World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(Research Article)

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Heavy metals analysis of *S. schall* and *O. niloticus* fish species sold at Otuocha Market, Anambra State, Nigeria

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World Journal of Advanced Research and Reviews, 2023, 19(02), 1536–1543

Publication history: Received on 12 July 2023; revised on 27 August 2023; accepted on 29 August 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.19.2.1695

Abstract

The potentials of heavy metal pollution in natural environments have necessitated the need to determine the presence of heavy metal pollutants in the surface waters and possible bioaccumulation in the inhabiting fish species and other aquatic organisms. This study thus aimed to determine the heavy metal species that occur outside their recommended ranges in the test fish species and two Cichlid fish species namely, *Synodontis schall* and *Oreochromis niloticus* were selected for the analysis. Water samples were taken to assess the concentrations of these heavy metals in the river while tissues and organs of the test fish samples were extracted for further analysis to determine the cumulative tendencies of these metals in the organisms. All the heavy metals (Cd, Zn, Mn, Cu, Pb) were detected in the fish organs except copper (Cu) which was not detected in the fillet/tissue. The metals occurred in the order; Pb > Zn > Cd > Cu > Mn in both fish species. The gill emerged as the organ with the highest concentration of heavy metal with a value of 100.92ppm of lead in *Synodontis schall* and 111.34 ppm in *Oreochromis niloticus*. The high concentrations of Zinc and Lead found in the fish organs were compared with the habitat and discovered that the concentrations were high compared to the lower levels found in the river water. Although Zn occurred at very high concentrations, they were still below the threshold recommended by WHO/FAO. However, Cadmium and Lead exceeded their permissible limits, Lead occurred in outrageous amounts while other metals tested (Mn, Zn, Cu) fell within acceptable ranges. Nonetheless, care should be taken and environmental laws and policies guiding the use and discharge of harmful substances put in place to check the influx and accumulation of these toxicants which pose as threat to man. Metal pollution results in abnormal physiological function of the body cells when they exceed these limits and so guidelines on the use of related products and industrial discharge of effluents should be adhered to and penalty measures taken out on defaulters.

Keywords: Aquatic pollution; Metal pollution; Heavy metals; *Synodontis schall; Oreochromis niloticus*

1. Introduction

The current trend in agricultural and industrial revolution is exposing almost all the water sources to contamination (Khare and Singh, 2002). Industrial effluents containing toxic and hazardous substances such as heavy metals (Gbem et al., 2001; Woodling et al., 2001) impact the aquatic ecosystem tremendously resulting to the pollution. For instance, the bioaccumulation of metals in body tissues and organs reflect the amount absorbed by impacted organisms, their distribution in the different tissues and the extent to which the metal is retained which is also determined by the organ or tissue type as well as species type.

Accumulation of zinc has attained a serious dimension causing health hazards as bad as Alzheimer's disease. The danger of toxic and trace metals in the environment is more serious than those of other pollutants due to their non bio-

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degradable nature, accumulative properties and long biological half lives. It is difficult to get rid of them completely from the environment once they enter (Aderinola et al., 2009). Heavy metal contamination may be associated with destructive impacts on the ecological balance of aquatic systems including the loss of biodiversity (Vosyliene and Jankaite, 2006; Farombi et al., 2007; Hayet and Javed, 2008).

Heavy metals tend to be more mobile in the soil where they can be easily taken up by plant via the roots and utilized for body building (Wuana and Okieimen, 2011) likewise, in the water, the metal uptake is dependent on the chemical form which influences its availability, mobility and toxicity (Shiowatana et al., 2001; Beukers, 2007 In: Okogbue, 2021). There could be dissociation and regrouping of metals to form complexes which may be predominantly toxic or less. The aquatic organisms absorb these nutrients and utilize them in growth and development processes thereby integrating them into their body tissues and organs (Okogbue, 2021). When the wrong metal cations are abundant in the environment, they cause heavy metal pollution which will be available for the organisms to uptake and utilize in response to growth. Generally, metal nutrients are required for body building and growth in both animals and plants however, when they occur in outrageous amounts, they become harmful to the living system. On the other hand, some of these metals do not have any beneficial use in the living organisms and so pose great health risks and hazards as they get biomagnified up the food chain. Examples are mercury, lead, silver and cadmium.

