Health Sciences Research
2014; 1(3): 35-39
Published online September 10, 2014 (http://www.aascit.org/journal/hsr)

American Association for Science and Technology

Assessment of heavy metals in the fish *Mormyrus deliciousus* and *Heterobranchus longifilis* from Alaro Stream in Ibadan, Nigeria

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Citation
Emmanuel Tyokumbur, Tonye Okorie. Assessment of Heavy Metals in the Fish *Mormyrus deliciousus* and *Heterobranchus longifilis* from Alaro Stream in Ibadan, Nigeria. *Health Sciences Research.* Vol. 1, No. 3, 2014, pp. 35-39.

Keywords
Heavy Metals,
Mormyrus deliciousus,
Heterobranchus longifilis,
Fish,
Ibadan

Received: August 24, 2014
Revised: August 30, 2014
Accepted: August 31, 2014

Abstract
A study was carried out on the assessment of heavy and trace metals in the tissues of two fish species, *Mormyrus deliciousus* and *Heterobranchus longifilis* in Alaro stream ecosystem in Ibadan, Nigeria. Cast nets with mesh sizes ranging between 30-50 mm with varying dimensional sizes were used for catching the two fish species over 3-month duration. Sixty-seven fishes were caught in the sampling with *M. deliciousus* making up 27 of the total while *H. longifilis* was 40 over 3-month duration. Fish dissections (*M. deliciousus*: n=27; *H. longifilis*:n=40) were carried out using dissecting set to remove the gills, gut, liver, fins and muscle which were oven dried at $105^{0}C$ for 6 hours. Pulverized tissues and organs were acid-digested for heavy and trace metal analyses using the inductively coupled plasma-mass spectrometer (ICP-MS). Percentage recoveries from the reference material were all above 70% with a range of 75. 25% (Pb) to 104. 54% (Ag). Mean of some heavy and trace metals were comparatively higher in *M. deliciousus*: Mg(10082ppm, fins), Co(8. 56ppm, muscle), Cu(175. 78ppm, liver), Zn(275. 2ppm, muscle), As(0. 963ppm, muscle), Se(11. 80ppm, liver), Mo(1. 73ppm, liver) and Pb(14. 12ppm, liver) while Na(12980ppm, bone), K(30912ppm, liver), Ca(203189ppm, bone), V(12. 98ppm, liver), Mn(752. 9ppm, liver), Fe(10092. 0ppm, liver), Cd(3. 208ppm, liver) were higher in *H. longifilis* whereas the following trace metals were the same for both species of fish: Nickel, Ni (5. 82ppm) and Silver, Ag (2. 33ppm). Most of the heavy metals were high due to their physiological requirement in the tissues of the fish species. As, Ag, Cd and Pb exceeded the recommended limits set by the World Health Organization for most of the tissues and organs in *M. deliciousus* and *H. longifilis.* This shows that these fish species caught in Alaro stream ecosystem are not safe for human consumption.

1. Introduction

Pollution of the aquatic ecosystem by heavy and trace metals from diverse sources could pose a public and environmental health challenge [1]. This is due to their potential to become biomagnified along the food chain to higher consumers such as human beings. Some of these heavy metals 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

[2]. The ever increasing influx of heavy metals into the aquatic environment could be detrimental to surface and groundwater quality, fish and shell fish quality [2]. Environmental health impact arises from the heavy metals that are toxic, carcinogenic, mutagenic or teratogenic in repeatedly small doses or mega doses of exposure [3]. Occasionally, smaller accumulated doses of the pollutants are taken up without the threat of an immediate side effect but become gradually toxic [3].

The bioavailability of heavy metals in natural waters depends on ecological factors that vary in time and space. These ecological factors can be pooled together to generate models for predicting into the future [4, 5]. The outbreak of Minamata disease in Japan in 1954 which claimed many lives that was attributed to the consumption of mercury-contaminated fish, cadmium poisoning or *itai-itai* disease brought about an elevated the awareness that other heavy metals also have the potential of contaminating water resources and causing harm to human beings either directly or indirectly through the food chain.

Monitoring of heavy metal levels in fish and shellfish is normally undertaken for the purposes of assessing possible hazards to human health.

