

Determination of Benzo(A)Pyrene and Heavy Metals Contamination in Smoked *Lates Niloticus* and *Oreochromis Niloticus* from Lake Victoria

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Abstract

Fish remains to be an important source of proteins in developing countries including Tanzania. Fish processing methods like smoking aim at improving the shelf-life of smoked fish as well as taste and aroma. During smoking, smoke by-products from different materials used as source of heat are deposited on the fish. The deposited by-products include the carcinogenic polycyclic aromatic hydrocarbons (PAHs) and heavy metals. Benzo(A)pyrene has been used as a marker for the occurrence of carcinogenic PAHs. The purpose of this study was to assess the different materials that are used in fish smoking practices, determine the levels of benzo(A)pyrene, mercury, cadmium and lead in smoked *Lates niloticus* (Nile Perch) and *Oreochromis niloticus* (Nile Tilapia) from different fish smoking areas in Mara and Mwanza regions. A total of 32 fish smokers were interviewed to assess the material used and how they use them to smoke their fish. This was followed by collection of 32 smoked fish samples for laboratory analysis of heavy metals (mercury, Cadmium and Lead) and concentration of benzo(A)pyrene. The findings of this study indicated that people engaged in smoking fish in the study areas are mostly using firewood and charcoal as their source of heat. There were no cases of the use of plastic materials. The laboratory results indicated that mercury and cadmium were not detected in all fish species while lead was detected at a mean concentration level of 0.28 µg/kg which is below the recommended level of 0.3 µg/kg as set by the EU. This indicated that smoked fish from Mara and Mwanza did not contain heavy metals to a harmful level. The Mean benzo(a)pyrene concentration detected was 4.79 µg/kg. This amount is higher compared to a level of 2 µg/kg set by the EU in 2014. There is therefore, a need for people who smoke fish to use other improved methods which will lower the levels of benzo(a)pyrene and the Government to have a continuous monitoring plan for these contaminants.

Keywords: Smoked fish, *Lates niloticus*, *Oreochromis niloticus*, heavy metals, and Lake Victoria.

Introduction

Fish is an important source of nutrients as it contains high amount of protein and several essential micronutrients (Bene *et al.*, 2015). It is the main source of animal protein for over 20% of the world's population (FAO, 2020). The total world fish production (inland and marine waters) has increased from 101 million tonnes in 1995 to over 179 million tonnes in 2018 with a total first sale value estimated at USD 401 billion (FAO, 2020). Africa contributing about 11 million tonnes whereas Tanzania contributed 0.31 million tonnes (FAO, 2020). The total production in

Tanzania includes fish from ocean, lakes, rivers and aquaculture. Aquaculture in Tanzania is dominated by freshwater fish farming in which small-scale farmers are practicing extensive and semi-intensive fish farming in small fish ponds of an average size of 150 to 500 m² (Shoko *et al.*, 2011). The main species farmed is the Nile Tilapia (*Oreochromis niloticus*) which is the dominant specie (99%) due to its superior growth compared to other species of the farmed fresh water fish (Rothuis *et al.*, 2014). Other farmed species include rainbow trout (*Oncorhynchus mykiss*), catfish (*Clarias gariepinus*) and milkfish (*Chanos chanos*) (Shoko *et al.*, 2011).

Moreover, fish are very perishable and they deteriorate rapidly under normal temperatures. The deterioration is influenced by several factors such as the habitat of the fish and nutritional composition. Bacteria grow on the outer and inner parts of the fish such as skin, gills and gastro-intestinal tract. Fish contain high protein (12-24%) and large amounts of non-protein-nitrogen (NPN) such as nucleotides, Trimethylamine Oxide (TMAO) and free amino acids. These serve as substrate for bacterial growth and upon decomposition, cause off odours and flavours. Also, fish have a lipid content of 0.1-22% which includes the long-chain, polyunsaturated fatty acids which are highly susceptible to hydrolysis and oxidation (Esteves *et al.*, 2016). In addition, the high-water activity (aw) of fish makes them more susceptible to spoilage. In order to maintain the quality of fish and assure the safety, preservation and processing measures are important (Adeyeye and Oyewole, 2016). Fish preservation aims at maintaining the quality and extending the shelf-life. Major fish preservation methods include drying, salting, freezing, chilling, fermentation and smoking (Assogba *et al.*, 2019).

