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The likely influence of size in the distribution and concentration of some metals in the organs of *Oreochromis niloticus* (l.) fish in a freshwaer pond

E.I. ADEYEYE[1] and M.K.O. ARIFALO[2]

[1]Department of Chemistry, University of Ado-Ekiti, P.M.B. 5363, Ado-Ekiti (Nigeria).
[2]Department of Chemistry, College of Education, P.M.B. 250, Ikere-Ekiti (Nigeria).

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ABSTRACT

Levels of cobalt, zinc, lead, manganese, copper, iron, sodium and potassium were determined by means of atomic absorption spectrophotometer in the fish organs (scales, gills, intestine, eyes, head and trunk muscle) of two different sized *Oreochromis niloticus* (L.) in a freshwater pond. Cobalt and copper were not detected in both the fish organs, stock water and associated sediments. Lead was not detected in water, sediments and smaller sized fish (SSF) but was found accumulated in some organs of the bigger sized fish (BSF). Metals most concentrated in the SSF were manganese and sodium while those most concentrated in the BSF were zinc, lead, iron and potassium. However, for all the metals detected, the BSF trunk muscle had the overall highest concentration but the lead level was below the deleterious level. Significant differences occurred in the sizes of the SSF and BSF; and also in the metal concentrations in the scales, gills, intestine, eyes, head, and trunk muscles of the two different sized fish at $p < 0.05$.

Keywords: Levels of metals, different sized *Oreochromis niloticus* fish.

INTRODUCTION

Fish, an important source of animal protein, is in increasing demand in Nigeria[1]. This increase in demand is due to a number of factors such as high population growth rate[2], increasing national income and increasing cost of meat and other sources of animal protein. Currently, about 40% of animal protein consumed in the country is derived from fish[3]. The relatively high per caput consumption of fish has been attributed to greater availability of this product at relatively cheaper prices[4]. The total demand for fish in Nigeria was estimated at 0.940, 1150 and 1450 million tonnes respectively in 1975, 1980 and 1985[5].

The mineral elements present in the body of tropical freshwater fishes have been little investigated. Some reported investigations included those of Odukoya and Ajayi[6,7], Olaleye and Akintunde[8], Ipinmoroti and Oshodi[9] and Adeyeye[10]. Some of these minerals might be involved in haematopoiesis while others might be involved in regulation of metabolic processes.

In tropical African waters, fishes of the Tilapia group are usually the most important in abundance and commercial value[1]. *Oreochromis niloticus* is an important member of this group which figure prominently in the fisheries of Nigeria. The importance of this fish warrants some biochemical studies.

In the present study, an attempt was made to determine some mineral elements in the fish parts (scales, gills, intestine, eyes, head and trunk muscle) of two different sized *Oreochromis niloticus* in a freshwater fish pond. Such attributes as flavour, odour, texture and even the appearances which govern the desirability of fish muscle as food are related directly or indirectly to the mineral composition[8].

MATERIAL AND METHODS

Sampling

Samples were taken from one artificial pond located at the Ondo State Government Hatchery Centre, Alagbaka, Akure. Grass was planted around the pond to prevent erosion and inwash of muddy water.

Grab water samples were taken just below the water surface in the fish pond using a one-litre acid-leached polythene bottle. This water was stored under deep freezing at-18°C. An auger was used to take soil sediment samples from the surface down to a depth of about 15cm at various locations. These were stored in polythene bags and kept in the deep freezer pending analysis. Two pieces of fresh fish (*Oreochromis niloticus)* of different sizes (two samples of small size and big size each) were caught from the pond with the assistance of the pond attendant. Each fish was briefly washed with distilled de-ionised water to remove any adhering contamination and then drained under folds of filter paper. Fish from the pond was identified, weighed, wrapped in aluminium foil and deep-frozen at-18°C.

Sample treatment

Temperature was measured with a simple thermometer demarcated in °C and the pH of water determined with a pH meter (Kent EIL 7020, Kent Industrial Measurement Ltd., UK) before the water was stored at-18°C.

