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Research Article

Clarias Gariepinus Parasites as Bioindicator For Assessing Water Quality in Omi Dam, Kogi State, Nigeria

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Abstract:

Studies using host-parasite dynamics as bioindicator of effects and accumulators of heavy metals for assessing environmental quality are still scarce, particularly in developing countries. This study aimed at elucidating the possible use of parasites of fish in monitoring and assessing water quality. 102 samples of 18 species of parasites of *Clarias gariepinus* were analyzed for copper, lead, manganese, iron, zinc and cadmium concentrations. Heavy metal concentrations were determined using atomic absorption spectrophotometer. Physico-chemical parameters were measured on sites using Hanna instrument. The nutrients and non-toxic constituents of water were also determined using methods by (American Public Health Association, 1999). The data obtained were analyzed using analysis of variance and significant differences accepted at $p \leq 0.05$. Duncan Multiple range test was used to compare the heavy metal accumulation in the parasites and sample t- test was used to compare the values of physico-chemical parameters, nutrients and non-toxic constituents of the water. The heavy metal concentrations in parasites of *C. gariepinus* were in the order of Lead>Cadmium>Copper>Iron>Magnese>Zinc. Bioindicating capacity of parasites were in the order Nematodes>Cestodes>Protozoan>Trematodes. All physico-chemical parameters of the water (pH, temperature, salinity, turbidity, electrical conductivity, total dissolved solid) except dissolved oxygen were within the permissible level of the WHO (2011) permissible limits. The water nutrients except fluoride were within the permissible limits of WHO (2011). The non-toxic constituents were within permissible limits except NH_4^+ in the control study sites and PO_4^{3-} in both study sites that was not within permissible limits. This study revealed that parasites can be ideal indicators for both effects and accumulation of heavy metals in aquatic environments. Findings from this study demonstrate the need for an ecosystem friendly approach towards sustainable management of dams and rivers. This will curb aquatic pollution which can directly and indirectly affect the structure and composition of fish parasite communities and also lead to a health risk in people consuming aquatic resources contaminated with heavy metals.

Keywords: Bioindicator, heavy metal, Omi dam, *Clarias gariepinus*, Physico-chemical

Introduction

Fish parasite species have been identified as being highly sensitive either in their physiological response to aquatic contaminants or in their ability to accumulate particular toxins in a dose-time dependent manner (Madanire-Moyo *et al*., 2012; Perez-I-Garcia *et al*., 2015). Bioindicators are organisms that can integrate and reflect the effects of heavy metals over an extended period of time. Bioindicators are differentially sensitive to environmental stressors and are therefore suitable tools for biomonitoring programmes (Justus *et al*. 2010). An indicator organism should at least have the following characteristics: (1) Taxonomic soundness, (2) low mobility, (3) well-known ecological characteristics, (4) wide distribution, (5) high sensitivity to environmental stressors, (6) numerical abundance, (7) suitability for laboratory experiments and (8) high ability for quantification and standardisation. Historically, the assessment of water quality using bioindicator organisms has been through the use of free-living biota such as fish, macroinvertebrates, and plankton **(**Ortega-Álvarez and MacGregor-Fors, 2011; Tweedley *et al*., 2014; Keke *et al*., 2017). Nevertheless, the use of free-living biota as bioindicators is characterized by several constraints; for example: (1) the sampling processes are sophisticated and require loads of funds;(2) sampling routine requires considerable amounts of samples to make any meaningful inference; (3) the composition of the organisms is affected by seasonal and temporal dynamics; (4) the large-scale heterogeneity in interpreting results occasioned by the use of sophisticated analytic tools and methods (Wright, 2010; Vidal-Martínez and Wunderlich, 2017).

Emphasis is shifting to the use of parasites as both effect indicators and accumulation indicators, given that they respond differently to a variety of pollutants in the environment (Vidal-Martínez *et al*., 2014; Vidal-Martínez and Wunderlich, 2017; Hassan *et al*., 2018; Al-Hasawi, 2019; Mehana *et al*., 2020). Helminth parasites of fish (e.g.trematodes, nematodes, cestodes and

acanthocephalans) have been employed as ideal tools for biomonitoring of heavy metal insults in aquatic ecosystem studies (Bush *et al*., 2001; Sures, 2003; Hassan *et al.,* 2018; AlHasawi, 2019).

