

RISK EFFECT OF WATER TREATMENT SLUDGE ON BIOACCUMULATION OF HEAVY METALS IN WATER, FISH (OREOCHROMIS NILOTICUS, AND CLARIAS GARIEPINUS) FROM RIVER CHANCHAGA MINNA NIGER STATE, NIGERIA**Oguh C. E^{1*}, Joseph P. S², Osuji C. A¹, Ubani C. S¹, Okunowo W. O³**¹Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.²Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria³Department of Biochemistry, College of Medicine, University of Lagos, Akoka-Yaba, Lagos, Nigeria<http://doi.org/10.35410/IJAEB.2019.4431>**ABSTRACT**

The safe disposal of Water Treatment Sludge has become one of the main environmental concerns throughout the world due to the enormous quantity of sludge generated. Typically, the sludge discharge to river bodies affect fish species. This study investigates the effect of water treatment sludge on bioaccumulation of heavy metals in water, fish (*Oreochromis niloticus*, and *Clarias gariepinus*) from river Chanchaga Minna Niger State, Nigeria and the human health risk associated with their consumption. Fishes and water samples were collected from river contaminated with sludge (River Chanchaga) and other samples from a private farm where there were no sludge served as control (Makolo farm). The water samples were collected and analyzed for physicochemical properties (dissolved oxygen, electrical conductivity, pH and temperature) using a standard method and concentrations of heavy metal both in fish and water, As, Cd, Cr, Cu, Hg and Pb were analyzed using Atomic Absorption Spectroscopy (AAS). The Bioaccumulation factor and potential risk (Daily intake, health risk index and Hazard index) from the consumption of these fish was assessed using standard methods and formula. All the samples collected were digested using a modified procedure from the Association of Official Analytical Chemists (AOAC) and were subsequently analyzed using Atomic Absorption Spectroscopy. The result of the physicochemical properties and Heavy metal concentration in water shows a significant difference ($P < 0.05$) between the C. river and M. pond. The mean of concentration of Heavy Metals (As, Cd, Cr, Cu, Hg, and Pb) in *O. niloticus*, and *C. gariepinus* from C. river were 2.66, 3.72, 1.84, 2.23, 3.68, 2.88, and 2.83, 3.87, 2.12, 2.66, 3.87, 2.61 mg/kg respectively and for M. pond were 0.69, 0.98, 1.04, 0.83, 0.54, 1.00 and 0.79, 0.98, 1.16, 1.02, 0.87, 1.16 mg/kg respectively. Fishes in C. river elicited higher ($P < 0.05$) heavy metals concentrations compare to Fishes in M. pond. The values were all greater than the maximum permissible limit of heavy metal in fish 0.01, 0.05, 1.5, 0.5, 0.01 and 0.3 mg/kg respectively recommended by FAO/WHO except Cr in the control farm which is within the limit. The result of health risk assessment shows that fishes from C. river have a higher risk from the daily intake, health risk index and hazard index with average values greater than one (2.845) and compared to fish in M. pond with average less than one (0.64). The study concludes that water treatment sludge discharge in Chanchaga river have deleterious effect on fishes and also bioaccumulate heavy metals in the fish. The risk assessment shows that fish in C. River are contaminated with heavy metals and could pose health risk from heavy metal Contamination when consume.

Keywords: Clariasgariepinus, Oreochromisniloticus, Heavy Metals, Water, Risk Assessment

1. INTRODUCTION

In Niger State, Nigeria because of rapid growing population, water treatment plants are facing the problem of treating highly loaded raw water with dissolved and suspended particles and have to use diverse chemicals in order to obtain drinking water which meets the fast growing population and acceptable health standards. Due to feeding and living in the aquatic environments fish are mostly vulnerable and heavily exposed to pollution because they cannot escape from the detrimental effects of pollutants i.e. they are cold blooded animals and survive only on water (Yarsanet *et al.*, 2013; Mahboob *et al.*, 2014). In aquatic environments, heavy metals (HMs) pollution results from direct atmospheric deposition, geologic weathering or through the discharge of agricultural, municipal, residential or industrial waste products, also via wastewater treatment plants. Fishes are considered to be most significant biomonitors in aquatic systems for the estimation of metal pollution level (Authman, 2008). Fish have been used for many years as indicators of environmental pollution status, and, thus, they are regarded as good indicators of metal pollution in marine environments (Marcus *et al.*, 2013). Studies from the field and laboratory works showed that accumulation of HMs in a tissue is mainly dependent on water concentrations of metals and exposure period; although some other environmental factors such as water temperature, oxygen concentration, pH, hardness, salinity, alkalinity and dissolved organic carbon may affect and play significant roles in metal's accumulation and toxicity to fish (Jitar *et al.*, 2014; Authman *et al.*, 2015). The pollutants such as HMs bioaccumulate in food chain and cause the antagonistic effects and even death, so fish among other animals are used to determine the health condition of aquatic ecosystem. Bioaccumulation of heavy metals means an increase in the degree of a metals in a living organism over time, compared with chemical's level in the environment.

Water treatment is the application of physical and chemical process to a water supply to remove taste, colour, odour, dirt and debris, to reduce hard elements, salt and to destroy harmful organisms. Consequently sludge produced from water purification processes contain high concentrations of those chemical removed by the purification processes (Uwimana *et al.*, 2010). Water treatment sludge (WTS) is produced in conventional drinking-water treatment processes as sedimentation including suspended and dissolved solids, organic matter, and other suspensions from the water. Due to these facts, the safe disposal of WTS has become one of the main environmental concerns throughout the world due to the enormous quantity of sludge generated. Typically, this WTS is discharge and channel back to the river close to the treatment and aquatic life such as phytoplankton, zooplankton, and crustacean, macro-algae and fish species are effected (Oguh *et al.*, 2019a).

Fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic or acute diseases (Al-Yousuf *et al.*, 2000). Aquatic systems are one facet of the impact of toxic substances on the natural environment (Mohammed *et al.*, 2016). The most important pollutants in aquatic life are HMs because of their toxicity, accumulation and bio-magnification by marine creatures. The high level of heavy metals in the river could indicate similar concentration in fishes by accumulation at concentrations causing

serious risk to human health through food chain when consumed. More important is that the very young stages of fish (larvae and juveniles) grow up in the near shore zone, where the water quality is heavily affected by the raw sludge (Zaqoot *et al.*, 2017). Accumulation pattern of HMs varies from fish species, some fish accumulate more heavy metal than others. The toxic effects of HMs can affect the individual growth rates, physiological functions, reproduction, and mortality in fish. Metals accumulate in fish bodies by three possible ways: gills, digestive track and body surface. The gills are considered as the significant site for direct uptake of metals from the water (Beijer and Jernelov, 1986), HMs in fish come mainly from their diet, though the body surface is normally estimated to take minor part in uptake of heavy metals in fish (Selda and Nurşah, 2012). Fish and bivalve molluscs are used in bioaccumulation tests because they are higher trophic level organisms and are usually eaten by human (Olaifaet *al.*, 2014).

The term heavy metal refers to any metallic chemical element that has a relatively high density greater than 5 g/cm³ and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl) and lead (Pb). The densities of Pb, Hg and Cd are 11.4 g/cm³, 13.6 g/cm³ and 8.65 g/cm³ respectively. Recent researchers have found that even low levels of mercury, cadmium, lead, aluminum and arsenic can cause a wide variety of health problems (Hassaanet *al.*, 2016). Heavy metals toxicity can result in damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, kidneys, liver and other vital organs. Long-term exposure may result in slowly progressing physical, muscular and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis. Allergies are not common and repeated long-term contact with some metals may cause cancer. Metals such as cadmium, mercury, arsenic and lead are non-essential and therefore have toxic effects on living organisms such as damage to the renal and nervous systems of fish as well as gill damage (severe destructive pathological changes, i.e. structural lesions) (Barkaet *al.*, 2001; Lars, 2003; Velchevaet *al.*, 2010; Deore and Wagh, 2012).

Lead exerts its effect, physiologically and biochemically as a mimetic agent substituting for essential elements participating in metabolism such as calcium, iron and zinc. For instance, it directly interferes with zinc and iron in the biosynthesis of heme. Lead binds to different kinds of transport proteins including, metallothionein, transferrin, calmodulin and calcium-ATPase (WHO, 1989 and Corpaset *al.*, 2002). The toxic effects of lead include anemia, proximal renal tubular damage, hypertension, cardiac disease, immune system suppression (antibody inhibition) and neurological damage. Lead severely damage the liver, kidneys, heart, brain, nerves and other organs. Exposure to Pb may also lead to reproductive disorders osteoporosis (brittle bone disease). Pb exposure causes increases in heart disease, anemia, high blood pressure, especially in men. Extensive exposure to Pb causes memory problems, behavioral disorders, and mental retardation while lesser levels of Pb damage the nerves and brain in fetuses and young children, resulting in lowered IQ and learning deficits (Kalayet *al.*, 1999). Cadmium is highly toxic metal that was the cause of death, serious illness, rheumatoid arthritis (RA), full skeletal deformities, depressed growth, hypertension, diarrhea, vomiting, stomach problems, fractures in bone, damage to DNA, failure in reproduction and fertility, cause damage to nervous system, damage to immune system, cancer and fetal deformity (Mansour and Sidky, 2002). The

International Agency Research on Cancer (IARC) has classified cadmium as human carcinogen (group I). It was found to be associated with prostate cancer and renal cell carcinoma (Stoeppler, 1991). Effect of chromium on humans by fish intake are faded immune system, Skin diseases, Cause ulcer and upset stomach, Respiratory track problem, Alteration in genetic material, Lung cancer, liver and kidney damage and Death. Low exposure to chromium can irritate the skin and cause ulceration. Long term exposure can cause kidney and liver damage. It can also cause damage to circulatory and nerve tissues. Mercury toxicity include visual field construction, behavioral changes, memory loss, headaches, tremors, loss of fine motor control, spasticity, hair loss, mental retardation to fetus and fetal deformity, cerebral palsy, blindness, deafness and muscular rigidity (Clarkson *et al.*, 2003). Arsenic is acutely toxic and intakes of large quantities result in adverse symptoms to gastrointestinal tract, severe disturbances of the cardiovascular and central nervous systems and eventually death. The latest WHO evaluation concludes that, exposure to arsenic via drinking water is causally related to cancer in the lungs, kidneys, urinary bladder and skin (WHO, 2001).

As a human food, Fish are considered as an excellent source of polyunsaturated fatty acids (predominantly omega-3 fatty acids), protein, Zn, iron and calcium (Toth and Brown, 1997). Seafood will be an even more important and safe source of food in future for protein and fatty acids for human intake and products made from aquaculture. Fish creates major sources of human beings food which is protein. Fish is a valuable and cheap food item, and also major part of the human diet. Sludge from water treatment plant, detergent, and dump sites present near the river water have high concentrations of heavy metals, which cause the disruption of the ecological balance of river water. Fish in such areas can be contaminated with HMs, consumption of this fishes by humans can cause accumulation of these metals in the food chain and many people could be at a risk of adverse health effect from consuming fishes in the river with WTS that is highly contaminated with toxic elements. Hence, this study was designed with the aim of determining the Effect of Water Treatment Sludge on Bioaccumulation of Heavy Metal (As, Cd, Cr, Cu, Hg, and Pb) in Water, *Oreochromis niloticus*, and *Clarias gariepinus* Muscles from River Chanchaga Minna Niger State, Nigeria.

2.1 MATERIALS AND METHODS

Two commonly consumed fish; African Tilapia (*Oreochromis niloticus*), and African Catfish (*Clarias gariepinus*), were selected for the study. The fishes used for the study were harvested fresh from river Chanchaga, Minna Niger State.

2.1 Study Areas

The study was carried out in river close to Niger State water board and a private farm (Makolo farm) at Chanchaga, Bosso Local Government Area in North-central, Niger State of Nigeria from May to June 2019. Chanchaga is situated at 9°34' North latitude, 6°33' East longitude, with an area of 72km² and a population of 201,429 at the 2006 census (Figure 1). River Chanchaga is originated from Tagwai Dam in Minna Niger State. It has a moderate climate with a very high temperature during the dry season and average rainfall during the rainy season. The river is used as water source in Niger State water board authority for water treatment. Makolo farm is a

major agricultural enterprise in Chanchaga whose operations include fish farming. It supplies about 1 tonnes of fish per day and is located at Kangiwa road Chanchaga Minna, Niger State.

