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

- 3
- 4

### 5 ABSTRACT

6 Bioaccumulation of some heavy metals (Cu, Zn, Cd and Cr) was determined in selected fish samples 7 from Onuimo River in Imo State. Concentrations of some heavy metals in water and fish samples were 8 determined using atomic absorption spectrophotometer (AA 500 PG) and the results obtained varied 9 significantly among the fish species. Bioaccumulation of heavy metals were found to follow the sequence 10 as Damsel fish > Cling fish > Dat fish > Tilapia fish > Cat fish. Bioconcentration factor model implored in 11 the study showed a decreasing order as follows; Damsel fish = Cling fish > Cat fish > Dat fish > Tilapia 12 fish and Damsel fish = Cling fish > Tilapia > Dat fish > Cat fish for copper and zinc metals respectively. 13 Those of Cadmium and chromium were not calculated due to the fact that there concentrations in water 14 samples were below detection limits. Results of analyses of heavy metals in selected water samples 15 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. 16

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

#### 19 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].

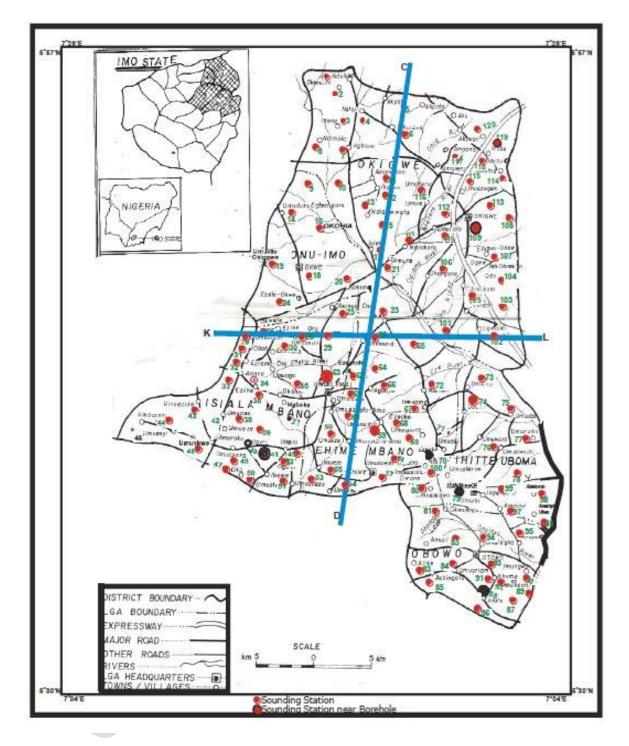
27 However, excess consumption of heavy metal contaminated fish can result in bioaccumulation of these 28 toxic wastes in the body over time. Fish which are relatively situated at the top of the aquatic food chain 29 can accumulate heavy metals in their tissues either from food, water, sediments or all. [2-4]. 30 Bioaccumulation of heavy metals in fish and their subsequent distribution at various organs of the fish are 31 greatly interspecific. Some factors that can influence metal uptake by fish may include the following; metal 32 type, fish species and tissue, sex, size, age feeding behaviour, swimming pattern, reproductive cycle and 33 geographical location etc [2, 5-7]. Entry of heavy metals into the organs of fish mainly takes place by 34 adsorption and absorption along kidney, liver, gut tract walls, muscular and gills surfaces. Thus, the rate 35 of accumulation becomes a function of uptake and depuration rates. This makes fish a good indicator of 36 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, 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, hematogic disorder, encephalopathy, pulmonary fibrosis, nasopharyngeal tumors, 48 nephrotoxicity, tremor, nausea, damage to fetus among others [19-21]. As a result of these effects, many inter-national monitoring programs have been established in order to assess the quality of fish for human 49 50 consumption and also to monitor the health of aquatic ecosystem [22-24].