In Nigeria, the establishment of the Federal Environmental Protection Agency (FEPA) in 1987 was intended to regulate the discharge of untreated wastes into the environment. The guidelines of FEPA were aimed at protecting the country's water resources [6].

The Alaro Stream which forms part of the hydro-ecological system of the Oluyole Industrial Estate receives effluents from diverse sources of trace element pollution. These industries and their potential pollutants are listed in Table 1.

Table 1. *Industrial activities and their potential pollutants in Oluyole industrial estate*

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

2. Materials and Methods

2.1. Study Area

Alaro Stream (latitude 7° 21'N -7° 22'N and longitude 3° 50'-3° 52'E) is part of the hydro-ecological system of the Oluyole Industrial Estate which receives effluents from diverse sources of heavy metal pollution forms the study area. 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 windblown materials during the dry season. 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 of a meat processing factory as its main tributary. The stream receives effluents from diverse industries.

2.2. Collection of Fishes and Identification

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

Cast nets with mesh sizes ranging between 30-50mm with 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 and 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.

Fish collected were identified using textbooks [7, 8]. Fish were sacrificed by hitting the back of their heads on the dissecting slab. 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 sachets and sealed prior to analyses.

2.3. Fish Digestion for Macro and Trace Element 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 digested sample was diluted to the 10ml mark using MilliQ water for inductively coupled plasma mass spectrometer (ICP-MS) analyses.

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

3. Results and Discussion

3.1. Fish Sampling Results

Sixty-seven fishes were caught in the sampling with *M. deliciosus* making up 27 of the total while *H. longifilis* was 40.

3.2. Standard Reference Materials and Quality Assurance

Percentage recoveries from the standard reference material (Table 2) 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
Na	100004	2	2000	2420	83
Mg	2759	1	552	601	92
K	44055	4	8810	9940	89
Ca	599	25	115	116	99
V	474. 69	0. 00	0. 09	0. 12	77. 19
Mn	48731. 9	0. 0	9. 7	10. 5	92. 8
Fe	868. 6	0. 9	173. 5	184. 0	94. 3
Co	1071. 46	0. 00	0. 21	0. 25	85. 72
Ni	1679. 65	0. 00	0. 22	0. 25	86. 50
Cu	733993. 80	119. 48	146. 77	160. 00	91. 73
Zn	471873. 0	1517. 8	94. 1	127. 0	74. 1
As	214. 8	0. 0	0. 0	0. 1	85. 9
Se	2773. 89	0. 00	0. 55	0. 73	76. 00
Mo	15591. 08	0. 00	3. 12	3. 50	89. 09
Ag	203. 85	0. 00	0. 04	0. 04	104. 54
Cd	2207. 431	0. 000	0. 441	0. 500	88. 29
Pb	664. 30	178. 93	0. 10	0. 13	75. 25

Table 3. *Mean heavy and trace metal levels in M. deliciousus (in parts per million, ppm) with standard deviation in parenthesis.*

Metal	Muscle	Liver	Bone	Gills	Fins
Na	524(50. 2)	672(40. 5)	12696 (523)	2567 (403. 6)	11098(287. 4)
Mg	1276(67. 8)	4356(100. 7)	562(42. 8)	6121(80. 5)	10082(254. 6)
K	448(12. 8)	522(21. 9)	13257(102. 7)	2912(45. 0)	12097(430. 0)
Ca	12542(235. 5)	4316(54. 9)	21087(432. 9)	42167(402. 1)	21765(23. 6)
V	0. 09(0. 01)	3. 24(0. 32)	10. 28(2. 10)	5. 28(1. 02)	1. 33(0. 61)
Mn	501. 7(5. 9)	1. 6(0. 21)	12. 6(1. 26)	4. 3(0. 7)	45. 9(2. 84)
Fe	9. 2(1. 3)	4. 8(0. 50)	6124. 8(23. 8)	6. 9(2. 1)	12. 3(2. 1)
Co	8. 56 (2. 23)	0. 01(0. 005)	2. 76(0. 55)	3. 76(0. 82)	6. 91(1. 33)
Ni	0. 86(0. 11)	5. 82(1. 20)	4. 87(1. 09)	1. 20(0. 5)	3. 47(0. 78)
Cu	12. 87(1. 25)	175. 78(5. 98)	43. 89(4. 98)	12. 00(2. 1)	6. 96(0. 54)
Zn	275. 2(5. 2)	43. 9(2. 89)	1. 6(0. 6)	3. 8(0. 4)	1. 1(0. 24)
As	0. 963(0. 12)	0. 896(0. 042)	0. 742(0. 091)	0. 213(0. 120)	0. 000(0. 000)
Se	0. 06(0. 01)	11. 80(1. 75)	10. 09(1. 87)	6. 76(1. 02)	4. 03(1. 25)
Mo	1. 10(0. 43)	1. 73(0. 80)	0. 00(0. 00)	1. 21(0. 90)	0. 68(0. 11)
Ag	2. 03(0. 05)	2. 33(0. 32)	0. 59(0. 11)	0. 97(0. 05)	1. 02(0. 22)
Cd	0. 189(0. 021)	3. 017(0. 621)	2. 091(0. 214)	0. 781(0. 120)	3. 217(1. 021)
Pb	5. 98(0. 52)	14. 12(2. 11)	7. 17(1. 22)	6. 04(1. 62)	2. 45(0. 29)