5cm^3 of concentrated hydrochloric acid was added to 250cm^3 of water samples and evaporated to 25cm^3. The concentrate was transferred to a 50cm^3 flask and diluted to the mark with distilled de-ionised water[12].

After air-drying, the soil sediments were sieved using 200mm mesh. 5g of the soil sediments were put into a 150cm^3 conical flask. 50cm^3 of 0.1M HCl was added and the flask was agitated on an orbital flask shaker (Gallenkamp) for two hours at 200 rev/min. The contents were filtered into 50cm^3 standard flask and made up to mark with 0.1M HCl. This extraction was used to determine heavy metals. 5g of soil sediments were put into another series of 150cm^3 conical flasks. Three extractions using 30cm^3 ammonium acetate solution each were carried out. The contents were filtered into a 100cm^3 standrd flask and made up to the mark[13]. The extraction here was used to determine the sodium and potassium in the soil samples.

The fish samples were given about two hours of defrosting before processing for analysis. After defrosting, the scales were removed, rinsed with de-ionised water and each fish separated into the head, eyes, gills, intestine, trunk muscle and scales. Different fish parts were dried at 105°C, blended and samples ranging from 0.8728g to 1.1577g were accurately weighed from digestion. The weighed fish samples were put in crucibles and ashed for three hours at a temperature of 540°C in the furnace (NEY.M – 525). The crucibles were then cooled to room temperature in a dessicator. Each ash sample was transferred to 50cm^3 beaker, crucible washed with 25cm^3 of 10% (v/v) nitric acid solution and then added to the ash sample in breaker and heated to boiling to break the ash. Each solution was carefully filtered into a 50cm^3 standard flask and made up to mark with distilled deionised water[14,15].

The concentrations of cobalt, copper, zinc, lead, manganese and iron in the three matrices were determined with an atomic absorption spectrophotometer (Perkin Elmer Model 306)[16]. Those of sodium and potassium were determined with a flame photometer (Corning Model 405)[16]. Earlier, the detection limits of the metals had been determined using the methods of Varian Techtron[15] with the following values in µg/cm^3: Cu (0.002), Mn (0.002), K (0.005), Na (0.002), Zn (0.002), Co (0.10), Fe (0.03) and Pb (0.02). All detection limits reported are for aqueous solution. The optimum analytical range was 0.1 to 0.5 absorbance units with a coefficient of variation of 0.87% to 2.20%. All chemicals used were analytical reagents grade obtained from British Drug Houses.

Based on the whole fish samples, they were labelled as bigger sized fish (BSF) and smaller sized fish (SSF).

All data generated were analysed statiscally[17]. From the average values obtained for metals in the pond water, concentration factors were calculated[18]. The metal concentrations in BSF and SSF were also subjected to the analysis of variance at p<0.05 significance level.

RESULTS AND DISCUSSION

The size distribution of the whole fish samples for this study is shown in Table 1. All the parameters considered like weight, length and trunk diameter varied among the fish samples. Weights varied between 85.60g to 54.0g, length varied between 16.50cm to 14.00cm, while the trunk muscle diameter varied between 6.00cm to 5.00cm. The coefficient of variation for weight is 22.64%, for length it is 8.40% while it is 9.0% for trunk diameter. The weight, length and diameter differences were significant at p<0.05. The fish samples were of the same female sex. The age of the SSF was estimated at about twelve months while the BSF was estimated to be about eighteen months.

The levels of the various metals and their distribution in the SSF are shown in Table 2. Both

Table 1: The size distribution of the samples available for study[a]

Description	Weight (g)	Length (cm)	Trunk diameter (cm)
Bigger sized fish (BSF)	85.60(1.10)[b]	16.50 (0.85)	6.00 (0.40)
Smaller sized fish (SSF)	54.00 (1.00)	14.00 (0.50)	5.00 (0.50)
Mean	69.80	15.25	5.50
±SD	15.80	1.50	0.50
CV (%)	22.64	8.20	9.09

SD = Standard deviation: CV = Coefficient of variation.