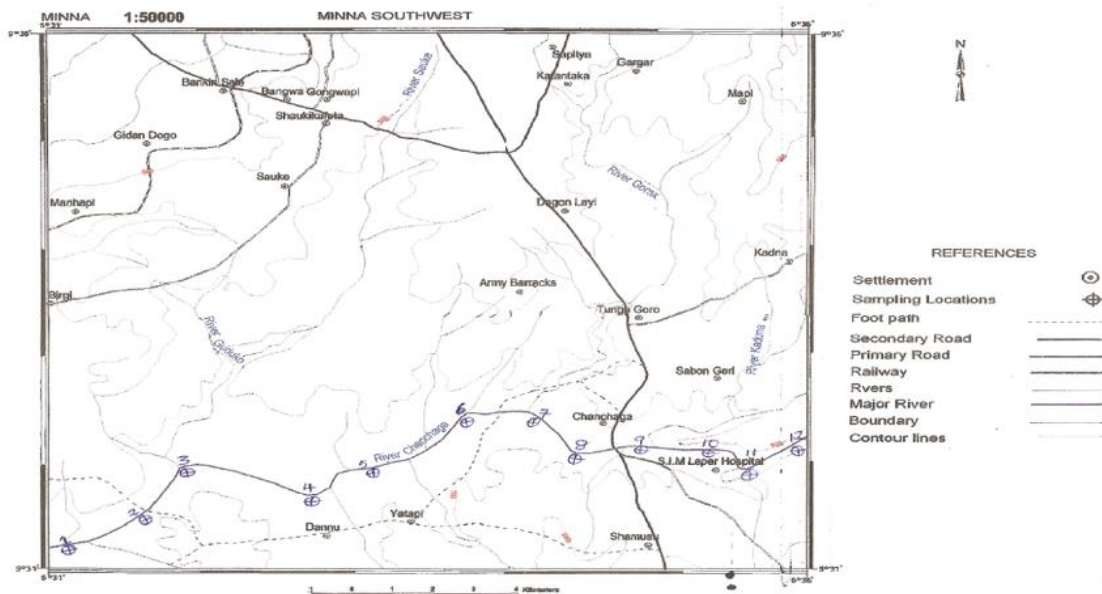


Figure 1: Topo-Map of Minna Niger State showing River Chanchaga with numbering 1-12

2.2 Study Design

The experiment was carried out under a Completely Randomized Design (CRD) with four samples and three replicate groups for each. The concentrations of the Heavy metals both in water and fish samples, were done in four groups, from group 1 to 4, which are samples from Chanchaga river and a control pond (where no activities) using two species of fish. Both water and fish sample were randomly collected and analyzed for water physicochemical properties and Heavy metals As, Cd, Cr, Cu, Hg and Pb.

2.3 Sample Collection

Sampling of Adult *O. niloticus* and *C. gariepinus* was carried out during February and June 2019 (dry and wet seasons respectively). Two experimental gill nets (measuring 30 m length, 1.5 m depth with stretch mesh size of 3 and 4 inches) were set up and left for three consecutive days. Each net was inspected every day for three days from morning until afternoon. The net was set up along the river that covers the most river pools. The total of 36 fish of similar weights was used from the study area and Makolo fish pond. The average length and weight of the samples were 38 cm and 1kg from the river and fish farm. Water samples were also collected from the study samples and the control in plastic containers, sealed and transferred to the laboratory in iced packs. Approximately 1ml of concentration HNO_3 is added to the water samples to prevent the microbial utilization of heavy metals. The fish samples were frozen in the laboratory in a clean ice Box before heavy metal analysis.

2.4 Fish Tissue Preparation

Fish samples were rinsed with distilled water to get rid of any remnants of trace metals on the outersurface of the fish, and the scales were removed. Muscle tissues of fish (dorsal muscle) was used in this study because it is the major target tissue for metal storage and is the most edible part of the fish. Fish tissues were cut and oven dried for 3 hours at 110°C to a constant weight. After ensuring of samples dryness they were removed and then were grounded into powder using ceramic mortar and sieved through 20µm mesh and then stored in polyethylene bottles at 30°C until analysis. A wet digestion method was used based on the Analytical Methods for Atomic Absorption Spectrometry.

2.5 Physico-chemical Properties

2.5.1 pH

The pH value which is a measure of the hydrogen or hydroxyl ion activity of the water system indicates whether the water is acidic, neutral or alkaline in reaction. Fishes suffers much both under very low as well as high pH. The instrument for pH measurement commonly used is a digital pH meters have single electrode assembly. The instrument being a potentiometer, the pH scale has to be calibrated before use with buffer solutions of known pH values. 75 ml of water sample is taken in a 100ml beaker. The suspension is stirred at regular intervals for 30 minutes and the pH is recorded. The suspension is stirred well just before the electrode are immersed and readings are taken. Three readings were observed and then mean of it was calculated.

2.5.2 Temperature

Water temperature was determined using a mercury-in-glass thermometer.

2.5.3 Dissolved Oxygen

Dissolved oxygen (DO) was performed by Winkler method(Boyd, 1979). Manganous sulfate solution (2 ml) and 2ml of alkaline potassium iodide was added to the water. The solutions were mixed thoroughly. 2 ml of conc. Sulfuric acid was added to it. From the above solution 200 ml was transferred into a conical flask. Few drops of starch indicator were added. Then it was titrated against sodium thiosulfate till blue color turns violet. The amount of titrants used gives DO value. Three readings were taken and mean of it was calculated.

The oxygen content of the water was obtained by calculation using the formula:

$$\text{D.O. content (mg/L)} = \text{Volume of original sample taken} / \text{Volume of sample titrated} \times A$$

Where A = Volume of the thiosulphate used in titration.

2.5.4 Electrical Conductivity

The electrical conductivity was determined by Richards, 1954. In this method, a 5 ml of sample water mixture is shaken for 30 minutes, allowed to settle then the conductivity measured with a temperature-compensated probe. An approximate soluble salts value may be derived from the conductivity using the empirical relationship:

$$\text{Soluble salts (\%)} = \text{Conductivity (dS/m)} \times 0.35.$$

Digestion of Water Samples

Water samples were analysed by a modified procedure from the Association of Official Analytical Chemists (AOAC, 1995). Nitric acid (10ml) was added to 50ml of each water sample and heated at 150°C for 30mins. 5ml of nitric acid was then added to each tube and heated for 30minutes at 200°C, to the mixture was added 2ml of hydrogen peroxide before further heating at 200°C for 30minutes. The resulting solutions were allowed to cool at room temperature and then the volume made up to 25ml with distilled water. Digested samples were analyzed for levels of As, Cd, Cr, Cu, Hg and Pb using Atomic Absorption Spectroscopy.

