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Heavy metals in fish species from lotic freshwater ecosystem at Afikpo, Nigeria

C.D. Nwani*[1,2], D.A.Nwachi[2], O.I. Okogwu[2], E.F. Ude[3] and G.E. Odoh[4]

[1]National Bureau of Fish Genetic Resources Canal Ring Road, P.O. Dilkusha, Lucknow - 226 002, India
[2]Applied Biology Department, [3]Department of Fisheries and Aquaculture,
Ebonyi State University P.M.B.053 Abakaliki, Ebonyi State, Nigeria
[4]Fisheries and Hydrobiology Research Unit, Department of Zoology, University of Nigeria, Nsukka, Nigeria

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Abstract: *A study was conducted between March 2006 and February 2007 to assess the concentration of Fe, Zn, Cu, Mn, Pb, and Cr in the gills and muscles of six fish species (Chrysichthys nigrodigitatus, Clarias anguillaris, Tillapia zillii, Mormyrus rume rume, Mormyrus macrophthalmus and Mormyrus tapirus) from lotic freshwater ecosystem at Afikpo South- East Nigeria using Atomic Absorption Spectrophotometer. In all the fish species studied the concentration of metals in the gills was significantly higher than that of the muscles (p<0.05). While the highest concentration (mg l^{-1}) of Fe (702.20±0.04), Zn (34.40±0.02), Cu (2.10±0.01), Mn (4.91±0.08) Pb (0.50±0.02) and Cr (1.12±0.07) were recorded in the gills of C. nigrodigitatus, that in the muscles were recorded in T. zillii (443.20±0.08, 23.30±0.06, 1.33±0.06, 3.09±0.02, 0.31±0.01 and 0.66±0.04 for Fe, Zn, Cu, Mn, Pb and Cr respectively). The lowest concentration of all the heavy metals in the gills was recorded in M. tapirus (309.00±0.07, 16.45±0.03, 0.92±0.04, 2.15±0.04, 0.21±0.01 and 0.50±0.06 mg l^{-1} for Fe, Zn, Cu Mn, Pb and Cr respectively) while the lowest in the muscles was recorded in C. anguillaris [Fe (186.00±0.07), Zn (14.20±0.08), Cu (0.56±0.03), Mn (1.30±0.02), Pb (0.10±0.01) and Cr (0.28±0.04)]. The order of heavy metals concentrations in both the gills and muscles was Fe>Zn>Mn>Cu>Cr>Pb. While the concentration of Zn, Cu and Pb both in the muscles and gills of all the six fish species studied were within the WHO and FEPA prescribed limits, that of Fe(except in the muscles), Mn and Cr were above the prescribed limits thus indicating contamination of the fish species by these metals. Fe has the highest bio-concentration factor (BCF) in both tissues while the least was Cu. Periodic monitoring of these metals in both fishes and river to ensure safety is advocated.*

Key words: *Heavy metals, Fish, Afikpo, Ecosystem, Nigeria*
PDF of full length paper is available online

Introduction

Fish constitutes an important and cheap source of animal protein to human beings and a large number of people depend on fish and fishing activities for their livelihood. Increasing human influences through heavy metal pollution have however led to the depletion of our fish resources and substantial reduction in the nutritive values (Srivastava and Srivastava, 2008). The danger of these heavy metals is their persistent nature as they remain in the biota for long period of time when they are released into the environment (Bolormaa *et al.*, 2006; Yoon *et al.*, 2008). As a result of these heavy metals pollution several endemic fish species have become threatened. Realizing this, concern for assessment of heavy metals in the tissues of fish species in most of our water bodies have increasingly been gaining ground throughout the world. Studies have also indicated that fish are able to accumulate and retain heavy metals from their environment and that accumulation of metals in tissues of fish is dependent upon exposure concentration and duration as well as other factors such as salinity, temperature hardness and metabolism of the animals (Cusimano *et al*., 1986; Heath, 1987; Allen, 1995; Karthikeyan *et al*., 2007). Pollutants enter the fish through five main routes: via food or non food particles, gills, oral consumption of water and skin. On absorption, the pollutant is carried in blood stream to either a storage point or to the liver for transportation or storage. Pollutants transformed in the liver may be stored there or excreted in bile or transported to other excretory organs such as gills, skin or kidneys for elimination or stored in fat which is an extra hepatic tissue (Heath, 1991; Nussey *et al*., 2000). Studies carried out in fishes have shown that heavy metals may have toxic effects, altering physiological activities and biochemical parameters both in tissues and blood (Hilmy *et al*., 1987; Olojo *et al*., 2005). Adeyeye *et al*. (1996) showed that the concentration of metals was a function of fish species and accumulate more in some fish tissues than others. Since the toxic effects of metals have been recognized, heavy metal levels in the tissues of aquatic animals are occasionally monitored to ensure that the level do not constitute health hazards to consumers.

