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Toxic and trace metal accumulation in *Clarias gariepinus* and *Tilapia zilli* from Alaro stream in Ibadan, Nigeria

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Abstract
A study was carried out on the toxic and trace metal accumulation in *Clarias gariepinus* and *Tilapia zilli* from Alaro stream in Ibadan, from January 2002 to December 2003. Organs assessed were muscle, liver, bone, gills, fins and gut, while the facility used for the assessment was the inductively coupled plasma-mass spectrometer (ICP-MS) for accuracy of the results. Toxic and trace metals assessed were Sodium (Na), Magnesium (Mg), Sodium (K), Calcium (Ca), Vanadium (V), Manganese (Mn), Iron (Fe),Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Selenium (Se), Molybdenum (Mo), Silver (Ag), Cadmium (Cd) and Lead (Pb). Toxic and trace metal levels in the liver of both fishes were significantly higher than the other tissues ($P>0.05$). Toxic and trace metal accumulation in the organs was found to be dependent on the concentration in the water, duration of exposure and rate of uptake of the metals. The muscle that is mainly consumed was found to be relatively low in the accumulation of the toxic and trace metals. Toxic metals such as As, Cd and Pb exceeded the guideline limit set for them by the World Health Organization (WHO). A biomonitoring program is recommended for Alaro stream due to the industrial effluents discharged into it.

1. Introduction

Health authorities in many parts of the world have become increasingly concerned about the presence of certain metal contaminants in food (WHO, 1972, Akan *et al*, 2012).

Monitoring of contaminant levels in fish and shellfish is normally undertaken for the purposes of assessing possible hazards to human health, the existing level of aquatic pollution for regional comparison and to test the effectiveness of measures taken for the reduction of aquatic ecosystem perturbation (Balls and Rowe, 1994). Pollution of the ecosystems by trace metals from diverse sources could pose a public and environmental health threats. This is due to the potential of toxic metals even in traces to bioconcentrate along the food chain to higher consumers such as humans (Tyokumbur, 2010). Some of these trace elements are essential to the body at optimum levels but become toxic when they reach a harmful threshold, while some are toxic at low or high concentrations. These pollution sources arise from industrial, agricultural, domestic and economic activities at the local, community, national or global levels (Mason, 1992; Tyokumbur, 1998). The ever increasing influx of trace elements into the aquatic environment could be detrimental to surface and groundwater quality, fish and shell fish quality. Environmental health impact could arise from the intake of these pollutants that are either toxic,carcinogenic,mutagenic or teratogenic in repeatedly small doses or mega doses of exposure. Occasionally, smaller accumulated doses of the pollutants are taken up without the threat of an immediate side effect but become gradually toxic as they accumulate. Trace metals are not biodegradable but are easily assimilated and bioconcentrated in the tissues and organs of living organisms. They have high densities and atomic weights and include all transition elements in groups III and V of the periodic table (Brady and Holum, 1988).

Previous regional studies were focused on the trace metal content in fish and shellfish in the Niger Delta (Kakulu *et al*, 1987) and on the effects of tannery effluents in *Clarias gariepinus* (Gbem *et al*, 2001). In this study, the tissue toxic and trace metal accumulation in *Clarias gariepinus* and *Tilapia zilli* from Alaro stream in Ibadan will be assessed because they are commonly caught and constitute a major source of fish protein.

2. Materials and Methods

2.1. Study Area

The study area is the Alaro Stream which forms part of the hydro-ecological system of the Oluyole Industrial Estate which receives effluents from diverse sources of trace metal pollution. Effluents from both natural and anthropogenic sources are discharged into it directly or indirectly through run-off, leaching or seepage especially during the rainy season or as wind blown materials during the dry season. Oluyole industrial estate is located between latitude 7^o 21’N - 7^o 22’N and longitude 3^o 50’-3^o 52’E.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 of a meat processing factory as its main tributary. The stream receives effluents from diverse industries.

2.2. Collection of Fishes

Fishes were collected from the sampling stations using the following techniques:

1. Cast nets with mesh sizes ranging between 30-50mm with varying dimensional sizes. These nets were left for about three minutes before retrieving with a drawing string to check for any entangled fish.
2. Gill nets with mesh sizes of 30-50mm of 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 and retrieved the following morning.

2.3. Fish Processing for Analyses

Fish dissections were carried out using dissecting set to remove the gills,gut,liver kidneys 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 satchets and sealed prior to analyses .

2.4. Toxic and Trace Metal Analyses

Tissue digestion was carried out by adding 2ml trace metal grade HNO_3 to 0.5g of each 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, boiled and left overnight. The digestate was 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) were used to obtain accurate values for fish tissue. The NIST number for the bovine liver was 1577.

3. Results

100 fishes were caught in the sampling with *Clarias gariepinus* making up 57 of the total while *Tilapia zilli* were 43.

