Pak. j. sci. ind. res., vol. 36, no. 8, August 1993

TRACE HEAVY METAL DISTRIBUTION IN THE FISH, *ILLISHA AFRICANA* ORGANS AND TISSUE II: CHROMIUM, ZINC, COPPER, IRON AND COBALT

E.I. ADEYEYE

Department of Chemistry, Federal University of Technology, P.M.B. 704, Akure, Nigeria

(Received March 20, 1993; revised August 8, 1993)

This work provides information on the levels of 5 essential trace metals found in the muscle tissue, internal organs, head and gills of the fish, *Illisha africana* commonly harvested from fresh-water ponds in Nigeria. The fishes were caught from 3 neighbouring man-made ponds and were analysed for their contents of Cr, Zn, Cu, Fe and Co by atomic absorption spectrophotometer. All determinations were made on dry weight basis. Cr was not detected in any samples. Both the coefficients of variation of the elements and their concentration factors were determined in the fish parts. Every part of the fish in the 3 ponds showed a sign of some degree of bioaccumulation.

Key words: Essential trace metals, Coefficient of variation, Concentration factor, *Illisha africana.*

Introduction

Among the several trace elements that have attracted the attention of nutrition scientists are Zn, Cu, Fe, Cr, I and Se. All the elements under consideration are transition elements and are all essential.

Once in the environment, these elements end up in the streams, rivers and the sea. From here, fishes and other aquatic organisms accumulate them into their muscle and organs from where they get to man.

The presence of metal pollutants in fresh water is known to disturb the delicate balance of the aquatic ecosystem. Fishes are notorious for their ability to concentrate heavy metals in their muscles [1] and since they play important role in human nutrition, they need to be carefully screened to ensure that unnecessarily high levels of some toxic trace metals are not being transferred to man through fishes. Little attention has been paid to the comparative study of metal concentration. Such studies are important to establish a baseline on existing levels and assess the level of pollution and nutritional value of fish.

This work provides additional information (on internal organs and muscle) and new information (on gills and head) on the levels of Cr, Zn, Cu, Fe and Co in *Illisha africana* which is a typical freshwater Tilapia fish caught in three neighbouring man-made ponds located in geographically identical sites.

Materials and Methods

The fish samples, *Illish africana* were obtained from three different man-made freshwater ponds separated by 4.6 meters of dyke covered with star grass. The grass is to prevent erosion and inwash of muddy water. The ponds are located in the college farm of the Ondo State College of Education, Ikere-Ekiti, Ondo State of Nigeria. The ponds were supplied with water from the same spring. Each fish was briefly washed with distilled water to remove any adhering contamination and then drained under folds of filter paper. Fish from each pond was identified, wrapped in aluminium foil and deep-frozen. The samples were given about 2 hrs of defrosting before processing for analyses.

After defrosting, the scales were removed, rinsed with de-ionised distilled water and each fish separated into the head, gills, internal organs (comprising the stomach, liver, kidney and intestine) and muscle tissue. The internal organs mentioned were used as a total subsample analyte because of the small size of the fish. Plastic knife was used to cut the muscle. Drying in the oven (using Gallenkamp Moisture Extraction Oven) was carried out for 8 hrs at 105°. Each part was grounded in glass mortar and pestle and sieved through 425 μm mesh.

The beakers used were washed, labelled and given a final clean by adding 20 cm^3 concentrated nitric acid (sg. 1.42) covering with watch glasses, then heating to 50° for 2 hrs. After cooling, the acid was poured out and the glassware rinsed well with de-ionised distilled water.

The homogenised samples were accurately weighed into the beakers. Digestion of the samples was the wet digestion method [2,3]. Digestion was carried out by adding 2.0 cm^3 nitric acid (sg 1.42) to each sample in a 50 cm^3 beaker. The beakers were covered with watch glasses and allowed to stand overnight in a fume cupboard before heating gently on a hot plate until any frothing has stopped and no solid material seen. The temperature was increased to 60° until brown fumes were no longer generated.