2.6 Digestion of Fish Samples

A 5 g of the dry powdered sample was put into a 50 ml beaker with 5 ml of HNO₃ and 5 ml of H₂SO₄. When the fish tissue stopped reacting with HNO₃ and H₂SO₄, the beaker was then placed on a hot plate and heated at 60°C for 30 min. After allowing the beaker to cool, 10 ml of HNO₃ was added and returned to the hot plate to be heated slowly to 120°C. The temperature was increased to 150°C, and the beaker was removed from the hot plate when the samples turned black. The sample was then allowed to cool before adding H₂O₂ until the sample was clear. The content of the beaker was transferred into a 50 ml volumetric flask and diluted to the mark with ultra-pure water. All the steps were performed in the fume hood. Digested samples were analyzed for levels of As, Cd, Cr, Cu, Hg and Pb using Atomic Absorption Spectroscopy. The above procedures in this section followed the guidelines from the Analytical Methods for Atomic Absorption Spectroscopy (Perkin Elmer 1996).

2.7 Bioaccumulation factor (BAF)

The ratio of the contaminant in the fish to the concentration in the ambient environment at a steady state, where the fish can take in the contaminant through ingestion with its food as well as through direct contact (U.S. Environmental Protection Agency, 2010). The transfer coefficient was calculated by dividing the concentration of toxic metals in fish by the total toxic metals concentration in the water. This index of water – fish transfer or intake of toxic elements from water through fish was calculated using:

$$BAF = C_{\text{fish}}/C_{\text{water}}$$

Where;

BAF represent the transfer factor of snail

C_{fish} = Toxic elements concentration in fish tissue, mg/kg fresh weight

C_{water} = Toxic elements concentration in water, mg/kg dry weight

BAF > 1 indicates that the fish are enriched in elements from the water (Bio-accumulation)

BAF < 1 means that the water excluded the toxic elements from water (excluder)

2.8 Risk Assessment of Heavy Metals via the Consumption of Fishes

Risk assessment was evaluated by considering only the edible part (muscles tissues) to determine daily intake of metal (DIM), health risk index (HRI) and hazard index (HI).

2.8.1 Daily Intake of Metals (DIM)

The daily intake of metals (DIM) was calculated to estimate the daily loading of metals into the body system (via the consumption of fish meal) of a specified body weight of a consumer according to (USEPA, 2011). This would entail the relative bioavailability of the studied metals in this study. The daily intake of metals (DIM) was determined by the following:

$$\text{ADDM} = \text{DI (kg/day)} \times \text{M}_{\text{fish}}(\text{mg/kg})/\text{BW (kg)}$$

Where;

ADDM = Average daily dose (mg/kg/d) of the metal into the body via the consumption of fish.

DI = The daily intake of fish for adult is $0.227 \text{ kg/day}^{-1}$ for food describe by Shakeriet *al.*, 2015

M_{fish} = The trace toxic elements (metals) concentration in the fish tissues (mg/kg)

BW = Represent the estimated average body weight of investigated in adult (60kg for adults).

2.8.2 Health Risk Index (HRI)

The health risk index (HRI) for the populations through the consumption of contaminated fish from sludge contaminated river was assessed based on the daily intake of metals (DIM) relative to reference oral dose (RfD) for each metal. This is an index justifying individual's risk of heavy metals. The HRI value of less than one implies safe trend and is considered acceptable; otherwise, the fish may pose heavy metals risk. The following equation describe by USEPA, 2017 is used to calculate the HRI of fish.

$$\text{HRI} = \text{ADDM} / \text{RfDM}$$

Where;

ADDM = represents the average daily dose (mg/kg/d) of the metal

RfDM = is the reference dose of the metal (mg/kg/d)

RfDM is define as the maximum tolerable daily intake of metal with no adverse effect.

HRI is the ratio between exposure and the reference oral dose (RfD). If the ratio is lower than one (1), there will be no obvious risk.

2.8.3 Estimation of Hazard Index (HI)

The hazard index (HI) was calculated to determine the overall risk of exposure to all the heavy metals via the ingestion of a contaminated fish (USEPA, 2002). The hazard index (HI) was calculated as the summation of the hazard quotient (HQ) arising from all the metals examined. The value of the hazard index is proportional to the magnitude of the toxicity of the fish consumed. $\text{HI} > 1$ indicates that the predicted exposure is likely to pose potential health risks. However, a hazard index >1 does not necessarily indicate that a potential adverse health effects will result, but only indicates a high probability of posing health risks.

$$\text{HI} = \Sigma \text{HQAs} + \text{HQCd} + \text{HQCr} + \text{HQCu} + \text{HQHg} + \text{HQPb}$$

2.9 Statistical Analysis

The data obtained were analysed using IBM Statistical Product and Service Solution (SPSS) version 20 and Microsoft excel 2013. The results were expressed as mean \pm standard deviation (SD). One way analysis of variance (ANOVA) was carried out as $P < 0.05$ considered statistically significant.

3 RESULTS

3.1 Physicochemical Properties of Chanchaga River and Makolo pond

A number of physico-chemical properties (dissolved oxygen, pH, Temperature, and electrical conductivity) of water in this study were determined shown in Table 1. The values of physicochemical properties of C. river and M. pond were dissolve oxygen(5.18 and 6.26 mg/l), pH (5.28 and 6.55), Temperature (35.83 and 28.10 °C), and electrical conductivity (680.04 and 583.61 mg/l) respectively which are significantly different ($P < 0.05$). The physicochemical changes in water parameter may be due to the sludgedischarge and other anthropogenic activities.

Table 1: Physicochemical Properties of Chanchaga River and Makolo pond

Physicochemical Properties	Water Samples		WHO, 2004**, 2010* Permissible Limit
	Chanchaga River	Makolo Pond	
Dissolve Oxygen (mg/l)	5.18 ± 0.01	6.26 ± 0.02	6 -8 mg/l*
pH	5.28 ± 0.02	6.55 ± 0.04	6.5 – 8.5*
Temperature (°C)	35.83 ± 0.05	28.10 ± 0.49	20 – 30 °C*
Electrical Conductivity (mg/l)	680.04 ± 5.65	583.61 ± 3.03	500 mg/l**

Results Expressed as Mean ± SD.

