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Clarias anguillaris as a biological indicator of metal contamination of selected aquatic habitats of North-Western Nigeria

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ABSTRACT

Fish is a natural source of vitamins and mineral elements that are important in growth and immune system. Because of its habitat, *Clarias anguillaris* was used as a biological indicator of metal contamination in pollution prone aquatic environment. Gills, muscles, and bones from *Clarias anguillaris* were digested, and selected metals were quantified by atomic absorption spectrophotometry. Values were compared with WHO acceptable limits for sea foods. Generally, lead and cadmium concentrations were higher in season I samples of Rivers Bunsuru and Rima while same metals were higher in concentrations in season II samples of River Gagare and Goronyo Dam. For other metals and mineral elements, season I samples had higher concentrations while in other cases season II samples had higher concentrations. Tissue samples of season II from Goronyo Dam had highest lead contents than other samples from other rivers. Bioaccumulation of lead in fish tissues had occurred in the study sites due to uncontrolled mining activities. Lead replaces ∞-aminolevulenic acid in haem biosynthesis and can cause anaemia in man being at the high trophic level of food chain.

Introduction

The effect of pollutants/contaminants on organisms can either be neurophysiological, behavioural or reproductive, and all the three are inter-related. The sub-lethal effect of the toxins results in the regression of the physiological or behavioural processes of the organism. If two or more toxins are present together in aquatic habitat, they may exert a synergistic or antagonistic effect on an organism. Toxic metals originate within the earth where they may be harmless until released by human interference. As the levels of heavy metals rise in the air, water and topsoil, they also rise within human bodies through bioaccumulation thereby contributing to chronic diseases, learning disorders, cancer and premature aging [14].

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Mineral elements and some metals perform synergistic functions that are essential for the existence of the organism in such a way that high intake of one affects the absorption of the other. Such example include, high intake of magnesium decreasing absorption of calcium from the intestine, while high phosphate in diet causes precipitation of calcium as calcium phosphate which cannot be absorbed from the gut and is excreted in faeces. In the same way deficiency of copper (hence deficiency of Ceruloplasmin) decreases the intestinal absorption and impairs the transport of iron [9]. The toxicity of metals is frequently the result of long – term low – level exposure to pollutants common in the environment: air, water, food and numerous consumer products. Acute toxicity is not difficult to recognize because the symptoms are usually severe, rapid in on–set and associated with known exposure or ingestion [11]. These symptoms are cramping, nausea and vomiting, pain, sweating, headaches, difficulty in breathing, impaired cognitive motor and language skills, mania and convulsions. Symptoms associated with chronic toxicity exposure are impaired cognitive and language skills, learning difficulties, nervousness and emotional instability, insomnia, nausea, lethargy and feeling ill [15]. Some mineral compounds are cofactors for enzymes in various metabolic pathways (e.g. K, Cu, and Mg), essential constituents of certain enzymes (e.g. Mo, Co, and Se) while others are integral components of biologically important compounds such haemoglobin (Fe), and insulin (Zn). Dietary deficiency of Fe causes anaemia while excess Fe causes hemosiderosis. In the same way deficiency and excess copper intake respectively cause Menke's and Wilson's diseases [3].

Materials and methods

Chemicals and reagents

All chemicals and reagents used for this research were of analytical grade, and include: concentrated HNO_3, H_2O_2, concentrated HCl, $HClO_4$, lead metal, nickel metal, calcium metal, iron fillings, chromium metal, copper metal, magnesium ribbon, NaN_3, KI, and 40%(v/v) hydrofluoric acid.

Equipment

The equipment used were: block digestor (Tecator, USA), electric oven (Gallenkamp, USA), distiller (Nottingham, UK), weighing balance (Mettler pc 4400, Gallenkamp, USA), fume cupboard (UK), furnace (Lenton Company, England), Atomic Absorption Spectrophotometer (Philips Model PU 9100, Country).