Smoking is the most common method of drying fish. The smoked fish are the most available form of fish in developing countries due to limited access to electricity to preserve fish (Tongo *et al.*, 2017). Smoking methods involve exposing fish directly to smoke from wood for several hours or days (2–3 days) which results in dehydration and deposition of combustion by-products on smoked fish (Forsberg *et al.*, 2012). The smoke gives the fish special taste, aroma and improves preservation due to its dehydrating and bactericidal properties. However, the deposited by-products include some potentially harmful combustion by-products such as Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metals (Tongo *et al.*, 2017). Polycyclic Aromatic Hydrocarbons (PAHs) refer to compounds which are chemically comprised of two or more benzene rings which are bonded in a linear, cluster or angular arrangements (Abdel-shafy & Mansour, 2015). They are ubiquitous and toxic to the environment and food processing contaminants produced by incomplete combustion or pyrolysis of organic

materials (Purcaro *et al.*, 2013). The PAHs are known to be mutagens and carcinogenic in mammals. Several studies confirmed that diet such as smoked fish is the major way of human exposure to PAHs (Forsberg *et al.*, 2012; Roseiro *et al.*, 2011). The PAHs can enter the food through smoking and cooking processes. Food becomes contaminated by PAHs due to incomplete combustion of the materials used during smoking (Visciano *et al.*, 2009). In Tanzania, smoked fish is one of the commonly consumed foods and it serves as a good source of proteins, but there is limited information on PAHs and heavy metals on smoked fish especially from Lake Victoria region. In Tanzania there are no established standards for PAHs in smoked fish and no routine monitoring procedures are in place for safeguarding public health. The consumers of smoked fish have limited knowledge about the presence of PAHs in the smoked fish. Thus, the objective of this study was to find out the materials used to generate heat for smoking and determine the levels of benzo(a)pyrene and heavy metals in smoked Nile Perch (*Lates niloticus*) and Nile Tilapia (*Oreochromis niloticus*) from different fishing communities around Lake Victoria.

Materials and methods

Study Area

The study was conducted in Mwanza and Mara regions (Figure 1 A and B respectively). Mwanza is located on the southern part of Lake Victoria about 1200 - 1400 metres above the sea level; and lies between latitudes 1°30' and 3°00' South of the Equator and between longitudes 31°45' and 34°10' East of Greenwich. The region is bordered by Lake Victoria in the North, Kagera and Geita regions in the West, Mara region on the East, while Shinyanga and Simiyu regions are located on the South and South-eastern side of the region (Mwanza Region Investment Guide, 2017). According to 2012 population census (NBS, 2013), Mwanza region has a population of 2,772,509 people and about 3.3% of economically active population are engaged in fishing. Mara region is located in the northern part of Tanzania Mainland and it lies between latitudes 10° and 20° South and longitudes 31°10' and 35°15' East. The region

is bordered by Kenya to the north, Simiyu region to the south, Arusha region to the east and Kagera region to the west (MRCO, 2005). The region has a population of 1,743,830 (NBS, 2013) and a small part of this population is engaged in fishing activities, including people who are living in the shores of the Lake Victoria (Mara Region report, 2007; NBS, 2013).

Sampling Techniques

The people engaged directly in fish smoking in Ilemela and Musoma were selected purposely. The fish smoking areas covered in Ilemela were Kirumba, Mwaloni, Kitangiri, Kiyungi, Igombe, Ibanda juu and Magomeni; and the areas covered in Musoma were Rukuba island and Bwai in Kiriba ward. Estimation of