[a]Values significant at p <0.05.

[b]Values in parentheses are standard deviations.

Table 2: Distribution of various metals in the smaller sized fish (SSF) organs of *Oreochromis niloticus* (mg/kg dry weight)

Parameter	Co	Zn	Pb	Mn	Cu	Fe	Na	K
Scales	ND	1.38	ND	0.50	ND	2.97	122.80	113.73
Gills	ND	0.27	ND	0.96	ND	3.39	198.10	12.75
Intestine	ND	0.55	ND	2.50	ND	15.93	63.16	66.89
Eyes	ND	0.49	ND	0.08	ND	1.27	541.32	19.61
Head	ND	1.39	ND	1.31	ND	10.68	392.98	11.11
Trunk muscle	ND	1.36	ND	1.00	ND	32.89	508.77	372.55
Mean	-	0.91	-	1.06	-	11.19	304.52	99.44
±SD	-	0.52	-	0.83	-	12.00	204.07	139.67
CV (%)	-	57.72	-	78.19	-	107.29	67.01	140.46

*Determinations were in triplicate.

ND = Not detected.

cobalt, lead and copper were not detected in any part of the SSF. Zinc was mostly concentrated in the head, scales and trunk in that order, the gills had the lowest value of 0.27 mg/kg. The overall mean for zinc in the SSF is 0.91±0.52 mg/kg and a coefficient of variation of 57.72%. Manganese was mostly concentrated in the intestine and least concentrated in the eyes, head and muscle trunk of SSF while potassium was mostly concentrated in the trunk muscle. Sodium was generally more concentrated in the various parts of SSF than potassium but potassium was unevenly distributed than sodium: while CV is 140.40% in potassium, it is 67.01% in sodium.

The metal concentrations and their distribution in the BSF are shown in Table 3. As observed in SSF, cobalt and copper were not detected in BSF. Lead was found in some organs of the BSF; the organs were gills, eyes and trunk muscle. The trunk lead concentration was 0.85mg/kg. Zinc values in the BSF were generally higher when compared with corresponding organs in the SSF. Zinc mean value in the BSF is 2.23± 1.14mg/kg with a CV of 51.32%. Manganese was not detected in the eyes of BSF but where detected, it is of higher concentration in comparison with the corresponding organs in the SSF. Because of the non detection of manganese in the eyes of BSF, the CV of manganese in the BSF is high with a value of 96.42%. Iron concentration was also high in the BSF particularly in the gills, intestine and the trunk muscle. Mean value of iron in the BSF is 16.37±16.07 mg/kg but a CV of 98.22% which is lower than 107.29% in the SSF showing that iron was less unevenly distributed in the BSF than in the SSF. Like the situation in the SSF, both sodium and potassium were highly concentrated in the BSF. As shown by the CV of both sodium and potassium in the BSF, both metals were unevenly distributed in the BSF than in the SSF. The trunk muscles in the BSF contained the highest concentrations of sodium and potassium.

Table 3: Distribution of various metals in the bigger sized fish (BSF) organs of *Oreochromis niloticus* (mg/kg dry weight)

Parameter	Co	Zn	Pb	Mn	Cu	Fe	Na	K
Scales	ND	2.14	ND	0.58	ND	2.85	105.26	35.26
Gills	ND	3.00	1.90	0.92	ND	20.43	57.14	43.14
Intestine	ND	1.36	ND	0.27	ND	32.90	484.21	45.10
Eyes	ND	0.72	0.16	ND	ND	0.43	81.58	5.88
Head	ND	2.21	ND	1.60	ND	4.57	312.50	71.08
Trunk muscle	ND	3.93	0.95	2.58	ND	37.01	635.09	494.12
Mean	-	2.23	0.50	0.99	-	16.37	279.30	115.76
±SD	-	1.14	0.71	0.96	-	16.07	240.17	186.53
CV (%)	-	51.32	141.61	96.42	-	98.22	85.99	161.13

*Determinations were in triplicate.