The study area

The study area is part of North-Western Nigeria, where active artisan lead mining activities are taking place. They include: Goronyo Dam established in 1984 by the State Government and located close to Keta village about twenty five kilometres east of Goronyo town and ninety kilometres away from Sokoto town. The Goronyo dam has a reservoir area of 200 km^2 and capacity of 942 million cm^3 at 288 m above sea level (M.A.S.L) full storage. The construction of the dam was completed across the Rima River and has Gagare, Bunsuru and Maradi rivers as the main tributaries. The dam supplies raw water to Sokoto State Water Board, stream bank (Fadama) irrigation through lifting, Wurno and Falalia irrigation schemes and fishing activities. The river Gagare has its source from Kaura Namoda through Moriki to Birnin Yero. River Bunsuru on the other hand comes from Zurmi, through Bafarawa. Both rivers (Gagare and Bunsuru) have their confluence at Attalawa, a village in Sokoto state. Local community is actively involved in fishing and irrigation activities around the two tributaries. Some of the crops grown by these schemes are rice, cassava, vegetables and garlic. River Rima is an overflow of Goronyo Dam, active fishing activities are taking place there too, and the water from the river is also used for irrigation (see Fig. 1). According to the Emir of Bukkuyum, no fewer than 300 children have died of lead poisoning at Yar'Galma village in Bukkuyum Local Government Area of Zamfara State, Nigeria. The aim of the research was to evaluate the concentrations of both toxic and trace metals in aquatic ecosystem using *Clarias anguillaris* as a biological indicator. This is because of illegal mining and tannery activities that are regularly carried out around the study sites.

Sample material and collection

Clarias anguillaris was chosen as a biological indicator for metal contamination in aquatic environment and by implication metal toxicity to man because of its availability throughout the year and a source of protein to the community. The fish sample was identified at the Department of Fisheries and Forestry, Usmanu Danfodiyo University Sokoto, Nigeria. Fresh specimen was collected in 2007 and 2009 from the local fishermen at the study sites either at the time of fishing operations or as the fishermen landed on the shores. The samples were kept in the ice packed containers for transportation to the laboratory where they were kept in the refrigerator till ready for digestion. The study sites are surrounded by lead mining activities and tanneries.

Sample digestion

Two gram (2.0) g wet-weight gills, muscles or bones sample were placed in separate and labelled reflux flasks and 20 ml of freshly prepared (1:1) nitric acid/hydrogen peroxide was added to each. The flasks were placed in the block digestor for

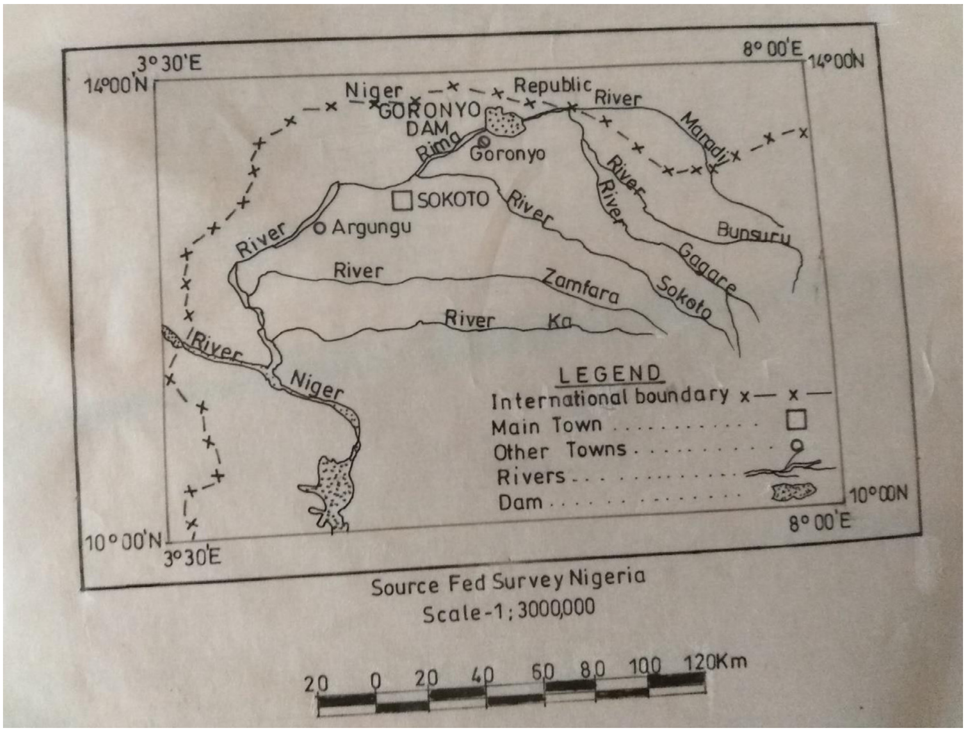

Fig. 1. Map of the study sites with adjourning rivers.