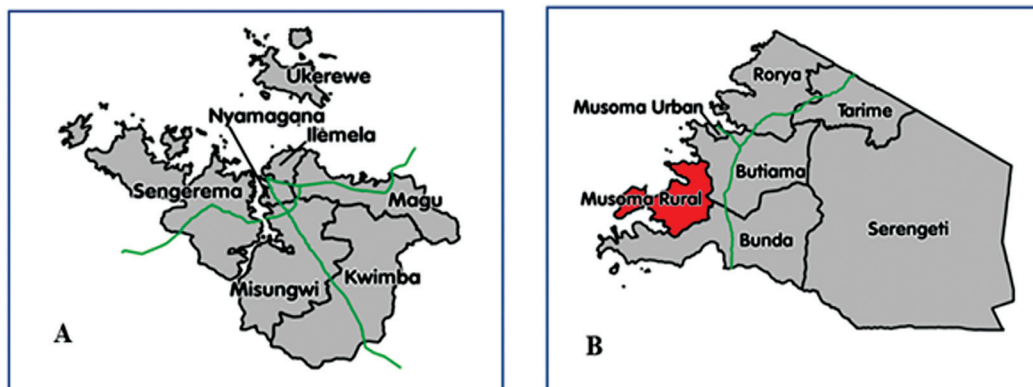


Figure 1. A. Mwanza region and B. Mara region (www.wikipedia.org).

Study Design

A cross-sectional research design was conducted where sociological and laboratory data were collected at one time. The design is flexible which minimizes bias and maximizes reliability and analysis (Kothari, 2004). Purposive sampling was used to select fish smoking dealers in Ilemela and Musoma Municipalities. People who are engaged in fish smoking activities were randomly selected and administered with structured questionnaire. Samples were collected for laboratory analysis and purchased at the same time from the randomly selected fish smokers.

Study Population and Inclusion and Exclusion Criteria

The study population comprised of selected people who were engaged in fish smoking activities, males and females in Ilemela and Musoma Municipalities. The selected people were those engaged in fish smoking activities and who were available at the time of data collection, willing to participate and ready to give the required information were included in the study.

the sample size was done by using the equation proposed by Kothari (2009).

$$N = \frac{Z^2 P(1 - P)}{D^2} \tag{1}$$

Whereas:

- N= estimated sample size
- Z=Confidence Interval
- D=Precision level (acceptable error)
- P=Estimated Prevalence

Samples of smoked fish were collected directly from the smoking premises and from the people who were interviewed after completing their questionnaire. The smoked fish samples were comprised of two species, *Lates niloticus* and *Oreochromis niloticus*. A total of 32 samples of smoked fish were collected from Mara and Mwanza (8 samples for each species from each region). The samples were then stored in properly-sealed plastic bags, labelled and then transported to Tanzania Food and Drugs Authority (TFDA) Lake Zone Laboratory in cooler box. Samples were then stored in a deep freezer at a temperature of -20 °C prior to analysis.

Questionnaire Administration Pre-Testing and Data Analysis

The questionnaire was pre-tested before commencing data collection at Nyatukara and Mtakuja wards in Sengerema District, Mwanza involving 5 fish smokers with the aim of checking the clarity and applicability of the questions. Questions which were unclear and difficult to answer were revised and others omitted. The revised questions were translated into Swahili for easy understanding by the majority of the people.

Laboratory Analysis (lead, cadmium and mercury)

Laboratory analysis of fish samples were carried out to determine the levels of three heavy metals namely lead, mercury and cadmium and the levels of benzo(a)pyrene contamination. On arrival of the samples to the laboratory, they were analysed for lead, cadmium and mercury contamination. The analysis was done using TFDA in-house method, 2018 (MP-AES manufacturer provided method) which involved preparation of stock solution, preparation of working standard and sample preparation prior to analysis.

Questionnaire and laboratory data were recorded using Microsoft Excel and later imported into SPSS version 20 for analysis. Descriptive statistics-frequencies, percentages, means and counts from the responses were used to determine distribution and magnitude of variables. Duncan's test and confidence intervals were used to compare variables where the differences were deemed significant when $P < 0.05$. Correlation analysis was done to test the association between different variables.

