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Full Length Research Paper

Interaction profile for As, Cd, Cr and Pb in tissues of fishes (*Tilapia gallier*, *Clarias lazera and Heterotis niloticus*)

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The levels of Arsenic (As), Cadmium (Cd), Chromium (Cr) and Lead (Pb) referred to as the quaternary heavy metals (QHMs) were investigated in fish tissues (gill, stomach, intestine and liver) of *Tilapia gallier*, *Clarias lazera and Heterotis niloticus* caught from Lake Alau, Nigeria with the aim of presenting the QHMs interaction profile and hence provide further supportive information to existing literature. The standard calibration method of flame atomic absorption spectrophotometry (FAAS) was employed in the determination of the QHMs. The results indicated presence of the QHMs in the all tissues and fish species analysed. Their concentrations varied for each metal in the different tissues and species, but only in few occasions were these variations statistically significant ($p < 0.05$). *T. gallier* accumulates the highest (39%) of Arsenic in the intestine, *C. lazera* with highest (29%) of Lead in stomach and *H. niloticus* showed highest (38%) of Cadmium in the liver. On the whole, the results of this study revealed that the concentrations of these QHMs are within safe limits for human consumption. The doughnut plot was found useful for profiling the interactions of the QHMs in fish tissue samples, provide supportive information to earlier findings and confirms that these metals do show varying concentrations at different facets of a particular system.

Key words: Gills, stomach, intestine, liver, doughnut plots, quaternary heavy metals.

INTRODUCTION

Studies of the interaction profile for Arsenic (As), Cadmium (Cd), Chromium (Cr) and Lead (Pb) have been carried out in various environmental matrixes following the toxicological concerns of these quaternary heavy metals (QHMs), which are strongly associated with anthropogenic activities leading to the corollary sequence of exposure, accumulation and pollution. The relatable study of the joint toxicity and interaction profile for As, Cd, Cr and Pb in the environment is that given by the Agency for Toxic Substance and Disease Registry, ATSDR (2004). However, the individual toxicological profiles for these quaternary metals have also been well documented in ATSDR (1999a) for Cd, ATSDR (1999b) for Pb, ATSDR (2000a) for As, and ATSDR (2000b) for Cr. In which these metals were reported to be frequently found in hazardous waste sites and implicated in several human health problems since they are easily accumulated in the environment; air, water, soil and the food chain (USEPA, 2000; ATSDR, 2004).

Fish species constitute a significant component of the food chain between the aquatic environment and man. Consequently, heavy metals studies are carried out on fish samples for aquatic environmental pollution monitoring (Fonkou et al., 2002; Zehra et al., 2003; Ashraf, 2006; Dimari et al., 2008) and toxicity studies (ATSDR, 2004; Yamaguchi et al, 2008) because they bioaccumulate these metals significantly in their tissues. A number of factors such as concentrations, the chemical forms of the metals in the aquatic environment, portal of access and physiology of organ where accumulation takes place have been attributed to the bioaccumulation processes of these metals in fishes (Protasowicki and Morsy, 1993; USEPA, 2000; Staniskiene1 et al., 2006; Vinodhini and Narayanan, 2008)

However, profile for these QHMs in the aquatic system of Lake Alau was earlier reported by Hati et al. (2008). Also the levels of seven metals in some fish samples caught from Lake Alau were investigated by Dimari et al.

