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Comparative Assessment of Heavy Metals in Orechromis NiloticusTilipia (From the Michael Okpara University of Agriculture Umudike) Fresh Water Fish Pond in Abia State with Those From Uzere Fresh Water Pond in Delta State of Nigeria

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Abstract: The nutritional value and heavy metals Zn, Fe, Mn, Pb, Pb, Hg and Cd in five different portions; head, scales, flesh, skeleton and internal organs of big size (10-20 cm) and small size (≤10cm) of oreochromis niloticus from the fish pond in Michael Okpara University of Agriculture Umudike (MOUAU) in Abia State and in Evicerated/descaled Orechromis niloticus from fresh water fish pond in Uzere Delata State were analyzed for comparism. Heavy metals concentrations were determined using UNICAM Atomic Absorption Spectrophotometer (AAS) while nutritional values were analyzed by standard methods. Results show detectible concentration in mg kg^{-1} dried wt of Zn (9.74-23.87), Mn (10.70-164.94), Fe (11.02-12.49), Cd (0.29-1.78) for the two *O. niloticus* from both locations, Hg and Pb were Below Detection Limit (BDL)for the University location while a range of Pb (7.07-7.39), Hg (147.00-200.16) for the Uzere location. The values for the Uzere location were generally higher than FDA/WHO safe limits while those for the University location were lower. On tissue accumulation the internal organs showed highest concentration (BDL-5.53) thereby reflecting their water concentration (BDL-0.6204 mg L^{-1}). A no significant variation in fat and water content was observed while protein, ash, carbohydrate and energy values varied significantly (p = t95). Fat and ash content increase with size. The toxicological implications of the result were discussed.

Key words: Orieochromus nilotucus, heavy metals, MOUAU, Uzere, FDA, WHO

INTRODUCTION

Fishes being one of the most ancient form of aquatic life, as a food item have been reported to have a two fold nutritional advantage of being able to provide high proportions of their dry weight as proteins of relatively good quality due to the presence of essential amino acids and also being easily digestible unlike those of beef and other livestock because of low collagen (Burk and Ezekeil, 1961). Pollution of aquatic environment from industrial, domestic and agricultural waste has exposed these important aquatic organisms to contaminants which not only endanger their lives (Horsefall and Spiff, 1999) but also eventually enter the food chain (Potter and Hotchkiss, 1995) leading to serious public health hazards (Owen and Pickering, 1994).

There have been reported cases of bio accumulation of trace elements Zn, Fe, Pb, Hg (Ovuru and Alfred, 2001) Pb, V and Ni, Cr (Okoye, 1991) in concentrations higher than the World Health Organization (WHO) standard for foods in Nigerian polluted fishes. The adverse effect of pollutants Pb, Hg and Cd are well documented. They do not have any useful biological function (Irish Sea Forum, 1995) and there is no level of exposure below which they are harmless (WHO, 1996a). They affect chemical synaptic transmission in the brain peripheral and central nervous systems (Winaham, 2002) Lead (Pb) in addition and causes hyperactivity in children, affecting their performance in attainment and ability test, even death (Owen and Pickering, 1994) and when remobilized in bones leads to impaired blood synthesis and hypertension (EDC, 2002) Mercury (Hg) disrupts the blood brain barrier leading to depression, irritability, blindness, paralysis and insanity and also disrupts the function of the liver and kidney 10. Cadmium (Cd) is extremely toxic and causes bone porosity and inhibition of bone repair and eventually immobility. Over load of iron (Fe) leads to cirrhosis and it deposition in the lungs, pancreas and heart (Carine and Lawrence, 1977). It is also associated with serious heart disease and tumor induction, pancreatic dysfunction and diabetics (Strause and Saetman, 2000; WHO, 1996b). The consumption of crude oil polluted fishes have been reported to induce cancer due to ingestion of carcinogenic polaromatic hydrocarbons (Nelson, 1979).

Fishes have been reported to feed extensively on different variaties of food and so it becomes important to control the concentration of likely pollutants in the

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aquatic habit so as to reduce to minimal their likely ingestion by fishes thereby rendering them unfit for human consumption. Well over 34 elements are known to affect fish and its nutritional values (Kakulu and Osibanjo, 1988; Kakulu *et al*., 1987). This research is centered on the MOUAU fresh water fish pond. Previous research on the MOUAU fresh water pond have been on water quality parameters (Nnorom, 1999) excluding heavy metals determination and on haematological parameters of some fish (Amadi, 1998; Alum, 1998).