The de-ionised water was added into each beaker and the temperature under the samples was increased to 80°. The acid fumed off until about 2-5 cm^3 was left in the beaker. The digest

was removed from the heat and covered with a watch glass which had been rinsed in de-ionised distilled water. The digests were made up to about 20 cm^3 using de-ionised distilled water and reduced for a second time down to 2-5 cm^3. The final solutions were washed into standard flasks (25 cm^3) and made up to volume using 0.1 M nitric acid.

Standards were prepared for each metal using suitable metal salts of each metal. All reagents used were of the analytical grade. All the metals analysed for, used the hollow cathode lamps and air-acetylene flame.

The samples were assayed for the various analytes using a calibrated Perkin Elmer Model 306, atomic absorption spectrophotometer (AAS – manufactured by Perkin Elmer) at standard operational conditions.

All data generated were analysed statistically [4]. From the average values obtained for metals in the pond water samples, concentration factors were calculated [5]. All determinations were on dry weight basis.

Results and Discussion

The results (Tables 1-3) show that Cr was absent (n.d.) in all the samples examined. For Zn the levels in the gills varied from 0.20 mg/kg dry weight in pond 1 to 0.34 mg/kg dry weight in pond 3, in the head it varied from 0.18 mg/kg dry weight in pond 1 to 0.23 mg/kg dry weight in pond 2, in the internal organs it varied from 0.18 mg/kg dry weight in pond 3 to 0.34 mg/kg dry weight in pond 1 while the value for Zn varied from 0.15 mg/kg dry weight in pond 1 and pond 3 to 0.22 mg/kg dry weight in the muscle tissue in pond 2. The levels of Cu in the gills ranged from 3.19 mg/kg dry weight in pond 1 to 10.06 mg/kg dry weight in pond 3, the value in the head ranged from 0.63 mg/kg dry weight in pond 1 to 2.75 mg/kg dry weight in pond 3, Cu value ranged from 2.06 mg/kg dry weight in pond 3 to 5.8 mg/kg dry weight in pond 1 for the internal organs while the value for the muscle tissue ranged from n.d. in pond 1 to 0.31 mg/kg dry weight in both ponds 2 and 3. For Fe the levels in the gills ranged from 3.44 mg/kg dry weight in pond 3 to 8.66 mg/kg dry weight in pond 2, in the head region Fe ranged from 2.11 mg/kg dry weight in pond 3 to 3.33 mg/kg dry weight in pond 1, the internal organs showed values ranging from 13.54 mg/kg dry weight in pond 3 to 21.76 mg/kg dry weight in pond 1 while the value in the muscle ranged from 2.00 mg/kg dry weight in pond 3 to 2.33 mg/kg in both ponds 1 and 2. For Co the values in the gills varied from n.d. in pond 2 to 0.29 mg/kg dry weight in pond 3, in the head it ranged from n.d. ponds 1 and 2 to 0.29 mg/kg dry weight in pond, for the internal organs it ranged from n.d. in ponds 1 and 3 to 0.29 mg/kg dry weight in pond 2 while the value of Co for the muscle ranged from 0.15 mg/kg dry weight in ponds 1 and 2 to 0.29 mg/kg dry weight in pond 3.

The highest concentration for Zn (0.34 mg/kg dry weight) was found in the internal organs and the gills while the lowest value (0.15 mg/kg dry weight) was found in the muscle; for Cu, the highest concentration (10.06 mg/kg dry weight) was found in the gills while n.d. was recorded in the muscle; for Fe, the

TABLE 1. DISTRIBUTION OF VARIOUS ELEMENTS IN *ILLISHA AFRICANA* IN POND 1 (mg/kg DRY WEIGHT).

Fish part	Cr	Zn	Cu	Fe	Co
Gills	ND	0.20	3.19	7.66	0.29
Head	ND	0.18	0.63	3.33	ND
Internal organs	ND	0.34	5.88	21.76	ND
Muscle tissue	ND	0.15	ND	2.33	0.15
Mean	–	0.22	2.43	8.77	0.11
Standard deviation	–	0.08	2.68	8.96	0.14
Coefficient of variation (%)	–	38.72	110.71	102.20	126.64

TABLE 2. DISTRIBUTION OF VARIOUS ELEMENTS IN *ILLISHA AFRICANA* IN POND 2 (mg/kg DRY WEIGHT).