Results and Discussion

Fish smoking practices and materials used in fish smoking

Demographic characteristics of the respondents

The demographic characteristics of the people who are engaged in fish smoking are presented in Figure 1. Female respondents form the majority (81%) of people engaged in fish smoking activities across all regions while the number of males is represented by 19%. The results show that the majority (47%) of the respondents are between the age of 25-34 years who constitute the largest proportion. Respondents with more than 35 years represent 34% while respondents with age below 24

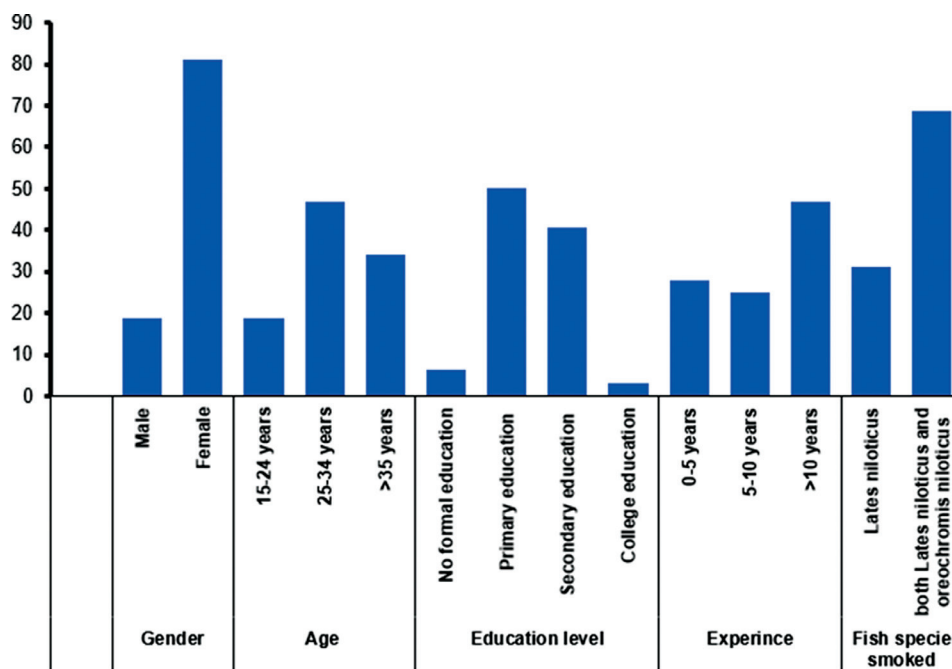


Figure 2: Socio-demographic characteristics of the respondents (N = 32)

years were 19%. The results show that 50% of the respondents had primary education, 41% secondary education, 6% no formal education and 3% college education. Almost half of the respondents (47%) had been in the fish smoking business for more than 10 years while 28% had been in business for less than 5 years. Twenty five percent (25%) of respondents had been engaged in the fish smoking business for a period of between 5 to 10 years.

This study showed that women are more engaged in smoking of fish than men across all regions (Fig. 1). Women have been reported to work in the fish industry in many parts of the world with different roles, depending on communities and type of fishing activity (Nwabueze, 2010). In most of these fishing communities, fishing has been viewed as men's role because it is tedious, sometimes being done at night. This reason causes women to focus more on the post-harvesting practices such as smoking, deep frying, drying and salting than on the night fishing activities (Nwabueze, 2010; Anihouvi *et al.*, 2012; FAO, 2015).

The findings of this study indicate that fish smoking is an important activity to women of Mara and Mwanza regions, and this is agreed with studies conducted by Onyango *et al.* (2017) who reported 85.2% of women engaged in fish processing compared to men who were 14.8%. Likewise, Medard *et al.* (2001) reported that over 76.5% of women participating in the fishery sector in Lake Victoria are involved in off-shore activities including fish smoking. Similarly, study conducted by Njenga & Mendum (2018) in Ghana showed that women comprised 100% of the fish smokers. The roles and contributions of women in the fisheries sector have been undervalued for a long time and they have been excluded in decision making (FAO, 2015).

Materials used and fish smoking practices

The materials used in fish smoking and safety knowledge of smoked fish are presented in Table 1. All fish for smoking are obtained from middlemen (100%) who buy the fish from fishermen and sell to people who smoke fish. It has been reported that middlemen are abundant in fish trade especially in developing countries (Surtida, 2000; Thuy *et al.*, 2019). In this case,

it is important for the middlemen to have safety knowledge of handling of fresh fish so that they remain safe until further processed.