Heavy metals are natural components of earth crust with a relative density greater than 5.0 g/cm<sup>3</sup>. The 51 can enter the environment through anthropogenic processes like mining, extraction and refining, 52 combustion and electro wining process, indiscriminate discharge of automobile and industrial wastes, 53 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 activates 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 four heavy metals in selected fish samples from Onuimo River namely; Moon fish (Citharinus citharus), 66 Tilapia fish (Oreochronus niloticus), Mud fish (Clarias anguillaris), Cat fish (Clarias gariepinus) and Carp 67 68 fish (Labeo coulbie).

#### 69 2. EXPERIMENTAL DETAILS

70 2.1. Site Description



71

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

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

#### 78 **2.2 Fish and Water Sampling**

Fifteen commercial fish samples were harvested with a local made net through the aid of local fishermen at the 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 prior to analyses. On the other hand, water samples were collected alongside where the fishes were harvested with separate acid pre washed water plastic containers, labeled, enclosed in a chest and transported to the laboratory where they were further refrigerated prior to analysis.

#### 86 2.3 Quality Control

All glass wares used for the analysis were all Pyrex and reagents of analytical grade. The glass wares prior to their various uses, were first washed with 10% nitric acid, 0.5% potassium permanganate and thoroughly with deionized water and air dried. Standard operating conditions were all observed to ensure 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 °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 ware all added to the 97 sample and heated on a hot plate in a fume cupboard at about 200 °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, 2<sup>nd</sup> edition, 2012 (APHA-AWWA-WEF) [38]. 111

#### 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.

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

Bioconcentration is the process by which a chemical substance is absorbed by an organism from the ambient environment only through its respiratory and dermal surfaces. The degree to which bioconcentration occur is expressed as the bioconcentration factor (BCF) and can only be measured under controlled laboratory conditions in which dietary intakes of the chemicals are deliberately not included. 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].

$$\frac{dC_{B}}{dt} = (k_{1}C_{WD}) - (k_{2} + k_{E} + k_{M} + k_{G})C_{B}$$
 Eq. 1

where  $C_B =$  chemical concentration in the organism (g.kg<sup>-1</sup>),

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

 $k_1$  = chemical uptake rate constant from the water at the respiratory surface (L.kg<sup>-1</sup>.d<sup>-1</sup>)

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

 $k_2, k_E, k_M, k_G$  = rate constants (d<sup>-1</sup>) 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 = 
$$\frac{C_B}{C_{WD}} = \frac{k_1}{(k_2 + k_E + k_M + k_G)}$$
 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].

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

<sup>123</sup> 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<sub>k</sub> = 
$$\frac{k_1}{k_T}$$
, where  $k_T = k_2 + k_E + k_M + k_G$  Eq. 4

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

S/N o	Regulatory Agency	Bioaccumulation endpoint	Criteria (log values)	Programme	
1	Environmental Canada	K <sub>ow</sub>	≥ 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 (8.7)	TSCA <del>I</del> , TRI
7	United States 'very bioaccumulative'	BCF	≥ 5000 (3.7)	TSCA, TRI
8	United Nations Environment Programme	Kow	≥ 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). <sup>†</sup>Registration, Evaluation and Authorization of Chemicals (REACH) Annex XII (European Commission 2001) <sup>‡</sup>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**

## Table 2. Levels of Heavy Metals in fish samples in River Onuimo compared to some International Standards

Standards				
Sample	Cu	Cd	Cr	Zn
Damsel fish				
A	30.88	2.00	2.61	79.55
В	28.99	1.36	3.20	80.10
С	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
$\overline{X} \pm SD$	28.99 <u>+</u> 1.89	1.36 <u>+</u> 0.64	2.61 <u>+</u> 0.59	79.5 <mark>±</mark> 0.55
Tilapia fish				
A	33.56	2.10	1.74	100
В	23.29	1.14	1.40	61.37
С	13.02	0.18	2.08	22.64
Range	13.02 - 33.36	0.18 – 2.10	1.40 – 2.08	22.64 – 100
$\overline{X} \pm SD$	23.29 <u>+</u> 10.27	1.14 ± 0.96	1.74 <mark>±</mark> 0.34	61.37 <mark>±</mark> 38.73
Cat fish				
A	30.36	2.15	0.88	40.12
	27.18	1.24	1.10	45.56
B 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
$\overline{X} \pm SD$	27.18 ± 3.80	1.24 <b>±</b> 0.91	0.88 ± 0.22	45.56 🛨 5.44
Dat fish	_	_	_	_
A	25.12	1.26	1.27	70.00
В	24.66	1.88	0.53	60.44
Č	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
$\overline{X} \pm SD$	24.66 <u>±</u> 0.46	1.88 ± 0.52	$1.27 \pm 0.74$	60.44 <u>±</u> 9.56
Cling fish	21.00 2 01.10			0000
A	30.77	0.21	0.41	79.35
B	29.10	1.12	0.32	80.20
C				
Range	27.10	2.03	0.23	79.10
$\overline{X} \pm SD$	27.10 – 30.77	0.12 - 2.03	0.23 – 0.41	79.10 – 80.20
Λ <u>T</u> 3D				