Fish part	Cr	Zn	Cu	Fe	Co
Gills	ND	0.28	9.25	8.66	ND
Head	ND	0.23	1.56	3.00	ND
Internal organs	ND	0.24	3.88	20.54	0.29
Muscle tissue	ND	0.22	0.31	2.33	0.15
Mean	–	0.24	3.75	7.38	0.11
Standard deviation	–	0.03	3.95	8.79	0.14
Coefficient of variation (%)	–	10.85	105.43	119.04	126.62

ND - Not detected.

TABLE 3. DISTRIBUTION OF VARIOUS ELEMENTS IN *ILLISHA AFRICANA* IN POND 3 (mg/kg DRY WEIGHT).

Fish part	Cr	Zn	Cu	Fe	Co
Gills	ND	0.34	10.06	3.44	0.15
Head	ND	0.20	2.75	2.11	0.29
Internal organs	ND	0.18	2.06	13.54	ND
Muscle tissue	ND	0.15	0.31	2.00	0.29
Mean	–	0.22	3.80	5.27	0.18
Standard deviation	–	0.08	4.30	5.55	0.14
Coefficient of variation (%)	–	38.72	113.33	105.27	75.84

ND – Not detected.

TABLE 4. CONCENTRATION FACTORS* FOR VARIOUS ELEMENTS IN POND 1.

Fish part	Concentration factors				
	Cr	Zn	Cu	Fe	Co
Gills	–	0.80	–	42.56	0.28
Head	–	0.72	–	18.50	–
Internal organs	–	1.36	–	120.89	–
Muscle tissue	–	0.60	–	12.94	0.15

*Ratio concentration in fish parts (µg/g to concentration in water, µg/g[5].

highest concentration (21.76 mg/kg dry weight) was found in the internal organs and the lowest value (2.00 mg/kg dry weight) was found in the muscle; for Co, the highest concentration (0.29 mg/kg dry weight) was found in the gills, internal organs, head and muscle. The highest concentrations of the metals were found most in the gills and the internal organs. This is probably due to the fact that since the gills are the organs of respiration, water must flow through them and in the process they filter the incoming water while the internal organs are the ultimate sink for all contaminants. Values for the muscles are generally the lowest. This agrees with the findings of Odukoya and Ajayi [6].

The results of Odukoya and Ajayi [6] showed the level of Cu in the muscle to be 0.07-0.63 mg/kg wet weight for fresh-water fishes, 0.05-0.21 mg/kg wet weight for lagoon fishes and 0.11-0.68 mg/kg wet weight for sea fishes. Zn had levels of 2.3-9.0 mg/kg wet weight in the muscles of fresh-water fishes, 1.5-4.6 mg/kg wet weight for lagoon fishes and 3.4-12.3 mg/kg wet weight for sea-fishes. In the organs, the liver had Cu levels of 2.4-62.2 mg/kg wet weight while the kidney had levels of 0.7-21.7 mg/kg wet weight. The levels of Zn in the liver were 5.6-38.4 mg/kg wet weight while the kidney levels were 1.3-58.8 mg/kg wet weight.

The coefficient of variation ranged from 10.85% in pond 2 to 38.72% in both ponds 1 and 2 for Zn, it ranged from 105.43% in pond 2 to 113.33% in pond 3 for Cu, the value for Fe ranged from 102.20% in pond 1 to 119.04% in pond 2 and for Co it ranged from 75.84% in pond 3 to 126.64% in pond 2 (Tables 1-3). The coefficient of variation is an indication of unequal concentration of metals in different organs of the fish.

TABLE 5. CONCENTRATION FACTORS FOR VARIOUS ELEMENTS IN POND 2.

Fish part	Concentration factors				
	Cr	Zn	Cu	Fe	Co
Gills	–	0.96	–	48.11	–
Head	–	0.79	–	16.67	–
Internal organs	–	0.83	–	114.11	0.21
Muscle tissue	–	0.76	–	12.94	0.11

TABLE 6. CONCENTRATION FACTORS FOR VARIOUS ELEMENTS IN POND 3.