two hours till the volumes were reduced to 10 ml. The digests were allowed to cool and filtered through Whatman No. 1 filter paper. The volumes were further made up to 100 ml. The procedure was repeated thrice for all tissue samples. The digests were then transferred to polythene bottles and stored till required for analysis [13].

Estimation of metals

Atomic Absorption Spectrophotometer (Philips model PU 9100, Country) was used for the estimation of Pb, Cd, Cr, Ni, Fe, Mg, Ca, Cu, Na and K as described in the Pye Unican Atomic Absorption data book (1984), and in the introduction to Atomic Absorption Spectrophotometry Scientific Analytical Equipment manual book by Whiteside [22].

Statistical analyses of results

Statistical Product for Social Solution (SPSS) software version 16 was used to analyze all the data. Results are expressed as Mean ± Standard Error of Mean (SEM) using one-way analysis of variance (ANOVA), followed by Turkey Duncan and Dunnet's multiple comparison test and the values of $P<0.05$ were considered to be statistically significant.

Results and discussion

The metal ions once in the body, compete with and displace the essential minerals such as zinc, copper, manganese and calcium and bind their receptor sites thereby affecting nerves, hormones, digestion and immune systems. In general, metals produce their toxicities by forming complexes or ligands with other organic compounds. These modified biological molecules lose their ability to function properly, and result in malfunction or death of the affected cells [17]. The most important common groups involved in ligand formation are oxygen, sulphur and nitrogen. In biological system, metal ions interact with DNA and nuclear proteins causing DNA damage and conformational changes that may lead to cell cycle modulation [8].

All seasonal I samples from Rivers Bunsuru and Rima had higher levels of lead in gills, muscle and bone of *Clarias anguillaris*, while same tissue samples of season II from River Gagare and Goronyo Dam had higher lead contents (Tables 1–4). This is in consonant with the work of Malhat [16] that investigated heavy metal residues in fishes from River Nile tributaries in Egypt. The nervous system is the most sensitive target of Pb exposure and according to ATSDR [4], lead exposed workers with blood lead levels (BLLs, 4–12) μ/dl have symptoms like fatigue, depression, irritability, diminished cognitive performance etc. Acute high Pb dose induced impairment of proximal tubular function manifest in aminociduria, glycosuria,

Table 1
Metal Concentrations (µg/g) in Tissues of *Clarias anguillaris* of River Bunsuru.

Season	Part	Pb	Cd	Ni	Fe	Mg	Cr	Cu	Ca	Na	K
Season I	Gills	0.48^{a}±0.08	0.41^{a}±0.06	0.36^{a}±0.06	30.70^{a}±2.93	34.91^{a}±4.87	0.21^{a}±0.03	2.40^{a}±0.22	162.45^{a}±10.62	3.37^{a}±0.51	2.25^{a}±0.31
Season II	Gills	0.07^{a}±0.03	0.02^{a}±0.01	0.03^{a}±0.01	7.34^{a}± 2.50	72.16^{a}±3.34	0.07^{a}±0.03	2.03^{a}±0.03	78.22^{a} ± 10.45	3.62^{a}±0.41	3.66^{a}±0.53
Season I	Muscles	0.46^{a}±0.07	0.40^{a}±0.03	0.36^{a}±0.07	29.38^{b}±3.17	35.08^{a}±5.11	0.19^{a}±0.03	1.72^{b}±0.10	169.01^{b}±10.50	3.39^{a}±0.41	2.43^{a}±1.66
Season II	Muscles	0.05^{b}±0.003	0.03^{a}±0.02	0.03^{a}±0.01	7.43^{a}± 2.17	77.69^{b}±8.13	0.06^{a}±0.03	2.06^{a}±0.02	66.98^{b} ±10.82	3.38^{b}±0.40	3.11^{b}±0.47
Season I	Bones	0.45^{a}±0.08	0.39^{a}±0.07	0.34^{a}±0.06	28.95^{c}±2.24	33.28^{b}±4.73	0.19^{a}±0.02	2.44^{b}±0.12	167^{c} ± 11.74	3.60^{a}±0.41	2.56^{a}±0.30
Season II	Bones	0.07^{a}±0.03	0.01^{a}±0.004	0.04^{a}±0.02	9.24^{a} ± 2.89	80.59^{c}±3.21	0.08^{a}±0.02	1.10^{b}±0.10	83.14^{c} ± 8.64	3.22^{c}±0.54	4.27^{c}±0.57