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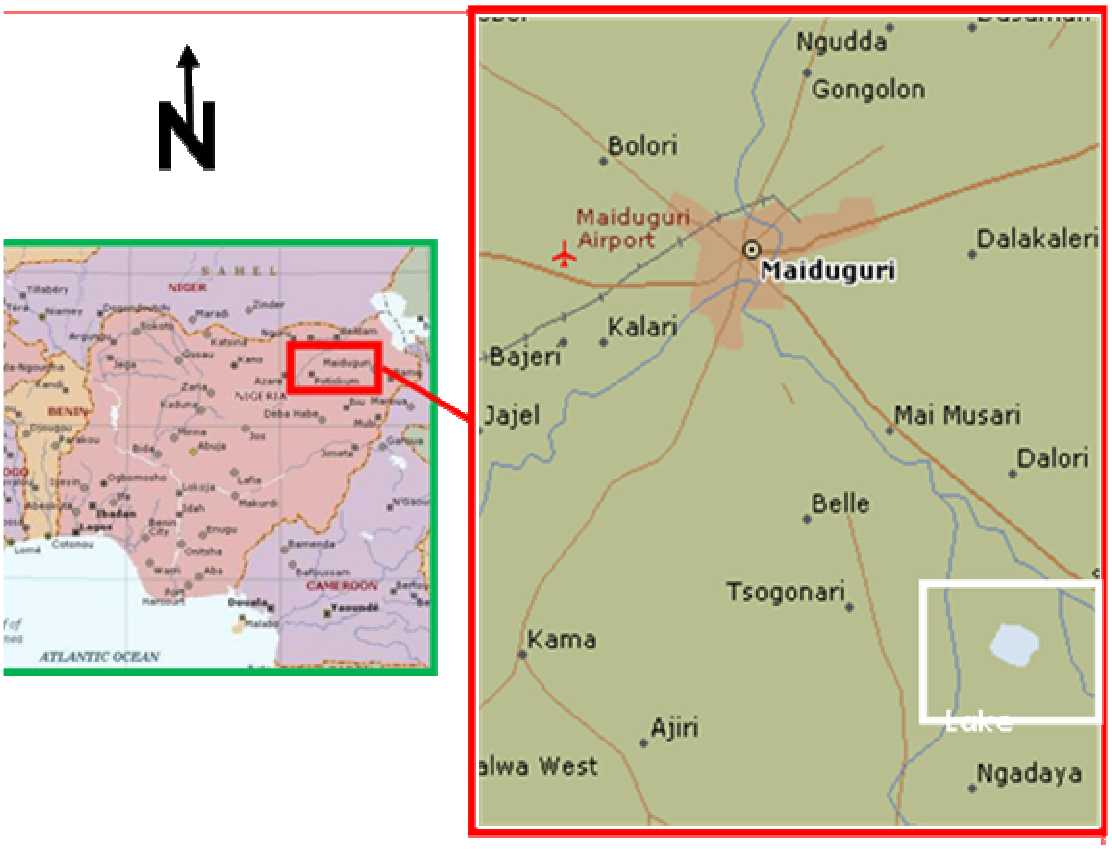

Figure 1. Location of lake Alau, Nigeria.

(2008). The later, however was not designed to center attention on the interaction profile for this QHMs. A major significance of presenting an interaction profile includes the vivid description of the QHMs allotment in the basic facets of a particular system. In this study, particular attention is given to the application of convenient doughnut plots to graphically describe the interaction profile, in addition to presenting information on the levels of this QHMs in the fish tissues of the different species (*T. gallier*, *C. lazera and H. niloticus*) studied and hence provide further supportive information to existing literature (ATSDR, 2004) for these QHMs in the three prominent fishes of Lake Alau not presented hitherto.

MATERIALS AND METHODS

Lake Alau (Figure 1) is located on latitude 11° 41′ N and longitude 13° 16′ E on the South Eastern (SE) part of Maiduguri, capital of Borno State, Nigeria (Idakwo and Abu, 2004; Google Earth, 2008). The lake supplies the municipal water treatment plant, the major source of drinking water supply to the capital, apart from sporadic water obtained from boreholes. Apart from sourcing construction sands, no industrial facilities exist around the lake. However, it supports a significant number of agricultural activities for an estimated population of 521,492 in Maiduguri, and immediate surroundings, Jere (211, 204) and Konduga (156, 564) (FRN, 2007). There are 16 species of fish in Lake Alau (Bankole and Mbagwu, 2000) and farming of several fresh vegetables (Uwah et al., 2007) takes place all year around, thus making Lake Alau a very significant water body to Maiduguri and Nigeria.

Three prominent fish samples (*T. gallier*, *C. lazera and H. niloticus*) found in Lake Alau were analysed in this study. They were selected and sampled following some of the basic criteria enumerated in USEPA (2000) such as sample type and size class. Random composite sampling technique was employed without discrepancies for sexes in species. Fishes were caught using gill nets and collected in a well labelled polytetrafluoroethylene (PTFE, Teflon) bags and transported to the laboratory. A total of 150 samples were collected over a period of eight months in 2008.