However, because of the ponds economic /research utilities, its proximity to the University farm and the link between the University drainage system and the Umudike-Ehimiri stream, the source of water supply to this pond, this study is focused on comparing the fish specie Oreochromis niloticus (Tilapia) from the pond with those from fresh water pond in Uzere -a fishing/farming/crude oil producing community in Delta state as regards bio accumulation of heavy metals and their effect on the nutritional states of the fishes.

MATERIALS AND METHODS

Sample collection and preparation: *Oreochromis niloticus* tilapia used in this researech were collected from the Michael Okpara University of Agriculture (MOUAU) in Abia State fresh water fish pond. The same fish species were obtained from a natural fish pond in the Niger Delta region of Uzere in Delta State for comparism. Water samples were collected from the MOUAU fresh water fish pond both at the point of entry and at the center of pond. The samples were sorted and separated according to sizes into 2 different groups-Big (10-20 cm) and small (≤10 cm). The measurement was done by taking the length from the tip of the mouth to the caudal tail. The samples were washed with distilled water and frozen prior to analysis in the laboratory at 4°C to prevent autolytic post-mortem changes after rigor mortis.

Crude protein determination: This was determined by a modification of the Kjeidahl gunnings procedure for organic nitrogen (Nnorom, 1999). Moisture content was analyzed using the method reported by James and involves drying at 80°C by evaporation of known weight of the samples to a constant weight and the moisture content determined as loss in weight (FDA, 2002).

Fat content: The methods used was that of exhaustive soxchlet extraction for meat products using the non-polar organic solvent-petroleum ether (B.p = 40°C c-60°C) (Amadi, 1998).

Ash content: This was determined by the triplicate burning off of the volatile organic residues like CNS as their oxides from known weight (Zg) of the homogenized dried whole fish sample leaving behind the grayish as using the muffle furnace at 450°C (James, 1995).

Total carbohydrates: This was determined by subtracting the % fat (F), % Crude Protein (Cp) and % as content (A) from 100 (Onyeike *et al*., 2000) i.e., % Total carbohydrates = [100 – (F+Cp+A)]%.

Estimation of energy (caloric) value: This was obtained by multiplying the percentage Crude Protein (CP), Fat (F) and Total Carbohydrate (TC) with their respective appropriate and water factor of 4, 9 and 4/Kcal per 100 g wt of fish sample and adding up to obtain the calorific value.
i.e., Calorific value = (4Cp +9F + 4TC)Kcal/100g wt.

Trace/Heavy metals determination: The method used is that of non-ashing as reported by James (1995). This involves the digestion of known weight (2 g) of the dried homogenized samples with conc. HCl in a fume cupboard to break down the carbohydrate and proteins. The resultant mixture was then filtered after allowing to cool and diluting to 50 mL in a volumetric flask. The filtrate and water sample from MOUAU's pond were sent for analysis.

Statistical analysis: T-test statistics at p = t95 was used for the rejection or acceptance of the null hypothesis (Onyeike *et al*., 2000)

$$t_{cal} = \frac{X1 - X2}{\sqrt{S^2 P\left[\frac{1}{n_1} + \frac{1}{n_2}\right]}}$$

RESULTS

The concentration of heavy metals observed for the two sizes of *O. niloticus* from the two locations are shown in Table 1. The results show that Fe, Zn and Mn were present in the two sizes of *O. niloticus* form the two locations, being found in all tissues or portions of those form MOUAU fish pond. However, Pb and Hg were below detection levels in (all tissues/portion of) all sizes of *O. niloticus* from MOUAU, but were accumulated in all sizes of *O. niloticus* from Uzere with Hg having the highest value of 200, 1625 mg kg^{-1}. Cd, was also observed for tilapia from the two locations with highest value of 1.707 mg observed for those from Uzere, though sparsely distributed in some tissues of those from MOUAU with a range of 0.485 mg Kg^{-1}- 0.2290 mg Kg^{-1} for the two sizes

Table 1: Accumulation (in mg kg^{-1} dry weight) of determined heavy metals in tilapia (*Oreochromis niloticus*) from mouau and uzere fresh water fish ponds

Heavy metals Tissue/ parts	BIG (10-20cm) *O. niloticus*						Small (≤ 10 cm) *O. niloticus*					
	Mn	Zn	Fe	Cd	Hg	Pb	Mn	Zn	Fe	Cd	Hg	Pb
IM	16.565	19.9460	90.4450	0.0820	BDL	BDL	22.9255	23.5470	128.8400	BDL	BDL	BDL
SM	4.6775	4.0895	5.7795	BDL	BDL	BDL	10.1790	4.9085	1.5935	0.0820	BDL	BDL
BM	4.5105	3.2020	3.8450	0.1060	BDL	BDL	10.1790	4.9085	1.5935	0.0820	BDL	BDL
HM	3.1600	3.3295	3.1155	0.0485	BDL	BDL	6.34465	7.9500	4.680	0.2055	BDL	BDL
FM	3.070	3.2045	5.5340	0.1210	BDL	BDL	2.6810	2.2920	1.5170	BDL	BDL	BDL
TM	10.6975	9.7360	12.4945	0.2755	BDL	BDL	19.2045	15.1505	17.7925	0.2875	BDL	BDL
TU	164.9420	20.9496	11.0212	1.7821	147.0000	7.0684	38.0415	23.8694	12.3728	1.77073	200.1625	7.3860

