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RESEARCH ARTICLE

EFFECTS OF SEASON AND FISH SMOKING ON HEAVY METAL CONTENTS OF SELECTED FISH SPECIES FROM THREE LOCATIONS IN BORNO STATE OF NIGERIA

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ABSTRACT

The present study was designed to determine the level of four toxic heavy metals: lead, cadmium, mercury and arsenic, in edible muscles of four fish species namely Tilapia nilotica (Tilapia), Synodontis guntheri (Kurungu), Heterotis niloticus (Bargi), and Clarias anguillaris (Catfish), harvested from three locations — Alua Dam, Doron Baga and Dabamasara within the Lake Chad Basin of Borno State, Nigeria. The main objective was to investigate the possible effects of dry and rainy seasons and smoking of the fish on the concentration of the toxic heavy metals in the fish following wet digestion. The toxic heavy metals, expressed in parts per million (ppm), were detected in all the fish species sampled during the dry and rainy seasons from the three (3) inland waters investigated. The overall mean concentrations of the heavy metals were significantly higher ($P \leq 0.05$) in fresh fish samples harvested during the rainy season than the dry season. Significantly different variations were also observed within fish types and between locations in the concentrations of the four heavy metals. Smoking of the fish also resulted in significant increases ($P \leq 0.05$) of the metals, especially in lead and mercury concentrations in smoked samples during the two seasons. Cadmium and arsenic were the lowest recorded metals in both fresh and smoked fish during the two seasons and in all the locations. Washing the smoked samples with double distilled water resulted in significant reduction of more than 50% of the metals in the smoked fish, indicating surface contaminants. The sequence of the heavy metals concentrations in all the fish samples was Pb>Hg>Cd>As. The concentrations of the metals in both the fresh and smoked samples from all the locations during the two seasons were however lower than the internationally recommended threshold levels. Follow up studies were recommended while it was concluded that fish from Lake Chad basin are still safe for human consumption in terms of their heavy metal contents.

INTRODUCTION

Globally, consumer demand for fish continues to climb (Doe, 2002; Akende and Odogbo, 2005), both amongst the affluent as well as the not so well to do sections of any population. Nearly one billion people, most of them in developing countries, currently depend on fish for their primary source of protein (Toth *et al*., 2012; Igwegbe, 2013). In addition, the harvesting, handling, processing and distribution of fish provide livelihood for millions of people across the globe (Al-jufaili and Opara, 2006). Fish and other seafood constitute an important and popular part of the diet of many Nigerians — particularly in regions where livestock is relatively scarce (Igwegbe, *et al*., 2014).

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Fish importation has been observed to be a source of serious foreign exchange drain on Nigerian economy (Akande and Odogbo, 2005). Nigeria is reported to spend over a billion naira annually on importation of fisheries products (Ahmed, 2007). Nigeria has a compact landmass of 923,762km^2; 860kms of coastline on a major gulf of the South Atlantic, abundant water resources with major rivers of the Niger and the Benue traversing its territory in addition to numerous smaller rivers and streams crisscrossing its vast terrains (Olaosebikan and Raji, 1998). It has vast fishing grounds of lakes, swamps, lagoons, deltas and estuaries. Fish supplies in Nigeria come from three main activities, which include artisans, commercial trawlers and fish farming (Igwegbe, 2013; Igwegbe *et al*., 2014). The gap between fish demand and supply is unfortunately widening due to increasing population, drop in meat and fish supply, thus prompting the search for methods of improving fish quantity and quality. Consequently, many methods have been used, including the application of herbicides for the control of Hyacinth, observed to have a profound effect on fish production attributed to the upsurge of available food for fish and increased nymphal proliferation at the post-application period (Ahmed, 2007). The application of chemical poison in fishing and during handling of fish may contribute to contamination of both the aquatic environment and fish and fish products with heavy metals among other contaminants (Mrosso and Werimo, 2005). Water quality parameters are essential for the survival, growth and reproduction of fish and other aquatic animals. However, increasing human activities in the vicinity of our lakes and rivers, particularly due to urbanization, industrialization, technological development, growing human population, indiscriminate sewage and waste disposal, agricultural activities, oil exploration and exploitation may lead to an increase in man-made pollutants in our aquatic environment. This in turn will result in elevation of the levels of organic contaminants such as nitrite, nitrate, ammonia and phosphates which ultimately increase the level of suspended solids making the water increasingly turbid (Ahmed, 2007; Akan *et al*., 2009; Igwegbe, 2013).

Contaminated water run-offs, mining and industrial activities such as textile, paper mills, tanneries, sugar and petroleum refineries have been reported to constitute sources of trace metal pollution to freshwater bodies in the developing countries including Nigeria (Obasohan and Eguavoen, 2008; Eneji *et al*., 2011; Ambedkar and Muniyan, 2011; Ashraf *et al*., 2006). Both terrestrial and aquatic food chains are capable of accumulating certain environmental contaminants up to toxic concentrations. In general, many of the thousands of chemicals produced by human industry may eventually end up in aquatic environment. Some of these chemicals are basically considered as part of seafood's natural environment (Akan *et* al., 2012), while others have anthropogenic sources (Igwegbe, *et al*., 1992 and 2014). Chemical contaminants can also come from industrial, municipal, or agricultural sources. In terms of organic chemicals, the best known examples of bioaccumulation in aquatic food chains are the polychlorinated biphenyls (PCBs), dioxins, and organo-chlorine pesticides such as dichlorodiphenyl trichloroethane (DDT).