3.2 Concentration of Heavy Metals in Water Samples

The mean concentration of heavy metals (As, Cd, Cr, Cu, Hg and Pb) indifferent water samples (Chanchaga river and Makolo pond) were 2.18, 3.81, 1.79, 2.24, 3.66, 2.86 mg/kg, and 0.84, 0.92, 1.05, 1.47, 0.76, 0.95 mg/kg respectively as shown in Table 2. The result of C. river shows a significant different ($P < 0.05$) between the M. pond. The result shows that water samples from C. river were more contaminated with Heavy metals compared to water from M. pond. The concentration of As, Cd, Cr, Cu, Hg and Pb were above the WHO/FAO, 2016 permissible limits of HMs in water 0.01, 0.003, 0.05, 1.0, 0.001, and 0.01 mg/kg respectively. The study also indicates that Cd in the C. river among the metals had the highest concentration, while Cr was the lowest.

Table 2: Concentration of Heavy Metals in Water Samples

Heavy Metals (mg/kg)	Water Samples		PL(mg/kg) by FAO/WHO, 2016)
	Chanchaga River	Makolo Pond	
As	2.18 ± 0.05	0.84 ± 0.09	0.01
Cd	3.81 ± 0.10	0.92 ± 0.04	0.003

Cr	1.79 ± 0.01	1.05 ± 0.04	0.05
Cu	2.24 ± 0.06	1.47 ± 0.10	1.0
Hg	3.66 ± 0.06	0.76 ± 0.07	0.001
Pb	2.86 ± 0.09	0.95 ± 0.04	0.01
Results Expressed as Mean ± SD. PL=Permissible limit, n=3			

3.3 Concentration of Heavy Metals in Fish Samples

Table 3 presents the summary of the mean concentrations (mg/kg) of heavy metals Arsenic (As), Cadmium (Cd) Chromium (Cr), Copper (Cu),Mercury (Hg) and Lead (Pb) analysed in *Oreochromis niloticus*, and *Clarias gariepinus* from Chanchaga river and Makolo pond. The concentration of HMs (As, Cd, Cr, Cu, Hg, and Pb) in *O. niloticus* and *C. gariepinus* from C.river were 2.66, 3.72, 1.84, 2.23, 3.68, 2.88 and 2.83, 3.87, 2.12, 2.66, 3.87, 2.61 mg/kg also from Makolo pond were 0.69, 0.98, 1.04, 0.83, 0.54, 1.00 and 0.79, 0.98, 1.16, 1.02, 0.87, 1.16 mg/kg respectively. The mean concentration of HMs in the fishes indicate bioaccumulation from the river. The result shows that fishes from C. river were more contaminated with HMs compared to the control from M. farm. The concentration of HMs on fish from C. river were all greater than the maximum permissible limit of HMs As, Cd, Cr, Cu, Hg and Pb in fish 0.01, 0.05, 1.5, 0.5, 0.01, 0.3 mg/kg respectively recommended by FAO/WHO (2016); FAO, 1993 and Saha et al., 2016. While the fishes from M. pond were also slightly greater than the maximum permissible limit except Cr which is below the recommended values. (Table 3).

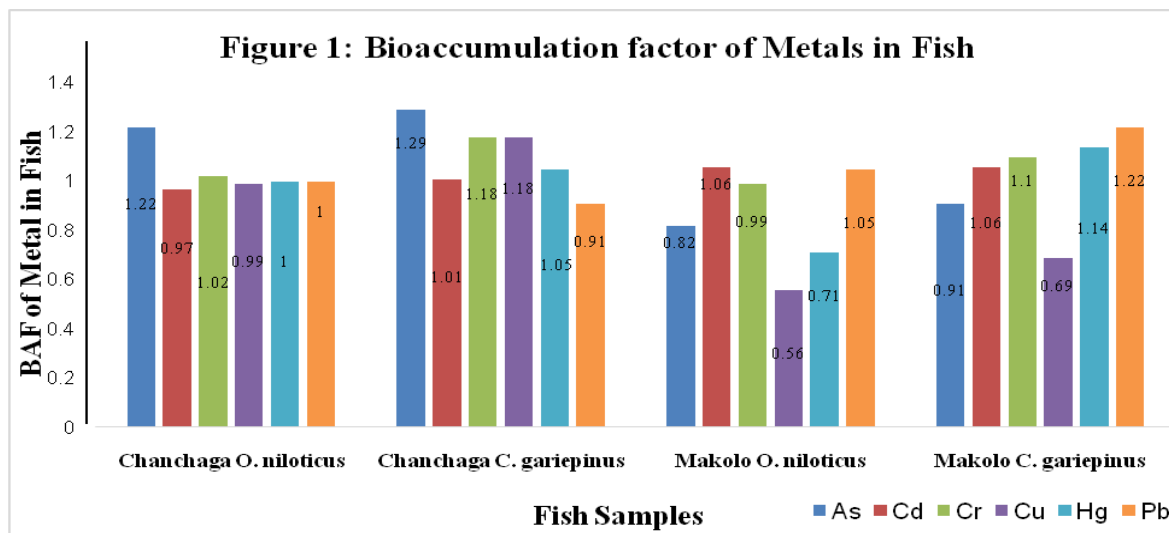
Table 3: Concentration of Heavy Metals in Fish Samples

Heavy Metals (mg/kg)	Fish Samples				PL(mg/kg) by FAO/WHO, 2016*, Saha et al., 2016**
	CO	CC	MO	MC	
As	2.66 ± 0.06 ^b	2.83 ± 0.06 ^a	0.69 ± 0.06 ^c	0.79 ± 0.03 ^c	0.01*
Cd	3.72 ± 0.04 ^b	3.87 ± 0.10 ^a	0.98 ± 0.02 ^c	0.98 ± 0.02 ^c	0.05*
Cr	1.84 ± 0.05 ^b	2.12 ± 0.02 ^a	1.04 ± 0.01 ^d	1.16 ± 0.01 ^c	1.5**
Cu	2.23 ± 0.02 ^b	2.66 ± 0.07 ^a	0.83 ± 0.09 ^d	1.02 ± 0.03 ^c	0.5*
Hg	3.68 ± 0.02 ^b	3.87 ± 0.09 ^a	0.54 ± 0.13 ^d	0.87 ± 0.10 ^c	0.01*
Pb	2.88 ± 0.03 ^a	2.61 ± 0.04 ^b	1.00 ± 0.01 ^d	1.16 ± 0.01 ^c	0.3*
Results Expressed as Mean ± SD. Mean values with different superscript letters on the rows are					

considered significant ($P < 0.05$). PL=Permissible limit, C: Chanchaga, O: *Oreochromis niloticus*, C: *Clarias gariepinus*, M: Makolo. n=3