	28.99 <mark>±</mark> 1.89	1.12 <mark>±</mark> 0.91	0.32±0.09	79.55 <mark>±</mark> 0.55
<sup>[42]</sup> FEPA(2003)	1.3	-	0.15	-
<sup>[43]</sup> WHO(2006)	3.0	-	0.15	-
<sup>[44]</sup> EU (2001)	1.0	-	1.0	-
<sup>[45]</sup> EU (2008)	0.5 – 1.0	0.5 – 1.0	2.0	-
<sup>[46]</sup> Indonèsia	80	-	-	200
<sup>[47]</sup> FAO (1983)	-	2.0	1.0	-

<sup>[42]</sup>Federal Environmental Protection Agency (2003)

<sup>[43]</sup>World Health organization (2006)

<sup>[44]</sup>European Union (2001)

<sup>[45]</sup>European Union (2008)

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

<sup>[47]</sup>Food and Agricultural Organization of the United Nation (1983)

129

Previous research had shown that escalating human population, economic development and poor waste management have affected adversely the ecosystem. This have significantly contributed to the current worldwide deterioration of water quality including seasonal accumulation of heavy metals like Cr, Cu, Zn, Cd, As, Hg, Fe, etc [23,24]. An insight on the levels of these heavy metals in aquatic organism especially fishes are of paramount important to basically for consumption by human and nature management. This research work documents results of bioaccumulation of some heavy metals in some fish samples 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 permissible limits like WHO (3.0mg/kg), FEPA (1.3 mg/kg), EU (2008) (1.0 mg/kg) and those reported in 142 143 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 fish. The various mean values as shown in Table 2 were also higher than permissible limits of EU (2008) 156 157 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 of160 chromium in Cling 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 level in the remaining fish samples were
higher than the permissible limits above and those reported in *Balistoides Vridiscens* [19].
Bioaccumulation of chromium in human can lead to the following health diseases; pulmonary fibrosis,
lung cancer (inhalation), cardiovascular, renal, gastrointestinal, hematological and neurological effects
[19].

- Levels of zinc in the investigated fish recorded least minimum value of 45.56 mg/kg in Cat fish and highest value of 79.55 mg/kg in Damsel and Cling fishes. A trend of decrease in mean values of zinc in the investigated fish samples can be seen as; Cat fish (45.56) < Dat fish (60.44) < Tilapia fish (61.37) <
- Damsel fish = Cling 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].

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

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

<sup>[54]</sup>World Health Organization (WHO, 2011)

<sup>[55]</sup>United Nation Environmental Programme (2007)

<sup>[56]</sup>United States Environmental Protection Agency (USEPA, 2011)

<sup>[57]</sup>European Commission Environment (ECE, 1998)

<sup>[58]</sup>Federal-Provincial-Territorial Committee on drinking Water (CDW), Health Canada (FTP-CDW, 2010)

<sup>[59]</sup>Australian Drinking Water Guidelines (2011)