There are also many evidences of bioaccumulation of metal compounds to potentially toxic levels in fish and their products (USGS, 2003; Hamed and Emara, 2006; Akan *et al*., 2009; Toth *et al*., 2012). If fish is not sold fresh, preservation methods are usually applied to extend its shelf-life. Consequently, the processing and preservation of fresh fish are of utmost importance, since fish is highly susceptible to deterioration immediately after harvest, in preventing economic losses (Okonta and Ekelemu, 2005). The preservation methods include freezing, smoking, drying, chemical preservation and heat treatment (canning, pasteurization, etc). The process of smoking fish from smouldering wood as a means of preservation and flavour enhancer is as old as civilization. Up to 70% of the total fish catch in developing countries is preserved by smoking (Clucas and Ward, 1996; Abolagba and Igbinevbo, 2010). Smoking usually extends the shelf life of fish due to the reduced moisture content and anti-microbial effects of phenolic compounds present in the smoke (Efiuvwevwere and Ajiboye, 1996). In addition, during hot smoking, high heat results in direct microbial destruction (Nickelson *et al*., 2001) as well as concentration of nutrients, which may include toxic heavy metals, due to the reduced moisture content (Igwegbe, 2013). The knowledge of the levels of contaminants in fresh and processed fish is of considerable importance because of its potential effects on the fish on one hand, and on the top-level predators that consume them, including humans, on the other hand. Although, the possibility of heavy metal contamination of lakes, ponds and rivers, and fisheries exists in Nigeria, the literature is still limited on the many possible factors, including processing method such as smoking, which may affect the level of the heavy metals on the processed fish in the country. This study was therefore designed to investigate the level of heavy metals — lead, cadmium, mercury and arsenic in smoked fish species harvested during the rainy and dry seasons, from three locations within the Lake Chad Basin in Borno State of Nigeria.

MATERIALS AND METHODS

Description of the Study Area

The Lake Chad Basin was selected for this study. The Basin is located on longitude 14^0 North and latitude 13^0 East in Borno State and shared by four West African countries: Chad, Nigeria, Niger and Cameroon. It lies within the high tropical regions of extreme scorching heat, where temperatures in the shade can reach 45-49^{0}C in most parts of the year. There are about 200 permanent and semi-permanent fishing communities and / or islands and over 40,000 fishermen on the Nigerian sector of the Lake Chad basin. It is the second largest basin in Africa after Congo basin (Akande and Odogbo, 2005; World Atlas, 2005; Ahmed, 2007); and one of the important sources of freshwater fish in Nigeria (Raji, 1992, Akande and Odogbo, 2005). It is located in the Sahel vegetational belt, south of Sahara with less than 600mm of rain annually, and contributes significantly to the domestic fish production, with an estimated annual yield of 70,000mt/year from Lake Chad and 870mt/year from Alau Dam (Ahmed, 2007). The inland waters of Lake Chad basin, which include Dabamasara and Dorobaga (Figure 1), occupy vast marshy swamps on the flood plains during the wet season (July – September).

The Basin is also endowed with shallow euthrophic water bodies, all produce approximately 13% of Nigerian inland fish (Raji, 1992), the predominant fish species in the area include *Clarias, Tilapia, Gymnarchus, Heterotis, Lates niloticus, Protopterus, Alestes, Synodontis, Citharius*, etc (Ahmed, 2007). The area is also characterized by unstable and sporadic rain patterns, drought, overfishing, mining and other human activities capable of contributing to environmental pollutions. Specifically, Lake Chad reportedly receives waste water from Komadugu Yobe River in Yobe State and the Ngadda and Yedzeram River Systems from Borno State (Figure 1). The Basin is also polluted by textile and tannery effluents in the upstream part of the lake on one hand, and from the waste water discharged from the settlements along Chari Lagoon and Kamadugu Yobe River course, from abattoirs, hotels, hospitals, mining wastes, agricultural wastes (used pesticides and agrochemicals through return flow of water, runoff and percolation from irrigated fields), on the other hand. The banks of these water bodies are dominated by intensive irrigational farming and fishing activities and serve as nesting ground for several species of birds as well as points for different types of livestock (cattle, camels, etc).