It is well known[19-22] that mineral elements are necessary for life. Iron's major role is in the formation of haemoglobin, cobalt (II) is a component of vitamin B_{12} (cyanocobalamin) which is essential for the prevention of anaemia, and copper and zinc are essential metals and play important roles in enzyme activities[23]. Both lead and cadmium are toxic even at very low concentrations and have no known function in biochemical processes. Sources of lead include storage batteries, ammunition and type metal, cable sheaths, solder, pigments and anti-knock compounds in petrol[24]. The onset of lead pollution of surface water in Nigeria had been reported[25], the source being the use of leaded gasoline. Lead is known to inhibit active transport mechanisms involving ATP, to depress the activity of the enzyme cholinesterase, to suppress celluar oxidation-reduction reactions, and to inhibit protein synthesis[26]. Virtually, all metals are toxic if the exposure is sufficiently high to exceed the tolerance level[20,23].

Meats and fish are excellent sources of some of the minerals, such as iron, copper, zinc and manganese, and play important role in the prevention of zinc deficiency and particularly of iron deficiency which is widespread[27]. Half of the iron in meat and fish is present as haeme iron (in haemoglobin). This is well absorbed, about 15- 35%, a figure that can be contrasted with other forms of iron, such as that from plant food, at 1-10%[27]. Not only is the iron of fish well absorbed but it enhances the absorption of iron from other sources, for example, the addition of fish to a legume/cereal diet can double the amount of iron absorbed and so contributes significantly to the prevention of anaemia, which is so wide-spread in developing countries like Nigeria[27,28]. Iron facilitates the oxidation of carbohydrates, proteins and fats. Zinc is present in all tissues of the body and is a component of more that 50 enzymes[27]. Zinc dietary deficiency has been found in adolescent boys in the Middle East eating a poor diet based largely on unleavened bread[27].

Families and individuals who may be using vegetable and cereal sources of protein may not be able to meet the zinc allowance per day. The zinc in these sources is not as available as animal sources[29]. Manganese functions as an essential constituent for bone structure, for reproduction and for normal functioning of the nervous system, it is

Table 4: Mean* concentration of metals in stock water and sediments (mg/kg)

Parameter	Co	Zn	Pb	Mn	Cu	Fe	Na	K
Water	ND	0.01	ND	0.04	ND	ND	4.95	0.04
Sediments	ND	0.41	ND	6.43	ND	78.95	6.67	1.56
Mean	-	0.08	-	3.24	-	39.48	5.81	0.80
±SD	-	0.07	-	3.20	-	39.47	1.36	0.76
CV (%)	-	87.50 -	98.80	-	99.97	23.41	95.00	

*Determinations were in triplicate.

Table 5: Bioconcentration factors* of the various metals in the smaller sized fish (SSF) organs

Parameter	Co	Zn	Pb	Mn	Cu	Fe	Na	K
Scales	-	138.00	-	12.50	-	-	24.81	2843.25
Gills	-	27.00	-	24.00	-	-	40.02	318.75
Intestine	-	55.00	-	62.50	-	-	12.76	1672.25
Eyes	-	49.00	-	2.00	-	-	109.36	490.25
Head	-	139.00	-	32.75	-	-	79.39	277.75
Trunk muscle	-	136.00	-	25.00	-	-	102.78	9313.75
Mean	-	90.67	-	26.46	-	-	61.52	2486.00
±SD	-	52.33	-	20.69	-	-	41.23	3491.83
CV (%)	-	57.72	-	78.19	-	-	67.01	140.46

*Ratio concentration in fish parts (ìg/g to concentration in water mg/1).

also a part of the enzyme system. Meat and poultry products contribute a little of this micromineral[30]; this observation agrees with this report. The fish samples are good sources of sodium and potassium. Potassium is primarily an intracellular cation, in large part this cation is bound to protein and with sodium influences osmotic pressure and contributes to normal pH equilibrium[31].

