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Determination of Some Heavy Metal Content in Tilapia and Cat Fish Species in Lake Njuwa, Adamawa State, Nigeria

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ABSTRACT: concentration (mg/kg) of Lead (Pb), Chromium (Cr), Copper (Cu), Nickel (Ni) and Cadmium (Cd) were measured in various organs (such as gills, bones and muscles) of Tilapia and Catfish collected from Lake Njuwa Adamawa state during dry season (February, 2017) and wet season (August, 2017). The results obtained were compared to permissible limits set by World Health Organization, WHO (1985), Food and Agricultural organization, FOA (2003), to ascertain its health implications. Comparison between the heavy metals concentration of dry and wet season were carried out using Mann Whitney u test, the relationship between the heavy metal concentration and the weight of the fish was carried out using Pearson correlation test. All the analysis were determined at significant level of p>0.05 using Microsoft excel 2010 and statistical package for social science (SPSS) 2.3. The results obtained shows that the heavy metals are more concentrated in the bones (2.30, 2.50, 1.37, and 1.40) than the gills (2.17, 2.20, 1.19 and 1.23) and the muscles (0.5, 0.7, 0.99 and 1.03) of both the Tilapia and Catfish during the wet season than the dry season.

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Fish is widely accepted because of its high palatability, low cholesterol and tender flesh, it is the cheapest source of animal protein and other essential nutrients required in human diet (Saduku and Olademeli, 1991). Fish may be sold and/ or affordable source of animal protein for poor house hold in urban and semi urban areas (Bene and Heck, 2005). The pollution has mainly been caused by industrial processes and industrial waste, typically from rubber and oil palm mills (Tariq *et al*., 1996). Heavy metals may accumulate in aquatic species, enter the food chain and cause serious harm to human health when contamination content and exposure are significant (Goyer 1997; Papagiannis *et al*., 2004; Turkmen *et al*., 2005; Fenandes *et al*., 2007) consequently, they have been listed by the US environmental Agency (USEPA) based on their potential for human exposure and health risk (Birungi *et al*., 2007). The levels of heavy metal accumulation in fish depend on the growth rate, metabolism, feeding pattern and ecological requirements of a given fish species (Yilmal *et al*., 2005, 2010). Another factor is the difference in life history patterns among species (including tropic and geographical distribution of life stages) which influence their exposure to heavy metal (Allen – Gil and Martynov, 1995). The harmful effect of trace metals when consumed above the recommended limit can be toxic (acute, chronic or sub-chronic) and heavy metals can be neurotoxic, carcinogenic, mitagenic or teratogenic. The general symptoms of human related to metal poisoning include vomiting, convulsions, paralysis, ataxia, gastrointestinal disorder, diarrhea, stomatitis, depression and pneumonia (McCluggate, 1991).

Several techniques have been used for determination of metal concentrations in fish species such as flame atomic absorption spectrometry (Abedin, 1986; El-Mehdi, 1987; Ahmet, 1992; Petisleam *et al*., 2003; Petisleam *et al*., 2007), graphite furnace atomic absorption spectrometry (Sperling, 1988; Botson *et al*., 2004), electro-thermal atomic absorption spectrometry (Perez, 2001; Mendez, 2002), inductive coupled plasma (Chirila, 1999; Petisleam *et al*., 2005) and mass spectrometry (Sanchez, 2003; Petisleam *et al*., 2005) Different digestion methods were used as sample preparation methods for determination of heavy metals in fish samples (Olaifa *et al*., 2004). This study aimed to assess the concentration (mg/kg) of Pb, Cr, Cu, Ni and Cd in the bones, gills and muscles of Tilapia and Catfish collected from Lake Njiwa Adamawa state during dry season (February, 2017) and wet season (August, 2017). The result obtained were then compared to permissible limits set by World Health Organization, WHO (1985), Food and Agricultural Organization, FAO (2003) to confirm its health implications.