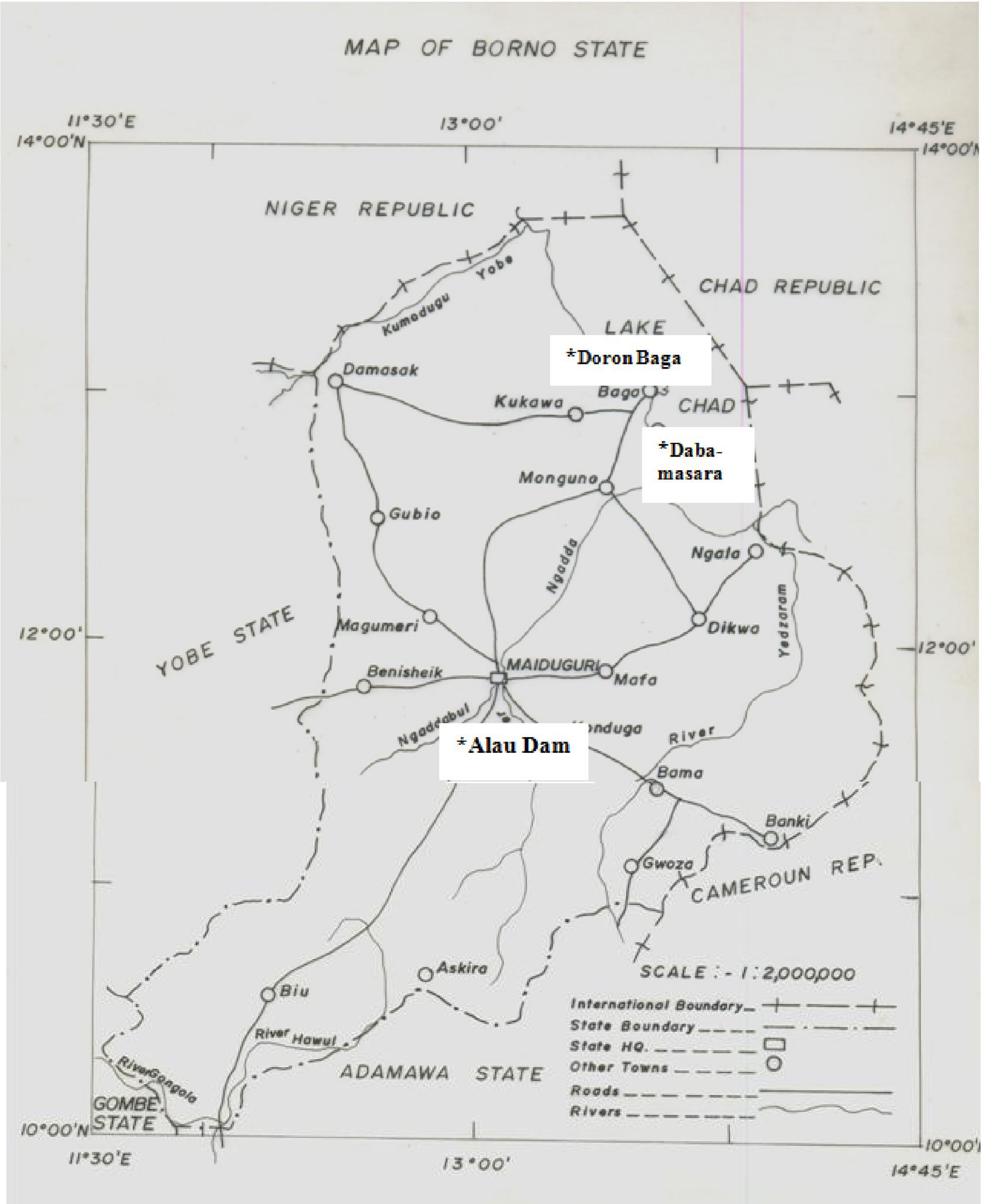

Figure 1. Base Map of Borno State showing the Sampling Locations from Lake Chad Basin (Igwegbe, 2013)

*Sampling Points: Alau Dam, Dobamasara and Doron Baga.

Salt mining is also a common feature in Lake Chad basin. Alau Dam, on the other side, also receives waste water from agricultural activities as a result of water flow from River Yedzram and River Gombole which meet at a confluence at Skambisha from where they flow, with large quantities of waste, as River Ngala into Alau Dam. There is therefore the need to periodically assess the burden of heavy metals in fresh fish harvested from those water bodies in the area so as to determine their suitability as seafood and sources of fish to the inhabitants of the immediate environment and beyond.

The possible effects of a traditional method of preservation, particularly smoking, on the concentration of the heavy metals in smoked and washed fish, were also investigated. Thus, the three major fish harvesting sites — Alau Dam, Doronbaga and Dabanasara, were chosen for this study.

Apparatus

Insulated plastic coolers (100 liters capacity), stainless steel cutting utensils (knives and spoons), a food-grade grinder with stainless steel cutting blades, Teflon glass beakers, volumetric flasks (50, 100, 1000mL capacity), graduated cylinders, watch glass, plastic funnels, polyethylene sheets, Whatman ashless filter papers 125mm, hot water bath, drying oven, Atona oven (Kiln) smoker, sawdust, and polyfloro-tetratheylene (PFTE) plastic containers with screw caps (100ml). All glass and plastic wares were soaked overnight in 10% (v/v) nitric acid, followed by washing with 10% (v/v) hydrochloric acid, and thoroughly rinsed with double distilled water (Wiersma *et al*., 1986; Khansari *et al*., 2005; BCS, 2006; Igwegbe, 2013; Igwegbe *et al*., 2014). A Buck 205 Atomic Absorption Spectrophotometer (AAS) and Inductively Coupled Plasma/ Optical Emission Spectrophotometer (ICP-OES), Buck Scientific Inc., USA, were used in the quantification of the heavy metals: lead and cadmium, mercury and arsenic, respectively.

Reagents

All reagents used were of analytical grades, and included concentrated nitric acid (sp. gr. 1.42 g/ 20^0C, 68-72% m/w; BHD Chemical Ltd., Poole, England), concentrated hydrochloric acid (sp. gr. 1.73 g/20^0C, 35 – 37.5%; May and Baker Ltd., Dagenham, England), concentrated sulfuric acid (sp. gr. 1.84 g/20^0C, 98% m/w; Fison Scientific Equipment, Loughborough, England. Oxides of lead, cadmium, mercury and arsenic (98.5 - 99.5%) metals (Reidel-de Haem, Germany). The metallic oxides were used to spike selected samples with measured concentrations of the heavy metals for the recovery and repeatability test.

Sample Collection and Preparation

Four (4) species of fish namely: *Tilapia nilotica* (Tilapia), *Synodontis guntheri* (locally known as *Kurungu*), *Heterotis niloticus* (locally known as *Bargi*), and *Clarias anguillaris* (Catfish) were collected directly from the locations due to their availability in large quantities in all the three sites: Alau Dam, Doronbaga, and Dabamasara (Figure 1). For the purpose of comparison, care was taken to select only the similar species, both in quantity and size, from all the three locations under investigation, and in sufficient quantities to guarantee representative sample of each species from each location. Sampling for the dry season commenced from September to March; while the sampling for the rainy season started from May to August, within the sample period. Samples were transported from sites to Food Science laboratory, University of Maiduguri, inside the previously cleaned insulated plastic coolers (100 liters capacity) containing ice blocks, where they were identified (with the help of Olaosebikan and Raji, 1998), sorted by location, species, and size. Fish packed in the insulated containers with ice blocks maintained its fresh quality for at least 72 hours.

Sample for each treatment consisted of 3 to 5 individual fish from each location. Fish were trimmed, eviscerated, scaled, washed and rinsed three times with double distilled water and stored in ice blocks until wet-digested with the concentrated acids (for the determination of the heavy metals in the fresh fish samples). A sample preparation technique was developed to prevent cross-contamination between samples as metals are found in slimes, blood and scales of fish. Only stainless steel cutting utensils were used during the trimming, evisceration and cleaning of the fish and, the preparation surfaces were covered with polyethylene sheets, with the sheets replaced between the preparations of each sample. Only the edible parts (the muscles) of the fish species were used and samples were treated and / or prepared as though they were for human consumption.

Fish Smoking

The smoking process was carried out in an Altona type oven (Clucas and Ward, 1996), available in the smoke house of the Food Science and Technology Department. The cleaned and rinsed fresh fish samples were allowed to drain properly at the prevailing ambient temperatures (30 – 39^0C or 25 - 29^0C during dry or rainy season, respectively) before smoking. The smoker was fueled by sawdust (Akande *et al*., 2005) purchased from Baga timber market, and smoking lasted for 8 to 12 hours at 50 to 70^0C (Bykowski and Dutkiewicz, 1996).