Season I (January–February).
Season II (August – September).
a = means on the same column, with same superscripts are not significantly different (P>0.05).
b, c = means on the same column, with different superscripts differ significantly (P<0.05).

Table 2
Metal concentrations (µg/g) in Tissues of *Clarias anguillaris* of River Gagare.

Season	Part	Pb	Cd	Ni	Fe	Mg	Cr	Cu	Ca	Na	K
Season I	Gills	0.27^{a}±0.06	0.11^{a}±0.03	0.20^{a}±0.02	48.90^{a}±5.41	24.79^{a}±2.79	0.20^{a}±0.03	1.12^{a}±0.04	247.8 ^{a}±18.48	3.46^{a}±0.51	2.15^{a}±0.31
Season II	Gills	0.37^{a}± 0.09	0.18^{a}± 0.09	0.12^{a}± 0.05	4.50^{a}±0.54	72.02^{a}±5.25	0.69^{a} ± 0.26	2.32^{a} ± 0.13	228.25^{a} ± 29.90	3.02^{a}±0.41	3.56^{a}±0.53
Season I	Muscles	0.24^{b}±0.06	0.15^{b}± 0.02	0.20^{a}± 0.03	34.59^{b}±3.01	26.03^{b}± 3.50	0.16^{b}± 0.02	2.13^{b}±0.02	209.54^{b}±16.94	3.12^{a}±0.41	2.23^{a}±1.66
Season II	Muscles	0.80^{b}± 0.15	0.31^{b}± 0.10	0.17^{b}± 0.09	4.31^{b}±0.33	83.13^{b}±13.54	0.80^{b}± 0.18	2.44 ^{b}± 0.17	147.51^{b} ± 19.24	3.15^{b}±0.40	3.01^{b}±0.47
Season I	Bones	0.18^{c}± 0.04	0.12^{a} ± 0.03	0.20^{a} ± 0.01	38.08^{c}±2.09	27.50^{c}± 3.36	0.17^{b} ± 0.02	1.12^{a} ± 0.04	241.01^{c}±17.73	3.23^{a}±0.41	2.36^{a}±0.30
Season II	Bones	0.56^{c}± 0.21	0.17^{a}± 0.04	0.12^{a}± 0.02	4.09^{c}±0.33	76.71^{c}±9.05	0.46^{c}± 0.14	2.48 ^{c}± 0.18	279.44^{c} ± 32.40	3.20^{c}±0.54	3.27^{c}±0.57

Season I (January–February).
Season II (August–September).
a = means on the same column, with same superscripts are not significantly different (P>0.05).
b, *c* = means on the same column, with different superscripts differ significantly (P<0.05).

Table 3
Metal concentrations (µg/g) in tissues of *Clarias anguillaris* of River Rima.