MATERIALS AND METHODS

Sites Description: The sampling area was Lake Njuwa in south Local Government Area of Adamawa state: it

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is located between 13^0S and 14^0S on longitude and 13^0W. It has a total square area of 56Km being located in the North east arid zone. The different species of fish were collected from the study site of the Lake by fisher men. The entire samples were collected based on availability and choice of consumption. The collected fish samples were properly labelled, packaged and transported to the laboratory for identification, preparation and analyses.

*Sample Preparation***:** Sampling was carried out during dry season (February, 2017) and wet season (August, 2017). Two sampling sections were selected along the Lake: Downstream and Upstream. Total of 20 individual samples were collected that is 10 during dry season (5 Catfish and 5 Tilapia fish) and 10 during wet season (5 Catfish and 5 Tilapia fish). (N = 5 fish nets × 1day × 2sampling station × seasons). All captured fish were labelled accordingly and placed in an ice chest before transported to Scientific and Basic Research Laboratory Department, National Research Institute for Chemical Technology, Basawa – Zaria, Kaduna state, Nigeria. In the laboratory, fish samples were identified to species on standard taxonomic keys (Mohsin and Ambak, 1991; Kottelate *et al*., 1993). The fish were also weighed and categorized based on the two species: The Tilapia and the Catfish.

Fish Preparation: Gills, Bones and Muscles tissues of the fish was used in this study. Fish tissues were cut and oven dry at 110^OC to a constant weight (Tuzen, 2003), after the oven dry the head of the fish was cut and the gills removed so also the fish was properly dissected to remove the existing required organ (bones) in the fish. The digestion was carried out based on the analytical methods for Atomic Absorbance Spectrometry. All the glass wares were thoroughly washed with distilled water, soaked in diluted nitric acid for 24 hours and then rinsed with distilled water. All the reagents used were of analytical grade obtained from Stevemor Chemical store, Zaria, Kaduna state, Nigeria.

*Ashing***:** 5 g of each of the organ samples were weighed and put into a platinum dish, then transferred in to muffle furnace, the temperature was raised to about 55^0C for 4-5 hours, after the sample has turned completely in to ash, it was removed and cooled in a desiccators

Digestion of Samples: The ash prepared samples (bones, gills and muscles) were put in to 50 ml beaker with 5 ml of HNO_3 and 5 ml of H_2SO_4. The beaker was then placed on a hot plate and heated at 60^OC for 30 minutes. After allowing the beaker to cool, 10 ml of HNO3 was added and returned to the hot plate to be heated slowly at 120^OC. The temperature was increased to 150^OC, and the beaker was removed from the hot plate when the sample turned black. The sample was then allowed to cool, adding H_2O_2 until the sample become clear. The content of the beaker was transferred in to 50 ml volumetric flask and diluted to the mark with distilled water. All the steps were performed in fume cupboard. The above procedures in this section followed the guidelines from the analytical methods for Atomic Absorption Spectrometry (Perkin Elmer, 1996).

*Analysis***:** Concentrations of the heavy metals (Cd, Ni, Cu, Cr, Pb) After digestion were analysed using Atomic Absorption Spectrometer (Shimazu- AA-t300). The results from the AAS were expressed as µg/g which was converted to mg/kg in the results obtained. All reagents used were of analytical grade.

*Statistical Analysis***:** The mean comparison of heavy metal concentrations among the fish species (Tilapia and Cat fish) collected in different season were carried out using Microsoft Excel and Statistical Package for Social Science, Mann-Whitney U-Test. The relationship between heavy metal concentrations among the fish species (Tilapia and Cat fish) collected in different season were carried out using Microsoft Excel: Regression and Correlation and Statistical Package for Social Science: Regression and Pearson Correlation. The relationship between the heavy metal concentrations and the weight of the fish were carried out by Pearson correlation test. All analysis was determined at significant level of $P>0.05$.