Sample Digestion (metal extraction)

Fresh fish samples were first macerated and then homogenized thoroughly in the food blender with stainless steel cutters, whereas the dried, smoked samples were crushed properly in the wooden mortar with the pestle. Three (3) grams of homogenized oven-dried fresh samples or crushed smoked samples were weighed, in triplicate, into the 50ml Teflon glass beakers and then passed through the digestion process. For the unwashed treatments, the smoked fish samples were simply cleaned of excess smoke or carbonized residues, before pulverizing. On the other hand, the washed samples were first cleaned of carbonized residues and then washing the sample three times before pulverizing it. The digestion process was as follows: 10ml of concentrated HNO_3 and 5ml of concentrated H_2SO_4 were slowly added to the previously weighed samples, covered with the watch glass, and allowed to digest overnight (Khansari *et al*., 2005; Voegborlo *et al*., 1999; Rahimi *et al*., 2010). The beakers containing the samples were then heated gently in the hot water bath to complete dissolution for 2 to 3 hours (appearance of clear solution). The beakers containing the samples were then cooled and the solution transferred quantitatively into 50ml volumetric flasks and made up to the mark with double distilled water; and then stored in the PFTE plastic containers until analyzed with the AAS. Blanks were prepared exactly in the similar manner but without the fish samples.

Validation of the method

To determine and verify the repeatability of the analytical methodology, standard stock solutions of lead, cadmium, mercury and arsenic were prepared by dissolving 2±0.1g of each of the metal oxides separately in 5ml of the concentrated HCl, quantitatively transferring into one liter volumetric flasks and making up the marks with the double distilled water. Homogenized fresh fish sample, Bargi from Alau Dam was spiked in triplicate for each metal, with 2ml of the stock solutions to yield 2ppm of lead, cadmium, mercury, and arsenic from each spiked sample. The spiked samples and blanks were then subjected to the digestion procedures (Wiersma *et al*., 1986; Khansari *el al.,* 2005). The resulting solutions were analyzed for the metal concentrations.

Statistical Analysis

Data obtained in this study were subjected to analyses of variance (ANOVA). The test for significant of differences between the factors investigated (otherwise known as mean separation), was conducted at 5% levels of significance, using the Duncan's Multiple Range Test (Montgomery, 1976; Gomez and Gomez, 1983). Correlation analysis was also conducted at 5%.

RESULTS AND DISCUSSION

The mean recovery for each of the four metals from the spiked fish samples is 100.20% for lead (ranging from 99.94 - 100.60%), cadmium 99.96% (ranging from 99.95 – 99.96%), mercury 99.41% (ranging from 99.40 – 99.41%), and arsenic 100.02% (ranging from 100.01 – 100.02%). The recovery of these heavy metals obtained in this study is highly comparable to those obtained through the similar techniques by other investigators including Ashraf (2006), Voegborlo *et al*. (1999), Khansari *et al*. (2005), Ekpo *et al*. (2008), Rahimi *et al*. (2010) and Olusegun (2011). Good recoveries of the metals from the spiked samples demonstrate accuracy of the analytical methods. Similarly, the comparison between the dry and rainy season heavy metal mean concentrations in the fresh fish samples from the three locations are presented in Table 1. Significant variations ($P \leq 0.05$) were mainly observed in mean lead concentrations among the fish types and between the locations. The seasonal mean concentrations of lead in fresh Bargi from Alau Dam were 0.00127ppm (dry season) and 0.00223ppm (rainy season). The corresponding values for the similar fish from Dabamasara and Doronbaga were 0.00167ppm (dry season) and 0.00127ppm (rainy season) and, 0.00253ppm (dry season) and 0.00143ppm (rainy season). Also, the mean lead concentrations recorded in catfish from Alau Dam, Dabamasara and Doronbaga were 0.00163 and 0.00183ppm; 0.00163 and 0.00143ppm and, 0.00167 and 0.00210ppm in dry and rainy seasons, respectively.

The highest mean concentration of lead, 0.00633ppm, was recorded in fresh Kurungu fish sampled from Doronbaga during the dry season; and the corresponding value obtained during the rainy season was 0.00236ppm (Table 1). Cadmium, mercury and arsenic seasonal mean concentrations among the fish types and between the locations were not significantly different ($P \geq 0.5$), though higher concentrations of the heavy metals were recorded in fresh fish samples collected during the rainy season than the dry season. These variations in the metal contents of the fish samples can be attributed to the different pollution levels at these locations. Lake Chad is reported to receive its water from various rivers in Nigeria, Chad, Cameroon and Niger Republic (Bdliya and Tagi, 2011). The rivers, whose peak flows are recorded during the rainy season are suspected to carry materials ranging from elements washed from the earth to effluents from domestic and farming activities as well as industries located along the banks of these rivers to different water bodies in the basin including Alau Dam, Dabamasara and Doronbaga.

Knowing when fish from a water body contain dangerous concentrations of metals is not a simple task. Metal concentrations in fish could be as results of complex interactions between the environment and living organisms. Differences in water chemistry and in the numbers and types of organisms present in a lake can also produce significant lake-to-lake variation in the amount of metal that moves from the water through the food chain to fish. Thus, fish in different water bodies may frequently carry different metal burdens even when the water bodies are located within the same geographic region. Chen *et al*. (2000) tracked and predicted the movement of potentially toxic metals through aquatic ecosystems to fish. They attributed a large part of the variation in metal accumulation in fish to differences among organisms in the lower levels of food web, particularly the tiny algae and other foods that fish eat. These foods, they explained, include smaller fish, zooplankton and insects, that can accumulate different quantities of metals due to metabolic differences and

Table 1. Comparison of Dry and Rainy Season Mean Heavy Metal Concentrations (ppm) in Fresh Fish Samples from the Three Locations*

