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Evaluation of heavy and trace metals in the fish species *Oreochromis niloticus* and *Alestes longipinnis* from Alaro Stream in Ibadan, Nigeria

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Abstract
An evaluation of the heavy and trace metals in the fish organs of *Oreochromis niloticus* and *Alestes longipinnis* from a tropical Alaro Stream in Ibadan, Nigeria was carried out. The aim of the study was to evaluate the concentration of heavy and trace metals in the organs of the fish species and to compare them with World Health Organization standards for human safety and exposure through consumption. Cast nets with mesh sizes ranging between 30-50mm of varying dimensional sizes were used for trapping the two fish species. Fish dissections were carried out using dissecting set to remove the gills, gut, bone, liver, fins and muscle which were oven dried at 105^{0}C for 6hours.Pulverized tissues and organs were acid-digested for heavy and trace metal analyses using the inductively coupled plasma-mass spectrometer (ICP-MS). The results showed that the mean concentration of heavy and trace metals were comparatively higher in *Oreochromis niloticus*: Mn(5612.7ppm,liver) and As (0.865ppm, muscle), while Na (15278ppm, liver), Mg (10245ppm, liver), K (30128ppm, liver), Ca (247186ppm, bones), V (13.82ppm, liver), Fe (10959.3ppm, liver), Co (8.56ppm, liver), Ni (5.82ppm, liver), Cu (175.78ppm, liver), Zn (274.6ppm, liver), Se (11.80ppm, liver), Mo (1.72ppm, liver), Ag (2.33ppm, liver), Cd (2.87 ppm, bone) and Pb (14.12ppm, gills) were higher in *Alestes longipinnis.* As, Ag, Cd and Pb exceeded the recommended limits set by the World Health Organization for most of the fish organs in *Oreochromis niloticus* and *Alestes longipinnis.* These results show that these fish species caught from Alaro stream which is polluted by industrial pollutants are not safe for human consumption.

1. Introduction

Bioconcentration refers to the uptake and accumulation of pollutants like trace metals in the organs or tissues of organisms [1]. In general, organisms have the ability to excrete or pass out any unwanted substances, while some may become inadvertently retained in their body systems as a result of physiological activities [1].Although animal protein remains the surest way of supply of a vast array of all the needed amino acids required for proper tissue formation, growth and repair, they also serve as route for the uptake of harmful heavy and trace metals from the food chain [2]. Trace metals are found naturally at various levels in the hydrosphere, and many are required for physiological and metabolic processes of organisms. These metals are also referred to as microminerals and are part of enzymes, hormones, and cells in the body. Insufficient intake of some required trace metals can cause symptoms of nutritional deficiency. Due to industrialization, the number of factories and human population has increased rapidly. The contamination of freshwaters with a wide range of pollutants has become a matter of concern over the last few decades [3-7]. Consumption of contaminated fish with some heavy and trace metals can result into hazardous effects on human health. Various pathways of metal accumulation in fish include ingestion of food, suspendedparticulate matter, metal ion exchange through gills and skin [8, 9]. Concentration of heavy metals in different tissues or organs of fishes is directly influenced by contamination in aquatic environment, uptake, regulation and elimination from the fish body [10]. Theoretically, the gills and liver accumulate trace metals in higher concentration than the muscles which exhibit the lowest levels of metal concentration. The liver in turn accumulates higher concentration of metals when compared to the other organs due to its detoxification role and has been used widely to investigate the process of bioconcentration. The gills also play a vital role in excretion of trace metal ions from the fish body [11]. Metallothioneins (MTs) are the metal-binding proteins accumulated in liver, whereas Romeo and colleagues [12] and Yilmaz [13] assert that metal accumulation in the muscles of fish is dangerous as they are the most edible part. *Oreochromis niloticus* (Linnaeus, 1758) and *Alestes longipinnis* (Günter, 1864) are two fish species frequently caught for consumption from Alaro stream in Ibadan. This stream is polluted by industrial wastes with potential heavy and trace metal sources that need to be analyzed and studied. *O.niloticus* has distinctive, regular vertical stripes extending as far down the body as the bottom edge of the caudal fin, with variable colorations. Adults reach up to 60 cm (24 in) in length and up to 4.3 kg (9.5 lb). It lives for up to 9 years and is native to Africa. It feeds mostly during the daytime hours. *A. longipinnis* is native to the western regions of Africa. It grows to at least five inches (12.5 cm) long although most specimens are smaller than this. The populations in the small streams are smaller than those in the big rivers [14].