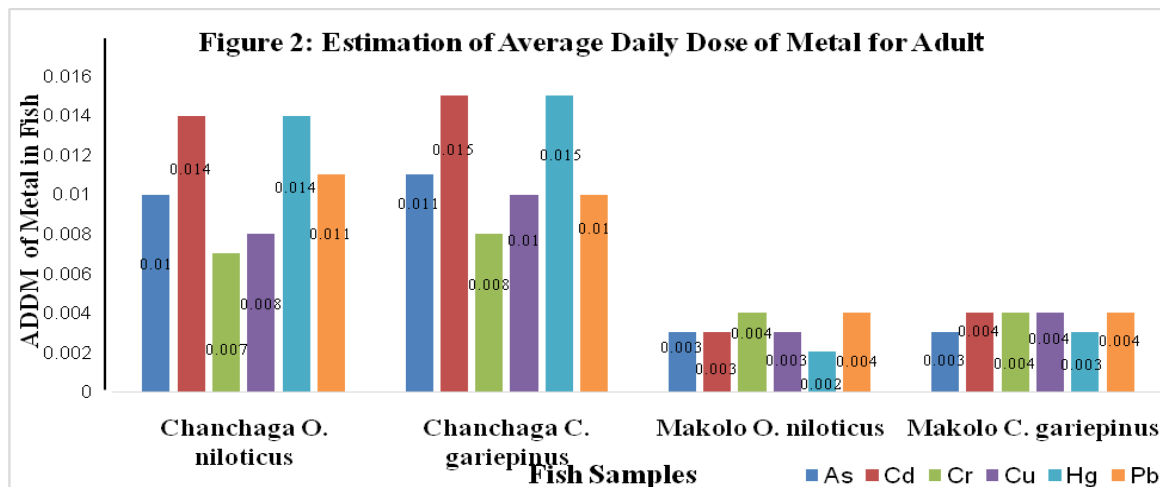
3.4 Estimation of bioaccumulation factor (BAF) of Toxic Element in fishes

The bioaccumulation factor (BAF) of HMs from the test river to fishes, which gives the ratio of the concentration of HMs in fish to the total concentration in the water. The BAF also shows the amount of elements that is transferred from water to fish. The BAF of metals As, Cd, Cr, Cu, Hg, and Pb obtained in fish (*O. niloticus*, and *C. gariepinus*) from C. river were 1.22, 0.97, 1.02, 0.99, 1.00, 1.00, and 1.29, 1.01, 1.18, 1.18, 1.05, 0.91. Fishes from M. pond were 0.82, 1.06, 0.99, 0.56, 0.71, 1.05 and 0.94, 1.06, 1.10, 0.69, 1.14, 1.22 respectively. Where the BAF is greater than one (>1) indicates that the fishes are enriched with the elements from the water (Bioaccumulators). Also where BAF is less than one (<1) means that the fishes exclude the elements from the water (excluders). In this study the BAF shows that Cat fish *C. gariepinus* bioaccumulate more HMs in both C. river and M.pond compared to Tilapia fish *O. niloticus* which shows that different species of fish have different rate of accumulation (Fig. 1).



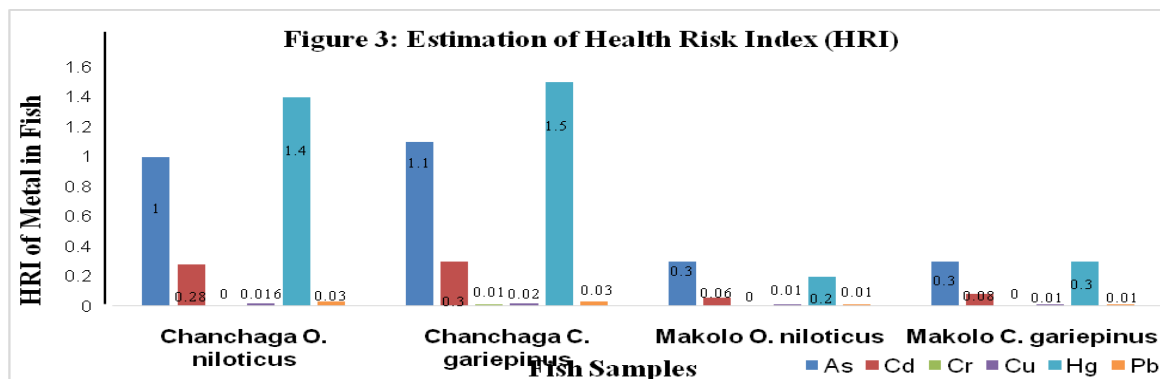
3.5 Estimation of average daily dose of metal (ADDM) for adult

The average daily dose of into the body system for adult (via the consumption of fish meal) was estimated according to the average fish consume through the food chain. The ADDM values for HMs (As, Cd, Cr, Cu, Hg, and Pb) in *O. niloticus*, and *C. gariepinus* from C. river were 0.010, 0.014, 0.007, 0.008, 0.014, 0.011 and 0.011, 0.015, 0.008, 0.010, 0.015, 0.010 and *O. niloticus*, and *C. gariepinus* from M. pond were 0.003, 0.003, 0.004, 0.003, 0.002, 0.004 and 0.003, 0.004, 0.004, 0.004, 0.003, 0.004 respectively for HMs. The result shows that fishes in C. river has higher values of ADDM compared to fish in M.pond (Fig. 2).