Afikpo (Lat5° 45' N and Log8°01' E) is a metropolitan city and one of the fastest growing cities in South- East Nigeria. Onu-Asu, (Lat 5°56' N and Long 7°30' E) Oziza (Lat5°30' N and Long7°40' E) and Ndibe (Lat5°40' N and 7°35' E) are important fresh water systems in Afikpo with rich fisheries production. At present, increasing

* **Corresponding author:** didigwunwani@yahoo.com

numbers of factories are established in the area as well as farmers using pesticides and fertilizers in their farm. The factory effluents (treated and untreated), pesticides and fertilizers as well as wastes from the nearby markets and workshops may finally get discharged into the water bodies. Oziza and Ndibe are tourist hotspots where washing bathing and fishing takes place. Diverse amount of wastes from these activities and numerous quarry companies engaged in mining and crushing stones are also constantly discharged to these freshwater ecosystems and there is the possibility of buildup of heavy metals in aquatic organisms especially fishes. The present study is thus to assess the levels of Fe, Zn, Cu, Mn, Pb and Cr in the tissues (muscles and gills) of six fish species of the fresh water ecosystem at Afikpo with a view to understanding whether the concentration level of these metals constitute health hazards to consumers.

Materials and Methods

Samples of fish species (*Chrysichthys nigrodigitatus*, *Clarias anguillaris*, *Tillapia zillii, Mormyrus rume rume, Mormyrus macrophthalmus* and *Mormyrus tapirus*) were collected randomly from Onu-Asu, Oziza and Ndibe sampling sites of the Afikpo freshwater ecosystem between March 2006 and February 2007 using gill nets, cast nets, hook and line and locally made hoop-fyke traps and transported immediately to the laboratory for analysis. Water samples were also collected along with fish samples for analysis. A clean washed high quality corrosion resistant stainless knife was used to cut 1 g wet weight of the fish tissue (muscle) along the lateral line. The operculum was opened and the gills removed. After dissection, all the samples were labeled according to their species. The entire sample (gills and muscles) were separately dried in a laboratory oven at 175°C for 3 hr. The dried samples were each ground with laboratory ceramic mortar and pestle to powder and sieved with 2 mm sieve. After the grounding, the samples were heated at temperature of 45°C in a muffle furnace till the aroma of the sample disappeared. The powdered samples were digested according to Rump and Krist (1988) with little modification. Each sample was treated with 1ml 60% perchloric acid and 10 ml 40% hydrofluoric acid. The contents were reheated to dryness on a sand bath to approximately 180^{0}C. After cooling, 15 ml 10% hydrochloric acid was added and the mixture was heated in a close crucible to dryness. The digested sample was diluted with de-ionized distilled water appropriately and filtered using 0.5 micron filter membrane. All samples were analyzed (APHA, 2005) in triplicate for heavy metals in a Hatch Model DR 300 Spectra Atomic Absorption Spectrophotometer. Bio-concentration factor (BCF) defined as the net result of the absorption, distribution and elimination of a substance in any organism after exposure via water was calculated as the ratio of metal concentration in the organism to the metal concentration in the medium (Lau *et al*., 1998). Thus $BCF_{fish} = C_{fish} / C_{water}$ where C_{fish} is the metal concentration in fish in mg/kg, C_{water} is the metal concentration in water in mg l^{-1} and BCF_{fish} is the bio-concentration factor for the organism (fish). Statistical analysis of the results was carried out by analysis of variance (ANOVA) (Ozdmar, 1999). Means of significance were separated using Duncan's t-test ($p<0.05$).