All values except TM are results of AAS determination. The values for TM were obtained by adding up those of the BM, HM and FM

Key: IM = Internal organs of tilapia from MOUAU, SM = Scales of tilapia from MOUAU, BM = Bony/Skeletal tissues of tilapia from MOUAU, HM = Head of Tilapia from MOUAU, FM = Fleshy tissues of tilapia from MOUAU, TM = Calculated value of dried MOUAU, TU = Dried eviscerated/discalced tilapia from Uzere location, BDL = Below Detection Limit

Table 2: Concentration (MgL^{-1}) of determined heavy metals in mouau fresh water fish pond

Heavy metals Site of pond	Zn	Mn	Fe	Cd	Hg	Pb
I	0.0596	0.1229	0.2753	BDL	BDL	BDL
II	0.0580	0.1950	0.6204	BDL	BDL	BDL

All values are results of AAS determination, Key: Site I = Point of entry of water into pond, Site II = Center of pond, BDL = Below Detection Limits

Table 3: Nutritional composition of *Oreochromis niloticus* from mouau and uzere fresh water fish ponds

Heavy metals Size	Moisture content (% wet. Wt)	Crude Protein (% dried wet)	Fat (% dried wet)	Ash (% dried wet)	Total carbohydrates (% dried wet)	Energy value (% dried wet)
TMS	78.94±0.06	38.8±0.10	7.50±2.50	11.17±0.26	45.51±2.52	392.82±3.55
TUS	78.44±0.40	32.96±1.02	4.65± 0.14	26.15±0.95	36.24±1.40	318.65±1.74
TMB	72.33±0.67	31.75±0.25	17.50± 2.50	12.77±0.27	37.98±2.53	436.42±3.57
TUB	74.44±0.10	34.45±1.25	16.09± 0.58	27.85±0.86	21.61±1.62	369.05±2.13

Results are mean of triplicate±standard deviation with the exception of moisture content, which is on wet weight basis, other nutritional compositions are on dry weight basis, Key: TMS = Small Tilapia from MOUAU, TUS = Small tilapia from Uzere , TMB = Big tilapia from, MOUAU, TUB = Big Tilapia from Uzere

of *O. niloticus* from there. The concentration of Fe, Zn and Mn were highest in the internal organs; the order being Fe> Zn> Mn though their order seems to be reverse for the calculated values for the discalced/eviscerated whole fish where the order is Mn (19.2045 mg Kg^{-1}> Zn (19.1505 mg Kg^{-1})> Fe (7.7925 mg Kg^{-1}) i.e., for the small sizes and Fe (12.4945 mg Kg^{-1})> Mn (10.6975 mg Kg^{-1}) > Zn (9.736 mg Kg^{-1}) for big sizes. This later trend was laso observed for *O. niloticus* form Uzere where the order was more uniform i.e. Mn> Zn> Fe still decreasing with size with a range of 11.0212 mg Kg^{-1} -164.9420 mg Kg^{-1}, the highest value (164.9420 mg Kg^{-1}) being recorded for Mn for the big size. The results of the freshwater analysis of the MOUAU pond in Table 2 shows the occurrence of the heavy metals; Fe, Mn, Zn in the order: Fe (0.6204 mg L^{-1})> Mn (0.1950 mg L^{-1})> Zn (0.0580 mg L^{-1}) while Cd, Hg and Pb were below detection levels. Table 3 shows, respectively the results of the proximate (nutritional) analysis for *O. niloticus* from MOUAU and Uzere. The results from the two locations shows an increase in fat content (the difference between them not being significant at 95% confidence level) with age with a resultant decrease in moisture content. However, carbohydrates content seems to decrease with age for *O. niloticus* from the two locations. Significantly ($P = t_{.95}$) higher as content (27.85±0.86 and 26.15±0.95) with a concomitant decrease in the crude protein content and calorific value were observed for *O. niloticus* from Uzere-Delta state compared to the ones form MOUAU pond.