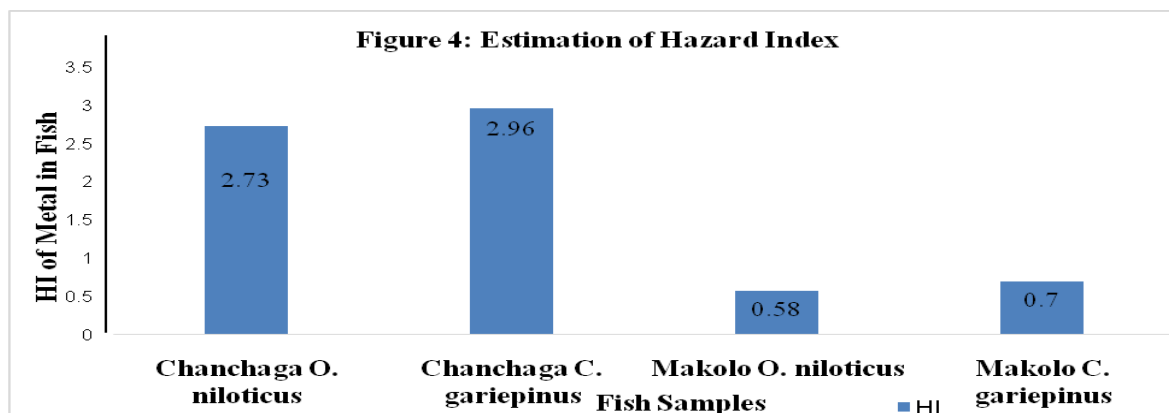
3.6 Estimation of Health Risk Index HRI

The HRI of metals through consumption of fish from C. river and M. pond were given in Fig.3. The HRI of HMs As, Cd, Cr, Cu, Hg, and Pb in *O. niloticus*, and *C.gariepinus* from C. river were 1.00, 0.28, 0.00, 0.016, 1.40, 0.03 and 1.10, 0.30, 0.01, 0.02, 1.5, 0.03. Fishes from M.pond were 0.30, 0.06, 0.00, 0.01, 0.20, 0.01 and 0.30, 0.08, 0.00, 0.01, 0.30, 0.01 respectively for HMs. The HRI for fishes from C. river recorded high HRI than that of fishes from M. pond. Metals As and Pb from C.river were greater than one, which indicate toxicity (Fig. 3).



3.7 Estimation of Hazard index (HI)

The overall risk of exposure to all the heavy metals via the ingestion of a contaminated fish is shown in Fig.4. the total Index of all metals in *O. niloticus*, and *C.gariepinus* from C. river were 2.73 and 2.96 respectively and M.pond were 0.58 and 0.70 respectively. The result shows that Fishes from C.river were highly contaminated with heavy metals compare to the fish in M. pond. HI > 1 indicates that the predicted exposure is likely to pose potential health risks.



4. DISCUSSION

The physicochemical properties (Dissolve oxygen, pH, Temperature, Electrical Conductivity) of water from C. river and M. pond were measured. The amount of dissolve oxygen in water has a major impact on fish behavior and survival. Dissolved oxygen (DO) refers to the level of free, non-bonded oxygen present in water. Chanchaga river has lower DO with 5.18 mg/l compared to Makolo fish pond water with 6.26 mg/l. The lower DO in C. river can lead to hypoxia. The recommended minimum dissolved oxygen require in fresh water is within 6-8 mg/l. The lower DO in C. river may be due to the waste discharge in the river which covers the surface of the river for oxygen penetration. The oxygen dissolves by diffusion from the surrounding air. Oxygen level can be reduced depending on pollutants present, dissolved minerals such as salt and temperature. Most fish species tolerate a drop below these minimum values for a short period of time and the period of time during which the oxygen levels drops below required limit, will cause fish to become stressed. The lower the concentration of DO, the greater the stress. The stress can cause fish death. C. river recorded low pH concentration than M. pond (5.28 and 6.55) respectively. Low pH level reduces the active nature of fish and also accelerate the release of heavy metals which is due to the waste water channel to the river and also runoff from polluted environment. The World Health Organization (WHO, 2010) recommends a pH value of 6.5 - 8.5 for drinking water to prevent corrosion. Fish are influenced by the temperature around them because they are unable to regulate their body temperature. River Chanchaga recorded higher temperature and electrical conductivity (35.83 and 680.04) than Makolo fish pond (28.10 and 583.61). High temperature do not hold enough oxygen for aquatic organisms to survive. Increase in water temperature means a decrease in dissolved oxygen available for the fish to breathe from the water. Water temperature according (Lenntech, 2014) affects the EC so that its value increase from 2 up to 3% per 1°C. The average limit of temperature in fish water is 20-30 °C. The increase in EC may be due to runoff, sewage waste, sludge, which also increases chloride, phosphate, and nitrate ions. Lower pH also increase electrical conductivity. The permissible limit of EC is 500 mg/l.

The result of Chanchaga river shows a significant different ($P < 0.05$) between Makolo pond. The result shows that water samples from C. river were more contaminated with Heavy metals compared to water from makolo farm. The sequence of occurrence for C.river $Cr < As < Cu < Pb < Hg < Cd$ and for M.pond $Hg < As < Cd < Pb < Cr < Cu$. Lead and cadmium levels were significantly higher in water samples from C. river than in the M.pond control samples. This is probably as a result of heavy metals from water treatment plant sludge discharge in the water, which are main pollutants in Chanchaga River. The heavy metal concentrations recorded in C. river were higher than standard limits for portable water and aquaculture (FAO/WHO, 2016). Aquatic life abound in the waters and can bioaccumulate these metals and subsequently transfer it to consumers via foodchain.

