Journal of Environmental and Occupational Science
www.jenvos.com
DOI: 10.5455/jeos.20150105105524

Original Research

ScopeMed

Trace metal contamination in tropical endemic fish: Factorial effect interactions and *in situ* quantitative risk assessment

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Received: December 13, 2014
Accepted: January 05, 2015
Published: January 14, 2015

ABSTRACT

Aim: Contamination of the Anambra River with heavy metals (arsenic, As; cadmium, Cd; chromium, Cr; copper, Cu; lead, Pb; nickel, Ni; and zinc, Zn) was examined in two preponderant fish species (*Synodontis clarias* and *Tilapia nilotica)* following earlier detection of the elements in water column. **Methods:** Levels of heavy metals were measured in both seasonal regimes (rainy and dry) at five selected locations with atomic absorption spectrophotometer. Factorial effects and interactions were explored on completely randomized block design. Quantitative risk of metal exposure through contaminated fish consumptions among the resident community population at the river was assessed to extrapolate the probable public health threat. **Results:** The result showed variations among heavy metal concentrations in fish and Zn and Cu recorded significant amounts with *S. clarias* recording higher concentrations than *T. nilotica*. Season, species of fish and location and their interactions had significant effects on the amounts of Cu and Zn accumulated in the fish tissues except season by species effect. Zinc recorded the highest concentrations at all locations measured, with Onono (location 5) producing the fish species with the highest amount of metals compared with other locations. The heavy metal concentrations were below the comparable international safe standards. Margin of exposure and exposure dose calculated for the heavy metals were all below reference standards and tentatively considered not to be of risk to public health. **Conclusion:** However, there is considerable concern of contamination of the fish species with heavy metals and recommended regular monitoring or examination of edible fish species.

KEY WORDS: Bioaccumulation, heavy metals, factorial effects, margin of exposure, exposure dose

INTRODUCTION

Heavy metal pollution of aquatic ecosystems has become a global phenomenon and have also drawn different dimensions of research to target the increasing scourge. Focus is now shifting to emerging metals and metalloids, but the known conventional heavy metal residues in environmental dispersion continue to attract attentions and spark up evolving physiological and public health effects, making the continuous research and database build-up on them inevitable. Biodiversity-rich freshwater ecosystems are presently declining faster than marine or land ecosystems making them the world's most vulnerable habitats [1,2]; their sustainability being threatened by anthropocentrism [3-6]. Anthropogenic activities have been the bane of environmental degradation and threats to its sustainability. Activities such as industrial, agricultural, domestic activities and urbanization processes give rise to pollutants, which are introduced into the surface waters through point and non-point sources [4] and much of the world still do not have access to clean, safe water [7,8]. These activities have resulted in the exponential increase of heavy metals in the water environment [9-11] and corresponding drastic effects on the physicochemical characteristics of the water and fish population [10,12-16]. Because heavy metals cannot decompose, its environmental persistence, mobility and redistribution provide exposure platforms for aquatic ecosystems and could alter the ecological balance and affect biodiversity [17,18].

Physicochemical properties of the water and various abiotic environmental variables have been shown to affect and upset the toxicity and accumulation of heavy metals in biota [19,20]. Toxicants can have dissimilar strengths depending on the variable physicochemical properties, presenting diverse risks for the same chemical in a different environment [21]. The trophic transfer of heavy metals through fish poses a threat to the public health in light of its position on the aquatic food chain and food menu [22,23]. Although some of these metals (e.g. Cu and Zn) are essential elements necessary for biological functionalities of aquatic life, elevated concentrations and intakes could produce aquatic toxicity [24].

Rivers are highly prone to material loadings that can result in pollution. Anambra River is a major freshwater system of Nigeria and a shallow fragile ecosystem that has suffered drastic changes in the past years from pollution of its waters [25]. Reconnaissance tour to various regions surrounding the river revealed crop agricultural and fishery production within the zone including the floodplains. About 15% of all irrigated cropland suffers from waterlogging and possibly, salinization due to drainage problems, thereby resulting in reduced crop yields [26]. Ojiako [27] earlier reported the pollution effects of irrigation drainage on quality of Anambra River and survivability of the occupant fish species. Soil fertility improvement is mostly based on application of inorganic fertilizer, especially during the dry season while natural spontaneous flooding takes care of crop yield during the rainy season along the floodplains [28]. Moreover, according to the latter author, the river is gradually becoming eutrophic. Use of agrochemicals was also evident and inefficient use of fertilizers and pesticides is also a major cause of pollution of both surface and ground waters [29]. Two major markets (Otuocha and Otu Nsugbe) are located at the bank of the river [30].

The most important and widely consumed fish species in the river are *S. clarias* and *T. nilotica* due to their preponderance, size, survivability and taste [31]. It is not known if the heavy metal bioaccumulation in these species has been undertaken. Considerable research has been done and further initiated and maintained at the Anambra River. Physicochemical properties and heavy metal loading of the water column of the river in various locations have been earlier evaluated [28,30,32,33]. However, bioaccumulation of the common heavy metals in preponderant fish species easily consumed by the local resident human population has not received detailed research to addressing public health risk and concerns of poisoned dietary intakes. *Quantitative risk assessment* is very essential in predicting the probability of an identified hazard to cause harm and also employed in part for site remedial surveys to delineate the magnitude to which location counteractive action is required. The risk assessment offers a numeric approximation of hypothetical risk or hazard, supposing no clean up takes place and it generally uses standard protective exposure assumptions when evaluating site risk [34]. To prioritize new and existing contaminants for toxicological studies and risk assessment, a better characterization of human exposure is required through data collection within realistic exposure scenario [21]. Studies on contamination of rural environments have been considerably low compared to its urban counterpart. In these areas, it is difficult to practice even elementary hygiene without sufficient quantities of water free of these contaminants [35]. As such, it is necessary to protect the water sources themselves from fecal, agricultural, and industrial contaminations. In developing countries, 90-95% of all sewage and 70% of all industrial wastes are dumped untreated into surface water [35]. Following these problems, the work was aimed to determine the heavy metals in two earlier identified fish species of the Anambra at different seasons and locations of probable varied anthropogenic impacts. In order to translate the metal bioaccumulation findings to human exposure risk, observed metal concentrations were compared to standard quality guidelines and non-carcinogenic quantitative risk related to exposure through dietary fish ingestion was extrapolated.