Results and Discussion

The heavy metal concentration in the muscles and gills of six fish species of freshwater lotic ecosystem at Afikpo, South-East Nigeria is presented in Table 1. The concentration of Fe in the gills and muscles (mg l^{-1}) of the fish species studied varied from minimum of 309.00±0.07 and 186.00±0.07 in *M. tapirus* and *C. anguillaris* to maximum of 702.20±0.04 and 443.20±0.08 in *C. nigrodigitatus*

Table - 1: Heavy metal concentrations (mg l^{-1}) in the gills and muscles of fish species in fresh water ecosystem at Afikpo, Nigeria

Fish	Tissue	Fe	Zn	Cu	Mn	Pb	Cr
C. nigrodigitatus	Gills	702.20±0.04^{a}	34.40±0.02^{a}	2.10±0.01^{a}	4.91±0.08^{a}	0.50±0.02^{a}	1.12±0.07^{a}
	muscles	197.60±0.03^{a*}	7.75±0.03^{a*}	0.59±0.03^{a*}	1.38±0.04^{a*}	0.14±0.03^{a*}	0.32±0.03^{a*c*}
C. anguillaris	Gills	469.55±0.13^{b}	16.75±0.14^{b}	1.46±0.08^{bc}	3.42±0.07^{bd}	0.30±0.02^{bc}	0.77±0.05^{bc}
	muscles	186.00±0.07^{b*}	14.20±0.08^{b*e*}	0.56±0.03^{a*}	1.30±0.02^{a*}	0.10±0.01^{a*c*}	0.28±0.04^{a*}
T. zillii	Gills	634.70±0.11^{c}	29.50±0.13^{a}	1.90±0.04^{ab}	4.43±0.06^{a}	0.44±0.02^{ab}	1.01±0.02^{ab}
	muscles	443.20±0.08^{c*}	23.30±0.06^{c*}	1.33±0.06^{b*}	3.09±0.02^{b*}	0.31±0.01^{b*}	0.66±0.04^{b*}
M. rume rume	Gills	391.00±0.04^{d}	33.20±0.20^{a}	1.17±0.02^{cd}	2.72±0.07^{c}	0.23±0.03^{c}	0.62±0.04^{c}
	muscles	245.50±0.10^{d*}	8.60±0.04^{a*}	0.74±0.03^{c*}	1.72±0.03^{c*}	0.11±0.01^{a*}	0.40±0.02^{c*d*}
H. macrophthalmus	Gills	360.55±0.04^{e}	33.25±0.07^{a}	1.37±0.05^{cd}	3.18±0.03^{d}	0.30±0.05^{bc}	0.73±0.06^{bc}
	muscles	214.60±0.03^{e*}	11.20±0.01^{d*}	0.64±0.01^{a*c*}	1.50±0.03^{a*}	0.11±0.03^{a*}	0.34±0.02^{a*c*}
P. tapirus	Gills	309.00±0.07^{f}	16.45±0.03^{c}	0.92±0.04^{d}	2.15±0.04^{ce}	0.21±0.01^{c}	0.50±0.06^{c}
	muscles	296.80±0.03^{f*}	14.50±0.03^{e*}	0.89±0.03^{d*}	2.07±0.02^{d*}	0.20±0.02^{c*}	0.48±0.04^{d*}

Means with the same letters within a column are not significantly different at $p<0.05$

Table - 2: Heavy metal concentrations (mg l^{-1}) in fresh water ecosystem at Afikpo, Nigeria

Sample	Seasons	Fe	Zn	Cu	Mn	Pb	Cr
Water	Dry Season	2.710±0.50^{a}	1.930±0.50^{a}	1.350±0.04^{a}	0.490±0.06^{a}	0.050±0.08^{a}	0.062±0.03^{a}
	Wet season	2.410±0.30^{b}	1.38o±0.70^{b}	0.660±0.60^{b}	0.400±0.03^{b}	0.052±0.04^{a}	0.064±0.06^{a}