RESULTS AND DISCUSSION

A total of 20 fishes were caught, 10 catfish: 5 wet season, 5 dry season. 10 Tilapia: 5 wet season, 5dry season. In this study, however, the heavy metal permissible limits (levels) were compared with standard such as WHO (1985) and FAO (2003) to ascertain the level of toxicity of the heavy metals in the fish because it's consumed in the area. The table above shows the concentrations of chromium in the bones and muscles of Tilapia fish were not detected in both the seasons. Bones and gills have the highest concentration of Pb both in dry and wet season. In the dry season, bones (2.4 kg/ma), gills (2.19 kg/mg) and (2.5 kg/mg) bones, gills (2.20 kg/mg) which are all above the critical limits set by the EC (2001), WHO (1985) and FAO (2003) while the muscles both in the dry and wet season were within the permissible limits. Lead poisoning is a type of metal poisoning caused by lead in the body (WHO, 2016). Symptoms may include: abnormal pain, constipation, headaches, irritability, memory problem, inability to have children and tingling in the hands and feet (LIW, 2013). It causes about 10 % of intellectual disability of otherwise unknown causes and can result in behavioural problems (WHO, 2016). Some of the effects are permanent (WHO, 2016). In severe cases anaemia, seizure, coma or death may occur (LIW, 2013; WHO 2016) causes includes exposure of lead via contaminated air, water, dust, food, consumer products (WHO, 2016). Therefore, it's not advisable

to consume the gills and bones of the Tilapia fish obtained from Lake Njiwa Adamawa state due to the concentration of the lead above the standard permissible limits. The study found that the concentrations of Cr in the bones and muscles both in the dry and wet season were not detected, while the concentration of Cr in the gills was found to be above the critical limit set by WHO (1985). Several in vitro studies indicated that high concentrations of chromium in the cell can lead to DNA damage (Eastmond *et al*., 2008), therefore, it is not advisable taking the gills of the Tilapia fish from Lake Njiwa due to its health implications. In this study, heavy metal concentrations were found to be high during the wet season (August, 2017) see (Fig 1). Fish collected during the wet season had significantly higher concentration of Pb, Cr, Cd, Cu and Ni ($P < 0.05$) compared to the dry season (Table 3). The high concentrations of heavy metals during the wet seasons can be attributed to the increases in surface run – off. Heavy rainfall leads accumulations of materials from the farm to the water bodies that is during the run- off large amount of pesticides containing heavy metal compounds are brought to the surface via run off from the near river and highly contribute to agricultural pollution, especially chemical fertilizers containing the heavy metals (Ni, Pb, Cu, e.t.c) However, several previous studies found that mean heavy metal concentrations in fish were higher in the dry season (Fufeyin 1998; Idodo-Umeli 2000; Oguzie, 2003; Obasohen and Equavoen, 2008). This is because high concentrations of metal in fish during the dry season were due to high temperature, which increases the activity, ventilation, metabolic rate and feeding seasons (Nussey *et al*., 2000). The low heavy metal concentrations in the wet season were due to the dilution of metal level associated with heavy rains (Obasohan and Equavoen 2008)

Table 1: Heavy metal concentration (mg/kg) of Tilapia fish collected in Dry and Wet season (Feb., and Aug., 2017)

Samples	Pb	Cr	Cu	Ni	Cd
Bones(D)	2.30	ND	0.34	0.060	0.020
Gills(D)	2.17	0.70	0.39	0.050	0.040
Muscles(D)	0.50	ND	0.68	0.060	0.036
Bones(W)	2.50	ND	0.41	0.080	0.020
Gills(W)	2.20	0.72	0.49	0.070	0.070
Muscles(W)	0.70	ND	0.86	0.079	0.037
G.M	1.73	0.24	0.53	0.330	0.037
Permissible limes(WHO,1985)	2.00	0.05	2.00	0.5 – 0.6	2.000
Permissible limits(FOA,2003)	0.20	0.05	0.20	-	0.050

Note: *D=Dry season; W=Wet season; G.M = Mean of means; ND=Not detected*

Table 2: Heavy metal concentration (mg/kg) of cat fish collected in dry and wet seasons (Feb, and Aug, 2017)

Samples	Pb	Cr	Cu	Ni	Cd
Bones(D)	1.37	0.71	0.30	0.039	0.05
Gills(D)	1.19	0.77	0.40	0.38	0.09
Muscles(D)	0.99	0.69	0.50	0.01	0.04
Bones(W)	1.40	0.84	0.34	0.05	0.07
Gills(W)	1.23	0.86	0.49	0.05	0.19
Muscles(W)	1.03	0.82	0.54	0.03	0.06
G.M	1.20	0.78	0.43	0.04	0.08
Permissible limits (WHO,1985)	2.00	0.05	2.00	0.5-0.6	2.00
Permissible limits (FOA,2003)	0.20	0.05	0.20	-	0.05