Before smoking, 59% of respondents store fresh fish on a wire mesh on the smoking kilns, 22% keep it on the ground, 13% are kept in cold storage and 6% are kept in plastic basins. Poor handling of the fresh fish prior to smoking may result in chances of contaminations in smoked fish (Igwegbe *et al.*, 2015). For example, storage of fresh fish on the ground (sand) exposes them to all forms of contaminations including heavy metals. The quality of the fresh fish is an important factor which determines the quality of the smoked fish product (Debbarma *et al.*, 2018). The current study found out that most of the fresh fish are stored in a wire mesh contained on the smoking kilns before smoking that reduce chances of contamination. This finding agrees that reported by Kabahenda *et al.* (2009) that shows before smoking fish products are usually placed in a rack in a kiln and allowed to drip to remove excess water for several hours. The results for fish size, smoking during and safety of materials used for smoking are shown in Table 1.

The current study shows that consumers are influenced by texture and appearance of the fish. For example, dry and moderately black or brownish smoked fish are the more preferred qualities. According to Abraha *et al.* (2018) texture and general appearance of smoked fish contributes to product acceptability by the consumers. In view of this, fish smokers may use other materials other than firewood which will result to dry, black or brownish fish such as plastic remains. The study found out that plastic materials and wastes were not used in fish smoking as the source of heat and smoke contrary to the study conducted by Kabahenda *et al.* (2009) that other materials such as cow dung was used as a source of fuel to smoke fish in Businga Island of Lake Victoria. Despite the fact that plastic materials were not used to smoke fish, majority of fish smokers admitted that sometimes these materials are used. The majority of the smokers can differentiate the firewood-smoked-fish from those smoked with plastic materials by physical appearances of the smoked fish.

Category		Region (frequency)		Total	Percent
		Mara	Mwanza		
Fish species smoked	<i>Lates niloticus</i>	5	5	10	31.3
	both species	11	11	22	68.8
Fish storage	Cold storage	2	2	4	12.5
	On the ground	4	3	7	21.9
	In a container	0	2	2	6.3
	On wire mesh	10	9	19	59.4
Fish smoking time	day time	12	10	22	68.8
	at night	0	1	1	3.1
	Both	4	5	9	28.1
Obtaining firewood	Not easy	0	4	4	12.5
	Easy	16	12	28	87.5
Smoking fish using materials	No	12	15	27	84.4
	Yes	4	1	5	15.6
Why use other materials other than firewood	When having small amount of fish	2	0	2	46.9
	Lack of firewood	1	2	3	25
	Use of firewood is tedious	1	0	1	3.1
Why not use other materials	Firewood is easy to get	4	11	15	46.9
	Firewood results into fish of good quality	6	2	8	25
	Firewood is easy to use	1	0	1	3.1
Other materials used	Charcoal	4	1	5	100
Source of other materials	Market	4	1	5	100
Time taken to smoke fish	Less than 3 hours	11	10	21	65.6
	4 hours	1	4	5	15.6
	5 hours or more	4	2	6	18.8
Preferred fish size for smoking	Small size	6	8	14	43.8
	Medium size	3	1	4	12.5
	Large size	6	1	7	21.9
	Both sizes	1	6	7	21.9
What makes smoked fish to be perceived of good quality?	Taste	2	1	3	9.4
	Keeping quality	14	15	29	90.6
What are customers looking for in smoked fish	moderately black fish	11	11	22	68.7
	less black smoked fish	1	1	2	6.3
	brownish fish	4	4	8	25
Awareness of any chemicals from the smoking materials	Not aware	16	16	32	100
Awareness of the safety of materials used to smoke fish	Not aware	16	16	32	100
Difference in appearance between fish smoked by firewood and by plastic materials	Yes	15	16	31	96.9

Category		Region (frequency)		Total	Percent
		Mara	Mwanza		
How they differ	No	1	0	1	3.1
	Fish smoked by other materials are reddish and not dry	2	1	3	9.7
	Fish smoked by wood are darker	1	2	3	9.7
	Fish smoked by wood are drier	12	13	25	80.6

Heavy metal levels in smoked *L. niloticus* and *O. niloticus*

The results for heavy metal contamination in smoked *Lates niloticus* and *Oreochromis niloticus* are presented in Table 2. Lead (Pb) concentrations ranged from 0 mg/kg to 1.21 mg/kg which was detected in smoked *Lates*

niloticus from Mwanza. Cadmium (Cd) and Mercury (Hg) were not detected in all samples.