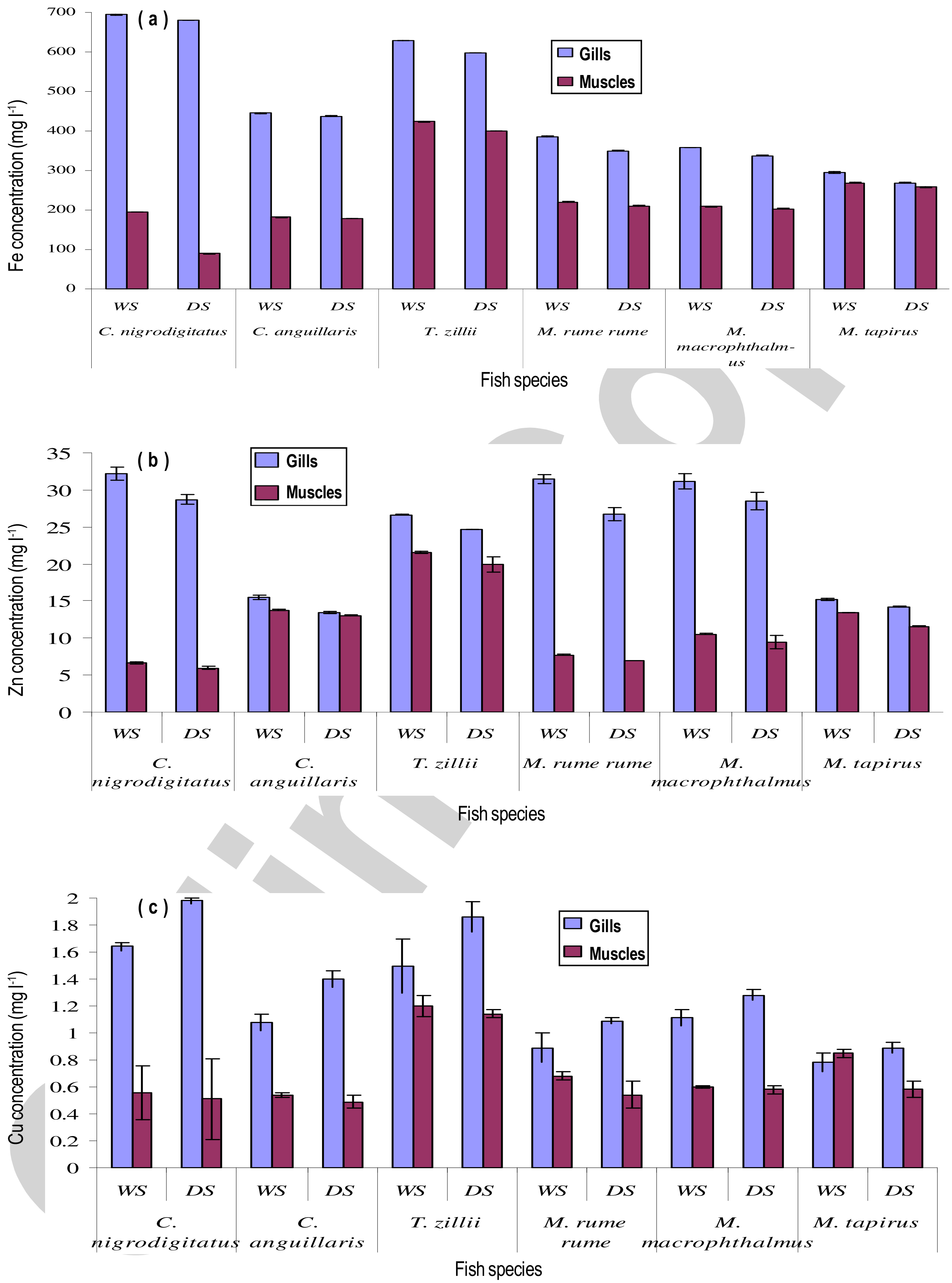
Means with the same letters within a column are not significantly different at $p<0.05$