Fish being at the higher level of the food chain accumulate large quantities of metals and the accumulation depends upon the intake and elimination from the body. There was a highly significant difference in the concentrations of HMs in fish's species (*O.niloticus* and *C.gariepinus*) from C.river and M. river. There was a significant increase of metal in *C.gariepinus* than in *O. niloticus* from C.river. The variation in the level of heavy metal accumulation in different fish species may support the view that there is a variation in ability of different fish to accumulate heavy metals. The presence of scales in *O. niloticus* also reduces the transfer of metals. The entire fish samples from C. river and M. pond contained detectable levels of the elements studied. The accumulation of these heavy metals in fish may represent a health risk, especially for populations with high consumption rates of fish (Oguh *et al.*, 2019c). One-way Analysis of variance (ANOVA) revealed a significant ($P < 0.05$) variation in the concentrations of HMs in C.river and M.pond, which is an indication of the extent of metal pollution from the river. Generally Chanchaga river had higher heavy metals concentrations (As, Cd, Cr, Cu, Hg, and Pb) than the controls, which were all above the FAO/WHO, 2016 permissible limit of 0.01, 0.05, 1.5, 0.5, 0.01, and 0.3 mg/kg respectively (Table 3). The mean values recorded at the control pond were also higher than the FAO/WHO acceptable value except Cr which is below the permissible limit of 1.5mg/kg. The mean concentration of heavy metals in *O.niloticus* and *C.gariepinus* from C.river is decrease in the following order $Cd > Hg > Pb > As > Cu > Cr$. The levels of chromium were comparatively lower in the fish samples which is in accordance with the findings of joy *et al.*, 2012. Heavy metals and nutrients absorbed by fish are usually translocated to different parts of the fish which could limit the concentrations in the river. However, availability of heavy metals in the water and continuous stay in the polluted water could lead to higher concentration in the fish.

The high concentration of As and Cd at C.river may be due to sludge from water treatment plant, atmospheric deposition, anthropogenic activities, combustion, etc. which translocated through the fishes. Arsenic affects almost all organs during its acute or chronic exposure. Liver has been reported as target organ of arsenic toxicity. Toxicity is due to arsenic's effect on many cell enzymes, which affect metabolism, DNA repair and brain problem. The most prominent chronic manifestations of As involve the skin, lungs, liver and blood systems. The levels of Cd recorded in this study was however much higher than the values of 0.27 mg/kg reported for fish by Hossam *et al.*, 2017. Cadmium is a dangerous element because it can be absorbed via the alimentary track; penetrate through placenta during pregnancy and damage membrane and DNA.

Significant concentration of Cd may have gastrointestinal effect and reproductive effect on livestock (Maobaet *al.*, 2012). Ndukwa *et al.*, 2008 reported that cadmium causes both acute and chronic poisoning, adverse effect on kidney, liver, vascular and the immune system.

High dose of chromium is observed to cause Bronchopneumonia, chronic bronchitis, diarrhea, emphysema, headache, irritation of the skin, itching of respiratory tract, liver diseases, lung cancer, nausea, renal failure, reproductive toxicity, and vomiting. Copper is indeed essential, but in high doses it can cause anaemia, diarrhea, headache, metabolic disorders, nausea, vomiting, liver and kidney damage, stomach and intestinal irritation on human health. The levels of Cu recorded in this study was however much higher than the values of 0.02 ± 0.002 mg/kg reported for *C. anguillar* by Hanyet *al.*, 2012. According to Maobaet *al.*, 2012) high levels of copper can cause metal fumes fever with flu-like symptoms, hair and skin decolouration, dermatitis, irritation of the upper respiratory tract, metallic taste in the mouth and nose.

Mercury poisoning symptoms include blindness, deafness, brain damage, digestive problems, kidney damage, lack of coordination and mental retardation. The ability of fishes to accumulate essential metals equally enables them to acquire other nonessential metals from the river or pond. Lead has no beneficial biological function and is known to accumulate in the body. Basapor and Ngabaza, 2015 reported that lead causes both acute and chronic poisoning and thus, poses adverse effects on kidney, liver, vascular and immune system. Lead can cause serious injury to the brain, nervous system, red blood cells, low IQ, impaired development, shortened attention span, hyperactivity, mental deterioration, decreased reaction time, loss of memory, reduced fertility, renal system damage, nausea, insomnia, anorexia, and weakness of the joints when exposed to high lead. The bio-accumulated metals on the fish may interact directly with biomolecules such as nucleic acid, protein, carbohydrate, disrupting critical biological processes, resulting in toxicity and the concomitant transfer of these metals through the food chain could ultimately pose risk to human life (Huang *et al.*, 2017; Oguh *et al.*, 2019b).

The BAF of HMs from water-fish is the key component of human exposure to HMs through the food chain. The BAF of *O. niloticus* and *C. gariepinus* from C. river were significantly different from M. pond. This indicates the high presence of metals in the C. river. The more the presence of metal in the water the more the bioaccumulation on the fish. The water-fish BAF of *O. niloticus* from C. river for Cd and Cu, were below one (<1) except As, Cr, Hg, and Pb which were up to and greater than one (>1). The BAF of *C. gariepinus* from C. river for As, Cd, Cr, and Hg were above one except Pb which is below one. The BAF of *O. niloticus* from M. pond were all below one except Cr and Pb while the BAF of *C. gariepinus* were above one except Hg and As which were below (<1). The BAF values below one (<1) indicate that the fish do not take up toxic element or exclude the element from the river or pond shown in Fig. 1. Where BAF > 1 indicates that the fish are enriched in elements from the water (Bio-accumulation).

As intake of fish is a possible source of metal accumulation in humans, there is an important interest in calculation of the daily and weekly consumptions of heavy metals through fish eating. The ADDM values for heavy metals were significantly little high in fishes from C. river than fishes from M. pond. The highest intakes of As, Cd, Cr, Cu, Hg, and Pb in fish were from C. river.

The Daily intake indicate the amount of HMs that will be taken in the body when such fish is consume.

The HRI values for Heavy metals were significantly high in fishes from C.river. The (HRI) values were significantly <1 ; in fishes from M.pond; indicating that consumers of fishes from these pond are not exposed health risk of As, Cd, Cr, Cu, Hg, and Pb. The HMs As and Hg were greater than one >1 Which indicate that consumption of fishes from C. river will lead to accumulation of As and Hg in the body, which can lead to severe health problem. Hazard index (HI) shows the overall risk of exposure to all the metal in fish from C. river and M.pond. The control fishes from M.pond were below <1 ; which indicates that the consumption of fish cannot pose health risk to consumer. The values of HI in fishes from C.river were above >1 ; it indicate that the consumption of all the fish can pose health risk to consumer through the intake of metal.

5. CONCLUSION

The present study concludes that heavy metals concentrations exceed the safe recommended value, which suggest that Chanchagar river is partly a heavy metal polluted river and the water, and fish are not fully safe for human health and ecosystem. The result also shows that *Clarias gariepinus* accumulate more heavy metals on fish tissue than *Oreochromis niloticus*. The community needs to be informed about the risk in consumption of these fishes.

6. ETHICAL APPROVAL

As per university standard guideline ethical approval has been collected and preserved by the authors.

7. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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