Fish part	Concentration factors				
	Cr	Zn	Cu	Fe	Co
Gills	–	0.77	–	9.56	0.11
Head	–	0.45	–	5.86	0.22
Internal organs	–	0.41	–	37.61	–
Muscle tissue	–	0.34	–	5.56	0.22

TABLE 7. COMPARISON OF LEVELS OF Cu AND Zn IN NIGERIA FISHES WITH OTHER COUNTRIES OF THE WORLD.

Country	Cu (mg/kg wet wt.)	Zn (mg/kg wet wt.)
1. Australia commercial fish [18]	0.10-2.4	0.50-24
2. U.S.A. Portsmouth Sea-fish (shell-fish)[8]	1.45-2.10	14.9-15.9
3. Papua New Guinea Sea-fishes [9]	0.27-0.67	3.0-4.5
4. Israel Haifa Bay Sea-fishes [10]	0.7-23.5	0.5-84.3
5. Gulf of Mexico and Bahama Islands [11]	0.23-1.15	3.17-4.0
6. West Malaysia Coastal Water-fishes [12]	–	2.3-6.5
7. Swedish EPB Lake fishes [13]		
Muscle	0.30-2.02	0.10-0.15
Liver	7.1-50.0	0.12-4.30
8. Nigeria [6]		
(a) Fresh-water		
Muscle	0.07-0.63	2.3-7.1
Liver	2.40-62.2	23.9-33.7
Kidney	0.70-1.90	1.3-33.2
(b) Lagoon		
Muscle	0.05-0.21	1.5-4.6
Liver	2.6-3.0	5.6-21.5
Kidney	1.2-1.8	8.7-14.6
(c) Sea		
Muscle	0.11-0.68	3.4-12.3
Liver	16.1	38.4
Kidney	21.7	58.8
9. Greece Sea-fishers [14]		
Polluted Areas		
Muscle	1.0-1.7 dry wt.	37-71 dry wt.
Liver	22-35 dry wt.	52-111 dry wt.
Unpolluted Areas		
Muscle	0.89-1.3 dry wt.	48-58 dry wt.
Liver	6.1-8.1 dry wt.	48-58 dry wt.
10. Nigeria (current study) dry wt.		
(a) Pond 1		
Gills	3.19	0.20
Head	0.63	0.18
Internal organs	5.88	0.34
Muscle	n.d.	0.15
(b) Pond 2		
Gills	9.25	0.28
Head	1.56	0.23
Internal organs	3.88	0.24
Muscle	0.31	0.22
(c) Pond 3		
Gills	10.06	0.34
Head	2.75	0.20
Internal organs	2.06	0.18
Muscle	0.31	0.15
Maximum permissible level		40.0

The higher such variation is, the higher the unequal concentration of such a metal.

From the average obtained for metals in pond water samples, concentration factors were calculated [5] and results are given in Tables 4-6. A look at the concentration factors showed that pond 3 had the least concentration factors which ranged from 0.34-0.77 for Zn while the highest values for Zn were found in pond 1 which ranged from 0.60-1.36. For Fe, the least concentration factor ranged from 5.56-37.61 in pond 3 while the highest value ranged from 12.94-120.89 in pond 1. For Co, least value ranged from 0.11-0.21 in pond 2 and highest value ranged from 0.15-0.28 in pond 1. No concentration factors were recorded for both Cr and Cu. This is due to the fact that both Cu and Cr were not detected in any of the water from the 3 ponds. Cr was not detected in any of the soils. Although the fish contained Cu from the various ponds, it is possible that the fish accumulated Cu from the source of the fingerlings and also from the bottom of the water since the soils in ponds 1 and 2 contained Cu, also the fishes might be bottom feeders. The concentration factors in the case of Fe for the 3 pond fishes is an indication that the Fe value in the freshwater is low while it is high in the fish organs, this is a good evidence of bioacumulation of the metal in the fish. Every part of the fish in the 3 ponds showed a sign of some degree of bioaccumulation as shown in Tables 4-6.