METHODS

Description of the Study Area

Anambra River in Nigeria lies between latitudes 6° 00'N and 6° 30'N and longitudes 6° 45'E and 7° 15'E. The river is at the South Central region of Nigeria [36]. Anambra River is approximately 207.4 km to 210 km in length [25,37], rising from the Ankpa hills (ca. 305-610 m above sea level) and discharging into River Niger at Onitsha [25]. The entire River basin drains an area of 14014km^2 [36] [Figure 1].

Experimental Design

The experiment for the quantification of heavy metals in fish was conducted under a 2 × 5 × 2 factorial in a completely randomized block design to test the effects of season, location and species together with their interactions on the concentration of heavy metals (Chromium [Cr], Cadmium [Cd], Arsenic [As], Zinc [Zn], Lead [Pb], Nickel [Ni] and Copper [Cu]) of fish species inhabiting Anambra River. Seasons were tested at two

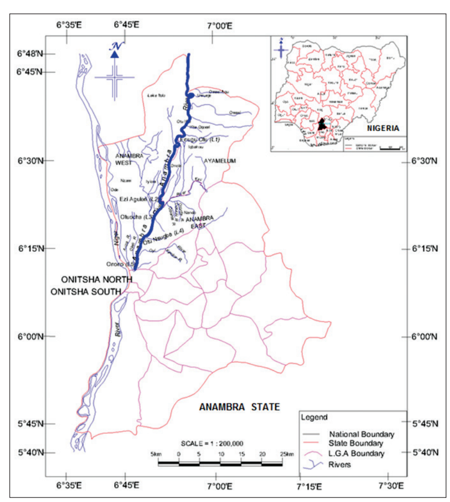

Figure 1: Map showing Anambra River and sampling location

levels *viz*.: Rainy season and dry season; location handled at five levels (Enugu Otu, Ezi Aguleri, Otuocha, Otu Nsugbe and Onono) and two major species of fish (*S. clarias*, Linnaeus, 1758 and *T. nilotica*, Linnaeus, 1757) inhabiting the river were used.

The model used is:

$$Y_{ijkl} = \mu + SS_i + L_j + S_k + SSL_{ij} + SSS_{ik} + LS_{jk} + SSLS_{ijk} + \varepsilon_{ijkl}$$

Where;

Y_{ijkl} = heavy metal (Cr, Cd, As, Zn, Pd, Ni and Cu) values that were observed due to:

μ = the population mean;

SS_i = the effect of $_{ith}$ season

L_j = the effect of $_{jth}$ location from where the samples were collected

S_k = the $_{kth}$species effect;

SSL_{ij}, SSS_{ik}, and LS_{jk} = are the interactions between season and location, season and species, and species and location, respectively.

$SSLS_{ijk}$ = the third level interaction between season, location and species.

ε_{ijkl} = is the error term associated with the experimentation.

Assumptions: Error term is independently, identically and normally distributed with zero mean and constant variance. That is, iind $(0, \sigma^2)$.

Total of 24 fishes, comprising 12 *S. clarias* and twelve *T. nilotica* were used for each location.

Sampling Locations and Campaign

The experimental site comprised of five distinct locations/ stations established to cover possible impacted and nonimpact area along the river course based on an earlier field reconnaissance tour. The locations (L_X) of the various measured stations are:
L_1: Enugu Otu
L_2: Ezi Aguleri
L_3: Otuocha
L_4: Otu Nsugbe
L_5: Onono

Following the experimental design, samples were collected in mid rainy (July) and dry (February) seasons. For each season, live *S. clarias* and *T. nilotica* of fairly similar live-weight were collected from Anambra River irrespective of the sex at the five stations using set nets, long-lines and traps. The collected fish were immediately classified, weighed and individual length determined (weights: 264.91 ± 2.28 and 260.13 ± 4.20; lengths of 29.16 ± 1.60 and 27.35 ± 1.42 for *S. clarias* ($P < 0.05$) and *T. nilotica*, respectively) before storage at temperature of −20°C. The relative distance between each station is approximately 12 km [Figure 1]. All the sample collections were made during the morning hours in both seasons.

Heavy Metal Analysis of Fish Samples

20 g of muscle tissues were removed from the samples with the help of a stainless steel surgery knife. Samples were freeze-dried and ground into homogenous mixture using a porcelain mortar and pestle. 10 g of sample was digested and analysed after the method adopted by Food and Agricultural Organization/Swedish International Development Cooperation Agency (FAO/SIDA) [38]. The heavy metal concentrations in each digested samples were determined by comparing their absorbance with those of standards (solution of known metal concentration) using Alpha- 4 Cathodeon Atomic Absorption Spectrophotometer. The obtained results were expressed as mg/kg wet weight.

Precautions were strictly taken in order to prevent contamination during investigation. Fish samples were washed by deionized distilled water prior cutting to remove adsorbed metals on the skin. All reagents used were of analytical grade; glassware were sterilised by soaking them in 10% nitric acid and rinsed with distilled water prior to use. Deionized water was used to prepare all aqueous solutions [15].