LOCATION:		Alau Dam		Dabamasara		Doronbaga	
Season:		Dry	Rainy	Dry	Rainy	Dry	Rainy
Type of Fish	Metal	$\times 10^{-3}$ ppm					
Bargi	Pb	1.27^{a}	2.23^{b}	1.67^{c}	1.27^{a}	2.53^{d}	1.43^{a}
	Cd	0.47^{a}	0.50^{a}	0.37^{ab}	0.30^{ab}	0.53^{a}	0.23^{b}
	Hg	0.93^{b}	0.87^{b}	1.63^{c}	0.60^{ab}	0.67^{ab}	0.80^{ab}
	As	0.30^{a}	0.30^{a}	0.43^{a}	0.23^{a}	0.20^{a}	0.30^{a}
Catfish	Pb	1.63^{a}	1.83^{a}	1.63^{ac}	1.43^{ac}	1.67^{ac}	2.10^{b}
	Cd	0.33^{a}	0.43^{ac}	0.33^{a}	0.23^{a}	0.27^{a}	0.67^{c}
	Hg	0.97^{a}	1.03^{a}	0.97^{a}	0.50^{c}	0.67^{c}	1.90^{b}
	As	0.37^{ac}	0.37^{ac}	0.37^{ac}	0.20^{a}	0.23^{a}	0.60^{c}
Kurungu	Pb	1.26^{a}	1.80^{d}	1.26^{a}	1.06^{a}	6.33^{b}	2.36^{c}
	Cd	0.33^{a}	0.43^{a}	0.33^{a}	0.23^{a}	0.30^{a}	0.73^{c}
	Hg	1.73^{a}	1.00^{b}	1.73^{a}	0.67^{c}	1.20^{b}	2.00^{d}
	As	0.47^{a}	0.43^{a}	0.47^{a}	1.00^{b}	0.12^{c}	0.60^{a}
Tilapia	Pb	1.13^{a}	1.60^{b}	1.13^{a}	1.70^{b}	1.47^{b}	2.53^{c}
	Cd	0.43^{a}	0.37^{a}	0.43^{a}	0.40^{a}	0.20^{a}	0.73^{b}
	Hg	1.00^{a}	0.83^{a}	1.00^{a}	1.00^{a}	0.93^{a}	1.00^{a}
	As	0.53^{a}	0.30^{a}	0.53^{a}	0.37^{a}	0.33^{a}	0.40^{a}

Standard Deviation for all means ranged from ± 0.000 to ±0.0002

*In any row, means bearing different superscripts are significantly different ($P \leq 0.05$)

environmental conditions in which they live. They concluded that fish from lakes where large-bodied species of zooplankton predominate tend to have more metals in their tissues than fish from lakes where small-bodied zooplankton predominate. In another study, Obasohan and Eguavoen (2008) attributed slight seasonal variations in mean concentration of some heavy metals, including lead and cadmium, in fresh fish, to lack of uniformity in the distribution and possible bio-availability of the metals in different sample stations.

They also suggested that low rainy season metal levels in fish muscles could be as result of low bio-availability due to reduced metal concentrations in rivers arising from dilution associated with heavy rains during the rainy seasons, whereas increased heavy metal concentrations in fresh fish samples observed during the dry season was attributable to changes associated with increased water temperatures during the season. Furthermore, the levels of the heavy metals recorded from the smoked fish samples during the dry and rainy seasons are presented in Tables 2 and 3, respectively, together with their statistical parameters. The smoked fish samples recorded higher mean concentrations of the four heavy metals than were observed in the fresh samples. The concentrations of the heavy metals in smoked fish samples were observed, in some cases, to be two folds higher than their concentrations in the fresh fish samples from the same locations during each of the two seasons (Tables 2 and 3). Significant variations (P≤0.05) were observed among types of fish and between locations in the concentrations of the metals except in cadmium in fish samples collected from the three locations during the dry season (Table 2); while significant variations (P≤0.05) were observed among fish types and between locations only in smoked fish samples from Alau Dam and in mercury concentrations in fish samples from Dabamasara (Table 3). Smoking process resulted in a greater increase in lead and mercury concentrations followed by cadmium and then arsenic. The highest concentration of lead, 0.00363ppm, was observed in smoked Kurungu sample from Alau Dam; while the lowest concentrations of arsenic, 0.00043ppm, was recorded in Bargi samples from Dabamasara and Doronbaga (Table 2). Washing the smoked fish samples three times with double distilled water resulted in a significant reduction (P≤0.05) in the metal contents of the fish species harvested from the three locations during the dry and rainy seasons (Tables 4 and 5). The results are also presented with their statistical parameters. Significant variations (P≤0.05) were observed among fish types and between locations in the concentrations of all the heavy metals in smoked and washed fish samples from Alau Dam; in cadmium and mercury in samples from Dabamasara; and in lead,

mercury and arsenic in samples from Doronbaga (Table 4); whereas significant variations (P≤0.05) were observed in lead concentrations in fish samples from all the three locations, and only in mercury and arsenic concentrations in fish samples from Dabmasara and Doronbaga, respectively (Table 5). It was also observed that the washing process reduced the concentrations of the metals to levels that are still higher, in some fish types, than the levels recorded in the corresponding fresh fish samples (Table 1).

Table 2. Concentration of Lead (Pb), Cadmium (Cd), Mercury (Hg) and Arsenic (As) in Smoked fish Samples collected from the three Locations during the Dry Season (ppm)

