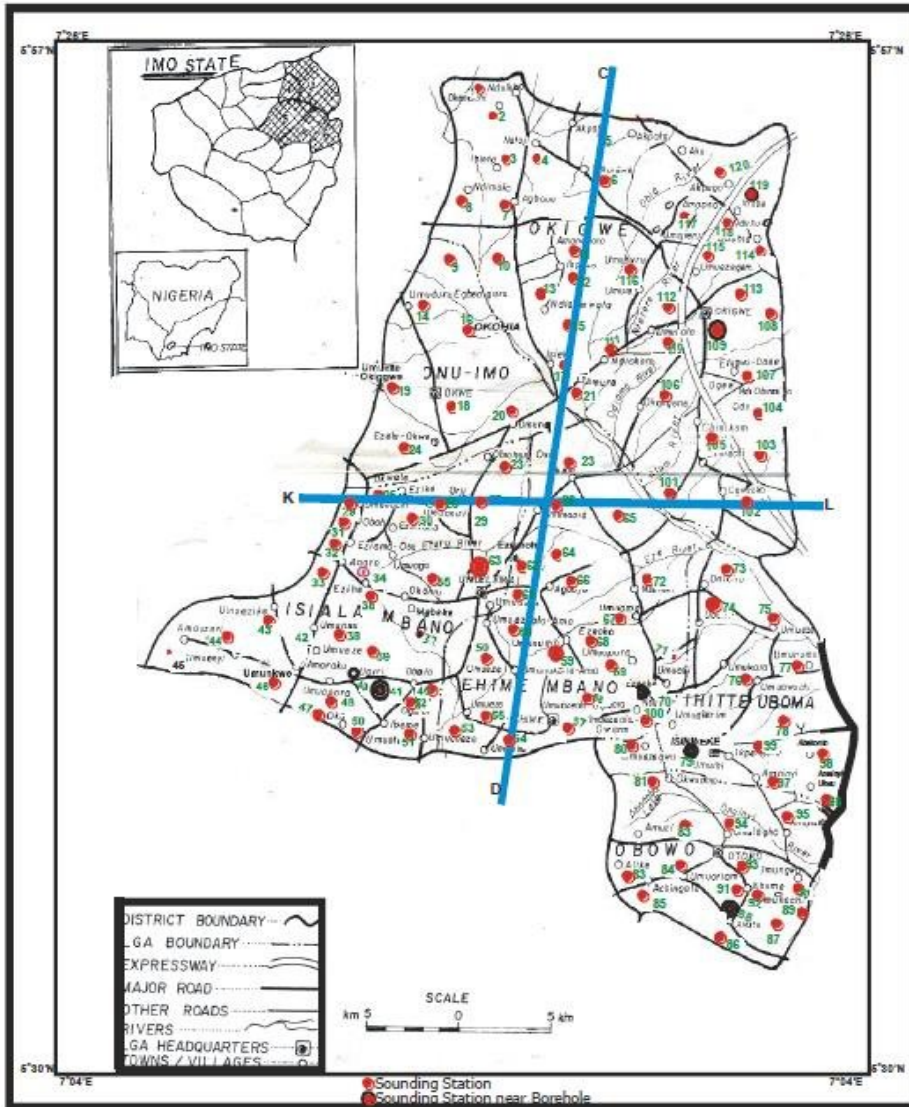
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42 metabolic, storage and detoxification mechanisms to counter metal uptake [15]. This eventually leads to
43 histopathological and physiological changes [16-17]. Heavy metals in fish can invalidate their health
44 benefits to humans. Several adverse effects of heavy metals in fish samples have been extensively
45 studied [18]. Some of the adverse effects are cardiovascular, renal and peripheral vascular diseases,
46 neurologic and non-behavioural disorders, cancer, proteinuria, liver and kidney failure, gastrointestinal
47 toxicity, nephropathy, hematologic disorder, encephalopathy, pulmonary fibrosis, nasopharyngeal tumors,
48 nephrotoxicity, tremor, nausea, damage to fetus among others [19-21]. As a result of these effects, many
49 inter-national monitoring programs have been established in order to assess the quality of fish for human
50 consumption and also to monitor the health of aquatic ecosystem [22-24].