Quality Control and Recovery Accuracy

In order to check the efficiency of sample digestion procedures and subsequent recovery of the metals, analytical quality was determined by analysis of standard reference material, fish flesh homogenate. Homogeneous mixtures of seven samples of fish muscles were spiked with solutions containing standard solutions of all seven metals considered in the current investigation [15]. The element solution was spiked in a manner to attain final concentrations of 1.0 and 3.0 mg/kg. A mixture without any metal was used as control and all mixtures were

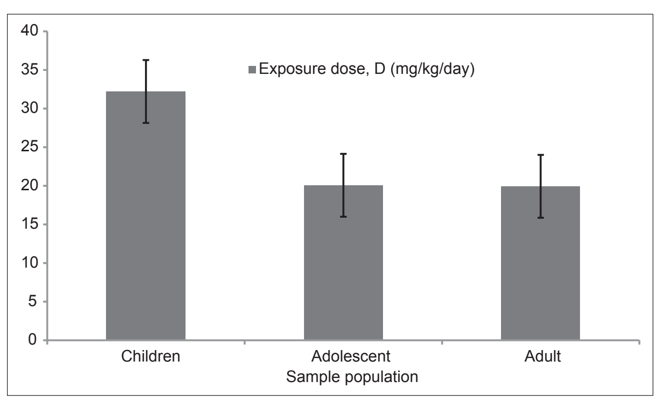

Figure 2: Relative exposure dose of heavy metals among resident population categories

then subjected to the digestion procedure. The resulting solutions were analyzed four times for metal concentrations according to the same procedures as the samples to establish confidence in the accuracy and reliability of data produced. The amount of metals recovered after the digestion of the spiked samples was used to calculate percentage recovery as follows: % recovery = ([t-c]/t) 100. Where t = concentration of a metal in treatment sample, and c = concentration of a metal in control sample.

Blanks and standard solutions were also included for quality control to confirm the accuracy and reproducibility of the results. Analytical results of the quality control samples indicated a satisfactory performance of heavy metal determination within the range of certified values 95-111% recovery for the metals studied [14].

Heavy Metal: Human Exposure Risk Assessment

Human population and fish consumption rate

A survey was conducted in order to characterize the rates of freshwater fish consumption and determine the average quantity of fish consumed per person among the Anambra River residents. The survey consisted of direct interviews with 300 shore-based community residents comprising of children, adolescent and adults irrespective of sex. The reason given for fishing was for food and indicated that they eat their catch and others given to them by friends and neighbors. Only 273 people responded to our interviews culminating into 91% of the survey respondents. Survey site was nominated based on observations of use by fishermen, population residency and domestic utilities of the river. Residents were asked specific questions concerning: Fishing and fish consumption habits on daily basis; perceptions of presence of contaminants in fish; and perceptions of risks associated with consumption of recreationally caught fish at various times of the day [39].

The survey actually employed "assumption of sharing"; the respondents who provided the data clearly indicated that they shared the fish that came into their households with every member of the household and gave the number of individual fish consumers. Respondents' consumption rates were based not only on the fish that they themselves had caught for consumption but also on the fish that other family members had brought into the household and shared with them, as well as fish that had been given to them by other individuals outside of the household [39].

Weights of the individuals were taken with flexible weighing equipment only by consent and average body weights of the population categories calculated. The daily intake of heavy metals from fish consumption per average individual population category was calculated by multiplying this value by the average concentration of each metal in analyzed fish as shown in the deduced formula expression;

Estimated daily intake = Heavy metals in fish × weight of consumed by average individual category

Exposure risk assessment

Risks associated with human consumptions of the potential contaminated fish with heavy metals measured in the study were derived by comparing the levels quantified in the muscles and International safe limits. Margin of exposure (MOE) was explored to assess the species-specific risk from consumption of contaminated fish with heavy metals as given by Watanabe *et al.* [40]:

$$\text{MOE}=\frac{\text{MCC}\times\text{CR}}{\text{BW}\times\text{RfD}}$$

Where MCC was the species-specific mean chemical concentration (mg/kg), CR was the consumption rate (kg/day), BW was the human body weight (kg) and RfD was the reference dose for chronic oral exposure of the specific heavy metal in mg/kg/day. MOE >1 indicates the exposure to a dose higher than the safe daily dose for chronic non-carcinogenic effects. The reference dose value adopted by the United States Environmental Protection Agency (U.S EPA)-Integrated Risk Information System [41-44] and used in this study were: As = 0.0003; Cd = 0.001; Cr = 0.003; Zn = 0.3; for Cu, Ni, and Pb, no information exist as per the RfD values. However, Cu is classified as human carcinogen under Groups 3a [45,46].

Exposure doses from ingestion of fish among the population were derived following the equation of Agency for Toxic Substances and Disease Registry [34] on calculation of exposure doses assuming that all fish consumed are caught from one contaminated water body:

$$\text{D}=\frac{\text{C}\times\text{IR}\times\text{EF}\times\text{CF}}{\text{BW}}$$

Where,

D = Exposure dose (mg/kg/day)

C = Contaminant concentration (mg/kg)

IR = Intake rate of contaminated medium (mg/day)

EF = Exposure factor (unitless) - the fish intake rate is a daily average, so the exposure factor is equal to 1

CF = Conversion factor (10^{-6} kg/mg)

BW = Body weight (kg)

Data Analysis

Data transformation and analysis were conducted using IBM SPSS Statistics 22 and Microsoft Excel (2010 version). Mean values ± standard deviation (the standard deviation of the mean) in mg/kg wet weight for metals and demographic indices were calculated. Significance and interaction effects on heavy metal concentrations detected in the fish samples were subjected to three factorial Analysis of Variance and the comparisons among group means were obtained using *post hoc* least significant difference. Student's *t*-test was used in comparing two means where appropriate. The results were evaluated at a probability level of $P < 0.05$.

RESULTS

Recovery Studies

Table 1 provides the data generated from the recovery studies carried out in the work. Recovery ranged from 96-99% to 95.33-99.67%, using solutions of 1 mg/kg and 3.0 mg/kg concentrations, respectively

Heavy Metal Concentrations in Fish from the Anambra River

Seasons, species and location effects on the heavy metal concentrations

Season, species, and location effects on heavy metal concentrations are presented in Table 2. The concentrations of the heavy metals observed varied in both seasons and locations. Season and location exhibited significant effects ($P < 0.05$) on the mean Cu and Zn concentrations detected in the fish samples. Concentrations of Cu and Zn were observed to be higher in dry season compared to rainy season. Again, Cu and Zn levels were high at Onono, followed by Otuocha and the least being at Ezi Aglueri and Enugu Otu. *S. clarias* was observed to record the higher concentration of heavy metals especially Zn.