Season	Part	Pb	Cd	Ni	Fe	Mg	Cr	Cu	Ca	Na	K
Season I	Gills	0.34^{a}±0.05	0.29^{a}±0.05	0.10^{a}±0.02	3.83.90^{a}±9.92	25.06^{a}±6.18	0.04^{a}±0.02	3.09^{a}±0.02	147.75^{a}±7.81	4.53^{a}±0.59	3.60^{a}±0.51
Season II	Gills	0.04^{a}±0.02	0.02^{a}±0.01	0.22^{a}±0.01	3.83^{a}±0.50	54.46^{a}±12.43	0.30^{a}±0.14	2.21^{a}±0.11	182.32^{a}±17.92	2.80^{a}±0.28	3.90^{a}± 0.16
Season I	Muscles	0.31^{b}±0.04	0.33^{b}±0.15	0.06^{b}±0.02	37.56^{b}±9.35	25.16^{a}±6.65	0.07^{b}±0.02	2.09^{b}±0.02	147.75^{a}±4.33	4.39^{b}±0.39	3.10^{b}±0.41
Season II	Muscles	0.10^{b}±0.05	0.02^{a}±0.01	0.19^{b}±0.02	4.08^{b}±0.43	47.17^{b}±11.45	0.50^{b}±0.25	2.03^{a}±0.02	144.26^{b}±18.57	2.96^{b}±0.40	3.86^{a}± 0.33
Season I	Bones	0.29^{b}±0.04	0.48^{c}±0.13	0.22^{c}±0.17	39.45^{c}±9.69	18.44^{b}±7.36	0.08^{b}±0.02	3.07^{a}±0.02	181.59^{b}±6.86	4.10^{c}±0.48	3.82^{c}±0.49
Season II	Bones	0.14^{b}±0.08	0.06^{b}±0.04	0.25^{c}±0.05	3.29^{c}±0.33	56.71^{c}±13.90	0.46^{c}±0.07	1.03^{b}±0.02	253.58^{c}±29.21	2.70±0.31	3.58^{c}± 0.30

Season I (January–February).
Season II (August–September).
a = means on the same column, with same superscripts are not significantly different (P>0.05).
b, c = means on the same column, with different superscripts differ significantly (P<0.05).

Table 4
Metal concentrations (µg/g) in tissues of *Clarias anguillaris* of Goronyo Dam.

Season	Part	Pb	Cd	Ni	Fe	Mg	Cr	Cu	Ca	Na	K
Season I	Gills	0.18^{a}±0.03	0.06^{a}±0.05	0.21^{a}±0.02	36.21^{a}±1.43	32.02^{a}±2.24	0.11^{a}±0.02	2.16^{a}±0.05	140.12^{a}±10.00	28.22^{a}±2.98	17.06^{a}± 1.54
Season II	Gills	5.69^{a}±0.38	0.39^{a}±0.08	0.003^{a}±0.003	5.18^{a}±0.25	105.67^{a}±5.43	0.06^{a}± 0.05	2.30^{a}±0.18	358.01^{a}±26.12	6.32^{a}± 0.90	5.82^{a}± 0.79
Season I	Muscles	0.16^{b}±0.02	0.05^{a}±0.04	0.18^{b}±0.02	30.37^{b}±1.47	27.01^{b}±1.55	0.10^{a}±0.02	2.14^{a}±0.05	114.15^{b}±7.98	22.56^{b}±2.81	15.87^{b}±1.45
Season II	Muscles	5.11^{b}±0.18	0.43^{b}±0.11	0.002^{a}±0.002	5.21^{b}± 0.19	99.82^{b}±5.84	0.01^{b}± 0.01	2.11^{b}±0.08	322.20^{b}±20.35	6.40^{a} ± 0.63	5.05^{b} ± 0.42
Season I	Bones	0.19^{a}±0.04	0.05^{a}±0.04	0.22^{a}±0.03	24.10^{c}±1.39	20.28^{c}±0.88	0.11^{a}±0.003	2.13^{a}±0.04	227.20^{c} ± 8.64	23.06^{c}±2.75	15.84^{b}±1.92
Season II	Bones	4.23^{c}±0.44	0.45^{c}±0.13	0.005±0.005	5.11^{c} ± 0.26	86.27^{c} ± 9.62	0.06^{a} ± 0.04	2.24^{c}±0.12	325.25^{c}±35.10	5.38^{c}± 0.85	5.46^{c} ± 0.13

Season I (January–February).
Season II (August–September).
a = means on the same column, with same superscripts are not significantly different (P>0.05).
b, *c* = means on the same column, with different superscripts differ significantly (P<0.05).

and hyperphosphaturia. Though some fish samples had high lead contents but were not up to the tolerable intake level of 10 µg/g set by WHO [23].

Like lead, high cadmium contents were found in season I samples of Rivers Bunsuru and Rima, and season II samples of Rivers Gagare and Goronto Dam (Tables 1–4). Ahmed et al. [1] also reported the presence of cadmium in some fishes collected from River Buriganga in Bangladesh. Sufficient Cd exposure leads to chronic renal failure manifested in glucosuria, hypercalcuria, polyuria and reduced buffering capacity for acids [7]. Values of Cd obtained from the study were below the EFSA limit of 2 µg/g [19].