Fish Type	Locations*											
	Alau Dam				Dabamasara				Doronbagar			
	Pb $x10^{-3}$	Cd $x10^{-3}$	Hg $x10^{-3}$	As $x10^{-3}$	Pb $x10^{-3}$	Cd $x10^{-3}$	Hg $x10^{-3}$	As $x10^{-3}$	Pb $x10^{-3}$	Cd $x10^{-3}$	Hg $x10^{-3}$	As $x10^{-3}$
Bargi	2.03^{a}	1.56^{a}	1.13^{a}	0.63^{a}	1.73^{b}	1.97^{a}	2.63^{a}	0.43^{a}	2.33^{a}	2.00^{a}	1.90^{a}	0.43^{a}
Catfish	2.23^{b}	1.76^{b}	1.27^{a}	0.60^{a}	2.53^{a}	1.97^{a}	2.03^{b}	0.63^{a}	2.33^{a}	1.90^{a}	1.50^{b}	0.67^{b}
Kurungu	3.63^{c}	1.60^{a}	1.27^{a}	1.17^{b}	3.03^{c}	1.77^{b}	1.50^{c}	1.00^{b}	1.67^{a}	2.00^{a}	2.00^{c}	0.90^{c}
Tilapia	2.03^{a}	2.00^{c}	1.70^{b}	1.13^{b}	2.50^{a}	1.87^{ab}	0.97^{d}	2.67^{d}	2.00^{a}	1.89^{a}	1.86^{a}	0.93^{c}
Mean	2.48	1.73	1.34	0.88	2.45	1.89	1.78	0.58	2.08	1.94	1.82	0.73
SE	0.05	0.05	0.10	0.06	0.12	0.06	0.09	0.10	0.13	0.12	0.13	0.05
LSD (5%)	0.17	0.19	0.36	0.21	0.41	0.19	0.32	0.34	4.53	0.43	0.44	0.18
CV (%)	3.50	5.40	13.60	12.10	8.40	5.10	9.10	29.60	10.90	11.10	12.20	12.20

SE = standard error; LSD = least significant difference; CV = coefficient of variation

Standard Deviation for all means (average of three determinations) ranged from **± 0.000** to **±0.0002**

***** In any column, means bearing similar superscripts are not significantly difference (**P≥0.05**).

Table 3. Concentration of Lead (Pb), Cadmium (Cd), Mercury (Hg) and Arsenic (As) in Smoked fish Samples collected from the three Locations during the Rainy Season (ppm)

Fish Type	Locations*											
	Alau Dam				Dabamasara				Doronbaga			
	Pb $x10^{-3}$	Cd $x10^{-3}$	Hg $x10^{-3}$	As $x10^{-3}$	Pb $x10^{-3}$	Cd $x10^{-3}$	Hg $x10^{-3}$	As $x10^{-3}$	Pb $x10^{-3}$	Cd $x10^{-3}$	Hg $x10^{-3}$	As $x10^{-3}$
Bargi	3.17^{a}	1.23^{b}	1.53^{c}	1.07^{d}	2.37^{a}	1.33^{b}	1.93^{d}	1.17^{b}	2.67^{a}	1.53^{b}	2.87^{g}	1.37^{h}
Catfish	2.57^{b}	1.23^{b}	1.83^{c}	1.03^{d}	2.93^{b}	1.43^{b}	1.90^{d}	1.23^{b}	2.57^{a}	1.40^{b}	2.60^{g}	1.13^{g}
Kurungu	2.53^{b}	0.93^{c}	2.13^{bc}	0.93^{d}	2.53^{a}	1.26^{b}	1.67^{e}	1.23^{b}	2.57^{a}	1.40^{b}	2.83^{g}	1.27^{gh}
Tilapia	2.53^{b}	1.33^{a}	2.20^{bc}	1.10^{d}	2.60^{a}	1.30^{b}	2.63^{f}	1.30^{b}	2.50^{a}	1.33^{b}	2.50^{g}	1.23^{g}
Mean	2.70	1.18	1.93	1.03	2.61	1.33	2.03	1.23	2.58	1.42	2.70	1.25
SE	0.06	0.05	0.14	0.15	0.12	0.07	0.05	0.05	0.10	0.15	0.11	0.07
LSD (5%)	0.20	0.16	0.49	0.50	0.41	0.25	0.19	0.16	0.33	0.52	0.39	0.23
CV (%)	3.80	6.90	12.60	24.30	8.00	9.40	4.60	6.50	6.40	18.3	7.30	9.30

SE = standard error; LSD = least significant difference; CV = coefficient of variation

Standard Deviation for all means (average of three determinations) ranged from **± 0.000** to **±0.0002**

***** In any column, means bearing similar superscripts are not significantly difference (**P≥0.05**).

Table 4. Effects of Washing on the Concentration of Lead (Pb), Cadmium (Cd), Mercury (Hg) and Arsenic (As) in Smoked Fish Species from the Locations, harvested during the Dry Season (ppm)

Fish Type	Locations*											
	Alau Dam				Dabamasara				Doronbagar			
	Pb x10^{-3}	Cd x10^{-3}	Hg x10^{-3}	As x10^{-3}	Pb x10^{-3}	Cd x10^{-3}	Hg x10^{-3}	As x10^{-3}	Pb x10^{-3}	Cd x10^{-3}	Hg x10^{-3}	As x10^{-3}
Bargi	1.20^{a}	0.70^{b}	0.47^{a}	0.20^{a}	1.47^{a}	0.97^{a}	0.83^{a}	0.53^{b}	1.37^{a}	0.43^{ab}	0.37^{a}	0.17^{a}
Catfish	0.93^{b}	0.30^{a}	0.30^{b}	0.17^{a}	1.73^{b}	1.00^{a}	0.93^{a}	0.33^{ab}	1.10^{b}	0.37^{a}	0.73^{b}	0.33^{b}
Kurungu	1.27^{a}	0.33^{a}	1.73^{c}	0.47^{b}	1.60^{ab}	1.00^{a}	0.20^{b}	0.10^{a}	0.90^{c}	0.43^{ab}	1.10^{c}	0.30^{b}
Tilapia	1.47^{c}	0.70^{b}	0.57^{a}	0.30^{c}	1.47^{a}	0.27^{a}	0.10^{b}	0.33^{ab}	1.17^{b}	0.53^{b}	1.00$^{c\,a}$	0.37^{b}
Mean	1.22	0.51	0.77	0.28	1.57	0.81	0.75	0.33	1.13	0.44	0.80	0.29
SE	0.05	0.06	0.14	0.03	0.07	0.04	0.08	0.09	0.07	0.05	0.05	0.04
LSD(5%)	0.16	0.21	0.49	0.12	0.25	0.15	0.26	0.31	0.23	0.16	0.19	0.14
CV (%)	6.60	20.50	32.30	21.20	8.20	9.00	17.60	47.00	10.30	18.50	11.80	23.60

SE = standard error; LSD = least significant difference; CV = coefficient of variation

Standard Deviation for all means(average of three determinations) ranged from **± 0.000** to **±0.0002**

* In any column, means bearing similar superscripts are not significantly difference (**P≥0.05**).