Seasons × species, seasons × location, and species × location interaction effects

The second level interaction effects on the mean concentrations of the heavy metals obtained are presented in Table 3. Seasons × location interaction showed significant ($P < 0.05$) effect on Cu and Zn only. Onono gave the highest concentration values of Cu and Zn in both rainy and dry seasons. Ezi Aguleri and Enugu Otu recorded least concentrations of the metals in rainy season, respectively. Species x location interaction also exhibited significant ($P < 0.05$) effects on Cu and Zn concentrations. S_1 (*S. clarias*) accumulated highest concentrations of Cu and Zn at Onono and least concentrations at Ezi Aglueri and Enugu Otu, respectively.

Similarly, *T. nilotica* (S_2) at location 1 concentrated least amount of Cu and Zn while exhibiting its highest concentration rate at the location 5 for both heavy metals [Table 3]. Seasons by species had no significant ($P > 0.05$) effects on these metals.

Seasons × species × location interaction effects

Third levels interaction effects on the heavy metal concentrations are presented in Table 4. It showed effects ($P < 0.05$) on the Cu and Zn concentrations. *S. clarias* in rainy season accumulated least amount of Cu and Zn at Ezi Aguleri and Enugu Otu, respectively with corresponding higher concentrations at Onono. Dry season maintained the same pattern of metal concentration distributions in various locations with that of rainy season. *T. nilotica* showed higher concentration values of Cu and Zn in rainy season at Onono and least values at Ezi Aguleri and Enugu Otu, respectively. At Onono, the species recorded higher concentrations for Cu and Zn, respectively for dry season (SS_2) with least concentration at Enugu Otu in the same season (SS_2).

Table 1: Recovery of metals from fish muscle samples

Heavy metal	Concentration of metal added (mg/kg)	Concentration of metal recovered (mg/kg)	Recovery %	Average %
Cd	1.0	0.99	99.00	97.84
	3.0	2.90	96.67	
Cr	1.0	0.98	98.00	96.50
	3.0	2.85	95.00	
Ni	1.0	0.99	99.00	97.50
	3.0	2.88	96.00	
Pb	1.0	0.98	98.00	96.67
	3.0	2.86	95.33	
Zn	1.0	0.99	99.00	98.17
	3.0	2.92	97.33	
As	1.0	0.96	96.00	97.84
	3.0	2.99	99.67	

Table 2: Seasons, species, and location effects on the mean heavy metal concentrations (mg/kg) in fish

Factor	Cd	Cr	Cu	Ni	Pb	Zn	As
SS_1	0.003±0.001	ND	0.095±0.11^a	0.0015±0.001	0.002±0.001	0.48±0.19^a	0.002±0.00
SS_2	0.006±0.002	0.001±0.0	0.139±0.16^b	0.0018±0.001	0.001±0.00	0.55±0.26^b	ND
S_1	0.004±0.002	0.001±0.0	0.130±0.17	0.002±0.001	0.002±0.001	0.55±0.21	0.002±0.0
S_2	0.001±0.00	ND	0.104±0.11	0.001±0.0	0.001±0.00	0.48±0.24	ND
L_1	ND	ND	0.037±0.008^a	ND	ND	0.33±0.07^a	ND
L_2	ND	ND	0.034±0.01^a	ND	ND	0.35±0.04^a	ND
L_3	0.003±0.001	ND	0.11±0.07^a	0.001±0.00	0.002±0.00	0.59±0.09^b	ND
L_4	ND	ND	0.034±0.10^a	ND	ND	0.43±0.10^b	ND
L_5	0.004±0.002	0.001±0.0	0.37±0.11^b	0.002±0.001	0.002±0.001	0.90±0.11^c	0.002±0.0

Mean values bearing different superscripts within the same column are significantly different ($P<0.05$; ND: Not detected below 0.001mg/kg); SS_1- Rainy season, SS_2- Dry season, S_1- S. *clarias*, S_2- T. *nilotica*; Lx- location

Table 3: Seasons×species, seasons×location and species×location interaction effects on the mean heavy metal concentrations (mg/kg) in fish

Factor		Cd	Cr	Cu	Ni	Pb	Zn	As
SS_1	S_1	0.0025±0.00	ND	0.11±0.12	0.002+0.00	0.003±0.001	0.51±0.17	0.002±0.0
	S_2	0.001±0.00	ND	0.08±0.11	ND	0.001±0.00	0.46±0.20	ND
SS_2	S_1	0.006±0.002	0.001±0.00	0.16±0.20	0.00±0.002	0.001±0.00	0.60±0.24	ND
	S_2	ND	ND	0.12±0.11	0.001±0.00	0.001±0.00	0.51±0.27	ND
SS_1	L_1	ND	ND	0.04±0.008^{a}	ND	ND	0.32±0.08^{a}	ND
	L_2	ND	ND	0.03±0.005^{a}	ND	ND	0.34±0.056^{a}	ND
	L_3	0.002±0.00	ND	0.07±0.01^{a}	0.001±0.0	0.002±0.00	0.54±0.11^{b}	ND
	L_4	ND	ND	0.029±0.01^{a}	ND	ND	0.44±0.06^{b}	ND
	L_5	0.002±0.001	ND	0.32±0.02^{b}	0.002±0.00	0.003±0.002	0.79±0.042^{c}	0.002±0.0
SS_2	L_1	ND	ND	0.04±0.009^{a}	ND	ND	0.35±0.07 ^{a}	ND
	L_2	ND	ND	0.04±0.005^{a}	ND	ND	0.36±0.021^{a}	ND
	L_3	0.004±0.00	ND	0.16±0.073^{b}	0.001±0.00	ND	0.65±0.025^{b}	ND
	L_4	ND	ND	0.04±0.009^{a}	ND	ND	0.42±0.125^{b}	ND
	L_5	0.007±0.00	0.001+0.0	0.42±0.14^{c}	0.003±0.002	0.001±0.00	1.00±0.04^{c}	ND
S_1	L_1	ND	ND	0.05±0.002^{a}	ND	0.005±0.002	0.41±0.015^{a}	ND
	L_2	ND	ND	0.034±0.004^{a}	ND	ND	0.39±0.009^{a}	ND
	L_3	0.003±0.001	ND	0.08±0.004^{a}	0.001±0.0	0.002±0.0	0.53±0.095^{b}	ND
	L_4	ND	ND	0.04±0.005^{a}	ND	ND	0.52±0.024^{b}	ND
	L_5	0.005±0.002	0.001±0.0	0.45±0.11^{b}	0.003±0.001	0.003±0.002	0.94±0.103^{c}	0.002±0.0
S_2	L_1	ND	ND	0.029±0.0^{a}	ND	ND	0.26±0.02 ^{a}	ND
	L_2	ND	ND	0.34±0.014^{b}	ND	ND	0.31±0.026^{a}	ND
	L_3	ND	ND	0.14±0.088^{a}	0.001±0.0	ND	0.66±0.015	ND
	L_4	ND	ND	0.025±0.006^{a}	ND	ND	0.34±0.045	ND
	L_5	0.001±0.0	ND	0.29±0.01^{b}	0.001±0.0	0.001±0.0	0.86±0.105^{c}	ND