Table - 3: Bioconcentration factors (BCF) in the gills and muscles of fish species in fresh water ecosystem at Afikpo, Nigeria

Fish	Tissue	Fe	Zn	Cu	Mn	Pb	Cr
C. nigrodigitatus	Gills	264.98±1.20^{a}	19.01±0.06^{a}	1.89±0.04^{a}	12.28±1.12^{a}	10.00±0.02^{a}	18.66±1.13^{a}
	muscles	74.56±0.40^{a*}	4.28±0.04^{a*}	0.53±0.05^{a*}	3.45±0.83^{a*}	2.80±0.03^{a*}	5.33±0.07^{a*}
C. anguillaris	Gills	177.19±0.80^{b}	9.25±0.14^{b}	1.32±0.07^{a}	8.55±0.09^{c}	6.00±0.04^{c}	12.82±0.12^{b}
	muscles	70.19±0.72^{a*}	7.86±0.54^{a*c*}	0.51±0.03^{a*}	3.25±0.06^{a*}	2.00±0.07^{a*}	4.67±0.03^{a*}
T. zillii	Gills	239.51±2.30^{c}	16.30±0.60^{c}	1.71±0.01^{a}	11.08±0.17^{a}	8.80±0.06^{ac}	16.83±1.12^{a}
	muscles	167.25±1.06^{b*}	12.87±1.00^{b*}	1.20±0.06^{b*}	7.73±0.07^{b*}	6.20±0.03^{b*}	11.00±0.83^{b*}
M.rume rume	Gills	147.55±2.40^{d}	18.34±0.03^{ac}	1.05±0.03^{b}	6.80±0.03^{c}	4.60±0.06^{c}	10.33±0.03^{b}
	muscles	92.64±0.70^{c*}	4.75±0.06^{a*}	0.67±1.02^{a*}	4.30±0.80^{a*}	2.20±0.12^{a*c*}	6.67±0.83^{a*}
H. macrophthalmus	Gills	136.06±3.20^{e}	18.37±0.06^{ac}	1.23±0.01^{ab}	7.95±1.16^{c}	6.00±0.08^{b}	12.17±1.12^{b}
	muscles	80.98±0.70^{a*}	6.19±0.30^{a*c*}	0.58±0.02^{a*}	3.75±0.79^{a*}	2.20±0.03^{a*}	6.81±0.93^{a*c*}
P. tapirus	Gills	116.60±0.80^{f}	9.09±1.01^{bd}	0.82±0.64^{c}	5.38±0.04^{b}	4.20±0.03^{b}	8.33±0.03^{c}
	muscles	138.05±1.10^{d*}	8.01±0.07^{c*}	0.80±0.42^{a*}	5.18±0.10^{c*}	4.00±0.70^{b*c*}	8.00±0.72^{c*}

Mean with the same letter within a column are not significantly different at $p<0.05$