173

Results of heavy metals in investigated water samples as shown in Table 3 depicts that levels of copper 174 (0.065 mg/L) and zinc (0.112 mg/L) were lower than permissible limits of World Health 175 176 Organization(2011). Cadmium and chromium levels were observed to be below detection limit (BDL). Results of Bioconcentration Factor (BCF) implored showed that copper has an increasing BCF values of 177 Tilapia fish (358) < Dat fish (379) < Cat fish (418) < Damsel = Cling (446). Also zinc has an increasing 178 179 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 showed that mean concentration of copper in the investigated water samples had a value of 0.0065 mg/L 185 which was observed to be lower than the permissible limits of World Health Organization (WHO, 2011) 186 (2.0 mg/L), United States Environmental Protection Agency (USEPA) (1.3 mg/L), European Commission 187 Environment (2.0 mg/L) and Federal-Provincial-Territorial Committee on Drinking Water, Health Canada 188 189 (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). Values of 190 cadmium and chromium were observed to be below detection limit. 191

#### 192 4. CONCLUSION

The results of the study provide valuable information on levels of heavy metals in water samples and their 193 194 bioaccumulation in some selected fish samples from Onuimo River, Imo State. The result also showed heavy metals sorption and bioaccumulation by fish varied with respect to species of fish and other 195 196 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 little contamination by heavy metals is safe for human, agricultural and industrial uses. Also rate of 203 204 disposal of heavy metal containing waste into the river and other anthropogenic activities which can contaminants the water should be discouraged. Better still, they should be adequately recycled before 205 206 disposal to avoid loading of heavy metals and other contaminants into the river.

#### 207 COMPETING INTEREST

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

#### 210 **REFERENCES**

- Kris Etheron P., Haris W., Appel L., Fish consumption, fish fish oil, omega 3 fatty acids and cardiovascular disease circulation 2002; 106 (21): 2747 2757.
- Zhao S., Feng C., Quan W., Chen X., Niu J., Shen Z., Role of living environments in the accumulated characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China. Marine Poll. Bull. 2012; 64 (6):1163 1171
- 2163. Yilmaz F., Özdemir N., Demirak A., Tuna A.L., Heavy metals levels in two fishes217species Cephalus and Lepomis gibbosus. Food Chemistry 2007; 100 (2): 830 835.
- Oguzie FA., Heavy metals in fish water and effluents of lower Ikpoba River, Benin City.
   Park J. Sci. Ind. Res. 2003; 46 (30): 156 160.
- 5. Mustafa C., Guluzar A., The relationship between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn)
  levels and the size of six Mediterranean fish species. Environmental Poll. 2003; 121 (1):
  129 136.
- 6. Korkmaz Gorur F., Keser R., Akcay N., Dizman S., Radioactivity and heavy metal
   concentrations of some commercial fish species consumed in the Black Sea Region on
   Turkey. Chemosphere 2012; 87: 356 361.
- Petrovic Z., Teodrovic V., Dimitrijevic M., Borozan S., Beukovic M., Milicevic D.,
   Environmental Cd and Zn concentration in liver and kidney of Erupean hare from
   different Serbian Region: Age and Tissue Difference. Bulletin of Cont. Toxicol. 2013; 90:
   203 207.
- 8. Oliveira Ribeiro CA., Vollaire Y., Sanchez-Chardi A., Roche H., Bioaccumulation and the effects of Organochlorine pesticides PAH and heavy metals in the eel (Anguilla Anguilla) at the Camargue Nature Reserve, France. Aquatic Toxicol 2005; 36: 243 – 252.