Mean values bearing different superscripts within the same column for season×location and species×location are significantly different ($P<0.05$)

Human Exposure Risk Assessment

Table 5 presents the demographic factors and fish consumption rates of the examined community population residing at the Anambra River. The daily intakes of heavy metals through fish consumption among children, adolescent and adult are shown. There were significant difference ($P < 0.05$) between the mean age, weight and consumption rates of the population with the highest in adult population.

Concentrations of heavy metals observed in the measured fish species and their estimated daily intakes through consumptions among children, adolescent and adult population are shown in Table 6. Metal concentrations were below safe limits stipulated in standard monographs of International compendia [47-49]. Human exposure risk estimation based on the MOE was negligible (below 1) for the entire population category [Table 7]. No data exist on reference dose for chronic oral exposure for Cu, Ni and Pb and their MOEs were not calculated. Conversely, heavy metal exposure doses for the community population from consuming fish are also presented in Table 7. Children had the highest exposure dose (ED) ($P < 0.05$) followed by adolescent and adult population recording the lowest values [Figure 2]. Exposure doses were all below reference doses for chronic oral exposure given by U.S EPA- Integrated Risk Information System

DISCUSSION

The studies analytically assessed the heavy metal concentrations in two endemic fish species of Anambra major freshwater system and are extensively consumed by the local populace on different factorial levels and effect interactions. The anthropogenic activities upsetting the delicate balance of the river environment are pointer facts for current strict monitoring and necessity for the quantitative risk assessment of human-metal exposure through dietary consumption.

Factorial Effects on the Concentrations of Heavy Metals

Effect of fish species

The study showed varying concentrations of heavy metals measured in fish species of the Anambra River. Occurrence levels of the heavy metals in the fish were relatively low and Cu and Zn concentrated at significant amounts in both seasons and species from different locations. Several factors characteristically affect the biological availability and accumulation dynamics of contaminants in aquatic organisms including fish to reach various tissue and molecular targets. Physicochemical factors and water chemistry such as temperature, salinity, and pH enhance the distribution and bioavailability of the aquatic heavy metals. Water chemistry characteristics of the Anambra River reported in our earlier investigations [30,33] are of considerable standards for most ecotoxicity to aquatic organisms and possible diversity in bioaccumulation observed in this study. Water temperature affects metal uptake by poikilothermic animals and also is generally regarded as one of the most crucial environmental element influencing toxicity of chemical contaminants to aquatic organisms [50,51]. Karakoc [52] observed an increase in the uptake of Cu in the liver, gill and muscle tissues of *T. nilotica* at low salinities,

Table 4: Seasons×species×location interaction effects on the mean heavy metal concentration (mg/kg) in fish

Factor			Cd	Cr	Cu	Ni	Pb	Zn	As
SS_1	S_1	L_1	ND	ND	0.043±0.018^{a}	ND	ND	0.390±0.02^{a}	ND
		L_2	ND	ND	0.030±0.01^{a}	ND	ND	0.400±0.08^{a}	ND
		L_3	0.002±0.001	ND	0.076±0.01^{a}	0.001±0.00	0.002±0.001	0.430±0.02^{a}	ND
		L_4	ND	ND	0.038±0.02^{a}	ND	ND	0.492±0.05^{a}	ND
		L_5	0.003±0.001	ND	0.340±0.10^{b}	0.002±0.00	0.004±0.001	0.834±0.20^{b}	0.002±0.001
	S_2	L_1	ND	ND	0.028±0.02^{a}	ND	ND	0.240±0.18^{a}	ND
		L_2	ND	ND	0.020±0.01^{a}	ND	ND	0.289±0.19^{a}	ND
		L_3	ND	ND	0.054±0.01^{a}	ND	ND	0.640±0.28^{b}	ND
		L_4	ND	ND	0.019±0.03^{a}	ND	ND	0.380±0.21^{a}	ND
		L_5	0.001±0.00	ND	0.300±0.21^{b}	ND	0.001±0.00	0.750±0.24^{b}	ND
SS_2	S_1	L_1	ND	ND	0.047±0.02^{a}	ND	ND	0.420±0.06^{a}	ND
		L_2	ND	ND	0.038±0.01^{a}	ND	ND	0.381±0.02^{a}	ND
		L_3	0.004±0.001	ND	0.084±0.03^{a}	0.001	ND	0.620±0.13^{b}	ND
		L_4	ND	ND	0.048±0.04^{a}	ND	ND	0.540±0.07^{b}	ND
		L_5	0.007±0.003	0.001	0.560±0.03^{b}	0.004±0.001	0.001±0.001	1.040±0.87^{c}	ND
	S_2	L_1	ND	ND	0.029±0.01^{a}	ND	ND	0.280±0.03^{a}	ND
		L_2	ND	ND	0.048±0.03^{a}	ND	ND	0.340±0.05^{a}	ND
		L_3	ND	ND	0.230±0.09^{b}	0.001	ND	0.670±0.24^{b}	ND
		L_4	ND	ND	0.030±0.01^{a}	ND	ND	0.290±0.07^{a}	ND
		L_5	ND	ND	0.280±0.06^{b}	0.001±0.001	0.001±0.00	0.960±0.26^{c}	ND