Fish samples were dissected and tissues (gill, intestine, liver and stomach) harvested according to procedures described by Dybern (1983) in FAO technical paper. This also consisted of weighing, drying of each separate tissues (10.0 g); dried at 105°C to constant weight, ashed at 550°C for 4 h in a muffle furnace, pulverized and digested with concentrated nitric acid and hydrogen peroxide (1:1 v/v) (Daziel and Baker, 1983; Vogel, 2000). The clear solution obtained following the filtration through Whatmann 541 filter paper was then subjected to the QHMs determinations.

The standard calibration method of flame atomic absorption spectrophotometry (FAAS) was employed in the determination of the QHMs (As, Cd, Cr, Pb) in the prepared samples according to procedures described by SC (2000). Shimadzu AA-6800 equipped with an ASC-6100 auto sampler and air-acetylene atomization gas mixture system was used for the analyses. Replicate samples made were run for each tissue prepared and results of determination were calculated on a wet weight basis in micro gram per gram (µg/g).

Results obtained were analysed using coupled Microsoft Excel + Analyse-it v. 2.12 (Analyse-it® 2007). Variations were considered significant at $p < 0.05$ following Shapiro-Wilk's tests for normality

Table 1. QHM concentrations (µg/g) in the Gills of fish species from lake Alau, Nigeria.

	As	Cd	Cr	Pb
Tilapia gallier	0.54 ± 0.02	0.38 ± 0.02	0.42 ± 0.02	0.53 ± 0.04
Clarias lazera	0.52 ± 0.01	0.36 ± 0.04	0.44 ± 0.01	0.48 ± 0.11
Heterotis niloticus	0.54 ± 0.02	0.35 ± 0.12	0.49 ± 0.14*	0.51 ± 0.05

*Significantly ($p < 0.05$) varied by ANOVA between fish species for metals within columns.

Table 2. QHM concentrations (µg/g) in the Stomach of fish species from lake Alau, Nigeria.

	As	Cd	Cr	Pb
Tilapia gallier	0.44 ± 0.01	0.22 ± 0.02	0.20 ± 0.02	0.40 ± 0.02
Clarias lazera	0.41± 0.05	0.19 ± 0.06	0.23 ± 0.01	0.38 ± 0.05
Heterotis niloticus	0.40 ± 0.03	0.28 ± 0.08*	0.20 ± 0.02	0.35 ± 0.20

*Significantly ($p < 0.05$) varied by ANOVA between fish species for metals within columns.

Table 3. QHM concentrations (µg/g) in the Intestine of fish species from lake Alau, Nigeria.

	As	Cd	Cr	Pb
Tilapia gallier	0.25 ± 0.02	0.09 ± 0.06	0.18 ± 0.03	0.12 ± 0.01
Clarias lazera	0.20 ± 0.01	0.10 ± 0.05	0.19 ± 0.01	0.13 ± 0.02
Heterotis niloticus	0.22 ± 0.08	0.32 ± 0.12*	0.15 ± 0.12	0.31 ± 0.16*

*Significantly ($p < 0.05$) varied by ANOVA between fish species for metals within columns.

Table 4. QHM concentrations (µg/g) in the liver of fish species from lake Alau, Nigeria.

	As	Cd	Cr	Pb
Tilapia gallier	0.27 ± 0.01	0.49 ± 0.04	0.26 ± 0.04*	0.40 ± 0.03*
Clarias lazera	0.25 ± 0.04	0.44 ± 0.11	0.25 ± 0.03	0.38 ± 0.03
Heterotis niloticus	0.24 ± 0.05	0.47 ± 0.05	0.19 ± 0.12	0.32 ± 0.12

*Significantly ($p < 0.05$) varied by ANOVA between fish species for metals within columns.

and analysis of variance (ANOVA) with Bonferroni post-hoc test.

RESULTS AND DISCUSSION

Summary results (mean ± standard deviations) of the QHMs concentration (µg/g) in the tissues, gills, stomach, intestine and liver of fish species are presented on Tables 1, 2, 3 and 4 respectively. The results indicated presence of the QHMs in all the tissues and fish species analysed. Their concentrations varied for each metal in the different tissues and species, but only in few occasions were there variations statistically significant ($p < 0.05$). Such as the concentrations of Cr in the gills of *H. niloticus,* was significantly higher than the other fish species. This trend was similarly observed for Cd in the stomach and intestine, and Pb in the intestine only. Cr and Pb concentrations in the liver of *T. gallier* were significantly higher than *H. niloticus* only.