All the metals detected in the fish parts are more concentrated in the trunk muscle than any part in the BSF. This pattern of metal concentration is not observed in the SSF except for iron and potassium (Tables 2 and 3). The metal concentration in the BSF may be an evidence of gradual accumulation of metals in fish as a fish ages. In fish, parts normally consumed mostly are the trunk muscles, although the head is now becoming a delicacy[32]. This means the bigger the fish, the likely is its ability to supply the human requirements of zinc, manganese, iron, sodium and potassium. The result of this study agrees with the results of Olaleye and Akintunde[8] who concluded that the factors which affect the quantity of mineral elements in the muscle tissue could be summarised as the size, state of maturity, sex and body location from where the samples were taken. The variable accumulation and fall in concentration of the various elements recorded in the muscle of fish is in agreement with the result of Thurston[33] and Love[34] who attributed such variation to changes in fish physiology associated with maturation processes and spawning. Other factors which might have affected the concentration of the elements in the various regions of the fish include the distribution of the dark and white muscle fibres each of which has different sizes[35] and which contained different amounts of different elements[34].

The metal concentrations in the stock water and the associated soil sediments are shown in Table 4. Cobalt, lead and copper were not detected in both water and soil sediments. Iron was not detected in the water but highly concentrated in the soil sediments. The concentrations of zinc, manganese, sodium and potassium were relatively low in the stock water. Comparing the analytical data for the sediment and fish with those of water, the concentration of most of the metals were several orders of magnitude greater than their corresponding values in water (Tables 5 and 6). Similarly the metal concentrations in the sediments were much lower than their corresponding values in the fish. These observations are in agreement with the results of Ipinmoroti and Oshodi[9], Adeyeye[10] and Okoye *et al.*[32]. In particular the relatively high values of potassium, zinc, sodium and manganese (in that order) in the fish would suggest that the fish is capable of concentrating these metals in their body from the aquatic environment. This is the case of bio-accumulation which though may be beneficial if the metal is essential but can be dangerous in case of toxic metals. Although lead was not detected in the water, soil sediments, SSF and some parts of BSF,

Table 6: Bioconcentration factors of the various metals in the bigger sized fish (BSF) organs

Parameter	Co	Zn	Pb	Mn	Cu	Fe	Na	K
Scales	-	214.00	-	14.50	-	-	21.26	881.50
Gills	-	300.00	-	23.00	-	-	11.54	1078.50
Intestine	-	136.00	-	6.75	-	-	97.82	1127.50
Eyes	-	72.00	-	-	-	-	16.48	147.00
Head	-	221.00	-	40.00	-	-	63.13	1777.00
Trunk muscle	-	393.00	-	64.50	-	-	128.30	12353.00
Mean	-	222.67	-	24.79	-	-	56.42	2894.08
±SD	-	114.27	-	23.91	-	-	48.52	4663.33
CV (%)	-	51.32	-	96.42	-	-	86.00	161.13

Table 7A: Size variation as it affects metal concentration in the fish organs

Metal	Parameter														
	Scales[a]					Gills[b]					Intestine[c]				
	SSF*	BSF*	Mean	±SD	CV(%)	SSF	BSF	Mean	±SD	CV(%)	SSF	BSF	Mean	±SD	CV(%)
Co	ND	ND	-	-	-	ND	ND	-	-	-	ND	ND	-	-	-
Zn	1.38	2.14	1.76	0.54	30.68	0.27	3.00	1.64	1.93	117.68	0.55	1.36	0.96	0.57	59.38
Pb	ND	ND	-	-	-	ND	1.90	0.95	1.34	141.05	ND	ND	-	-	-
Mn	0.50	0.58	0.54	0.06	11.11	0.96	0.92	0.94	0.03	3.19	2.50	0.27	1.39	1.57	112.95
Cu	ND	ND		-	-	-	ND	ND	-	-	-	ND	ND	-	-
Fe	2.97	2.85	2.91	0.08	2.75	3.39	20.43	11.91	12.05	101.18	15.93	32.90	24.42	11.99	49.10
Na	122.80	105.26	114.03	12.40	10.87	198.10	57.14	127.62	99.67	78.10	63.16	484.21	273.69	297.73	108.78
K	113.73	35.26	74.50	55.49	74.48	12.75	43.14	27.95	21.49	76.89	66.89	45.10	56.00	15.41	27.52

a,b,c are significant at $p<0.05$. *See Table 1.