Mean values bearing different superscripts within the same season and the same species along the columns are significantly different ($P<0.05$)

Table 5: Fish consumption rate among the measured population of the Anambra River

Demographic indices					Consumption rate (g/day)
Population	No of individuals	Age group (years)	Mean age (years)	Weight (kg)	
Children	93	5-10	7.35±1.69^{c}	22.46±2.37^{c}	151.89±1.37^{c}
Adolescent	86	11-19	15.50±2.25^{b}	51.08±6.49^{b}	167.61±2.12^{b}
Adult	94	20-60	44.46±14.09^{a}	58.74±0.96^{a}	191.54±5.43^{a}

Mean values with superscripts are significantly different ($P<0.05$)

Table 6: Concentrations and EDI of heavy metals (mg/day) among the human population at the Anambra River through fish consumption

Heavy metals	Concentration^{a}	Children	Adolescent	Adult
Cd	0.0025±0.002	3.797×10^{-4}	4.190×10^{-4}	4.789×10^{-4}
Cr	0.001±0.00	1.519×10^{-4}	1.676×10^{-4}	1.915×10^{-4}
Cu	0.117±0.0184	1.777×10^{-2}	1.961×10^{-2}	2.241×10^{-2}
Ni	0.0015±0.0007	2.278×10^{-4}	2.514×10^{-4}	2.873×10^{-4}
Pb	0.0015±0.0007	2.278×10^{-4}	2.514×10^{-4}	2.873×10^{-4}
Zn	0.515±0.0495	7.822×10^{-2}	8.632×10^{-2}	9.864×10^{-2}
As	0.002±0.00	3.038×10^{-4}	3.352×10^{-4}	3.831×10^{-4}

[a]Average of the two fish for both rainy and dry seasons, measured in mg/kg wet weight, EDI: Estimated daily intakes

since a decrease in salinity from 20% to 50% caused an increase in the metal uptake. Conversely, Karakoc and Dincer [53] recounted highest accumulation of Zn in kidney tissue at 15°C and 30°C for different concentrations, which was followed by gills and liver. In all tissues, Zn accumulation increased with increasing temperatures. Similar trend of linear temperature dependent chemical sensitivity and uptake rate of Cd has been documented in *Daphnia magna* [54]. The Anambra River has temperature of 26.22 ± 0.71 - 28.84 ± 1.38°C in earlier surveys [30,33] and could attest to the accumulation patterns between the fish species

Observable difference was recorded between the two fish species in the amounts of heavy metals accumulated. The disparity in the bioaccumulation of the two species could be an intrinsic factor characteristic to each species especially differences in the metabolic pathways necessary for heavy metal sequestration or elimination [55] and *S. clarias* seems to be a better predictor of heavy metal pollution in freshwater than *T. nilotica*. Species effect on bioaccumulation of heavy metals has been extensively reported in other studies [9,56]. Certain organisms possess physiological regulatory mechanisms necessary in detoxification of heavy metals. In our studies, based on this homeostatic mechanism, *T. nilotica* presumably have efficient metal removal process compared to *S. clarias*, alternatively, other factors and biological variables could modify the nature and magnitude of aquatic metal exposures and species accumulation differentiation such as age, length and body weight, reproductive status, seasonal changes, feeding habits and ecological lifestyles of the fish [55,57]. *S. clarias* in Nigerian freshwaters are euryphagus and bottom feeders, which depend on size of fish, season, water temperature, location, age and sex [57,58]. This particular fish can switch food to different items in different seasons ranging from phytoplankton, zooplankton to detritus and also depending on availability of the materials [59]. *T. nilotica* has feeding habit affected by several factors related to *S. clarias* such as age, sex and environmental factors. However, phytoplankton form major part of their diets [60] especially in dry season while low amount of detritus and sand particles in wet season. Following the feeding characters

Table 7: Concentrations of heavy metals, safe limits, MOE and ED for measured community population

Heavy metals	Concentration*	Standard	Children		Adolescent		Adult	
			MOE	ED	MOE	ED	MOE	ED
Cd	0.0025±0.002	0.05[a]	1.691×10^{-2}	1.014×10^{-10}	8.203×10^{-3}	4.922×10^{-11}	8.152×10^{-3}	4.891×10^{-11}
Cr	0.001±0.00	0.73[b]	2.254×10^{-3}	4.058×10^{-11}	1.094×10^{-3}	1.969×10^{-11}	1.087×10^{-3}	1.956×10^{-11}
Cu	0.117±0.0184	30[a]	-	4.747×10^{-9}	-	2.303×10^{-9}	-	2.289×10^{-9}
Ni	0.0015±0.0007	-	-	6.086×10^{-11}	-	2.953×10^{-11}	-	2.935×10^{-11}
Pb	0.0015±0.0007	0.5[a]	-	6.086×10^{-11}	-	2.953×10^{-11}	-	2.935×10^{-11}
Zn	0.515±0.0495	30[a]	1.161×10^{-2}	2.090×10^{-8}	5.633×10^{-3}	1.014×10^{-8}	5.598×10^{-3}	1.008×10^{-8}
As	0.002±0.00	1.3[c]	4.508×10^{-2}	8.115×10^{-11}	2.188×10^{-2}	3.938×10^{-11}	2.174×10^{-2}	3.913×10^{-11}