Generally, with the exception of Cd in the liver, the levels of the other metals were higher in the gill tissue of all fish species analysed. In terms of Shapiro-Wilk's tests, the data on Tables 1 - 4 showed that all QHMs in the respective tissues of fish species fell within a normal distribution, that is., they are a single population. However the Box-Whisker plot (Figure 2), which shows the overall

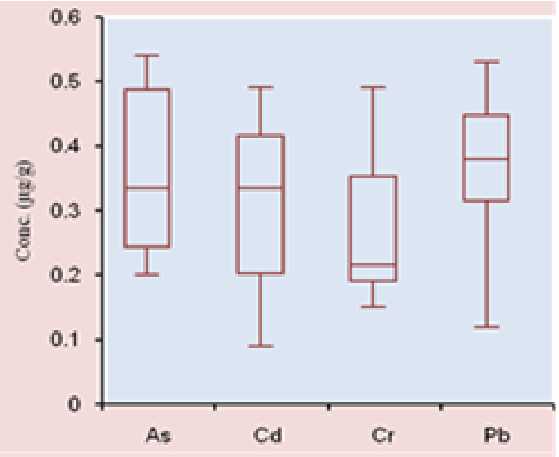

Figure 2. Box-Whisker plot of QHMs concentration spread in all fish species.

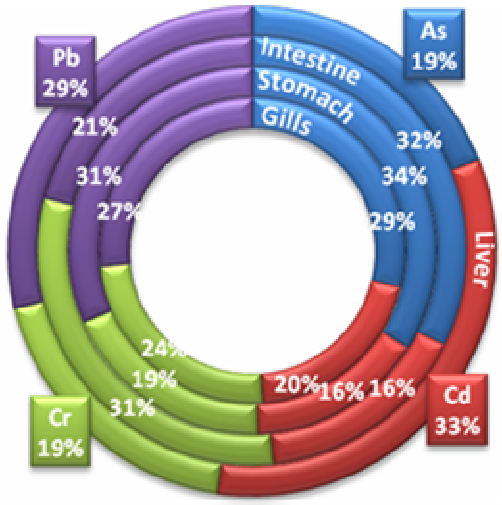

Figure 4. Interaction profile for As, Cd, Cr and Pb in tissues of *Clarias lazera* from lake Alau, Nigeria.

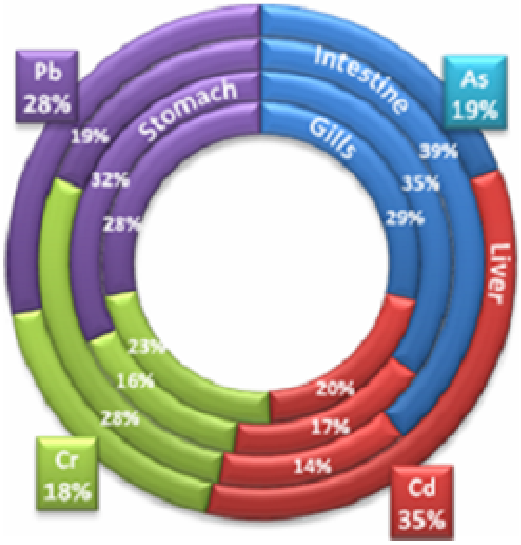

Figure 3. Interaction profile for As, Cr and Pb in tissues of *Tilapia gallier* from Lake Alau, Nigeria.

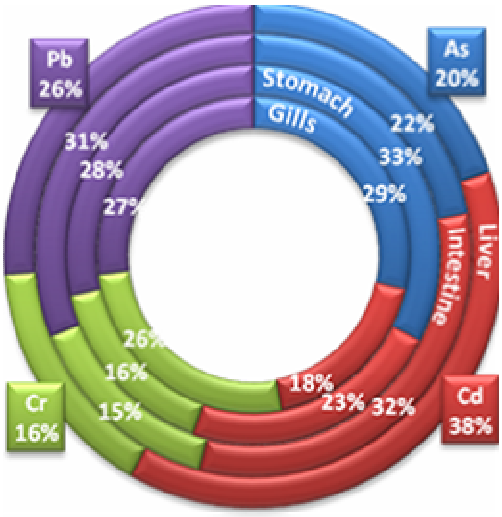

Figure 5. Interaction profile for As, Cd, Cr and Pb in tissues of *Heterotis niloticus* from lake Alau, Nigeria.