Table 7B: Size variation as it affects metal concentration in the fish organs

Metal	Parameter														
	Eyes[d]					Heads[e]					Trunk Muscle[f]				
	SSF*	BSF*	Mean	±SD	CV(%)	SSF	BSF	Mean	±SD	CV(%)	SSF	BSF	Mean	±SD	CV(%)
Co	ND	ND	-	-	-	ND	ND	-	-	-	ND	ND	-	-	-
Zn	0.49	0.72	0.61	0.16	26.23	1.39	2.21	1.80	0.58	32.22	1.36	3.93	2.65	1.82	46.31
Pb	ND	0.16	0.08	0.11	137.50	ND	ND	-	-	-	ND	0.95	0.48	0.67	85.90
Mn	0.08	ND	0.04	0.06	150.00	1.31	1.60	1.46	0.21	13.13	1.00	2.58	1.79	1.12	62.57
CU	ND	ND	-	-	-	ND	ND	-	-	-	ND	ND	ND	-	-
Fe	1.27	0.43	0.85	0.59	69.41	10.68	4.57	7.63	4.32	56.62	11.19	37.01	24.01	18.26	76.05
Na	541.32	81.58	311.45	325.09	104.38	392.98	312.50	352.74	56.91	16.13	508.77	635.09	571.93	89.32	15.62
K	19.61	5.88	12.75	9.71	76.16	11.11	71.08	41.10	42.41	103.91	372.55	494.12	433.34	85.96	19.84

d, e, f are significant at $p<0.05$.

it was detected in the gills, eyes and trunk muscle of the BSF thus suggesting that the use of fish to assess the level of water pollution from such bio-accumulated metals may be misleading if not carefully interpreted. This same observation has been made fir cadmium and copper by Ipinmoroti and Oshodi[9]. The level of lead concentration in the BSF trunk muscle is lower than the maximum permissible level (MPL) for metals in fish muscle set by the US Food and Drug Administration[6] which is 2.0mg/kg wet weight for lead. When it is realised that the determination is on dry weight basis then the present report is still within the safe level.

Table 8: Summary of metal concentration (on average basis) in the fish samples

Observation	Inference
Total number of metals determined	Eight (8)
Metals better concentrated in SSF	Mn, Na
% metals better concentrated in SSF	25%
Metals better concentrated in BSF	Zn, Pb, Fe, K
% metals better concentrated in BSF	50%
Metals not detected in both SSF and BSF	Cu, Co
% metals not detected in both SSF and BSF	25%

The size variation as it affects the metal concentration and distribution in the fish organs pairwise is shown in Table 7. An examination of the metal concentration can be done at a glance in this Table. In the majority of the fish organs, the pairwise comparison showed that most of the metals were more concentrated in the BSF than in the SSF. This observation is also depicted in the CV which is more than 50% in most cases. The analysis of variance showed that significant differences occurred in the metal concentrations between the SSF and the BSF in the scales, gills, intestine, eyes, heads and trunk muscles at a significant level of $p<0.05$.

CONCLUSION

In a survey carried out by Adeniyi[1] he reported that when consumers were asked to indicate the sources of animal protein they preferred most, 60.2% of them indicated that they preferred fish to any other sources of animal protein. Fish have thus become the major source of animal protein to the average Nigeria citizenry. There is a reasonable level of the beneficial metals (zinc, iron, manganese, sodium and potassium) in the fish samples studied and the metal concentrations appear enhanced by the bigger size of the fish. A summary of the various concentrations in SSF and BSF is shown in Table 8.

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