and *T. zillii* respectively. While *C. nigrodigitatus* recorded the highest concentration of Fe in the gills among all the fish species studied, *T.zillii* recorded the highest concentration of Fe in the muscles. The mean values obtained for Fe in the muscles even though higher than any of the other metals in the muscles of the fish species studied were still within the 300 mg l^{-1} recommended limits for food fish by WHO (1994) and FEPA (2003) except in the muscles of *T. zillii*. The contrast was the case in the gills where the mean Fe concentration in all the fish species studied exceeded the 300 mg l^{-1} recommended values. High mean concentration of Zn was also observed in all the fish species studied and the mean concentration in the gills and muscles varied from minimum of 16.45±0.03 and 7.75±0.03 in *M. tapirus* and *C. nigrodigitatus* to maximum of 34.40±0.02 and 23.30±0.06 in *C. nigrodigitatus* and *T. zillii* respectively. The values obtained for Zn in both the gills and muscles were below the 75 mg l^{-1} limit prescribed for food fishes indicating that these fishes were free from Zn toxicity. The mean concentration of Cu in the gills of the fish species varies from a minimum of 0.92±0.01 in *M. tapirus* to a maximum of 2.10±0.01 in *C. nigrodigitatus*. In the muscles the mean Cu concentration varies from a minimum of 0.56±0.03 in *C. anguillaris* to a maximum of 1.33±0.06 in *T. zillii*. The mean values obtained for Cu in this study in both the gills and muscles were below the 3.0 and 1.0-3.0 mg l^{-1} prescribed limits for Cu in food fish respectively, thus indicating that the fishes examined were free from Cu related toxicity. The mean value obtained for Mn in both the gills and muscles varied from minimum of 2.15±0.04 and 1.30±0.02 in *M.tapirus* and *C.anguillaris* to maximum of 4.91±0.08 and 3.09±0.02 in *C. nigrodigitatus* and *T. zillii* respectively. The values obtained for Mn in both the gills and muscles in all the fish species in the present investigation were above the 0.50 mg l^{-1} recommended by WHO (1994) and FEPA (2003) for Mn in food fishes. This implies that all the fishes examined could pose manganese related health problems to consumers. The mean concentration of Pb in the gills and muscles of the six fish species studied varied from minimum of 0.21±0.01 and 0.10±0.01 in *M. tapirus* and *C. anguillaris* to a maximum value of 0.50±0.02 and 0.31±0.01 in *C. nigrodigitatus* and *T. zillii* respectively. The values obtained for Pb in both the gills and muscles were below the Pb prescribed standard safe limits of 2.0 mg l^{-1} for food fish. This implies that the fishes examined were free from Pb related toxicity. The values obtained for Pb in this report is in line with the report of Daka *et al*.(2008) who obtained 0.01-0.06 mg l^{-1} in fish species from Azuabie Creek in the Bonny Estuary, Nigeria. Our report is also in line with Oguzie (2003) who reported Pb concentration of 0.007-0.03 mg l^{-1} in fishes from Ikpoba River Nigeria but differed from Obasohan (2007) who reported Pb concentration of 2.67-7.33 mg l^{-1} in fishes from Ogba River Nigeria. The mean Cr concentration in both gills and muscles of the fish species indicate that Cr varied from minimum values of 0.50±0.06 and 0.28±0.04 in *M. tapirus* and *C. anguillaris* to maximum values of 1.12±0.07 and 0.66±0.04 in *C. nigrodigitatus* and *T. zillii* respectively. The values obtained for Cr in the gills and muscles in all the fish species studied were above the Cr recommended permissible value of 0.15 mg l^{-1}, thus indicating that these fishes may pose Cr related health hazard like cancer of the lungs and kidneys to the consumers (Jackson and Morris, 1989). While the highest concentration of all the heavy metals in the gills of the six fish species studied was recorded in *C. nigrodigitatus* that of the muscles was recorded in *T. zillii*. The lowest concentration of all the heavy metals in the gills was recorded in *M. tapirus* while the lowest in the muscles was recorded in *C. anguillaris*. The differences in the level of concentration of heavy metals in the fishes could be attributed to differences in their metabolic rates. Ademoroti (1996) had reported that different organisms have different metabolic rates and different food requirements and organism with high food intake tend to accumulate more metals. This may explain why the gills and muscles of *C. nigrodigitatus* and *T. zillii* respectively recorded the highest concentration of all the heavy metals while the least were recorded in the gills and muscles of *M. tapirus* and *C. anguillaris* respectively. In all the fish species studied the concentration of metals in the gills was significantly higher than that of the muscles ($p<0.05$). The gill tissues play an important role in ion regulation, gas exchange, acid balance, nitrogenous wastes and excretion which signifies the key role it play at the interface with the environment (Karthikeyan *et al*., 2007). This is in agreement with the report of Agrahari and Gopal (2007) who noted that the gills and liver showed highest accumulation of Pb and cadmium while the brain and kidney