Materials and Methods

Use of Parasites of *Clarias gariepinus* as Biological indicators

Heavy metal Analysis

Wet digestion method

Approximately 0.5 grams of parasites sample was placed in a porcelain crucible and dissolved in 2 ml concentrated nitric acid HNO_3 and heated at 100°C for 6hours. Upon cooling, it was filtered through Whatman No. 42 filter paper into a 50 ml volumetric flask and made to volume with triple distilled water of the sample. A blank solution was also prepared using similar experimental procedure (Haipeng *et al*., 2019).

Instrumentation

The heavy metal content was determined by employing Atomic Absorption Spectrophotometer (AAS), Analyst 700 Perkin Elmer, USA (Perkin, 1996) with air-acetylene burner for flame. A liquol of the digested solution was aspirated to the instrument for the determination of metals/mineral iron (Fe), Copper (Cu), Lead (Pb), Zinc (Zn), Manganese (Mn) and Cadmium (Cd). Calibration of AAS was done using the working standard prepared from commercially available metal/mineral standard solutions 1000 g/ml. The most appropriate wavelength hallow cathode lamp was selected as given in the instrument user's manual. The measurement was made within linear range of working standard used for calibration (AOAC, 2022).

Water Quality

Water quality parameter from fish sampling site i.e. physico-chemical parameter such as temperature, pH, Electrical Conductivity (S/m), salinity (‰), dissolved oxygen (mg/l), Total Dissolved Solid(mg/l) and Turbidity (NTU) were used.

Determination of the Physico-chemical Parameters of Omi Dam Water

Temperature (^{0}C) , pH, electrical conductivity, turbidity, total dissolved solid and salinity of the water was measured by dipping a Hanna instrument model HI 98129 in water for 1-2 minutes then the reading was recorded (APHA, 1999).

Determination of dissolved oxygen

100 ml of Water sample was poured into a 300 ml Biological Oxygen Demand (BOD) bottle, Two Millilitre (2 ml) of M_nSO_4 and 2ml of alkali –iodide azide reagent and then stoppered with care to exude air bubbles. It was thereafter mixed by inverting the bottles a number of times until a clear supernatant was observed. It was then allowed to settle for 2 minutes after which 2 ml of Concentrated H_2SO_4 was added by allowing the acid run down the neck of the bottle. It was stoppered again by gentle inversion until the solution was complete. Exactly, 100 ml of the prepared solution was transferred into a conical flask, titrated with 0.0125N of $Na_2\ So_2\ 0_3.5H_2o$ solution to a pale straw/yellow colour. Approximately 1 ml of freshly prepared starch was added. Titration was continued by adding thiosulphate until the blue colour disappeared.

$$Do_2 = \frac{100 \times 8 \times n \times v^{-1}}{V}$$

Where V^{-1} = Volume of thiosulphate used (Titre value)
n = normality of thiosulphate (0.013IN)
v= volume of sample used (200ml)

Determination of chloride

Exactly 100 ml of water sample was transferred into a conical flask, 2-3 drops of Potassium Chromate were added, and content was swirled for few minutes and then titrated against Silver nitrate solution until a dirty reddish precipitate was obtained. Chloride was calculated using Clmg/1 = volume of $AgNo_3$ x 10 (APHA, 1999).

Determination of fluoride

Approximately 20.0ml of standard solution of NaoH to 100 ml, 10 to 25 ml of Nacl in 100ml beakers, the samples in room temperature, equal volume of buffer added to each beaker. Calibration was set up at 10 meter using standard solution. Standard solution already diluted with the buffer was supplied with meter to avoid stirring before immersing an electrode a direct instrument used for reading (APHA, 1999).