Table 3: mean comparison of heavy metal concentrations among Tilapia fish collected in different seasons

S/N	Pb	Cr	Cu	Ni	Cd
D1	2.30	ND	0.340	0.060	0.020
D2	2.17	0.70	0.390	0.050	0.040
D3	0.50	ND	0.680	0.060	0.036
W1	2.50	ND	0.410	0.080	0.020
W2	2.20	0.72	0.490	0.070	0.070
W3	0.70	ND	0.860	0.079	0.037
P-value Excel	0.12	0.42	0.07	0.00028	0.4037
p-value SPSS	0.86	0.985	0.540	0.13	0.55

Table 4: mean comparison of heavy metal concentrations among Cat fish collected in different seasons

S/N	Pb	Cr	Cu	Ni	Cd
D1	1.37	0.71	0.30	0.039	0.05
D2	1.99	0.77	0.40	0.038	0.09
D3	0.99	0.69	0.50	0.010	0.04
W1	1.40	0.84	0.34	0.05	0.07
W2	1.23	0.86	0.49	0.05	0.19
W3	1.03	0.82	0.54	0.03	0.06
P-Value Ms-Excel	0.008163	0.0128	0.07688	0.037	0.222
p-value SPSS	0.823	0.12	0.534	0.285	0.353

Note: D = Dry season; Wet season

The table above shows that bones and gills of the catfish both in the wet and dry season has the highest concentrations of Pb but all the concentrations of the Pb were within the Permissible limits set by WHO (1985) and FOA (2003) testifying the safe for the consumption of the parts (Gills and the Bones including the muscles). Cr was found to be above the permissible limits set by WHO (1985) in which the bones has chromium concentration of 0.84 kg/mg, gills 0.86 mg/kg and muscles 0.84 kg/mg during the wet seasons, so also it concentration in the dry seasons were above the set permissible limits. Therefore, due to the health effect of chromium to human as result of it accumulation it is recommended not to eat the bones, muscles and the gills of the catfish caught from Lake Njiwa Adamawa state. the Cu has the highest value in the muscles of 0.5 mg/kg in the dry season and 0.54 kg/mg in the wet seasons including the other values all falls within the permissible limits set by the WHO (1985) both the Ni and the Cd concentration in the catfish during both dry and wet seasons were found to be within the permissible limits with bones and gills has the highest concentration of Ni 0.05 mg/kg each in the dry and wet season while the Cd has the highest concentration in the gills of 0.19 mg/kg.

The results also showed that the concentrations of the heavy metal were higher in the wet season compared to the dry season (Fig 2). Therefore, the fish collected during the wet season has significantly high concentration of heavy metal ($P < 0.05$) in comparison with the obtained during the dry season (Table 4). Table 3 shows the mean comparison of heavy metal concentrations among Tilapia fish collected in different season using Ms-Excel and SPSS, the p-value of pb, cr, cu, cd with the exception of Ni which are greater than the alpha value 0.05 implies that there is no significant difference between the heavy metal concentrations of the dry and wet season this was also proven with SPSS