51 Heavy metals are natural components of earth crust with a relative density greater than 5.0 g/cm^3 . The
52 can enter the environment through anthropogenic processes like mining, extraction and refining,
53 combustion and electro wining process, indiscriminate discharge of automobile and industrial wastes,
54 fertilizer, pesticides and herbicides application in farm lands, river run off like hydrothermal vent [26] and
55 volcanic eruptions [27]. Due to great benefits of fish consumption, there is an urgent need for humans to
56 ascertain the level of heavy metals in fish species that are consumed daily by both humans and animals
57 in other avoid health hazards that come with consumption of heavy metals contaminated fishes. Some
58 researchers have reported presence of toxic metals in various fish samples in water bodies in Nigeria [24,
59 28-35]. Onuimo has been one of the fresh water rivers in the South Eastern part of the country. The river
60 plays important role in water supply (domestic and industrial), flood control and fisheries and agricultural
61 purposes to the rural communities that her tributaries cut across. Presence of massive economic and
62 agricultural activities around the river is believed to be the major sources of pollution through river run
63 offs. Hence presence of contaminants like heavy metals and its bioaccumulation in water, soil, sediments,
64 and aquatic organisms especially fishes in river could cause adverse health risk to people who consume
65 products from the river [36-37]. The main objective of this work is to investigate the bioaccumulation of
66 four heavy metals in selected fish samples from Onuimo River namely; Moon fish (*Citharinus citharus*),
67 Tilapia fish (*Oreochromis niloticus*), Mud fish (*Clarias anguillaris*), Cat fish (*Clarias gariepinus*) and Carp
68 fish (*Labeo coulbie*).

69 **2. EXPERIMENTAL DETAILS**

70 **2.1. Site Description**



71

72 **Figure 1. Geological Map Showing Onuimo River**

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

78 **2.2 Fish and Water Sampling**

79 Fifteen commercial fish samples were harvested with a local made net through the aid of local fishermen
80 at the river. The harvested fish samples were first washed with clean water at the point of harvest,
81 separated according to species and preserved in an ice chest. They were then taken to the laboratory
82 and kept frozen at $-20\text{ }^{\circ}\text{C}$ in a refrigerator prior to analyses. On the other hand, water samples were
83 collected alongside where the fishes were harvested with separate acid pre washed water plastic
84 containers, labeled, enclosed in a chest and transported to the laboratory where they were further
85 refrigerated prior to analysis.

86 **2.3 Quality Control**

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

91 **2.4 Determination of Heavy Metal Contents in Fish and Water Samples**

92 The fish samples were brought out of the refrigerator, rinsed thoroughly with deionized water and dried in
93 an electric oven at a temperature range of $70 - 80\text{ }^{\circ}\text{C}$ for three days. After drying, they were all separately
94 brought out and crushed to fine particle size using acid pre-washed mortar and pestle. Exactly 1 g of the
95 grounded fish sample was accurately weighed and transferred into a 250 mL conical flask. 10 mL of
96 digestion mixture in the ration of (1:2:2) of perchloric, nitric and sulphuric acids were all added to the
97 sample and heated on a hot plate in a fume cupboard at about $200\text{ }^{\circ}\text{C}$ for 30 minutes until white fumes
98 formed disappeared indicating complete digestion. The sample was allowed to cool after which 20 mL of
99 distilled water was added to bring the metals into solution. Sample was later filtered with whatman filter
100 paper under gravity into a 100 mL volumetric flask. The filtrate was made up with deionized water up to
101 the graduated mark of the volumetric flask. The following metal (Cu, Zn, Fe, Pb, Cd and Hg) contents
102 were analyzed using atomic absorption spectrophotometer (AAS) of model AA500PG after selecting the
103 various wavelengths at which the heavy metals were determined. Also an analytical blank was prepared
104 in a similar method which was used in the calibration of the AAS machine. The analyses were validated
105 by diluting the salt solutions of the investigated heavy metals (Zn, Cu, Cr, Cd) in various concentrations of
106 0.2, 0.4, 0.6, 0.8 and 1.0 ppm to enable the spectrophotometer measure the concentrations of the
107 investigated heavy metals from their sample solutions [30]. The same procedure was also repeated for all
108 the fish samples and all analyses were done in triplicate. Refrigerated water samples were brought out
109 allowed to defrost and transferred into separate beakers. It was followed by digestion of samples
110 according to the procedure stated by Standard Methods for Examination of Water and Waste water, 2nd
111 edition, 2012 (APHA-AWWA-WEF) [38].