Table 4. *Mean heavy and trace metal levels in H. longifilis (ppm) with standard deviation in parenthesis*

Metal	Muscle	Liver	Bone	Gills	Fins
Na	9012(37. 5)	2678(45. 6)	12980(102. 5)	735(19. 8)	10298(50. 8)
Mg	7652(13. 2)	3021(40. 7)	501(12. 4)	9826(54. 9)	709(12. 8)
K	21827(102. 1)	30912(201. 9)	205(10. 5)	12087(101. 2)	1672(23. 9)
Ca	24155(210. 7)	127829(579. 2)	203189(827. 1)	56213(415. 2)	590(12. 9)
V	0. 95(0. 23)	12. 98(3. 89)	5. 97(1. 28)	6. 92(1. 7)	1. 97(0. 78)
Mn	1. 7(0. 4)	752. 9(48. 2)	6. 9(2. 1)	2. 8(1. 2)	100. 2(10. 7)
Fe	9. 9(2. 1)	10092. 0(42. 0)	287. 9(12. 6)	65. 8(2. 12)	100. 9(9. 8)
Co	0. 05(0. 01)	8. 50(2. 31)	1. 27(0. 75)	6. 97(1. 95)	3. 80(1. 21)
Ni	0. 50(0. 08)	5. 82(1. 72)	3. 40(1. 20)	5. 27(2. 21)	0. 20(0. 08)
Cu	0. 75(0. 10)	170. 25(12. 8)	21. 95(3. 99)	2. 63(1. 00)	0. 52(0. 12)
Zn	2. 75(0. 85)	274. 6(21. 7)	56. 9(6. 92)	23. 1(4. 7)	1. 9(0. 56)
As	0. 960(0. 126)	0. 657(0. 162)	0. 128(0. 021)	0. 615(0. 086)	0. 098(0. 010)
Se	0. 06(0. 01)	11. 50(3. 27)	4. 53(1. 04)	8. 90(2. 07)	5. 21(2. 01)
Mo	0. 56(0. 12)	0. 98(0. 23)	1. 72(0. 65)	1. 09(0. 75)	0. 28(0. 08)
Ag	0. 09(0. 02)	2. 33(0. 95)	2. 09(0. 54)	2. 06(1. 28)	0. 97(0. 13)
Cd	0. 000(0. 0)	3. 208(0. 102)	1. 029(0. 782)	2. 098(0. 978)	0. 895(0. 0915)
Pb	0. 98(0. 21)	12. 09(2. 81)	13. 98(3. 09)	4. 56(1. 93)	1. 28(0. 59)

3.3. Heavy and Trace Metal Levels in Fish

Results of the mean heavy and trace metals in *M. deliciousus* are shown in table 3 while that of *H. longifilis* is shown in table 4.