2. Materials and Methods

2.1. Study Area

Alaro Stream forms part of the hydro-ecological system of the Oluyole Industrial Estate which receives effluents from diverse sources of trace metal pollution. The Alaro stream flows into Oluyole in a west–south east direction from its source at Agaloke near Apata in Ibadan. It joins River Ona at the south east end where a meat processing factory is located beside this main tributary. The stream receives effluents from diverse industries as shown in table 1. Effluents from both natural and anthropogenic sources are discharged into Alaro stream directly or indirectly through run-off, leaching or seepage especially during the rainy season or as windblown materials during the dry season that contribute heavy and trace metals into it. The Oluyole industrial estate is located between latitude 7^{o} 21'N -7 ^{o} 22'N and longitude 3 ^{o} 50'-3 ^{o} 52'E. Table 1 shows the types of industries that discharge effluents into Alaro Stream and their potential pollutants.

Table 1. *Industrial activities and their potential pollutants in Alaro Stream*

Industry	Number of industries	Potential pollutants
Food processing		
i. carbonated beverages	2	Alkalis, phenols, suspended solids, detergents, fermented starches, pathogens, nitrates, trace metals from oiling machine parts and organic wastes
ii. confectionery and biscuit	2	Organic wastes (solids and suspended), heavy metals, pathogens, total suspended solids(TSS)
iii. animal husbandry and meat processing	1	Organic wastes, heavy and trace metals
Iron and fabrication		
i. steel	2	Trace metals, cyanide, fluorides, chromates, thiocyanates, naphthalenes
ii. metal foundry	2	Diverse trace metals
iii. crown corks	1	Metal filings, heavy and trace metals
Wood processing	1	Waste lignin, organic sulphur, mercury, magnesium, sulphides, terpenes, mercaptans, heavy metals

2.2. Sampling Strategy and Identification

Fish were collected from the entire Alaro stream downstream of the effluent outfall for three months from June to August 2003. Fish were collected using the following techniques: Cast nets with mesh sizes ranging between 30-50mm of varying dimensional sizes were used .These nets were left for about three minutes before retrieving with a drawing string to check for any entangled fish. In addition, gill nets with mesh sizes of 30-50mm with varying dimensions were tied to stakes with a lead weight on the stream bed and maintained vertically in water with the aid of floats overnight. Forty-five (45) fish were caught in the sampling of Alaro stream with *Oreochromis niloticus* making up 22 of the total while *Alestes longipinnis* were 23.

Fish collected were identified using the textbook by Moses [14] .The dissections were carried out using dissecting set to remove the gills, fins, gut, liver, bones and muscle .These tissues were oven dried at 105^{0}C for 6hours.

Each organ or pooled organs were pulverized separately by means of a porcelain mortar and pestle. The pulverized samples were kept in sample sachets and sealed prior to analyses.

2.3. Fish Digestion for Heavy and Trace Metal Analyses

Tissue digestion was carried out by adding 2ml trace metal grade HNO_3 to 0.5g of each fish organ sample in Teflon digestion tubes which were heated at 105 ^{0}C for 1 hour in a heat block. The clear solution was then allowed to cool down, followed by addition of 1ml H_2O_2. After the simmering, it was boiled and left overnight. The digested sample was then diluted to the 10ml mark using MilliQ water for inductively coupled plasma mass spectrometer (ICP-MS) analyses.

Standard Reference Materials (SRM) comprising of bovine liver from the National Institute of Standards and Technology (NIST-1577) were used to obtain accurate values for fish tissue through reproducibility.

3. Results and Discussion

3.1. Standard Reference Materials and Quality Assurance

The results of the NIST SRM liver standard for the quality assurance of the results is shown in Table 2. Percentage recoveries from the reference material were all above 70% with a range of 75.25% (Pb) to 104.54% (Ag). The results were also corrected for errors using MilliQ water as the blank.