Concentration spread of the QHMs in both tissue and fish samples indicated Pb and Cd with the widest concentration spread (minimum and maximum) while As and Cd showed the least, but showed the widest 1st - 3rd quartile range of concentrations, and the narrowest was Pb. The doughnut plots of Figures 3, 4 and 5 show the interaction profile for the QHMs in each of the fish species, *T. gallier, C. lazera and H. niloticus* respectively. The plots show concentration values for the QHMs in terms of percentage ratios of each metal in the different tissues, represented by a ring, for a particular fish spe-cies. The inner most ring being for the gills, followed by stomach, intestine and liver (outer most ring). Thus, in *T .gallier* (Figure 2), indicates that %As is most accumulated in the gills and intestine, while %Pb and %Cd in the stomach and liver respectively. In *C. lazera* (Figure 3), the gills, stomach and intestine accumulated most of %As, with highest %Cd in the liver. Similarly, the highest %Cd accumulation was in the liver of *H. niloticus* (Figure 3), also with highest %Cd in the intestine and As in the gills and stomach.

The species of fishes (*T. gallier, C. lazera and H. niloticus*) analysed in this work are species of high commercial value, marketed fresh or smoked and a major source of protein in the diet of indigenes of this region in Nigeria. These species are freshwater native to tropical Africa (Teugels and Sudarto, 2001; GSMFC, 2003; Encarta, 2008).

The result of this study revealed that the QHMs concentrations in the tissues of f ish species analysed, with

with exception of Pb in the gills, were within safe limits for human consumption (FDA, 1998).

That the gills showed highest accumulations of the QHMs is due to the fact that the gill is an important portal of heavy metals transport into the fish, which also has been found to provoke lesions and gill damage (Bols et al., 2001). Also, the generally high and wide concentration variations of Pb are very likely associated with the forms of agricultural practices, domestic waste load and the peculiar Saharan dust laden with this metals that are released into the lake body (Hati et al., 2008). However, the concentrations of Cd and As in this study show correspondence with the work of Zyadah (1999) in term of *Tilapia* spp of Egypt. Also Pb and Cr concentration in this work showed general similarity to that found in the study of Abu Hilal and Ismail (2008). These depict aquatic environments that are less polluted with these metals.

As enumerated earlier, a major significance of presenting an interaction profile includes the vivid description of the QHMs allotment in the basic facets, which in this case are the tissues, and for a particular system, the fish species. For instance, from the doughnut plots it is easier to conclude that the liver accumulates more of Cd, and that it is least observed in *C. lazera* of the three fish species analysed. It becomes very easy to make reference to the fact that though fish diversity are being reduced considerably (Bilong-Bilong et al., 1997) but that certain species like *C. lazera* resist pollution (Fonkou et al., 2002) to a certain extent and are regularly catched by the population. Thus both the physiological characteristic of the fish species in respect of the activity of each organ or tissue and the individual metallic properties of the QHM are better appreciated. Such reports (Filipovic and Raspor, 2003; Abou EL-Naga et al., 2005; Virbickas and Sakalauskienë, 2006) shows that the organisms develops protective defense against the deleterious effects of these QHMs, including essential heavy metals and other xenobiotics that produce degenerative changes like oxidative stress in the body. On the other hand, while with the plots it is much easier to draw out an order for each of these QHMs in the tissues analysed for a particular species. It has been noted that the presence of these metals in a particular tissue is dependent on the chemical nature (ATSDR, 2004).

Conclusion

The results of this study revealed that the concentrations of these QHMs are within safe limits for human consumption. The doughnut plot was found useful for profiling the interactions of the QHMs in fish tissue samples, provide supportive information to earlier findings and confirms that these metals do show varying concentrations at different facets of a particular system. Further profiling of these QHMs is suggested in significant tissues of fishes that serve as aquatic environmental pollution monitoring tools and toxicity studies.

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