The results of the NIST SRM liver standard for the quality assurance of the results is shown in Table 1.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 1. *Results of liver standard (srm) for fish tissue analysis*

	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

The results of the mean organ toxic and trace metal concentration (parts per million, ppm) in *Clarias gariepinus* and *Tilapia zilli* are shown in tables 2 and 3 respectively.

Table 2. *Mean toxic and trace metal concentration in Clarias gariepinus (ppm)*

	Muscle	Liver	Bone	Gills	Fins	Gut
Na	15168	980	652	1098	125	956
Mg	9087	457	1097	168	525	334
K	21346	3456	586	12086	2168	10253
Ca	25789	6257	1289	9821	5637	8076
V	0.01	12.32	10.86	7.97	5.32	6.52
Mn	2.6	1.9	56.7	6.3	3.7	2.2
Fe	56.8	1092.8	278.6	21.9	109.9	73.5
Co	0.08	7.95	2.88	6.01	2.90	4.12
Ni	5.09	5.81	4.36	1.95	0.19	2.33
Cu	0.36	173.22	57.08	79.19	5.14	33.9
Zn	1.4	34.8	12.9	76.8	0.9	7.2
As	0.943	0.856	0.612	0.753	0.121	0.532
Se	0.07	1.27	4.98	2.19	0.12	2.10
Mo	1.07	0.57	1.72	0.97	1.03	1.01
Ag	2.01	2.33	1.09	1.02	0.97	2.22
Cd	0.095	0.179	0.816	3.071	2.082	1.642
Pb	12.22	10.91	5.89	2.86	4.29	8.06

Table 3. *Mean toxic and trace metal concentration in Tilapia zilli (ppm)*

	Muscle	Liver	Bone	Gills	Fins	Gut
Na	14209	15007	880	986	12009	10382
Mg	8906	437	10269	7615	8901	9012
K	21658	329	5148	12926	19123	12014
Ca	120928	56189	214278	643	7829	40218
V	0.66	12.08	14.08	7.78	2.56	5.77
Mn	1.9	6172.9	526.7	8.9	102.6	1006
Fe	9.6	10092.8	267.9	6278.7	10.2	493.7
Co	8.23	8.54	0.07	6.74	2.19	5.33
Ni	5.61	2.87	0.98	0.61	3.94	4.21
Cu	167.45	154.98	0.97	7.89	5.23	122.98
Zn	252.9	272.8	12.4	9.5	6.9	178.23
As	0.854	0.923	0.128	0.716	0.078	0.762
Se	0.08	11.60	3.87	6.97	1.07	5.44
Mo	1.63	1.73	0.09	1.26	0.19	1.34
Ag	1.09	2.30	0.56	1.27	0.92	2.11
Cd	0.812	3.209	2.065	1.092	0.313	3.002
Pb	1.98	10.86	7.05	6.09	0.05	9.33

In *Clarias gariepinus,* Na was highest in the muscle (15168ppm) with the least as 125ppm in the fins. The muscles showed highest Mg concentration of 9087ppm with the least in the gills (168ppm).The least K level of 586ppm was recorded in the bone while the highest was 21346ppm in the muscle. The highest Ca level was 25789ppm in the muscle while the bone was the least with 1289ppm.V was least in the muscle with 0.01ppm, while the liver had the highest value of 12.32ppm.The bone had the highest Mn (56.7ppm) while the liver had the least value of 1.9ppm.The least value of Fe was 21.9ppm in the gills while the highest was 1092.8ppm in the liver. The highest Co level was recorded in the liver (7.95ppm) with the least as 0.08ppm in the muscle. Ni was highest in the liver (5.81ppm) with the least in the fins (0.19ppm).The liver had the highest Cu concentration of 173.22ppm while the least was in the muscle (0.36ppm).Zn was highest in the gills (76.8ppm) with the least in the fins (0.9ppm).As was highest in the muscle (0.963ppm) while the least value was 0.121ppm in the fins. Se was highest in the bone (4.98ppm) while the least was 0.07ppm in the muscle. Mo was highest in the bone (1.72ppm) with the least 0.57ppm in the liver. Ag had the highest concentration in the liver at 2.33ppm with the least in the fins (0.97ppm).Cd had the highest level of 3.071ppm in the gills with the least value of 0.095ppm in the muscle. Pb had the highest value of 12.22ppm in the muscle while the least was in the gills (2.86ppm).