The aquatic life such as fish and the water itself remains the most impacted by any form of water-based pollution. Fish and other aquatic organisms give rise to products of great economic importance all over the world. Fish is one of the most important animal protein sources available in the tropics and has been generally accepted as a good source of protein and other similar elements for the maintenance of healthy body (Al-jufalli and Opera, 2006). In 2010- 2012 according to FAO (2014), a third or more of the population of Africans suffered from malnutrition and fish generally have been established to be good source of vitamins and minerals; it supplies the body with fluorine and iodine which are essential for the development of strong teeth and the prevention of goitre in man (Andrew, 2007). Fish is also rich in selenium and other vitamin based antioxidants that protect the body cells against damage (Okogbue and Ibegbulem, 2020). Hence the need to study and proffer solutions on pollution prevention to avoid deleterious effects on the aquatic community.

This study therefore tends to appraise some health hazards of consuming some fresh fish species harvested from Omambala River which are usually sold at Otuocha market in Anambra State of Nigeria. The potentials of heavy metal pollution in the river has necessitated the need to determine the presence of heavy metal pollutants in the River and possible bioaccumulation in the inhabiting fish species. Thus, two Cichlid fish species namely, *Synodontis schall* and *Oreochromis niloticus* were selected for the analysis. The study also tends to determine the heavy metal species that occur outside their recommended ranges in the selected fishes species.

2. Material and methods

2.1. Data collection

Fresh fish samples were randomly collected from a fish landing site located in the Otuocha community forth nightly (every 2-weeks interval) and taken to the Spring Board Laboratory, Udoka Estate in Awka, Anambra State for analysis.

The fish samples were identified to species level using standard keys with taxonomic descriptions and indices by Sikoki and Francis (2007). The local names were obtained from the local artisanal fishermen at the landing site which helped to identify their common names and eventually the scientific names using the keys.

2.2. Water Analysis for Heavy Metals

Water samples from the surface water were collected using a Ruttner water sampler. 5ml of concentrated Hydrochloric acid (HCL) was added to 250ml of the water sample and evaporated to 25ml. The concentrate was transferred to a 50ml flask and diluted to mark with distilled water. Metal contents were determined using 304 u/c Atomic Absorption Spectrophotometer (AAS) model VarianAA240 to determine the presence of heavy metals.

2.3. Fish tissues analysis for Heavy Metals

Metals concentration in the fish sample was determined in a laboratory using the Atomic Absorption Spectrophotometer (AAS). The wet digestion method was used and the procedure involved grinding a portion of the fresh fish sample and weighing out 5g into Teflon crucible digestion flask. 20ml of freshly prepared concentrated acid mixture comprising of 650 ml Conc. HNO_3, 80 ml Perchloric acid and 20 ml Conc. H_2SO_4 was added to the sample in the Teflon crucibles. The crucibles were covered with watch glass for initial reaction to subside. The samples in the crucibles were heated in the oven at a temperature not exceeding 150 °C for 2hours until the solution became clear and completely digested. The samples were cooled and 10 ml of distilled water was added to each of the samples. The samples were filtered using Whatmans filter paper of 0.56μm into 250 ml volumetric flask. The samples were made up to 250 ml with distilled water and used for heavy metal analysis using Varian AA240 Atomic Absorption Spectrophotometer (AAS). Presence of metals deposits were then analyzed and recorded.

2.4. Data Analysis

Data were analyzed statistically and presented graphically in the following tables.

3. Results and discussion

The water samples were analyzed for cadmium, zinc, manganese, copper and lead. The results are presented in Table 1 below.

Table 1 Heavy metals in water samples from River Omambala

Heavy metal	First sampling	Second sampling	Third sampling	Fourth sampling	Fifth sampling	Pooled mean±SD	LSD ($p<0.05$)	E.U. STANDARD
WATER SAMPLE								
Cadmium (Cd) ppm	0.02^{b}	0.03^{a}	0.02^{b}	0.02^{b}	0.001^{c}	0.02±0.01	0.00078	2.0
Zinc (Zn) ppm	4.62^{b}	4.58^{d}	4.60^{c}	4.70^{a}	4.54^{e}	4.61±0.06	0.00055	10-75
Manganese (Mn) ppm	ND	ND	ND	ND	ND	ND	-	-
Copper (Cu) ppm	0.05^{b}	0.08^{a}	0.05^{b}	0.04^{c}	0.04^{c}	0.05±0.02	0.00077	1-3
Lead (Pb) ppm	0.90^{b}	1.0^{a}	0.90^{b}	0.80^{c}	1.0^{a}	0.92 ±0.08	0.00449	0.2

Mean values with different superscript letters within the same row are significantly different ($p<0.05$), LSD- Least Significant Difference, ND- Not Detected.