Comparison of the levels of Cu and Zn in Nigerian fishes with those from other countries of the world (Table 7) shows that Nigerian fishes have generally lower levels in these metals than fishes from other parts of the world.

The level of metals in fishes is an indication of the level of heavy metal pollution of the waters from which they are caught. The comparison (Table 7) therefore indicates a lower level of pollution of Nigerian man-made ponds compared to waters in developed countries [7-14].

It has been suggested that the trace metal levels were a function of species rather than of weight, although within a given species the trace metal level might depend upon the weight of the fish [15]. Table 8 lists the U.S. recommended daily dietary allowances (RDA) supplied by a 100 g serving of fish muscle [16].

TABLE 8. U.S. RECOMMENDED DAILY DIETARY ALLOWANCES (RDA) SUPPLIED BY A 100 g SERVING OF FISH MUSCLE.

Metal	Concentration (mg)
As	0.003
Cd	0.014
Hg	0.008
Pb	0.300
Ni	0.010
Zn	2.600
Fe	0.5-2.0
Cu	2.0-3.0
Cr	0.05-0.20
Mn	2.5-5.0

Conclusion

The levels of the metals in the gills, internal organs and head are higher than in the muscles (Tables 1-3). The metal mostly concentrated in the fish studied is Fe followed by Zn and Co. The maximum permissible level (MPL) for Zn in fish muscle set by the U.S. Food and Drug Administration is 40.0 mg/kg wet weight for Zn while non is found to be recommended for the other metals.

For the fish studied (*Illisha africana*), the dry weight determination of the metals is a good indication of what the greater percentage of the consumers might be consuming since facilities for preservation in most homes are just not there. The best way to preserve the fish is through drying. Most consumers remove and throw off the internal organs and the gills when processing fish for consumption. It is gratifying to note that the levels of the metals are low in the muscles. However, there is a reasonable level of these essential and beneficial elements in the fish studied and values are below the safety limits. Based on the quantitative information (Table 8), the size of serving that is safe for human consumption could be calculated. The data for Nigerian fishes (Table 7) indicate that most fish species can be consumed safely.

References

1. B.C.O. Okoye, Intern. J. Environmental Studies, **37**, 285 (1991).
2. B.J. Harper *et al.*, *Methods of Analysis for Trace Metals in Marine and Other Samples*, Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Lowest of Aquatic Environment Protection, Analytical Methods No. 3 (1989).
3. T. Varian, *Basic Atomic Absorption Spectroscopy - A Modern Introduction* (Dominican Press, Australia, 1975).
4. R.G.D. Steel and J.N. Torrie, *Principles of Procedures of Statistics* (McGraw - Hill, 1960).
5. K.C. Pillai, Proc. COST, Seminar on Pollution and Human Environment, Bombay (1970).
6. O.O. Odukoya and S.O. Ajayi, Nigerian J. Nutri. Sci., **8** (1), 41 (1987).
7. G.N. Rebbington, Aust. J. Mar. Fresh-Water Res., **28**, 277 (1977).
8. J.B. Palma and G.M. Raud, Bull. Environ. Contao. Toxicol., **18** (4), 512 (1977).
9. J.R. Powell, R.E. Powell and D.R. Fielder, Water, Air and Soil Pollution, **16**, 143 (1978).

10. I. Roth and H. Hornuing, Environ. Sci. Tech., **18** (3), 265 (1977).
11. D.D. Taylor and T.J. Bright, Department of Marine Resources, Information Centre for Marine Resources. Tax. A.M. Univ. Tech. Rept. TAMU - SG, 73 (1973).
12. A.S. Babji, M.S. Embong and W.W. Woori, Bull. Environ. Contam. Toxicol., **23**, 830 (1979).
13. R. Uhrberg, Swedish Environmental Protection Board Tech., Rept. (1979).
14. A.P. Grimanis, D. Zafiropoulos and V. Orimanis, Environ. Sci. Tech., **12**, 723 (1978).
15. M. Ashraf and M. Jaffer, Fisheries Research, **8**, 287 (1990).
16. F.M. Teeny *et al.*, J. Agric. Food Chem., **32**, 852 (1984).