In *Tilapia zilli,* the highest Na level was recorded in the liver (15007ppm) while the least was in the bone (880ppm).The highest Mg was in the bone (10269ppm) with the least in the liver (437ppm).The highest K was in the muscle (21658ppm) and the least in the liver (329ppm).Ca was highest in the bone (214278ppm) with the least in the gills(643ppm).V was highest in the bone (14.08ppm) while the least was the muscle (0.66ppm).Mn was highest in the liver (6172.9ppm) while the least was in the muscle (1.9ppm).Fe was highest in the liver (10092.8ppm) while the least was in the muscle (9.6ppm).Co was highest in the liver (8.54ppm) with the least in the bone (0.07ppm). Ni was highest in the muscle (5.61ppm) while the least was in the gills (0.61ppm).Cu was highest in the muscle (167.45ppm) with the least in the bone (0.97ppm).The highest Zn was recorded in the liver (272.8ppm) with the least in the fins (6.9ppm).As was highest in the liver (0.963ppm) with the least in the fins (0.078ppm).Se was highest in the liver (11.60ppm) with the least in the muscle (0.08ppm). Mo was highest in the liver (1.73ppm) while the least was in the bone (0.09ppm).Ag was highest in the liver (2.30ppm) with the least in the bone (0.56ppm).Cd was highest in the liver (3.209ppm) while the least was in the fins (0.313ppm). Pb was highest in the liver (10.86ppm) with the least in the fins (0.05ppm).

Comparatively, *Clarias gariepinus* had the highest tissue concentration of Na (15168), Ni (5.81), Cu (173.22), As (0.943), Ag (2.33) and Pb (12.22) while *Tilapia zilli* had higher levels of Mg (10269),K (21658),Ca (1 20928),V (14.08),Mn (6172.9),Fe (10092.8),Co (8.54),Zn (252.9),Se (11.60),Mo (1.73) and Cd (3.209).Toxic metals such as As, Pb and Cd exceeded the World Health Organization guideline limits set for them (WHO,1972).

4. Discussion

Trace metal accumulation in the tissues and whole body of fish has been observed to be directly related to the concentration in water and the duration of exposure (Offem, 1983; Oladimeji and Ologunmeta, 1987; Agboola, 1998, Akan *et al*, 2012).

This study found the liver to have accumulated macro and trace metals significantly higher than other tissues ($P<0.05$).High accumulation of metals in the liver can be attributed to its role in storage and detoxification. In addition, fishes are known to have the metal-binding protein metallothionein; a sequestering agent that detoxifies metals in fish (Friberg *et al*, 1971).Hence, elimination by excretion is not a critical method of detoxification. It is worthy of note that the fish's ability to produce this metal binding protein is limited ,hence when its abilities to excrete metals in copious solution and binding the toxic metals are exceeded detoxification by calcification in the scales as in the case of *Tilapia zilli* may be explored. Exceedingly high levels of macro and trace metal accumulation or other pollutant that cannot be further regulated may immobilize the fish and lead to death .Variability in tissue metal accumulation could be attributed to competition for protein-binding sites both at the mucosal, tissues and organ level because when metals occur in association they interact through antagonism and synergism thereby altering their critical concentration and toxic effects (Tyokumbur,1998).Mechanisms of metal-binding interaction may include formation of complexes among metals and metalloids, induction of metal-binding protein and interchange of metal bound proteins which affects transport of metal ions (Chmielnicka,1983).

This study shows a high accumulation of toxic and trace metals in the gills and gut which are the main routes of uptake into the body. This is in accordance with Annune and Iyaniwura (1993) and, Badsha and Goldspink (1982) who reported high uptake through these routes. Smith *et al* (1996) has suggested a relationship between toxic and trace metal accumulation in fish and the amount in food, sediments, water and the feeding habit. Being a benthic feeder, *Clarias gariepinus* has the potential to accumulate metals from sediments, food and water while the feeding habits of *Tilapia zilli* could also favour metal uptake. The bioavailability and bioaccessibility of these toxic and trace metals could also be enhanced by recharges from the sediments which are the major sinks of metals in the aquatic environment.

This study also found toxic and trace metal accumulation to be low in the muscles which might be due to the growth factor since growth has the potential of diluting toxicant concentration especially if growth is faster than uptake and accumulation.

However, the muscle of *Clarias gariepinus* accumulated higher Na, Mg, K, Ca, As and Pb than the other tissues which may be due to permeability differences to metal uptake and accumulation.Similarly, the muscle of *Tilapia zilli* had higher levels of Cu, Ni, Na, Mg and K. This study corroborate findings of trace metals in the muscles of fish by Beveridge *et al* (1985) and Marcus *et al*, (2013) .The muscle is a major tissue of interest because it is the most consumed in terms of quantity in the human diet.

5. Conclusion

This study shows that Alaro stream is polluted and fish caught from it is not safe for human consumption. This is because toxic metals such as As, Cd and Pb exceeded the guideline limits set for them by the World Health Organization. Since fish caught from it, is not safe for human consumption, a biomonitoring program is recommended for Alaro stream due to the industrial effluents discharged into it.

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