River Rima season II samples consistently had high nickel concentration in all the tissues while tissue concentrations of nickel from Season I samples of Rivers Bunsuru, Gagare and Goronyo were higher than the corresponding season II sample concentration. Youssfzai et al. [26] reported similar findings in *Wallago attu* and *Labeo dyocheilus* in aquatic habitats of some Pakistani towns. Nickel is a cofactor in the absorption of iron from intestine and high level of Ni may impair Fe utilization or absorption when Fe status is low.

Iron contents of tissues from all season I samples were significantly higher than the corresponding values from season II samples (Tables 1–4). Authman et al. [6] in a bio-monitoring research of metal contamination of aquatic ecosystem reported high iron contents of fishes studied. Iron is required for basic metabolic processes such as oxygen transport, DNA synthesis, cytochrome P450 enzyme oxidative metabolism, and electron transport. Iron is an essential component of haem moiety of cytochrome P450, and therefore would be expected for cytochrome P450 synthesis. But Fe levels in the liver are correlated to NADPH-dependent lipid peroxidation, and increased lipid peroxidation has been associated with decreased drug metabolism [10].

In general, all season II samples had higher Magnesium content in tissues than the corresponding sample of season I. There was no consistency in high Mg content as Mg was highest (105.67±5.43) in gills of samples collected from Goronyo Dam, in muscle (83.13±13.54) of River Gagare, and in bones of River Gagare (Tables 1–4). Mohan [18] reported magnesium accumulation in *Oratosquilla nepa*, and related it to reduce lead content of the tissues as lead content is inversely correlated to other metal contents. Magnesium intoxication causes depression of neuromuscular system, lethargy, hypotension, respiration depression, bradycardia and weak tendon reflexes.

Season I samples of River Bunsuru, and Goronyo Dam had higher concentrations of chromium while season II samples of Rivers Gagare and Rima had higher concentrations of chromium (Tables 1–4). Ahmed et al. [2] reported high chromium concentration in *Heteropneustes fossils* and asserted that it caused genotoxicity in the fish samples. Chromium tends to accumulate in brain, kidney and myocardial tissues in mammals, although accumulation, storage, retention depend on its chemical form, route of entry, and amount administered [25].

In addition to muscle tissue of Goronyo Dam season I sample, copper was high in Rivers Bunsuru and Rima season I samples. In the same way, in addition to muscle tissue of River Bunsuru season II sample of copper was higher in River Gagare and Goronyo Dam (Tables 1–4). This agrees with the Saleh and Marie [20] that reported high copper concentration in tissues of *Arius thalassinus* fish from the Red Sea coast of Yemen. The degree to which copper is absorbed in the gastrointestinal tract depends on its chemical state and other compounds like zinc [21]. ATSDR [5] has set a limit for copper at 2 µg/g in seafoods.

In addition to muscle tissue of River Rima season I sample, Rivers Bunsuru and Gagare season I samples had high calcium content while season II samples of River Rima and Goronyo Dam had high calcium concentration in addition to River Gagare bone sample of season II (Tables 1–4). Jabeen and Chaudhry [12] reported similar findings when they examined the mineral uptake in *Oreochromis mossambicus* from an industrial river in Pakistan. Calcium plays a role in the biochemistry of the cell, particularly in signal transduction pathways, the skeleton acts as its major storage site and releases Ca^{2+} into the blood stream under controlled conditions.

Sodium and potassium as mineral elements are present in all tissues from the study sites in WHO acceptable limits of ≤2 g/day and 200 mg/day, respectively [24].

Conclusion

Different tissues *of Clarias anguillaris bioaccumulated* and stored metals and trace elements in different concentrations. It is therefore recommended that individuals around the study sites that fed on the fish samples be screened for the levels of these metals in their blood, and body fluid samples to ascertain the level of bioaccumulation and hence toxicity. Thereafter medication intervention can be sort for.

Conflict of interest

The authors have no conflict of interest as the work was done by the lead author as part of the Ph.D. work that was supervised by the other three co-authors.

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