Determination of calcium

Exactly 50 ml of sample was taken into a conical flask with the help of a pipette. 1 ml of NaoH was added to produce pH of 13-14 and 0.2 g muroxide was added as indicator and stirred. It is titrated with O.1N EDTA solution until a colour change from pink to

purple is observed. The volume of EDTA was recorded as that of the value of Calcium (APHA, 1999).

Determination of magnesium

The values of total hardness and Calcium hardness was determined by Ethylene Diamine Tetra Acetic Acid (EDTA) and Magnesium was calculated (APHA, 1999).

Calculation

Mg Mg/L = (TH as mg $CaCO_3$ /L – Calcium) Hardness as mg $CaCO_3$/L) x 0.243

TH = Total Hardness, Mg $CaCO_3$/L

Determination of potassium

Photometry flamecell method was used. Instructions of flame photometer was followed for selecting proper photocell wave length, slit width adjustments, fuel gas and air pressure, step for warm up a blank and potassium calibration standard was prepared ranges, 0-100, 10 or 0-1 mgK/L remission at 766.5 nm was measured and calibration curve. Potassium concentration of the sample was determined from the curve calculation

MgK/L =mgK/L from the calibration x Dilution

$$\text{Dilution} = \frac{\text{mL sample + mL distilled water}}{\text{mL Sample}}$$

Determination of sodium

Photometry flamecell method was used. Instructions of flame photometer for selecting proper photocell, wavelength, width adjustment, fuel gas and air pressure, steps for warm up was followed. A blank solution was prepared and Sodium calibration, in any of the ranges 0-100, 0-10, 0-1 mg Na/L. Instrument was set at zero with standard containing sodium emission, calibration curve at 589 nm was measured. Sodium concentration was determined by the sample, diluted sample or from the curve calculations

Mg Na/L = mgNa/L from calibration curve x Dilution

$$\text{Where: Dilution} = \frac{\text{mL sample + mL distilled water}}{\text{mL sample}}$$

Determination of non-toxic constituents of the omi dam water

Non-toxic constituents of the water such as Total Nitrogen (Inorganic Nitrogen), Nitrate, Nitrite, Ammonium and Phosphate were determined using the method of APHA (1999).

Data Analysis

Values are Mean± SD, data were analyzed using one-way Analysis of Variance (ANOVA) and Duncan Multiple range test to separate the means and compare the heavy metal parameters. Data were analyzed using independent sample t-test is to compare the parameters of the physico-chemical parameters, nutrients and non-toxic constituents of the study area and control at <0.05 level of significance.

Results

Table 1: Parasites of *Clarias gariepinus* as Biological Indicators

	Fe	Cu	Cd	Zn	Mn	Pb
(Control)Ichthyophthirius multifilis	0.008±0.001^{a}	0.015±0.007^{a}	0.115±0.000^{a}	0.001±0.000^{a}	0.005±0.001^{a}	0.025±0.007^{a}
Acanthostomum imbutiforme	0.039±0.016^{b}	0.298±0.065^{b}	0.316±0.010^{c}	0.030±0.010^{b}	0.041±0.005^{bc}	0.571±0.155^{cde}
Agamospirura sp.	0.067±0.018^{e}	0.300±0.036^{b}	0.398±0.003^{cd}	0.025±0.003^{ab}	0.064±0.008^{e}	0.503±0.086^{cde}
Apophallus muehlingi	0.052±0.016^{d}	0.399±0.162^{b}	0.293±0.003^{bc}	0.030±0.003^{b}	0.049±0.010^{d}	0.402±0.049^{b}
Bucephalus polymorphus	0.059±0.022^{d}	0.263±0.071^{b}	0.398±0.008^{d}	0.025±0.008^{ab}	0.058±0.007^{de}	0.447±0.117^{bc}
Capillaria brevispicula	0.045±0.006^{c}	0.360±0.113^{b}	0.629±0.012^{fg}	0.033±0.012^{b}	0.044±0.006^{bc}	0.494±0.076^{cde}
Chloromyxum mucronatum	0.047±0.001^{c}	0.375±0.090^{b}	0.255±0.006^{ab}	0.030±0.006^{b}	0.045±0.005^{bc}	0.527±0.139^{cde}
***Diphyllobothrium latum* larva**	0.046±0.011^{c}	0.331±0.146^{b}	0.390±0.009^{d}	0.030±0.009^{b}	0.041±0.012^{bc}	0.646±0.304^{e}
Eimeria rivirei	0.040±0.021^{bc}	0.360±0.013^{b}	0.298±0.007^{c}	0.029±0.007^{b}	0.036±0.009^{b}	0.509±0.098^{cde}