- Voegborlo RB., Atta A., Agorku ES., Total mercury distribution in different tissues of six
   species of freshwater fish from the Kpong hydroelectric reservoir in Ghana.
   Environmental modeling and Assessment 2012; 184 (5): 3259 3265.
- 10. Annabi A., Said K., Messooudi I., Cadmium: bioaccumulation, histopathology and
   detoxifying mechanisms in fish. Am. J. Res. Commun. 2013; 1: 60 70.
- 11. Tüzen M., Determination of heavy metals in fish samples of the MidDame Lake Black
   Sea (Turkey) by graphite furnace atomic absorption spectrometry. Food Chem.2003; 80:
   19 123.
- 12. Järup L., Hazards of heavy metal contamination. British Med. Bull. 2003; 68: 167 182
- 242 13. Eisler r., Cadmium hazard to fish, wildlife and invertebrates: A Synoptic Review, U.S fish
  243 wild L. Serv. Biol. Rep. 1985; 85: 1 30.
- 14. Fernandes C., Fontainhas Fernendes A., Cabral D., Salgado M.A., Heavy metal in
   water, sediments and tissues of Liza Saliens from Esmoriz- paramos lagoon, Portugal.
   Environmental monitoring Assessment. 2008; 136: 267 275
- 247 15. Obasohan E.E., Oronsaye JAO, Eguavoen OI., A comparative assessment of heavy
   248 metal loads in the tissues of a common Catfish (*Clrias gariepinus* from Ikpobaand Ogba
   249 River in Benin City, Nigeria. Journal of African Science. 2018; 9: 13 23.
- 16. Vinodhini R., Narayanan M., Heavy metal induced histopathological alterations in
   selected organs of the Cyprinus Carpio nL., (common Carp). Int'l. J. Envioron. Res.
   2009; 3:95 100.
- 17. Gorgiera E., Velcheva i., Yancheva V., Stoyanova S., Trace metal effects on gill
   epithelium of common carp *Cyprinus Carpio L. (Cyprinitae).* Acta Zool. Bulgarica. 2014;
   66: 277 282.
- 18. Castro-González MI., Mendez-Armenta M., heavy metals: implications associated to
   fish consumption: Environ. Toxicol. Pharmacol. 2008; 26: 263 17.
- 19. Hakami MO., Risk assessment of heavy metals in fish in Saudi Arabia. American J.
   Environ. Sci. 2016; 12 (6) :341 357.
- 20. Al-Busaidi M., Yesudhasan P., Al-Rhini WAK., Al-Harthy KS., Al-Mazooei NA., Al-Habsi
   S.H., Toxic metals in commercial marine fish in Oman with reference to National and
   International Standards. Chemosphere 2011; 85: 67 73 Doi:
   10.3844/ajessp.2016.341.357
- 264 21. Rahman MS., Molla AH., Saha N., Rahman A., Study on heavy metals levels and its risk
   265 assessment in some eligible fishes from Bangshi River Savar, Dhaka, Bangladesh. Food
   266 Chem. 2012; 134: 1847 1854.
- 267 22. Meche A., Martins MC., Lofrano BESN., Hardaway CJ., Merchant M., Verdade L.,
   268 Determination of heavy metals by inductively coupled plasma optical emission
   269 spectrometry in fish from the Piracicaba River in Southern Brazil. Micro J. 2010; 94:171
   270 174.
- 23. Rajeshkumar S., Li X., Bioaccumulation of heavy metals in some tissues of fish species
  from the Meiliang Bay, Taihu lake China. Toxicology Reports 2018; 5: 288 295.
  https://doi.org/10.1016/j.toxrep.2018.01.007
- 274 24. Bioaccumulation of heavy metals in some tissues of fish in Lake Geriyo, Adamawa
  275 State, Nigeria. Journal of Environmental and Public Health Vol 2018, Article ID 1854892,
  276 7 pages <u>https://doi.org/10.1155/2018/1854892</u> (2018)