Table 5. Effects of Smoking and washing on the concentration of Lead (Pb), Cadmium (Cd), Mercury (Hg) and Arsenic (As) in different fish species from the Locations harvested during the Rainy Season — washed samples (ppm)

Fish Type	Locations											
	Alau Dam				Dabamasara				Doronbagar			
	Pb x10^{-3}	Cd x10^{-3}	Hg x10^{-3}	As x10^{-3}	Pb x10^{-3}	Cd x10^{-3}	Hg x10^{-3}	As x10^{-3}	Pb x10^{-3}	Cd x10^{-3}	Hg x10^{-3}	As x10^{-3}
Bargi	1.87^{a}	0.50^{b}	1.03^{c}	0.50^{d}	1.23^{a}	0.43^{b}	1.13^{c}	0.37^{d}	1.53^{e}	0.77^{f}	1.57^{g}	0.70^{a}
Catfish	1.47^{b}	0.47^{b}	0.93^{c}	0.37^{d}	1.60^{b}	0.67^{b}	1.23^{c}	0.60^{e}	1.53^{e}	0.60^{f}	1.57^{g}	0.37^{b}
Kurungu	1.53^{b}	0.27^{b}	1.03^{c}	0.40^{d}	1.67^{b}	0.50^{b}	1.07^{c}	0.47^{ed}	1.20^{f}	0.70^{f}	1.53^{g}	0.50^{b}
Tilapia	1.37^{b}	0.60^{b}	1.36^{c}	0.40^{d}	1.37^{a}	0.53^{b}	1.50^{d}	0.60^{e}	1.57^{e}	0.60^{f}	1.13^{h}	0.43^{b}
Mean	1.56	0.46	1.09	0.42	1.47	0.53	1.23	0.51	1.46	0.67	1.45	0.50
SE	0.10	0.14	0.09	0.11	0.07	0.09	0.06	0.06	0.06	0.09	0.10	0.06
LSD (5%)	0.33	0.48	0.32	0.37	0.26	0.30	0.22	0.20	0.22	0.31	0.36	0.21
CV (%)	10.60	52.10	14.70	44.40	8.70	28.50	9.00	19.90	7.00	23.30	12.30	21.30

SE = standard error; LSD = least significant difference; CV = coefficient of variation

Standard Deviation for all means (average of three determinations) ranged from **± 0.000** to **±0.0002**

*In any column, means bearing similar superscripts are not significantly difference (**P≥0.05**).

Table 6. Comparison of Mean Heavy Metal Concentrations in Smoked, Washed and Unwashed Fish, Sampled from the Locations during the Dry Season (ppm)*

LOCATION:		Alau Dam		Daban Masara		Doronbaga	
Treatment:		washed	unwashed	washed	unwashed	washed	unwashed
Type of Fish	Metal	x10^{-3} ppm					
Bargi	Pb	1.20^{a}	2.03^{b}	1.47^{c}	1.73^{d}	1.37^{e}	2.33^{f}
	Cd	0.70^{a}	1.56^{b}	0.97^{c}	1.97^{d}	0.43^{e}	2.00^{f}
	Hg	0.47^{a}	1.13^{b}	0.83^{c}	2.63^{d}	0.37^{a}	1.90^{f}
	As	0.20^{a}	0.63^{b}	0.53^{d}	0.43^{d}	0.17^{a}	0.43^{d}
Catfish	Pb	0.93^{g}	2.23^{h}	1.73^{i}	2.53^{j}	1.10^{k}	2.33^{l}
	Cd	0.30^{g}	1.76^{h}	1.00^{i}	1.97^{j}	0.37^{g}	1.90^{l}
	Hg	0.30^{g}	1.27^{h}	0.93^{i}	2.03^{j}	0.73^{k}	1.50^{l}
	As	0.17^{g}	0.60^{h}	0.33^{g}	0.63^{h}	0.33^{g}	0.67^{h}
Kurungu	Pb	1.27^{m}	3.63^{n}	1.60^{p}	3.03^{q}	0.90^{r}	1.67^{p}
	Cd	0.33^{m}	1.60^{q}	1.00^{p}	1.77^{q}	0.43^{m}	2.00^{s}
	Hg	1.73^{m}	1.27^{n}	0.20^{p}	1.50^{q}	1.10^{n}	2.00^{s}
	As	0.47^{m}	1.17^{n}	0.10^{p}	1.00^{q}	0.30^{m}	0.90^{s}
Tilapia	Pb	1.47^{a}	2.03^{b}	1.47^{a}	2.50^{c}	1.17^{d}	2.00^{b}
	Cd	0.70^{e}	2.00^{f}	0.27^{g}	1.87^{h}	0.53^{e}	1.89^{h}
	Hg	0.57^{g}	1.70^{h}	0.10^{i}	0.97^{k}	1.00^{k}	1.86^{h}
	As	0.30^{a}	1.13^{b}	0.33^{a}	2.67^{c}	0.37^{a}	0.93^{d}

Standard Deviation for all means ranged from **±0.000 to ±0.0002**

*In row, means bearing the same superscript are not significantly different (P≥0.05)

When compared with the smoked unwashed samples from the same location and during the same season, the reduction in metal concentrations by washing was greater than 50% in some fish types (Tables 6 and 7). The inability of the washing process to remove all the metal concentrations from the smoked samples may indicate that some of the smoke constituents might react with the metals in fresh fish during the smoking process, forming water insoluble complexes that may not be readily removed by washing; or the metals may be present in the fish tissues in a strongly bounded form that could not be easily removed by washing. In addition, the efficiency and ability to reduce the heavy metals from fish through washing can be possible only when these metals occur as surface contaminants on the fish, and this may be affected by the initial levels of contamination with the metals as observed in studies on heavy metal contents in fruits and vegetable crops by Igwegbe *et al*. (1993 and 2013). The fact that the smoking process resulted in increased concentrations

Table 7. Comparison of Mean Heavy Metal Concentrations in Smoked, Washed and Unwashed Fish, Sampled from the Locations during the Rainy Season (ppm)*