(a)
Fe concentration (mg l-1)
700
600
500
400
300
200
100
0
Gills
Muscles
WS DS WS DS WS DS WS DS WS DS WS DS
C. nigrodigitatus
C. anguillaris
T. zillii
M. rume rume
M. macrophthalm-us
M. tapirus
Fish species
(b)
Zn concentration (mg l-1)
35
30
25
20
15
10
5
0
Gills
Muscles
C. nigrodigitatus
C. anguillaris
T. zillii
M. rume rume
M. macrophthalmus
M. tapirus
Fish species
(c)
Cu concentration (mg l-1)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Gills
Muscles
C. nigrodigitatus
C. anguillaris
T. zillii
M. rume rume
M. macrophthalmus
M. tapirus
Fish species

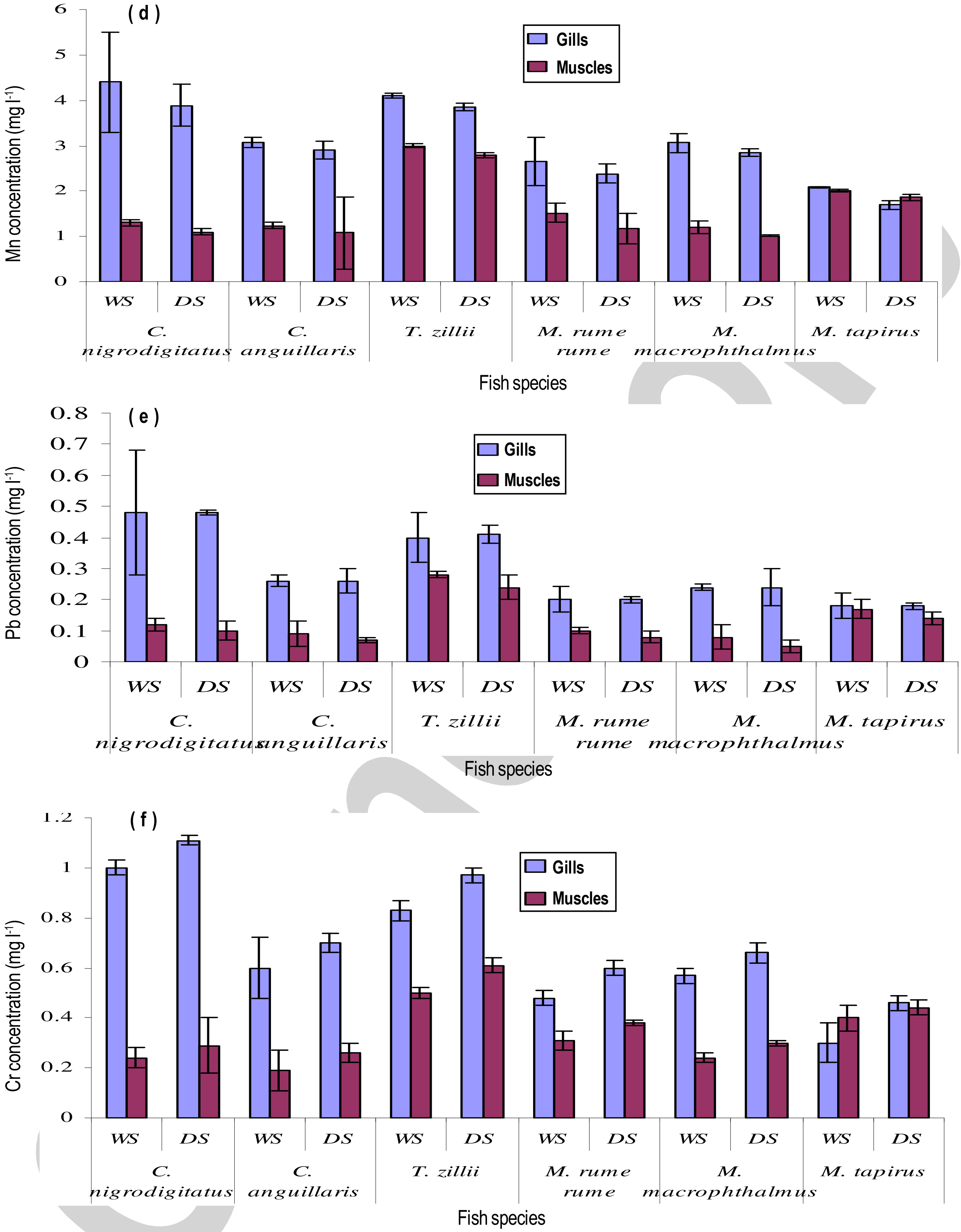


Fig. 1: Seasonal variation of metals (a = Fe, b = Zn, c = Cu, d = Mn, e = Pb, f = Cr) in gills and muscles cells in fish species at Afikpo, Nigeria (WS = Wet season and DS = Dry season)