- 277 25. Ekeocha CI., Nwoko CI., Onyeke LO., Impact of automobile repair activities on
  278 Physicochemical and microbial properties of soils in selected automobile repair sites in
  279 Abuja, Central Nigeria. Chemical Science Int'l J. 2017; 20 (2): 1 15 Doi:
  280 10.9734/CSJI/2017/36065
- 281 26. Minic Z., Serre V., Herve G., Adaptation of organisms' to extreme conditions of deep –
  282 sea hydrothermal vents. Comptes Rendus Biol. 2016; 329: 527 540 Doi:
  283 10.1016/j.crvi.2006.02.001
- 284 27. Calabrese S., D'Alessandro W., Bellomo S., Brusca L., martin R.S., Characterization of
   285 the Etna Volcanic emissions through an active biomonitoring technique (moss-bags):
   286 Part 1 Major and trace element composition. Chemosphere. 2015; 119; 1447 1455
- 287 28. Olaifa FE., Olaife AK., Adelaja AA., Owolabi AG., Heavy metal contamination of *Claris* 288 *gariepinus* from a lake and fish farm in Ibadan, Nigeria. African J. Bio. Res. 2004; 7:
   289 145 148
- 290 29. Obot OI., Isangedighi AI., David GS., Heavy metal concentration in some commercial
   291 fishes in the lower Cross River Estuary, Nigeria. J. Agricult. Food & Environ. 2016; 12(4):
   292 218 223
- 30. Adeosun F.I Akinyemi AA., Idowo AA., Taio IO., Omoike A., Ayomide BJO., The effect of
   heavy metals concentration on some commercial fish in Ogun River, Opeji, Ogun State,
   Nigeria. African Journal of Environ. Sci. Tech. 2005; 9(4):365 370 Doi:10.5897/AJEST
- 31. Nwani CD., Nwoye VC., Eyo JE., Assessment of heavy metal contamination in the
   Tissue (Gills and Muscles) of six commercially important fresh water fish species of
   Anambra River South Environ. Sci. 2009; 11(1): 7 12.
- 32. Ojutiku R.O., Okojevoh FI., Bioaccumulation of some heavy metals in three selected
   fish species from Chanchaga River, Minna, Niger State. Nigeria. Nigerian Journal of
   Fisheries and Aqua culture 2017; 50(10):44 49.
- 302 33. Ujah II., Okeke DO., Okpashi VE., Determination of heavy metals in fish Tissues, water
  and sediments from the Onitsha segment of the River Niger, Anambra State, Nigeria. J.
  Benviron. Anal. Toxicol. 2017; 7(5):507 509. Doi:10.4172/2161-0525.1000507
- 305 34. Olowu RA., Ayejuyo OO., Adewuyi IA., Denloye AAB., Babatunde AO., Ogundajo AL.,
   306 determination of heavy metals in fish tissues, water and sediments from Epe and
   307 Badagry Lagoons, Lagos State , Nigeria E Journal of Chemistry 2010; 7(1): 215 –
   308 221.
- 309 35. Wangboje OM., Ekome PC., Efendu UI., Heavy metal concentration in selected fishes
  and water from Orogodo River, Agbor, Delta State in Nigeria. Asia J. Environ. Ecol.
  2017; 3(1): 1-10. Doi:10.9734/AJEE/2017/33608
- 36. Tao Y., Yuan Z., Xiaona H., Wei M., Distribution and bioaccumulation of heavy metals in
   aquatic organisms of different tropic levels and potential health risk assessment from
   Taihu Lake, China. Ecotoxicol. Environ. Saf. 2012; 81:56 64.
- 37. Bao L., Wang D., Li Y.,, Zhang G., Wang G., Zhang S., Accumulation and risk
  assessment of heavy metals in water sediments: and aquatic organisms in rural rivers in
  the Taihu lake Regions of China. Environ. Sci. Pollut. Res. 2015; 22:6721 6731.
- 318 38. Standard Method for the Examination of Water and Wastewater, 22<sup>nd</sup> Edition, Edited by 319 Rice EW., Baird RB., Eaton AD., Clesceri LS., American Public health Association