LOCATION:		Alau Dam		Dabamasara		Doronbaga	
Treatment:		washed	unwashed	washed	unwashed	washed	unwashed
Type of Fish	Metal			$x10^{-3}$ ppm			
Bargi	Pb	1.87^{a}	3.17^{b}	1.23^{c}	2.37^{d}	1.53^{e}	2.67^{f}
	Cd	0.50^{a}	1.23^{b}	0.43^{a}	1.33^{b}	0.77^{c}	1.53^{d}
	Hg	1.03^{b}	1.53^{c}	1.13^{d}	1.93^{e}	1.57^{f}	2.87^{g}
	As	0.50^{c}	1.07^{d}	0.37^{c}	1.17^{d}	0.70^{e}	1.37^{f}
Catfish	Pb	1.47^{b}	2.57^{c}	1.60^{a}	2.93^{d}	1.53^{b}	2.57^{c}
	Cd	0.47^{c}	1.23^{d}	0.67^{b}	1.43^{a}	0.60^{b}	1.40^{a}
	Hg	0.93^{d}	1.83^{a}	1.23^{c}	1.90^{a}	1.57^{b}	2.60^{e}
	As	0.37^{a}	1.03^{b}	0.60^{d}	1.23^{c}	0.37^{a}	1.13^{b}
Kurungu	Pb	1.53^{c}	2.53^{a}	1.67^{c}	2.53^{a}	1.20^{b}	2.57^{a}
	Cd	0.27^{a}	0.93^{b}	0.50^{d}	1.26^{c}	0.70^{e}	1.40^{c}
	Hg	1.03^{b}	2.13^{d}	1.07^{b}	1.67^{c}	1.53^{c}	2.83^{a}
	As	0.40^{d}	0.93^{c}	0.47^{d}	1.23^{b}	0.50^{d}	1.27^{b}
Tilapia	Pb	1.37^{a}	2.53^{b}	1.37^{a}	2.60^{b}	1.57^{c}	2.50^{b}
	Cd	0.60^{c}	1.33^{b}	0.53^{c}	1.30^{b}	0.60^{c}	1.33^{b}
	Hg	1.36^{b}	2.20^{c}	1.50^{b}	2.63^{a}	1.13^{d}	2.50^{a}
	As	0.40^{d}	1.10^{b}	0.60^{c}	1.30^{a}	0.43^{d}	1.23^{ab}

Standard Deviation for all means ranged from **±0.000 to ±0.0002**

*In any row, means bearing the same superscript are not significantly different ($P \geq 0.05$)

of the four heavy metals investigated in the present study is in agreement with the findings of Essuman (2005). He recorded high levels of lead and mercury in smoked Tilapia fish ranging from 10 to 30ppm and 14 to 53pp, respectively. In another study, Ashraf (2006) observed that the level of heavy metal contaminants could increase in food from time to time during commercial handling and processing. Also, Moore (2000) noted that skinning and trimming fish neither significantly reduced the mercury concentration in fish fillets nor was the mercury removed during the cooking process. In fact, as cooking and smoking processes remove moisture, metals could be concentrated in the fish. The results of this study is also in consistence with that of Serile *et al*., 2006 who observed that lead and cadmium concentrations in muscle of vacuum packaged fish species ranging form 0.001 to 0.036ppm. Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity and accumulation by marine organisms. While lead, cadmium, mercury and arsenic can be tolerated at extremely low concentrations, they are extremely toxic to humans.

The ingestion of food is an obvious means of exposure to these metals, not only because they are natural components of foodstuffs but also because of environmental contamination and contamination during processing. With the exception of occupational exposure, fish are acknowledged to be the single largest source of particularly mercury for man. In some instances, fish catches were banned for human consumption because their total toxic metal contents exceeded the maximum limits recommended by the FAO and WHO. Toxic heavy metals may influence the role of essential metals as cofactors for enzymes or metabolic processes. For instance, lead has been observed to interfere with the calcium-dependent release of neuro-transmitters (Annua and Cuomo, 1988; Bellinger *et al*., 1992). Also, lead, cadmium and vitamin D have been shown to have a complex relationship affecting mineralization of bone, and there exists a more direct influence involving impairment of 1-25-dihydroxy vitamin D synthesis in the kidney (Lutz *et* al., 1996; Needleman *et al*., 1979). In general, the concentrations of lead, cadmium, mercury and arsenic recorded in this study are within the ranges, and below the values obtained in some similar studies in Nigeria and around the world (Luczynskan and Bruck-Jastrzebska, 2006; Obasohan and Eguaveon, 2008; Abdel-Baki *et al*., 2011; Ambedkar and Muniyan, 2011). Many previous literatures have shown that the occurrence of toxic elements in fish is related to length of time in water, weight, age, sex of fish, their feeding habits and bio-availability of the specific metal (Khansari *et al.,* 2005; Ashraf, 2006; Ekpo *et* al., 2008; Akan *et al*., 2009 and 2012; *Rahimi et al*., 2010; Voegborlo *et al*., 1999). Season and location are also important in the levels of toxic elements accumulation in various species of fish (Moore, 2000; Rahimi *et al*., 2010; Igwegbe, *et al*., 2014).

Conclusion

The four toxic heavy metals, lead, cadmium, mercury and arsenic investigated in this study were detected in both the fresh and smoked fish samples. Smoking resulted in greater concentrations of the heavy metals, though the concentrations were still lower than the maximum permissible limits and guidelines set by WHO and FAO, and adopted by many countries for fish and fish products. The study reveals that eating both fresh and smoked fish obtained from rivers and lakes in the Lake Chad Basin is safe, and does not pose any immediate threat to the health of the fish-consuming public for now. However, it is important to note that the presence of trace heavy metal pollutants in diets could create serious health problems ranging from neuro-, nephro-, carcino- to immunological disorders, if ingested over a long period of time. It is therefore recommended that assessment of heavy metal content in fresh and processed fish harvested from inland waters of Lake Chad Basin should be continued. This should be done at least twice annually and the analysis should be limited to lead, cadmium, mercury and arsenic. Samples of water from the various inland waters within the Lake Chad Basin should also be analyzed regularly to determine their heavy metal contents.

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