112 **2.5 Statistical Analysis**

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

115 **2.6 Bioconcentration of Heavy Metals in Investigated Fish Samples**

116 Bioconcentration is the process by which a chemical substance is absorbed by an organism from the
117 ambient environment only through its respiratory and dermal surfaces. The degree to which

118 bioconcentration occur is expressed as the bioconcentration factor (BCF) and can only be measured
 119 under controlled laboratory conditions in which dietary intakes of the chemicals are deliberately not
 120 included. The competing uptake and elimination processes resulting in bioconcentration can be
 121 represented mathematically by an organism-water-two-compartment model where the organism is
 122 considered to be single compartment in which the chemical is homogeneously mixed [39].

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

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 ($\text{L.kg}^{-1}.\text{d}^{-1}$)

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;

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

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].

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

123 The steady state calculation also referred to as the "Plateau Method" is only valid if the steady 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}),

$$\text{BCF}_k = \frac{k_1}{k_T}, \text{ where } k_T = k_2 + k_E + k_M + k_G \quad \text{Eq. 4}$$

124 **Table 1. An Overview of Regulatory Bioaccumulation Assessment Endpoint and Criteria [39]**

S/N	Regulatory Agency	Bioaccumulation endpoint	Criteria (log values)	Programme
1	Environmental Canada	K_{ow}	≥ 100000 (3)	CEPA (1999)*
2	Environmental Canada	BCF	≥ 3000 (3.7)	CEPA (1999)
3	Environmental Canada	BAF	≥ 3000 (3.7)	CEPA (1999)
4	European Union	BCF	≥ 2000 (3.3)	REACH*
5	European Union	'very bioaccumulative'	≥ 3000 (3.7)	REACH

6	United States	BCF	1000 – 3000 (3.7)	TSCA ‡, TRI
7	United States	'very bioaccumulative' BCF	≥ 3000 (3.7)	TSCA, TRI
8	United Nations Environment Programme	K _{ow}	≥ 100000 (3)	Stockholm convention †, ‡
9	United Nations Environment Programme	BCF	≥ 3000 (3.7)	Stockholm convention

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

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

‡Currently 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).

125

126 3.0 EXPERIMENTAL RESULTS AND DISCUSSION

127 **Table 2. Levels of Heavy Metals in fish samples in River Onuimo compared to some International**
128 **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±1.89	1.12±0.91	0.32±0.09	79.55±0.55
^[42] FEP(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	-

^[42]Federal Environmental Protection Agency (2003)

^[43]World Health organization (2006)

^[44]European Union (2001)

^[45]European Union (2008)

^[46]Indonesia Decree of General Director of Food and Drug Supervision No.03725/B/SK/VII concerning, maximum limits of metals in food.

^[47]Food and Agricultural Organization of the United Nation (1983)

129

130 Previous research had shown that escalating human population, economic development and poor waste
 131 management have affected adversely the ecosystem. This have significantly contributed to the current
 132 worldwide deterioration of water quality including seasonal accumulation of heavy metals like Cr, Cu, Zn,
 133 Cd, As, Hg, Fe, etc [23,24]. An insight on the levels of these heavy metals in aquatic organism especially
 134 fishes are of paramount important to basically for consumption by human and nature management. This
 135 research work documents results of bioaccumulation of some heavy metals in some fish samples
 136 together with their bioconcentration factor.

137 Results of the study conducted as shown in Table 2 above reveals that copper level in the investigated
 138 fish samples ranges as follows; Damsel fish (27.10- 30.88) mg/kg, Cat fish (24.00- 30.36) mg/kg, Tilapia
 139 fish (13.02-33.56) mg/kg, Dat fish (24.20-25.12) mg/kg and Cling fish (27.10-30.77) mg/kg. A trend of
 140 mean concentrations of copper in mg/kg can be written as Tilapia (23.29) < Dat (24.66) < Cat (27.18) <
 141 Damsel = Cling (28.99) mg/kg. These mean values were found to have been higher than some standard
 142 permissible limits like WHO (3.0mg/kg), FEPA (1.3 mg/kg), EU (2008) (1.0 mg/kg) and those reported in
 143 *Cyprinus Carpio* and *Pelteobagrus Fluridraco* [23], *L.Coubie* and *M. Tapirus* [31]. Indo-pacific king
 144 Mackerel and Tiger tooth Crocker [48]. Although copper is recognized as an essential element, excessive
 145 intake of it can lead to poisoning, nausea, nausea, diarrhea and fever, acute stomach pain and death
 146 [23].