In *M. deliciousus*, Na was highest in the bones while the least was in the muscle. The fins had the highest Mg concentration with the least in the bone. K was least in the liver while the highest was in the bone. Ca was highest in the gills, with the least value was in the liver. The bone had the highest V concentration while the least was in the muscle. Mn was highest in the muscle with the liver having the least value. Fe was highest in the bone with the least in the liver. The highest mean Co was recorded in the muscle with the least in the liver. Ni was highest in the liver while the least was in the muscle. Cu was highest in the liver with the least in the fins. The highest value of Zn was in the muscle with the least in the fins. As was the highest in the muscle while the fins was the least. The highest value of Se was in the liver while the least was in the muscle. The liver recorded the highest Mo level while the least was in the bone. Ag was highest in the liver while the least was in the bone. Cd was highest in the liver while the least was in the muscle. The highest Pb level was recorded in the liver while the least was in the fins.

In *H. longifilis*, Na was highest in the bone while the least was in the gills. The highest Mg was in the gills while the least was in the bone. The highest K was recorded in the liver with the least in the bone. The bone had the highest mean Ca with the least in the fins. V was highest in the liver with least in the muscle. Mn was highest in the liver while the least was in the muscle. Fe was highest in the liver while the least was in the muscle. Co was least in the muscle with the highest in the liver. Ni was highest in the liver with the least in the fins. Cu was highest in the liver with the least in the fins. The highest Zn level was in the liver with the least in the fins. The highest As level was in the muscle with the least in the fins. Se was highest in the liver with the least value in the muscle. Mo was highest in the bone while the least was in the fins. Ag level was highest in the liver with the least in the muscle. The least Cd was in the muscle while the highest was in the liver. Pb was highest in the bone while the least was in the muscle.

3.4. Comparison of the Heavy and Trace Metal Levels in the Two Fish Species

The following mean heavy and trace metals were comparatively higher in *M. deliciosus*: Mg(10082ppm, fins), Co(8. 56ppm, muscle), Cu(175. 78ppm, liver), Zn(275. 2ppm, muscle), As(0. 963ppm, muscle), Se(11. 80ppm, liver), Mo(1. 73ppm, liver) and Pb(14. 12ppm, liver) while Na(12980ppm, bone), K(30912ppm, liver), Ca(203189ppm, bone), V(12. 98ppm, liver), Mn(752. 9ppm, liver), Fe(10092. 0ppm, liver), Cd(3. 208ppm, liver) were higher in *H. longifilis*. The following were the same for both species of fish: Ni (5. 82ppm) and Ag (2. 33ppm).

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. 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 concentration as shown in some tissues and organs exceeding the maximum tolerable limits given by World

Health Organization [9].

4. Conclusion

As, Ag, Cd and Pb exceeded the recommended limits set by the World Health Organization for most of the tissues and organs in *M. deliciousus* and *H. longifilis*. For instance, bone, liver, muscle of *M. deliciousus* exceeded the 0. 5ppm set for As, while the liver, bone, gills and fins also exceeded the 0. 5ppm set for Cd, whereas all tissues and organs analyzed exceeded the 1. 0ppm set for Pb. In *H. longifilis*, only muscle and liver exceeded the As minimum level, while Cd was not detected in the muscle and this organ also did not exceed the WHO limit set for Cd. The high As levels can be attributed to natural background concentrations while electronic wastes and leaded fuel would account for Cd and Pb respectively. Similar results have been shown for other species of fish from other studies such as studies of heavy metal contamination of *Sarotherodon melanotheron* in Lagos Lagoon [10], heavy metals in fish of the lower Ikpoba River in Benin [11], review of trace metals as a potential threat to the Nigerian Fishing Industries [12], measurement of heavy metals in fish from the Tajan River [13], heavy metal levels in fish species from Saudi Arabian Markets [14], heavy metals concentration in edible fish from South East Coast of India [15], heavy metals in fish from Pakistan[16] and heavy metals in Tilapia fish from Malaysia[17].

In addition , heavy and trace metals such as Na, Mg, K, Ca, V, Mn, Fe, Co, Ni, Cu, Mo and Zn were high in the tissues of the fish due to their physiological requirement as corroborated by Opaluwa [18] and Kousar and Tawed [19]. Since most of the heavy and trace metals exceeded the World Health Organization set limit in the fish organs, it therefore means that fish from Alaro stream is not fit for human consumption due to public health consequences.

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