DISCUSSION

Zn, Mn and Fe have been reported to be essential in fishes and other organism (Reuter, 1995). This probably explains their occurrence in all sizes of *O. niloticus* from the two locations. Also their occurrence n the water also explains this since bio accumulation of metals in fishes have been associated with occurrence probably due to pollution in the aquatic habitat (Kakulu and Osibanjo 1988, 1992; Kakulu *et al*., 1987; Odukoye and Arayi, 1983). Similar reason also accounts for the non-occurrence of Pb and Hg and comparatively lower value for Cd, observed for *O. niloticus* from MOUAU and also the comparative higher value of Hg> Pb> Cd observed for *O. niloticus* from uzere. Occurrence of heavy metals Hg, Pb, Cd, Ni, Cr etc have been reported in aquatic organism from the Niger-Delta region (Odu Koye and Arayi, 1983; Nnorom, 1999; Kakulu *et al*., 1987; Kakulu and Osibanjo, 1992;

Ovuru and Alfred, 2001). These have been associated with crude oil pollution. Fresh water fishes been reported to accumulate Hg well above 10 mg kg^{-1} (Reuter, 1995).

The occurrence of Cd in some tissues, despite its BDL value in the freshwater for *O. niloticus* from MOUAU could be ascribed to the fact that certain metals uptake have been associated with the geochemical (sediments etc) and dietary composition (Rueter, 1995). Pasternak had earlier noted that the degree of contamination of aquatic environment with metals cannot always be precisely evaluated on the basis of their water content as part of them are distributed with suspended matter and accumulated in bottom sediments (Pastermark, 1974). This accounts for fish in some aquatic environments, having higher metals levels than the water sample (Udiodiong, 1983). Occurrences of Cd have been associated with untreated organic sewages or waste applied to farmlands (FDA, 2002; Ademoroti, 1996). The preponderance of Cd in the bony/skeletal tissues and freshly tissues (for MOUAU) could be associated with Cd^{2+} competing with Ca^{2+} for active binding site in bones and Zn^{2+} for active binding sites in metallopretin and enzyme thus interfering with protein synthesis (Bottino *et al*., 1980). The relatively higher fat content for *O. niloticus* from the 2 locations compared to values of 7.03±0.21 reported for unpolluted aquatic environment (Onyeike *et al*., 2000) could be associated with Agricultural activities and crude oil pollution (for *O. niloticus* from Uzere-Delat State). Increase in fat content have been associated with pesticides leached from farm lands, an Polychlorinated Biphenyls (PCB) (from crude oil) accumulated in adipose tissues which increase the activity of Mixed Function Oxidase (MFO) with the resultant increase in lipogenesis (Payne and Penrose, 1975; Prottey, 1976; Aveeva and Muronova, 1981). Seasonal fluctuations have also been associated with fat content in fishes (Bottino *et al*., 1980). The higher value recorded for bigger sizes could be associated with increase accumulation of fat with age (Bottino *et al*., 1980). The high ash content values (26.15±0.95-27.85±0.86) obtained for the two locations were higher than those reported earlier (11.39±0.4) for unpolluted aquatic environment (Onyeike *et al*., 2000) and could be ascribed to heavy metals uptake from the aquatic environment (Vinogradov *et al*., 1953). The value was even much higher for *O. niloticus* from crude-oil producing community of Uzere. This was reflected on the reduced crude protein content (34.45±1.25-38.8±0.10]% in contrast with the range of 50-55% for tilapia (Onyeike *et al*., 2000). However, the value incidentally falls within the range of 30.0±3.2-54.0±5.8 recorded for members of the Cichlidae family (Ukoka and Olatunde, 1988). The occurrence of Cd probably gives raise to this because of its reported interference with protein synthesis (Aveeva and Muronova, 1981). Starvation and gonad maturity have been reported to reduce protein content in fishes (Onyeike *et al*., 2000). The relatively higher total carbohydrates content could be associated with reduced crude protein value. Increased glucose content in fishes has been ascribed to stress probably due to increasing heavy metal load, salinity or acidity (Garry and Williams, 1997). Similar reason of reduction in crude protein could be associated with the observed variations in the calorific values (318.65±1.74-369.05±7.13) obtained for *O. niloticus* from Uzere when compared with those (392.82±3.55- 436.42±3.57) from MOUAU.

CONCLUSION

The bioaccumulation of heavy metals in fish tissues increases with their increased occurrence in the aquatic environment whereas all tissues are susceptible to heavy metals. Pollution in the aquatic environment the degree of susceptibility is of order internal organs> Scale > skeleton> Head>Flesh.

There is need to maintain the low level of Cd observed in MOUAU fish pond and its *O. niloticus* which is below the safe limit of 0.5ppm FDA (2002). Precautions should therefore be taken to avoid overshooting this safe limit with time from the increasing use of fertilizers and untreated animal dung's from the livestock farm as source of organic manure by the practical year students of the University.

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