Lead (Pb) exceeded the permissible limit by E. U. standard. Other metals fell within the tolerable ranges (Table 1). Manganese was not detected in the river water environment though it is present in the fish tissues (Table 4) but at permissible limits. From Tables 2 and 3, the concentrations of these metals in the fish body exceeded what was detected in the river water. The high concentrations of Zinc and Lead found in the fish organs compared to the lower level found in water (Table 1) may be attributed to the fact that aquatic organisms such as fin-fish and shell-fish accumulate metals to concentrations many times higher than present in water. The study is consistent with the findings of Khare and Singh, (2002) who stated that fish can take up different metals and then stores them in their various body organs and tissues. Cadmium, zinc and lead were above the FEPA (2013) prescribed limits in some fish organs of the two fish species. Continuous consumption of these heavy metal contaminated fishes could pose serious health risks.

The fish species used for this study were *Synodontis schall* and *Oreochromis niloticus*. The results of the heavy metals analysis in the fresh fish harvested from River Omambala are presented in the following tables.

Table 2 presents the heavy metals concentration in different organs of one of the test fish species, *Synodontis schall*. All the heavy metals tested were found in the gills and intestine of the fish while the liver and the tissue showed variance in the occurrence of the heavy metals. Cadmium and lead were not detected in the liver while Manganese and Copper were not detected in the tissue of the fish but were seen in the liver. The highest concentration of zinc (84.82ppm) and cadmium (4.30ppm) were found in the intestine. The highest concentration of manganese (0.016ppm) was found in the gill but it was not detected in the tissue. Copper had the highest concentration value of 1.460ppm in the liver. Conversely, lead had the maximum mean concentration of 111.34ppm in the gill but was not detected in the liver. The analysis of variance showed that there was significant difference ($p<0.05$) in the various heavy metal mean concentrations obtained.

Table 2 Heavy metals in fresh *Synodontis schall* from River Omambala

Heavy metal	Cadmium ppm	Zinc ppm	Manganese ppm	Copper ppm	Lead ppm	Pooled mean±SD
Synodontis schall						
Gill	1.29[ab]	51.30[c]	0.016[a]	0.042[a]	111.34[a]	32.80±49.12[a]
Liver	ND	0.17[d]	0.004[c]	1.460[a]	ND	0.54±49.12[a]
Intestine	4.30[a]	84.82[a]	0.014[b]	0.892[a]	22.84[bc]	22.5±41.58[a]
Fillet/Tissue	1.20[ab]	78.76[b]	ND	ND	44.22[b]	24.80±33.62[ab]
LSD	1.0953	5.0571	0.00374	0.6649[a]	14.626	

Mean values with different superscripts within the same row are significantly different ($p<0.05$), ND - Not Detected, LSD – Least Significant Difference.

In Table 3, the heavy metals in fresh *Oreochromis niloticus* from Omambala River are presented. All the heavy metals (Cd, Zn, Mn, Cu, Pb) were detected in the fish organs except copper (Cu) which was not detected in the fillet/tissue. The organ with the highest concentration of heavy metal (lead) was the gill with a value of 100.92ppm. Cadmium had a highest mean concentration of 4.02ppm in the tissue and lowest concentration of 0.00 ppm in the liver. Also zinc had a high mean concentration of 79.14ppm in the intestine and low concentration of 0.17ppm in the liver. In the analysis, manganese had a low concentration in all the fish organs with a highest value of 0.016ppm found in the intestine and least values of 0.002ppm detected in the liver and tissue. In summary, gill had the highest pooled mean concentration (32.50±45.39ppm) followed by the intestine (25.50±35.36ppm) while the liver had the least pooled concentration of 0.42±0.58ppm.