- (APHA), American Water Works Association (AWWA) and Water Environment
   Federation (WEF), Washington D.C, USA (2012)
- 39. Arnot JA., Gobas FAPC. A review of bioconcentration factor (BCF) and bioaccumulation
   factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev.
   2006; 14:257 297. Doi:10.1139/A06-005
- 40. USEPA 1996a. Fish BCF. US Environmental protection Agency, Washington D.C., USA.
- 41. OECD 1996. Bioconcentration: Flow- through fish test. OECD guidelines for the testing
   of chemicals N0. 305 E. Organizations for Economic Co-operation and Development,
   Paris, France. Pp 23
- 42. Federal Environmental Protection Agency (FEPA). Guidelines and Standards for
   Environmental Pollution Control in Nigeria, Page 238.
- 43. World Health organization (WHO) Guidelines for Drinking Water Quality,
   Recommendation volume 1, WHO, Geneva, page 130, (1985).
- 44. European Union (EU), Commission Regulation as Regards Heavy metals, Directive,
   2001/22/EC, No: 466, (2001)
- 45. European Union (EU), Commission regulation (EC) No: 629/2008. Setting maximum
   levels for certain contaminants in food stuffs. Official journal of the European Union L
   173 (2008)
- 46. Indonesia Decree of General Director of food and Drug Supervision N.
   03725/B/SK/VII/89 Concerning maximum limits of metals in food.
- 47. Food and Agriculture Organization (FAO), 1983. Compilation of legal limits for
   harzadous substances in fish and fishery products. FAO fishery circular 1983; 463: 5 100
- 48. Dobaradaran S., Naddafi K., Nazmanra S., Ghaedi H., Heavy metals (Cd, Cu, Ni, Pb)
  content in two fish species of Persian Gulf in Bashehr Port, Iran. African J. Biotech.
  2010; 9(37): 6191 6193.
- 49. Gupta BN., Mathur AK. Toxicity of heavy metals. Ind. J. Med. Sci. 1983; 37:236 270.
- 50. Larson Sc., Orsini N., Wolk A., Urinary cadmium concentration and risk of breast cancer.
  A systematic review and dose- response meta-analysis. Am. J. Epidemol. 2015; 182 (5):
  375 380 Doi:10.1093/aje/kwv085.
- 350 51. Gallagher CM., Chen JJ., Kovach JS. Environmental cadmium and breast cancer risk.
   351 Aging 2: 804 814 PMID: 21071816
- 52. Sekar CK., Chang NS., Kamala TC., Raj DSS., Rao AS., Fractionation studies and
  bioaccumulation of sediment-bound heavy metals in Kolleru lake by edible fish. Environ.
  Int. 2003; 29: 1001 1008.
- 355 53. Mendil D., Uluozlu OD., Determination of trace metal levels in sediment and five fish
   356 species from lakes in Tokat, Turkey. Food Chem. 2007; 101: 739 745.
- 54. Rahmanian N., Ali SHB., Homayoonfarrd M., Ali NJ., Rehan M., Sadef Y., Nizami AS.
  Analysis of physiochemical Parameters to Evaluate the Drinking Water Quality in the
  State of Perak, Malaysia. Hindawi Publishing Corporation. Journal of Chemistry volume
  2015, Article ID 716125, <u>http://dx.doi.org/10.1155/2015/716125</u>
- 55. Khan SL., Din ZU., Ihsanullah, Zubair A., Levels of selected Heavy Metals in Drinking
   water of Peshawar City. Int'l J. Sci. Nat. 2011; 2(3): 648 652.

363	56. United Nation Environmental programme (UNEP), Global drinking Water Quality Index
364	Development and Sensitivity Analysis Report, 2007
365	57. Total dissolved solids (Guidelines for drinking-water quality). Health criteria and other
366	supporting information. World Health organization, Geneva, 1996, 2 <sup>nd</sup> edition, volume 2.
367	58. World Health organization (WHO). Guidelines for drinking water quality, 4 <sup>th</sup> edition,
368	WHO, Geneva, Switzerland, 2011.
369	59. Mahugija JAM. Levels of heavy Metals in Drinking Water, Cosmetics and Fruits Juices
370	from selected Areas in Dar ES Salam, Tanzania. Tanzania J. Sci. 2018; 44 (1): 1-11,
371	60. United States Environmental Protection Agency (2011). National primary Drinking Water
372	Regulations. Retrieved from <u>http://water.epa.gov/drink/contaminats/index.cfm#List</u>
373	(Verified January, 2019)
374	61. European Commission Environment (1998). Council Directive 98/83/EC of 3 <sup>rd</sup>
375	November, 1998. Retrieved from <u>http://eur-lex.europa.eu./lexUriServ/LexUriServ.do</u> ?
376	Uri = OJ:L1998:330:0032:0054:EN PDF (verified January, 2019).
377	
378	
570	
379	
380	
381	
382	
202	
383	
384	
385	
386	
500	