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

158 Chromium another environmental pollutant showed an increasing trend in mg/kg as follows, Cling fish
 159 (0.32) < Cat fish (0.88) < Dat fish (1.27) < Tilapia fish (1.74) < Damsel fish (2.61). Mean values of
 160 chromium in Cling and Cat fishes (Table 2) were observed to be lower than permissible limit of FAO

161 (1983), EU (2008) and some literature values [52-53]. Chromium level in the remaining fish samples were
 162 higher than the permissible limits above and those reported in *Balistoides Vridiscens* [19].
 163 Bioaccumulation of chromium in human can lead to the following health diseases; pulmonary fibrosis,
 164 lung cancer (inhalation), cardiovascular, renal, gastrointestinal, hematological and neurological effects
 165 [19].

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

171 **Table 3. Level of Heavy Metals in Water, Bioconcentration Factor (BCF) of Investigated Fish**
 172 **Samples**

Metal	Conc. of water (mg/L)	[54] [55] [56] [57] [58] [59]						Bioconcentration Factor				
		WHO (2011) limits (mg/L)	UNEP (2007) limits (mg/L)	USEPA (mg/L)	ECE (1998) (mg/L)	FTP- CDW (mg/L)	ADWG (mg/L)	Dam sel	Tila pia	Cat	Dat	Cling
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)

173

174 Results of heavy metals in investigated water samples as shown in Table 3 depicts that levels of copper
 175 (0.065 mg/L) and zinc (0.112 mg/L) were lower than permissible limits of World Health
 176 Organization(2011). Cadmium and chromium levels were observed to be below detection limit (BDL).
 177 Results of Bioconcentration Factor (BCF) implored showed that copper has an increasing BCF values of
 178 Tilapia fish (358) < Dat fish (379) < Cat fish (418) < Damsel = Cling (446). Also zinc has an increasing
 179 BCF values of Cat fish (407) < Dat fish (540) < Tilapia (548) < Damsel = Cling (710). These values were
 180 found to be lower than the criteria (log value) of ≤ 2000 (3.3) for European Union bioaccumulative and
 181 criteria (log values) of 1000 (3) – 5000 (3.7) for United States bioaccumulative and other international
 182 regulatory assessment endpoints and criteria as shown in Table 1. Bioconcentration factors of chromium
 183 and cadmium were not calculated because there levels in water samples were below detection limits.

184 Levels of heavy metals in water samples were also investigated and reported (Table 3). The results
 185 showed that mean concentration of copper in the investigated water samples had a value of 0.0065 mg/L
 186 which was observed to be lower than the permissible limits of World Health Organization (WHO, 2011)
 187 (2.0 mg/L), United States Environmental Protection Agency (USEPA) (1.3 mg/L), European Commission
 188 Environment (2.0 mg/L) and Federal-Provincial-Territorial Committee on Drinking Water, Health Canada
 189 (FTP-CDW, 2010) (1.0 mg/L). Mean concentration of zinc (0.112 mg/L) was also lower than permissible
 190 limits of WHO (3.0 mg/L), USEPA (0.5 mg/L), FTP-CDW (50 mg/L) and UNEP (3.0 mg/L). Values of
 191 cadmium and chromium were observed to be below detection limit.

192 **4. CONCLUSION**

193 The results of the study provide valuable information on levels of heavy metals in water samples and their
194 bioaccumulation in some selected fish samples from Onuimo River, Imo State. The result also showed
195 heavy metals sorption and bioaccumulation by fish varied with respect to species of fish and other
196 specific factors like feeding pattern, weight and age. A trend of bioaccumulation of heavy metals by fish
197 samples can be deduced as follows; Damsel fish > Cling fish > Dat fish > Tilapia fish > Cat fish.
198 Bioconcentration factor model implored in the study showed a decreasing order as follows; Damsel fish =
199 Cling fish > Cat fish > Dat fish > Tilapia fish and Damsel fish = Cling fish > Tilapia for copper and zinc
200 metals respectively. Also bioconcentration (BCF) values obtained were lower than the standards of some
201 International regulatory bodies. Levels of heavy metals in investigated water samples were also lower
202 permissible limits of WHO, USEPA, UNEP and FTP-CDW. These results imply that River Onuimo having
203 little contamination by heavy metals is safe for human, agricultural and industrial uses. Also rate of
204 disposal of heavy metal containing waste into the river and other anthropogenic activities which can
205 contaminants the water should be discouraged. Better still, they should be adequately recycled before
206 disposal to avoid loading of heavy metals and other contaminants into the river.

207 **COMPETING INTEREST**

208 The authors have declared that there is no conflict of interest regarding the publication of this
209 research work.

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