Table 3 Heavy metals in fresh *Oreochromis niloticus* from River Omambala

Heavy metal	Cadmium ppm	Zinc ppm	Manganese ppm	Copper ppm	Lead Ppm	Pooled mean±SD
Oreochromis niloticus						
Gill	3.94[a]	57.64[c]	0.010[ab]	0.002[b]	100.92[a]	32.50±45.39[a]
Liver	0.0	0.17[d]	0.002[b]	1.084[a]	0.0	0.42±0.58[a]
Intestine	3.86[a]	79.14[a]	0.016[a]	0.042[b]	44.44[b]	25.50±35.36[a]
Filler/Tissue	4.02[a]	68.66[b]	0.002[b]	ND	28.96[c]	25.41±31.55[a]
LSD	0.9183	2.6964	0.0034	0.06385	21.2654	

Mean values with different superscripts within the same row are significantly different ($p<0.05$),ND-Not detected, LSD – Least Significant Difference.

From the result, the intestine presented to be an organ that is highly bioaccumulative and retains metals in its tissues when compared with the other organs analyzed. The Intestine recorded the highest amount of Cadmium, Zinc and also Copper alongside in the Liver. Manganese and Lead were also high in the intestine as was detected during the analysis. The intestine impacted by toxicants has been reported to respond to environmental stressors being an osmo-regulatory organ in diverse ways such as osmo-regulatory disruptions, bioaccumulation of toxicants, gastrointestinal disruptions, etc. Metals accumulate in intestines via absorption along gut tract walls to higher levels than background concentrations.

The gill recorded the highest concentration of lead (Pb) among all the organs tested. A number of pollution tests have qualified the gill as a good aquatic pollution parameter. Apart from bioacculutative capacity as seen in this study, the gill is used for both lethal and sub-lethal tests analysis. From histological tests to respiratory distress assessments. The report showed that the gill bioacumulated more metals followed by the intestine while the liver recorded the lowest bioccumulative capacity among the four organs tested. The gill as a site for respiration and osmo-regulation is in constant contact with water as well as toxicants in the water bodies, thus making it a good parameter for toxicity monitoring. The active transport at the gill site which involves special salt absorbing cells in the gill epithelium and ventilator movement of water expose the gill to toxicants as ion exchange take place here leaving the susceptible and delicate gill with stress when exposed to environmental stressors like heavy metal salts. Thus, bioaccumulation of these metals is possible as seen in this work. For example, Copper forms complex salts with anions making it more toxic and also clogs gills in fish and any amount above 3 ppm has been discovered to be toxic to aquatic life (Odiete, 1999).

The liver accumulated the highest amount of Copper in both fish species tested while Cadmium and Lead were not detected in the liver of both species as well. The liver is a site for detoxification and so is faced with high incidence of toxic effects with constant cleaning and high cleansing capacity to maintain the sanctity of the hepatic system. This also makes it sensitive to water pollution and a good parameter for pollution assessment. This effect on the long run could lead to hepatoxicity which means, intoxication of the liver and could also involve bioaccumulation of persistent toxicants in the organ causing injury or damage to the liver as well as oxidative stress (Jaeschke et al., 2002). Again, the liver not detecting lead Pb could be as a result of constant excretion of Pb in the bile. Also, excess lead is unabsorbed in the intestine and so expelled in the feaces (Odiete, 1999).

The use of the skin and body tissues in histological diagnosis and environmental pollution monitoring has been established since one of the major routes of pollution is through absorption via skin surfaces exposing the organism directly to toxicants (Vogt, 1987 In: Okogbue, 2021). Zinc, Zn was high in the fish tissue as well as Lead. Pb. However, Manganese, Mn and Copper, Cu were not detected in the tissues.

Cumulative tendencies in fish depend a lot on the concentration of the heavy metal in the surrounding water and sediments as well as the feeding habits of the organism (Eneji *et al.*, 2011 In: Kelle *et al.,* 2018). The authors also stated that other factors such as age of fish, the tissue lipid content and mode of feeding also affect the accumulation of heavy metals in fish. Thus, fish species are seen as good accumulator of organic and inorganic pollutants (King, and Jonathan, 2003) and so this study buttresses this assertion by other authors having seen high levels of metals in the various body organs tested.

The ratio of pollution value to background value indicates the pollution index (Shifaw, 2018). Hence, bioaccumulation of metals and other toxicants occur when the ratio in the organism is higher than it is in the background as could be seen in this study. Fish concentrate these pollutants from their environment (water) and store them in their fatty tissues (where they cause harm e.g. organ damage) especially, for the lipid soluble toxicants such as organochlorides and heavy metals.