Table 2. *Results of liver standard (SRM) for fish tissue analysis using ICP-MS*

Metal	Average	Blanks	PPM	Theory	% Recovery
Sodium (Na)	100004	2	2000	2420	83
Magnesium(Mg)	2759	1	552	601	92
Potassium (K)	44055	4	8810	9940	89
Calcium (Ca)	599	25	115	116	99
Vanadium (V)	474.69	0.00	0.09	0.12	77.19
Manganese (Mn)	48731.9	0.0	9.7	10.5	92.8
Iron (Fe)	868.6	0.9	173.5	184.0	94.3
Cobalt(Co)	1071.46	0.00	0.21	0.25	85.72
Nickel (Ni)	1679.65	0.00	0.22	0.25	86.50
Copper(Cu)	733993.80	119.48	146.77	160.00	91.73
Zinc (Zn)	471873.0	1517.8	94.1	127.0	74.1
Arsenic (As)	214.8	0.0	0.0	0.1	85.9
Selenium (Se)	2773.89	0.00	0.55	0.73	76.00
Molybdenum (Mo)	15591.08	0.00	3.12	3.50	89.09
Silver (Ag)	203.85	0.00	0.04	0.04	104.54
Cadmium (Cd)	2207.431	0.000	0.441	0.500	88.29
Lead (Pb)	664.30	178.93	0.10	0.13	75.25

3.2. Fish Heavy and Trace Metal Levels in the Organs

Table 3. *Mean heavy and trace metal levels in the organs of Oreochromis niloticus (parts per million, ppm)*

Metal	Muscle	Liver	Bone	Gills	Fins
Na	12802	6802	808	1029	528
Mg	9812	278	678	6236	57
K	21927	23019	6785	6512	1009
Ca	499	2198	19285	1078	15278
V	2.33	10.87	13.09	5.68	2.10
Mn	1.9	5612.7	12.9	10.8	3.2
Fe	12.8	10559.9	42.8	1256.9	1.2
Co	8.43	6.98	2.12	0.08	0.12
Ni	0.05	5.78	4.21	0.78	1.08
Cu	12.97	134.98	1.89	5.86	0.97
Zn	1.5	2.7	217.0	65.9	7.8
As	0.865	0.129	0.764	0.096	0.108
Se	0.05	10.98	5.07	3.12	2.58
Mo	1.52	1.28	0.89	0.96	1.09
Ag	0.78	2.11	2.23	1.08	1.03
Cd	2.318	2.517	1.098	0.826	0.092
Pb	1.21	10.28	14.02	5.96	6.07

Results of the mean heavy and trace metal levels in the tissues and organs of *Oreochromis niloticus* are shown in table 3.

In *Oreochromis niloticus,* Na was highest in the muscle (12802ppm) while the least was in the fins (528ppm). Similarly, Mg was highest in the muscle (9812ppm) while the least was in the fins (57ppm). K was highest in the liver (23019ppm) with the least in the fins (1009ppm). Ca was highest in the bone (19285ppm) with the least in the muscle (499ppm). V was highest in the bone (13.09ppm) and least in the fins (2.10ppm). Mn was highest in the liver (5612.7ppm) and least in the muscle (1.9ppm). The highest Fe was in the liver (10559.9ppm) with the least value in the fins (1.2ppm). Co was highest in the muscle (8.43ppm) while the least was in the gills (0.08ppm). Ni was highest in the liver (5.78ppm) while the muscle was the least (0.05ppm). Cu was highest in the liver (134.98ppm) while the least was in the fins (0.97ppm). Zn was highest in the bone (217.0ppm) and least in the muscle (1.5ppm).As was highest in the muscle (0.865ppm) and least in the gills (0.096ppm). Se was highest in the liver (10.98ppm) and least in the muscle (0.05ppm).Mo was highest in the muscle (1.52ppm) while the least was in the bone (0.89ppm). Ag was highest in the bone (2.23ppm) while the least was in the muscle (0.78ppm). Cd was highest in the liver (2.517ppm) with the least value in the fins (0.092ppm). Pb was highest in the bone (14.02ppm) while the least mean value was recorded in the muscle (1.21ppm).

Results of the mean heavy and trace metal levels in the tissues and organs of *Alestes longipinnis* are shown in table 4.

Table 4. *Mean heavy and trace metals in the tissues and organs of Alestes longipinnis (parts per million, ppm)*

Metal	Muscle	Liver	Bone	Gills	Fins
Na	12982	15278	478	2780	5127
Mg	9812	10245	692	782	612
K	23189	30128	24156	12097	7189
Ca	178290	201928	247186	52198	102728
V	0.98	13.82	3.67	8.12	9.12
Mn	23.1	78.5	1092.8	109.2	54.9
Fe	9.2	10959.3	5627.8	421.9	25.9
Co	8.30	8.56	6.9	1.8	0.9
Ni	4.52	5.82	3.21	1.27	0.79
Cu	0.39	175.78	65.21	54.90	12.07
Zn	1.6	274.6	125.9	1.2	63.2
As	0.642	0.612	0.102	0.619	0.064
Se	0.06	11.80	4.50	7.89	0.17
Mo	0.06	1.72	1.29	0.98	0.53
Ag	2.10	2.33	1.95	0.97	0.01
Cd	0.064	0.867	2.870	1.091	1.005
Pb	0.10	7.85	2.10	14.12	1.97