Euclinostomum heterostomum	0.045±0.019^{c}	0.340±0.081^{b}	0.334±0.004^{cd}	0.032±0.004^{b}	0.047±0.014^{cd}	0.454±0.167^{d}
Gyrodactylus latus	0.040±0.011^{bc}	0.397±0.164^{b}	0.579±0.009^{f}	0.031±0.009^{b}	0.053±0.017^{de}	0.517±0.069^{cde}
Ichthyophthirius multifilis	0.036±0.013^{b}	0.344±0.142^{b}	0.489±0.009^{ef}	0.027±0.009^{ab}	0.050±0.010^{d}	0.618±0.132^{de}
Myxidium oviforme	0.040±0.012^{bc}	0.383±0.085^{b}	0.373±0.012^{cd}	0.070±0.012^{c}	0.044±0.011^{bc}	0.530±0.086^{cde}
Myxosoma branchialis	0.063±0.034^{de}	0.296±0.088^{b}	0.509±0.008^{f}	0.024±0.008^{ab}	0.053±0.010d^{e}	0.573±0.085^{cde}
Philometra intestinalis	0.047±0.011^{bc}	0.339±0.069^{b}	0.683±0.004^{g}	0.024±0.004^{ab}	0.053±0.007^{de}	0.459±0.170^{d}
Philometra rischata	0.054±0.016^{d}	0.311±0.034^{b}	0.403±0.006^{e}	0.029±0.006^{b}	0.059±0.013^{de}	0.585±0.068^{cde}
Scolex pleuronectis	0.052±0.021^{d}	0.355±0.084^{b}	0.575±0.012^{f}	0.025±0.001^{ab}	0.052±0.012^{de}	0.630±0.208^{de}
***Triaenophorus nodulosus* larvae**	0.041±0.013^{bc}	0.288±0.089^{b}	0.436±0.006^{ef}	0.034±0.006^{b}	0.048±0.011^{cd}	0.635±0.053^{de}
Trypanosoma cobitis	0.043±0.019^{c}	0.340±0.115^{b}	0.287±0.006^{bc}	0.032±0.006^{b}	0.045±0.012^{bc}	0.578±0.252^{cde}
WHO 2011	0.3	0.05	0.003	5	0.05	0.010

Means with the same alphabets are not significantly different at $p < 0.05$

Table 2: Physico-Chemical Parameters of the Water in Omi Dam

	Sample	**Control**	**p-values**	**WHO 2011 permissible limits**
Temperature(°C)	6.30±0.00	6.76±0.01	0.000	20
Ph	7.95±0.07	7.55±0.07	0.030	6.5-8.5
Turbidity(NTU)	9.75±0.35	13.00±0.00	0.006	40
Salinity(‰)	0.06±0.01	0.09±0.00	0.018	0.6
Electrical Conductivity(S/m)	0.71±0.01	0.92±0.01	0.003	4
Total Dissolved Solid(mg/L)	40.25±0.35	99.95±0.07	0.000	600
Dissolved Oxygen(mg/L)	10.35±0.21	8.25±0.07	0.006	5

Table 3: Nutrients of the Water in Omi Dam

	Sample	**Control**	**p-values**	**WHO 2011 Permissible limit**
Chloride(mg/L)	7.05±0.14	2.98±0.00	0.001	250
Fluoride(mg/L)	6.83±0.18	2.86±0.00	0.000	1.5
Na(mg/L)	2.70±0.14	9.20±0.14	0.001	200
K(mg/L)	6.80±0.14	15.70±0.28	0.000	200
Mg(mg/L)	12.35±0.21	40.80±0.00	0.000	50
Ca(mg/L)	26.55±0.21	102.15±0.07	0.000	100