Generally, when the concentration of a metal such as Pb in the aquatic environment is higher, and exceeds the capacity of fish body to discharge it, parallel accumulation of Pb in the fish body (gills, liver, muscle) will be the outcome (Huang *et al*., 2022).This is toxicokinetics which involves the biotransformation and distribution processes and pathways of toxic substances that influence elimination or bioaccumulation. Some get biotransforemed to more toxic or less toxic substances, some get excreted out of the system or easily subjected to depuration while some get absorbed into the fatty tissues of the organisms because they are oleophilic in nature and thus lead to bioaccumulation.

Looking at the permissible limit of these heavy metals in foods and fish flesh (Table 4), metal pollution result in abnormal physiological function of the body cells (Onuoha, 2009) when they exceed these limits, this is used to compare the results obtained in this work.

Table 4 Heavy metal concentrations in fresh Cichlid fish species from River Omambala and the FAO/WHO Permissible Limit

HEAVY METAL	RANGE OCCURED IN SAMPLE ORGANS (ppm)		FAO/WHO Permissible limits (Eneji *et al.*, 2018)
	Synodontis schall	*Oreochromis niloticus*	
Cadmium (Cd)	1.0953 - 4.30	0.0 - 4.02	0.05
Zinc (Zn	0.17 - 84.82	0.17 - 68.66	150
Manganese	0.004 - 0.016	0.002 -0.016	0.2 - 0.4
Copper	0.0042 - 1.460	0.002 - 1.084	10
Lead	14.626 - 111.34	0.0 - 100.92	0.5

Lead biomagnifies in the food chain and thus accumulates in individual organism. It is highly toxic to freshwater organisms and is considered safe when it occurs at levels less than 300 ppm in garden soil (Raskin and Ensley, 2000) because, it taken up by plants via the soil. Lead causes adverse haematological changes and cellular alterations which can result to mortality. In this study, lead was the highest accumulated metal in the tested fish species, *O. niloticus* and *Synodontis sp*. and these high concentrations were seen in the gills where they exceeded the permissible limit making it potentially harmful for consumption.

Cadmium and Lead were 0.0 ppm in the liver while Copper was not detected in the tissue as was also the case in *S. schall.* Cadmium is one of the 'big three' heavy metal poisons that do not have any known biological function. The toxicity increases as pH decreases (Campbell, 2006) and is known for its persistence in the environment and also remains resident in organism for many years when absorbed (Adebanjo, n.d.). A case of its bio-accumulative capacity which is swift but slow to depurate has been reported by Okocha and Adedeji (2005). The presence of this metal toxicant in body organs leads to a host of histopathological and health degenerative conditions in impacted organisms (Dangre *et al*, 2010). Cd bioaccumulates in organs such as the liver and kidney and causes disfunctions and can bioaccumulate up to 200 mg/kg in the environment (Odiete, 1999). Zinc is toxic at relatively low concentrations to so many aquatic life (Wuana and Okieimen, 2011). Some fish accumulate zinc in their bodies when they live in polluted aquatic bodies which biomagnify up the food chain and excesses in the medium may increase the acidity of the medium and retard breakdown of organic matter (Greany, 2005).

Copper is the third most used metal in the world according to VCI (2011). Copper has been reported by Martinez and Motto (2000) not to biomagnify in the food chain or bioaccumulate in the body but rather can cause anemia, organ damage such as in liver and kidney, etc. (Wuana and Okieimen, 2011). This may not be completely true because, the impact on organ leading to damage could be due to its persistence however, copper is observed to fall below the permissible limit in both species in this paper (Table 4)

4. Conclusion

From the findings of this study, it can be inferred that the presence of heavy metals in the fish samples is largely attributed to contamination of water bodies which is the immediate environment of the fish species. This concentration observed in fish organs are within the permissible limits except for Cadmium and Lead. The concentrations of these heavy metals tested were higher in the fish samples than in the surrounding waters. It was noted that the metals that exceeded their limits which are lead and cadmium were the ones bioaccumulated by these fish species to outrageous amounts. This summarizes the fact that these toxicants should be kept at levels within permissible limits to avoid bioaccumulation by aquatic organisms which forms part of man's diet and thus expose him to health hazards.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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