In *Alestes longipinnis,* Na was highest in the liver (15278ppm) with the least as 478ppm in the bone. The highest Mg was in the liver (10245ppm) while the least was 612ppm in the fins. The liver had the highest K level of 30128ppm while the fins had the least (7189ppm).Ca had the highest level in the bones (247186ppm) with the least as the gills (52198ppm). The muscle showed the least V concentration of 0.98ppm while the highest was 13.82ppm in the liver. The highest Mn was 1092.8ppm in the bone while the least was 23.1ppm in the muscle. The least Fe was 9.2ppm in the muscle while the highest was 10959.3ppm in the liver. Co was highest in the liver (8.56ppm) with the least in the fins (0.9ppm). Ni was highest in the liver (5.82ppm) while the least was in the scales (0.79ppm). Cu was highest in the liver (175.78ppm) while the least was 0.39ppm in the muscle. The highest Zn level was in the liver (274.6ppm) while the least was in the gills (1.2ppm). The highest As level was recorded in the muscle (0.642ppm) while the least was the fins (0.064ppm). Se concentration was highest in the liver (11.80ppm) while the least was 0.06ppm in the muscle. The highest Mo level was in the liver (1.72ppm) with the muscle as the least (0.06ppm). The liver showed highest Ag concentration of 2.33ppm while the fins were the least with 0.01ppm.The bone showed highest Cd concentration of 2.870ppm with the muscle as the least (0.064ppm). The highest mean Pb was recorded in the gills (14.12ppm) while the least was the muscle (0.10ppm).

3.3. Discussion of Mean Heavy and Trace Metals between *O. Niloticus* and *A. Longipinnis*

The following mean heavy and trace metals were comparatively higher in *Oreochromis niloticus*: Mn (5612.7ppm, liver) and As (0.865ppm, muscle), while Na (15278ppm, liver), Mg (10245ppm, liver), K (30128ppm, liver), Ca (247186ppm, bones), V (13.82ppm, liver), Fe (10959.3ppm, liver), Co (8.56ppm, liver), Ni (5.82ppm, liver), Cu (175.78ppm, liver), Zn (274.6ppm, liver), Se (11.80ppm, liver), Mo (1.72ppm, liver), Ag (2.33ppm, liver), Cd (2.87 ppm, bone) and Pb (14.12ppm, gills) were higher in *Alestes longipinnis.* The high level of heavy and trace metal levels could be due to its role as a homeostatic and detoxification organ. The high Na, Mg, K, Ca, V, Mn, Fe, Co, Ni, Cu, Mo and Zn levels in the tissues of the fishes could be due to their physiological requirement in the proper functioning of the organism as these are required nutrients. Similar high heavy and trace metals in fish had been observed in fish from the similar ecological zone by Akan and colleagues [11]. On the other hand, As, Ag, Cd and Pb are not required in the functioning of the fish and therefore constitute a hazard even at low concentrations as shown in some tissues and organs exceeding the minimum 0.5-1.0ppm recommended tolerable limits. This study also supports assertions of the impact of industrial effluents on fish trace metal levels as stated by Oguzie [15] and Adeogun [16]. In this study for example, Cd in the muscle, liver, bone and gills of *Oreochromis niloticus* exceed the 0.5ppm recommended by World Health Organization (WHO) [17, 18] and FAO [19] while mean Pb in all the tissues and organs exceeded the 1.0ppm limit. This study corroborates findings from other studies on fish heavy and trace metals showing elevated levels of concentration from impacted ecosystems [20-25].

4. Conclusion

The occurrence or absence of trace metals in any given location and in fish tissue or organ is influenced by human activities such as agricultural land uses, industrial activities and ecological factors as corroborated in previous studies and in this study on Alaro Stream .These results are of public health significance because fish is consumed as a food source while some of the trace metals are toxic to human health when they exceed the recommended limits. In *A. longipinnis*, mean Cd and Pb in the liver, bone, gills and fins exceeded the set limit, thereby making the fish unfit for consumption due to the public health consequences associated with acute and chronic uptake of cadmium and lead. Similarly, As, Cd and Pb also exceeded the recommended limits in *O.niloticus* thereby making it an exclusion from the in human diet. This study also shows that fish caught from Alaro stream have to be regularly screened for toxic heavy and trace metals in order to safeguard public health.

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