*Average of the two fish for both rainy and dry seasons, measured in mg/kg wet weight, [a]FAO (mg/kg) [47], [b]IAEA (mg/kg) [48], [c]NRCC (mg/kg) [49], ED: mg/kg/day, ED: Exposure dose, MOE: Margin of exposure, FAO: Food and Agricultural Organization, IAEA: International Atomic Energy Agency, NRCC: National Research Council of Canada

of the two fish species, *S. clarias* apparently would accumulate aquatic contaminants more than *T. nilotica* hence, the higher concentrations of heavy metals in *S. clarias*. Other studies have assented influence of feeding habit on bioaccumulation of trace metals in fish [14,61]. Biometric indices could play a crucial role in facilitating the bioaccumulation dynamics of the fish species [62-65]. Fish species had significant length difference with *S. clarias* being higher. Fish were selected and examined irrespective of age and gender and therefore could not be integrated empirically into the present studies for inferential conclusion. Researches considering those factors in the Anambra River are highly needed for more understanding of the heavy metal behavior in bio-systems and their linkages with the biometrics. In addition, the concentrations of metals detected in our study are below those earlier documented [12,13,15,16].

Season effect

Season is another major factor that affects the concentration of heavy metals in freshwater ecosystem and invariably accumulation in fish. Emoyan *et al*. [66] have reported variability of heavy metal concentrations with season in Nigerian freshwater. It could be that as water level drops, the concentration of heavy metals in the ecosystem increases, hence higher level of heavy metals were observed in the fish during dry season. However, it could also be attributed to the reduction in capacity of the freshwater to naturally filter the increased influx of fresh inland waters from the adjoining water bodies such as Rivers Oyi, Ezu and other tributaries, which drain the neighboring locations and discharge its contaminants into the Anambra River, making them available to the aquatic biota. Malik *et al*. [9] obtained highest concentrations of metals in summer and the lowest in monsoon season, which they attributed to dilution effect of the receiving medium. Similar studies on date differences in heavy metal bioaccumulations were made on Cu and Cd concentrations in fish [67]. They found higher metal concentrations in the fish in summer than autumn and probabilistically referred it to respiratory rate changes and feeding ecology (on plant and grasses) as these habits tend to be higher during the summer. Higher bioavailability of heavy metals were demonstrated in sediment of the Monjolinho River, Brazil during the dry season period, effect authors theoretically linked to lower dilution of pollutants [68]. Analogous report was made by Jain and Sharma [69] while working on the distribution of trace metals in the Hindon River system, India. Season metal concentration differential observations in water column have also been confirmed in the Anambra River [30,33].

Location effect

Onono (location 5) consistently gave the highest concentration of pollutants in fish followed by Otuocha (location 3). It could stem from the burgeoning population, industrial, marketing, and agricultural activities surrounding the areas unlike Enugu Otu and Ezi-Aguleri locations. It is remarkable that Onono is close to Onitsha metropolis and mouth of River Oyi and Niger, which enlarge the pollution level of the Anambra River. Contaminant concentration at a particular location appears to be specifically influenced by proximity to the pollution and dilution effect of the receiving medium.

The various anthropogenic activities mentioned have been shown to increase the heavy metal loading of aquatic environment [66,70-73]. The detection of cadmium in the fish, although not significant, could be ascribed to rural/urban effluents along the river course and atmospheric precipitation [74]. Minute concentrations of Cr and As were observed and significant Cu obtained in tested animals from the study area. Probable explanation to the accumulations could be related to dumping of wood treated with chemicals made from salt of As, Cr and Cu in mixed soluble preparation (as copper-chrome-arsenate preservative). These chemicals are being used to prevent fungi and pest attack, and could provide a potential source of chemical spills and drainage from the treated wood within and around the processing plant [75]. Additional input of Cd could be via agrochemicals used by farmers and that it occurs together with Zn, Pb and Cu [76,77]. Chromium, As, and Cd are toxic metals and have no biological essentialities to human and animals and are reportedly carcinogenic and mutagenic. Copper is an important component of certain enzymes and crucial for synthesis of hemoglobin [78]. However the necessity of Cu, high intake is biologically damaging. The low levels of Pb and Ni in fish sourced from the study area, is an indicator to the natural distribution of the elements in surface water due to weathering of minerals and atmospheric deposition [74,79]. The concentrations could also be accelerated by industrial and other technical uses such as chemical pigments and alloy production, and burning of fossil fuel. These activities have been demonstrated to cause such elemental increase in aquatic environment [80,81]. The significant Zn levels obtained from the study area could stem from high incidence of iron (Fe) in Nigerian soil. Zinc occurs in nature with other metals of which Fe and Cd is the most common [82]. It is notable that most of these heavy metals could be made available in the freshwater system not only through industrial and domestic effluents but also through dumping of refuse [83].

Heavy Metal: Human Exposure Risk Assessment

Comparison with international standards

The concentrations of the heavy metals detected in current eco investigation were low compared to stipulated international standards [47-49]. It appears no uniform limits exist for most heavy metals in fish and there is no identical limit for most heavy metals except mercury [12]. Our studies compared with standards compiled from different international guidelines and which had been long revised considering the level of anthropogenic activities in recent times and emerging contaminants. Ingestions of heavy metals at low concentrations, however, are potential threat to public health in a long exposure. The fleshy part and muscle of fish is chiefly consumed by the population and muscle is not an active tissue of metal accumulation and biotransformation [15]. However, heavy metals present in fish can pose a health risk to the inhabiting fish of the river, to their predators and to human population dependent on them for food [12].

MOE and fish consumption

MOE can be used to prioritize diverse contaminants, providing that a regular approach has been adopted. Its acceptability depends on its magnitude and is ultimately a risk management decision [84]. Margin of exposure data derived in this work for the heavy metal residues were below the reference standard of systemic toxicity for individual and/or population and showed that the metals in fish species do not pose a risk to frequent consumers. However, no data were obtained for Cu, Ni and Pb as the RfD values were not available because no evidence of threshold below which a non-harmful intake could be allowed [12]. Generally, "RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" [42-44]. The values are mostly dependent on toxicological studies and outcomes of various metal residues. The reference dose for Pb is not available as it was considered inappropriate to develop since the degree of uncertainty about health effects of Pb is quite low when compared to most other environmental toxicants [85]. Animal model has demonstrated carcinogenicity of Ni [86].