Table 4: Non-toxic Constituents of the Water in Omi Dam

	Sample	**Control**	**p-values**	**WHO 2011 permissible limit**
Total Nitrogen(mg/L)	0.014±0.000	0.060±0.005	0.006	10
NO_3^-(mg/L)	0.024±0.003	0.061±0.005	0.012	10
NO_2^-(mg/L)	0.11±0.01	0.17±0.16	0.681	1.0
NH_4^+(mg/L)	0.31±0.04	0.56±0.00	0.014	0.5
PO_4^{3-}(mg/L)	6.91±0.10	6.38±0.05	0.021	5.0

Discussion

The findings of this study have revealed the order of heavy metal accumulation Pb>Cd>Cu>Fe>Mn>Zn. Lead (Pb) had the highest

accumulation compared to the other metals. This may be attributed to the source water/river that supplies the dam and the proximity of the dam to a rice mill where expired batteries and tyres are dumped into the river that supplies the dam. Also, higher level of Pb observed in parasites might be attributed to runoff from agricultural lands which contain agrochemical such as fertilizers and pesticides (Banat *et al*., 1998). The findings in this current study are consistent with the report of previous researchers like Tenora *et al*. (2000); Lohan *et al.* (2001); Abdel-momem (2001); Sures and Reimann (2003); Thielen *et al*. (2004); Eira *et al*. (2009); Jankovska *et al*. (2010) and Shahtat *et al.* (2011) with the similar order of heavy metal accumulation (Pb>Cd>Cu).

The findings by Ogri (2004) and Abraham *et al*. (2015) were not consistent with this study, who reported Fe as most abundant metal and Keke *et al*. (2020)'s work also disagrees with heavy metal accumulation in their study being in this order Fe>Zn>Mn>Cu>Pb.

Parasites that recorded the highest accumulation of heavy metal were *Philometra intestinalis* followed by *Diphyllobothrium latum, Triaenophorus nodulosus, Scolex pleuronectis* and *Capillaria brevispicula.* Bioindicating capacity of these parasites was in this order of Nematodes> Cestodes> Protozoans> Trematodes. Therefore, nematodes are believed to have higher accumulation capacity of heavy metals than the other parasites. The findings of this study are in consonance with the findings of other authors in this order: Acanthocephalan and Nematodes>Cestodes >Trematodes in Nigeria (Ugokwe and Awobode, 2015; Keke *et al*., 2020), Egypt (El-Lamie and Adel-Mawla, 2018; Al-Hasawi, 2019), Iran and Saudi Arabia (Hassan *et al*., 2018), South Africa (Erasmus *et al*., 2020; Pretorius and Avenant-Oldewage, 2021).

Comparing metal accumulations in different helminthic taxa, helminthes utilized their gut for higher metal accumulation capacity because cestodes absorb their nourishment through their tegument and nematode larvae take up their nutrients through two routes; their digestive system and tegument.

To the best of my knowledge, Parasitic protozoans have never been employed as effect or accumulative indicators in any study before now, but were reported in this study suggesting a major breakthrough.

The concentration of iron (Fe) in fish parasites was below the permissible limit recommended by WHO (2011). The concentration of copper (Cu) in this study was less than 0.05 $mgkg^{-1}$ concentration recommended by WHO (2011) and 2.25 $mgkg^{-1}$ by WHO (2017). The concentration of cadmium (Cd) was higher than the 0.003 $mgkg^{-1}$ recommended permissible limit (WHO, 2011). It has been reported that exposure to cadmium enhances hypertension and kidney damage in humans (Yujun *et al.,* 2011). The concentration of zinc (Zn) in this study was lower than the tolerable level of 5.0 $mgkg^{-1}$ specified by WHO (2011). Manganese (Mn) concentration was found to be below the WHO Permissible limit of 0.50 mg/kg^{-1}. The concentration of Mn in this study appears considerably low, as excessive exposure or intake of it may lead to a condition known as Manganism, a neurodegenerative disorder that causes dopaminegic neuronal death and symptoms similar to Parkinson's disease (Silva *et al*., 2013).