had the lowest in *C. punctatus*. Our result however differed from the report of Olaifa *et al*. (2004) who recorded lower concentration of heavy metals in the gills and bones than muscles and intestines in *C.gariepinus* in lake and fish farm in Ibadan Nigeria. The concentration of the heavy metals in the gills and muscles of the fish species studied show seasonal variations (Fig. 1a-f). The concentration of Fe, Zn, Cu and Mn were significantly higher ($p<0.05$) in the wet than dry season in the gills and muscles in all the fish species. There was however no significance difference ($p>0.05$) in the concentration of Pb in both seasons in the gills but in the muscles Pb was significantly higher in the wet than dry season ($p<0.05$). Cr in the gills of the fish species studied also showed no significance seasonal difference but was significantly higher in the dry than wet season ($p<0.05$) in the muscles. The values obtained for the heavy metals in the tissues (gills and muscles) of the fish species studied far exceeded the values of the heavy metals in the Afikpo water system (Table 2) thus indicating biological concentration of these metals in the fishes. Canterford *et al*. (1978) reported that it is useful to express results of bioaccumulation in terms of biological concentration factor (BCF) when comparing the order of uptake of metals. In this study high BCF were obtained in all the fish species (Table 3) and were generally higher in the gills than muscles in all the six fish species studied. This is agreement with Kargin (1996) who noted that the gill, bile, skin and mucus are generally the elimination routes in fishes. Muscles are not active tissue regarding bioaccumulation when compared to the gills and liver (Bajc *et al*., 2005; Ekpo and Ibok, 1999; Filazi *et al*., 2003; Shukla *et al*., 2007). Laws (1981) had noted that differences in concentration of metals in different parts of an organism could be attributed to the tendency of metals to bind to various molecular groups found within the cells of organisms as well as the degree of exposure to metal as influenced by its metabolic characteristics and position in the food chain. This may explain why the gills in all the fish species have more concentration of the metals than the muscles. The order of heavy metal concentration in both gills and muscles in the six fish species studied was Fe>Zn>Cu>Mn>Cr>Pb. These heavy metals which form a high proportion of the industrial, municipal and domestic wastes are also found in large proportion in pesticides fungicides and fertilizers used in agriculture in the Afikpo area of South- East Nigeria. It is possible that metals from these substances along with those from human activities like sand mining and carwash near the water body may have been washed into the water system during the wet season thus accounting for their high proportion in the fishes examined during the wet season. Essien *et al*. (2009) had attributed the high wet season concentration of heavy metals in the sediments of Cross River Estuary Nigeria to intensive fishing activities, sewage drainage and industrial activities. The high dry season concentration of Cr recorded in the gills of the fish species may be attributed to more bioaccumulation of Cr arising from reduced water volume.

This study shows that while the concentration of Zn, Cu and Pb both in the muscles and gills of all the fish species were within the WHO (1994) and FEPA (2003) prescribed limits that of Fe (except in the muscles), Mn and Cr were beyond the limits. The BCF was highest in Fe but lowest in Cu both in the gills and muscles. These metals could pass to humans through the food chain and thus predispose the consumers to possible health hazards. Periodic monitoring of these and other heavy metals in both the fishes and river system to ensure continuous safety of people in the area is recommended. Safe disposal of domestic wastes and industrial effluents should be practiced and where possible recycled to avoid these metals and other contaminants from going into the environment. Further studies on the concentration of heavy metals in other fish tissues (brain, liver, kidney, intestines and heart) are recommended.

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