Our current discoveries showed MOEs for heavy metals to be lower than values made earlier in freshwater fish risk survey at the Guaiba Lake, Southern Brazil [13]. The observable difference could be linked to dissimilarity in MOE model indices. While the authors quantitatively estimated the risk of heavy metal exposure through fish consumption, adopting the theoretical model used by Watanabe *et al.* [40], our studies empirically incorporated field validated data in quantifying the real risk of the aquatic contaminants. Other factors such as pollution level of the freshwater and demography could impact the inferential outcomes of the quantitative contaminant-exposure marker. Correspondingly, the MOE values were also lower than those documented in most fish species of lower Mississippi River, USA [40]. The model used is pointedly relevant for constructing a reliable framework of assessing consumer exposure. Interestingly, to translate our findings from the model-specific scenario into wide spectrum of exposure situations that is found in the real world, reliable data on different fish species, other aquatic organisms and food groups need to be established. We only attempted the determination of specific fish species consumption rate and body weights of the population living around the Anambra River for proper risk quantification of feeding on potential toxic chemicals through that particular foodstuff. Highest body weight of the population categories was for adult with 58.74 kg, which was lower than 70 kg theorized by Watanabe *et al*. [40]. Consequently, consumption rate of fish differs among individuals and likely depends on age and weight and these could explain the difference in two interrelated studies.

Toxicity from feeding on contaminated fish would depend on the quantity consumed and other factors such as physicochemical characteristics of the metals, ingestion rate, weight and physiological activities of individuals necessary for bioaccumulation and biotransformation of the metals ingested. The quantitative risk assessment study was piloted in dry season, a period when peak fishing is anticipated to be low compared to rainy season of high water. This leads to the possibility that fish intake rates calculated for the population categories may devalue individuals' habitual fish consumption [39]. Conversely, principal occupation among the poor population at the study location is fishing and they probably consume more fish and other freshwater animals following the field tours and consumption analysis. Differences in fish consumption as a function of socioeconomic factors and potential risk to public health have been recorded in developed countries [40,63]. Accounting for weight of the human population categories in risk estimation, highest value at Anambra River was lower than the one used in the MOE calculation [40] and if weight could play a crucial role in metal accumulation and toxicity, basis on the existential difference in the model parametric values (weight), we can presume that lower concentrations of the heavy metals studied could easily elicit biological toxicity to the fish consumers in the study area. However, concentrations of metals observed and as deduced by the MOE may not pose immediate danger to the human population at the Anambra River feeding on the fish diet contaminated with it; but we cannot rule out probable eventualities resulting from long-time exposure of low acting concentrations. Community health effects of feeding on Zn and other heavy metal contaminated fish have been earlier shown by Ogwuegbu *et al*. [87] and Fosmire [88].

Exposure dose and daily fish consumption

Exposure and daily intake of heavy metals was estimated on the basis of the concentrations measured in fish muscle and daily fish consumption rate among the population categories studied as consumption of fish is a conceivable source of metal accumulation in humans [15].

We provided the exposure doses for all the metal residues studied and they were below the reference doses for chronic oral exposure of the specific heavy metals [41-44]. Assessment of the public health risk from consumption of contaminated food requires statistics on the quantities of contaminated foodstuffs consumed and the extent of contamination present in foodstuffs [34]. Our work only focused on the prevalent fish (*S. clarias* and *T. nilotica*) dietary ingestions among the resident population and attempted direct measurements of metal concentrations as fish appeared to be a significant pathway for human exposure along the food chain. Moreover, the ED estimation did not consider the relatively complex physiological and chemical processes that occur once a substance enters the body, rather followed laid-down standard procedural steps [34]. Our consumption rate values were reported as uncooked fish weight. Contaminant concentrations in fish are generally measured and reported in the uncooked samples [39]. Supposing that cooking results in some reductions in weight such as through loss of moisture, and the mass of the contaminant in the fish tissue remains constant, then the contaminant concentration in the cooked fish tissue will increase. Although actual consumption may be overestimated when intake is expressed in an uncooked basis, the net effect on the dose may be cancelled out since the actual concentration could be underestimated when it is based on the uncooked sample [39]. The variance in current data of ED and standard RfD presumably resulted from these alterations.

Children recorded the highest level of ED followed by adolescent with adult category recording the lowest. In dermal exposure to contaminants, children, adolescent and adults are expected to have different exposure frequency and duration. Children and younger adolescent would have an increased exposure frequency because they tend to retain soil on their skin after coming indoors [34,39]. Adults would have a decreased exposure frequency because they tend to have less time to be exposed to outdoor soil [39]. The hypothesis that children had the highest exposure doses for the heavy metals through ingestions in current study could be explained by age, and body weight factor in the ED risk equation.

CONCLUSION

Aquatic toxicological studies are well-maintained on fish species, underlining the budding inclusion of fish model in ecotoxicology and human hazard assessment and identification. Season, species, and location are key factors that determine the concentration and distribution of heavy metals in freshwater ecosystems. Of all the heavy metals analyzed, Cu and Zn were observed to be high and significant, indicating elevated source input into the water environment than other elements. High concentrations of Zn and Cu in some locations (Onono with the highest amounts) are indication of location proximity to sources of the metals and as such, diffused contamination of the inhabiting biota. Estimated MOEs and exposure doses for the metal residues were below reference standards and tentatively pose no threat to the consumers. Nonetheless, the detection of metals in the resident biota of the river is a clear evidence of contamination and need for proactive management measures.

Public health and quantitative risk assessments through interaction with the surroundings are essential in forestalling environmentally-mediated epidemics. Supplementary wide-ranging assessment of human exposure to metals through aggregation of diverse sources and pathways using non-target screening approaches, and reflection on the complexity of freshwater environment and its dynamic interactions are basic stepladders in management and risk valuation of this fragile ecosystem.

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Source of Support: Nil, Conflict of Interest: None declared.