Lead concentration was higher than the recommended concentration of 0.010 $mgkg^{-1}$ of WHO (2011).

Also, anthropogenic activities around the dam could lead to lead accumulation above the permissible limit. The study area is an agro-industrial base community. Lead is one of the toxic heavy metals which have no known metabolic use. Lead accumulation in humans could lead to loss of concentration, delusions and hallucinations manifesting as poor memory and irritability mostly in children, mental capability reduction, difficulties in learning, growth reduction, anemia, severe stomach ache, weakness of muscle and brain damage (Izah *et al.,* 2015).

Temperature of Omi dam was within the WHO Permissible limit of 0-20^{0}C. Aquatic organisms have a range of temperature at which optimal growth, general fitness and reproduction occur (Dallas and Day, 2004).

pH of Omi dam was within permissible limit of 6.5-8.5 (WHO, 2011). The pH values obtained in this study were within this range that is 7.55-7.95 which is slightly alkalinic. The **pH** measures the concentration of the hydrogen ions (H^+) and alkalinity by the concentration of hydroxyl, bicarbonate and carbonate ions in the water (Palmer *et al*., 2004).

The turbidity of Omi dam was within permissible limit of 40 NTU (WHO, 2011), while that of the control (study site) was above the permissible limit (FEPA, 2004). Increase in turbidity has been associated with a loss of fish body condition and vision and leading to a decrease in ability to capture food. (Gray *et al*., 2011).

The salinity of Omi dam was 0.06‰ and control study site was 0.09‰. Salinityis a term used to refer to the saltiness of the water (Dallas and Day, 2004). The electrical conductivity of the dam was within permissible limit of WHO (2011). Electrical conductivity is the ability of water to conduct an electrical current. The total dissolved solids of Omi dam were within the permissible limit (WHO, 2011). The TDS concentrations that are too high or too low may limit growth and may lead to the death of aquatic organisms (Dallas and Day, 2004).

Dissolved oxygen of Omi dam was above permissible limit of (WHO) 2011. These values exceeded permissible limit of 5.00 $mg\ell^{-1}$. According to Palmer *et al*. (2004), an increase in water temperature decreases oxygen solubility which may increase the toxicity of certain chemicals to aquatic life. The DO concentrations recorded in this study would not adversely affect the functioning and survival of biological communities because DO concentration value was below 5 $mg\ell^{-1}$ recorded during this study period.

The values for chloride were less than permissible limit of 250 $mg\ell^{-1}$, Fluoride was above the permissible limit of 1.5 $mg\ell^{-1}$ (WHO, 2011). Sodium was within permissible limit for sodium in aquatic ecosystem (WHO, 2011). The concentration of potassium in Omi dam was within the permissible limit (WHO, 2011). Magnesium was within permissible limit (WHO, 2011). The concentration of Calcium in Omi dam study site was within permissible limit of <100 $mg\ell^{-1}$ and above permissible limit in control study site (WHO, 2011). Calcium is a vital element for all living organisms (Dallas and Day, 2004

Total Nitrogen was within permissible limit of (WHO) 2011. Ammonium in Omi dam was within permissible limit and control

study site was above permissible limit of 0.5 $mg\ell^{-1}$ (WHO, 2011). Ammonium concentrations recorded in the control study site was high and may thus result in mortalities of aquatic organisms. The high concentration of ammonium could be attributed to agricultural runoffs from farmlands. However, no mortalities of fish were recorded during the sampling period.

Nitrate was within the permissible limit of 10 $mg\ell^{-1}$ (WHO, 2011). Nitrite was within the permissible limit of 0.1 $mg\ell^{-1}$ (WHO, 2011). Phosphates in Omi dam and Control study site respectively found to exceed permissible limit of 5.0 $mg\ell^{-1}$ (WHO, 2011). Phosphate is a crucial nutrient for living organisms and exists in water bodies in both dissolved and particulate forms.

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