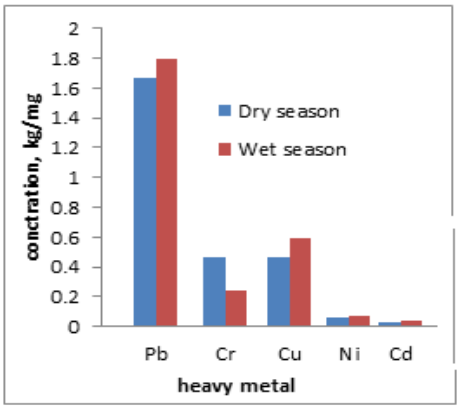

Fig 1: Tilapia fish collected in dry and wet season

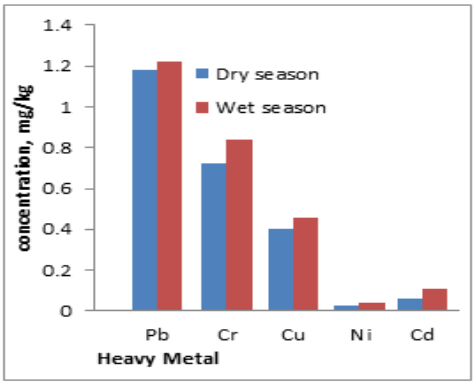

Fig 2: Catfish collected in dry and wet season

Relationship between heavy metal concentrations among tilapia fish collected in different season: Fig 3 shows that the population is normally distributed. R value, 0.996 of regression and correlation using Microsoft Excel signifies that, there is strong relationship between the heavy metal concentration of wet and dry season among the Tilapia fish collected in different season, this was conformed to R value, 0.991 of the correlation using Statistical Package for Social Science. The p-value using Microsoft Excel and Statistical Package for Social Science $1.55*10^{-15}$ and 0.00 respectively, implies that there is significant difference between the heavy metal concentration among Tilapia fish collected during dry and raining season

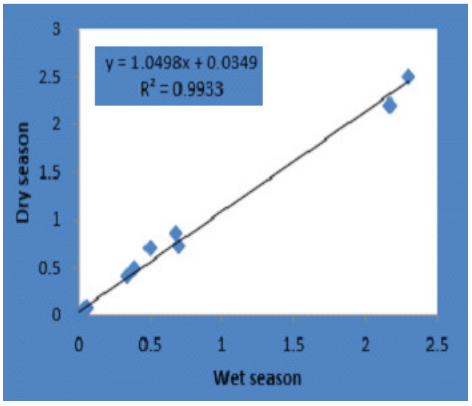

Fig 3: Relationship between dry season and wet season among Tilapia fish

The table above shows mean comparison of heavy metal concentration among Cat fish collected in different season using Microsoft excel and SPSS, which revealed the p-value of Pb, Cr, Ni are less than the alpha value this implies that, there is significant difference between the heavy metal concentration among the cat fish collected in different season while the Cu and Cd has p-value greater than the alpha which implies that, there is no significant difference between the heavy metal concentration of Cu and Cd among the Cat fish collected in different season which agreed with the SPSS analysis

Relationship between heavy metal concentrations among cat fish collected in different season: Fig 4 shows that the population is normally distributed. The R value, 0.99628 of regression and correlation using Microsoft Excel signifies that, there is strong relationship between the heavy metal concentration of wet and dry season among the Tilapia fish collected in different season, this was conformed to R value, 0.996 of the correlation using SPSS. The p-value of Microsoft Excel and SPSS $3.27*10^{-15}$ and 0.00 respectively, proves that there is significant difference between the heavy metal concentration among Tilapia fish collected during dry and raining season

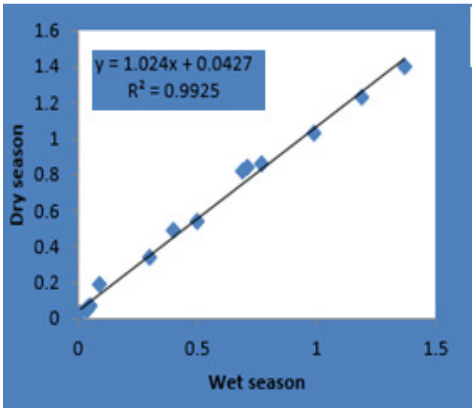

Fig 4: Relationship between dry season and wet season among Cat fish

Relationship between Heavy Metal Concentrations and Weight Fish The significant value of the Pearson Correlation in Table5 revealed that, there is no significant relationship between the weight of the fish and the heavy metal concentrations in the fish

Table 5: correlation of metal concentration and weight of the fish

		Weight of Fish	metal con
Weight of Fish	Pearson Correlation	1	.332
	Sig. (2-tailed)		.585
	N	5	5
metal con	Pearson Correlation	.332	1
	Sig. (2-tailed)	.585	
	N	5	5

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