The minimum, maximum and mean concentrations levels of Pb, Cd and Hg from the two fish species are indicated in Table 3. The levels of Pb observed in all samples were not significantly different ($P > 0.05$).

Table 2: Levels of Pb, Cd and Hg in smoked *L. niloticus* and *O. niloticus*

Sample Code	Fish Specie	Lead (mg/kg)	SD	Cadmium (mg/kg)	SD	Mercury (mg/kg)	SD
MS O1	<i>O. niloticus</i>	0.79	0.18	n.d	0.15	n.d	0.01
MS O2	<i>O. niloticus</i>	n.d	0.16	n.d	0.09	n.d	0.02
MS O3	<i>O. niloticus</i>	0.31	0.19	n.d	0.08	n.d	0.02
MS O4	<i>O. niloticus</i>	0.32	0.18	n.d	0.03	n.d	0.02
MS O5	<i>O. niloticus</i>	n.d	0.05	n.d	0.07	n.d	0.02
MS O6	<i>O. niloticus</i>	0.02	0.06	n.d	0.18	n.d	0.04
MS O7	<i>O. niloticus</i>	n.d	0.06	n.d	0.11	n.d	0.01
MS O8	<i>O. niloticus</i>	n.d	0.25	n.d	0.05	n.d	0.02
MS L1	<i>L. niloticus</i>	n.d	0.64	n.d	0.07	n.d	0.02
MS L2	<i>L. niloticus</i>	n.d	0.57	n.d	0.08	n.d	0.02
MS L3	<i>L. niloticus</i>	0.89	0.39	n.d	0.02	n.d	0.02
MS L4	<i>L. niloticus</i>	0.32	1.78	n.d	0.05	n.d	0.04
MS L5	<i>L. niloticus</i>	0.69	0.01	n.d	0.04	n.d	0.05
MS L6	<i>L. niloticus</i>	0.96	0.26	n.d	0.18	n.d	0.04
MS L7	<i>L. niloticus</i>	0.22	0.03	n.d	0.04	n.d	0.03
MS L8	<i>L. niloticus</i>	n.d	0.69	n.d	0.09	n.d	0.04
M O1	<i>O. niloticus</i>	n.d	0.55	n.d	0.06	n.d	0.03
M O2	<i>O. niloticus</i>	n.d	0.33	n.d	0.05	n.d	0.04
M O3	<i>O. niloticus</i>	0.22	0.13	n.d	0.03	n.d	0.01
M O4	<i>O. niloticus</i>	n.d	0.14	n.d	0.01	n.d	0.01
M O5	<i>O. niloticus</i>	n.d	0.01	n.d	0.03	n.d	0.01
M O6	<i>O. niloticus</i>	0.06	0.01	n.d	0.04	n.d	0.04
M O7	<i>O. niloticus</i>	n.d	0.46	n.d	0.11	n.d	0.03

Table 2: Levels of Pb, Cd and Hg in smoked *L. niloticus* and *O. niloticus*

Sample Code	Fish Specie	Lead (mg/kg)	SD	Cadmium (mg/kg)	SD	Mercury (mg/kg)	SD
M O8	<i>O. niloticus</i>	0.79	0.28	n.d	0.02	n.d	0.02
M L1	<i>L. niloticus</i>	1.21	0.02	n.d	0.05	n.d	0.05
M L2	<i>L. niloticus</i>	0.62	0.14	n.d	0.1	n.d	0.05
M L3	<i>L. niloticus</i>	n.d	0.89	n.d	0.08	n.d	0.03
M L4	<i>L. niloticus</i>	n.d	0.06	n.d	0.05	n.d	0.13
M L5	<i>L. niloticus</i>	0.94	0.03	n.d	0.04	n.d	0.01
M L6	<i>L. niloticus</i>	0.26	0.26	n.d	0.07	n.d	0.03
M L7	<i>L. niloticus</i>	n.d	0.18	n.d	0.06	n.d	0.02
M L8	<i>L. niloticus</i>	0.72	0.17	n.d	0.08	n.d	0.04

MS=samples from Mara, M=samples from Mwanza, n.d=not detected, L=Lates, O=Oreochromis, SD=Standard Deviation. Values are means of three replicates

Table 3: Levels of Pb, Cd and Hg in smoked *L. niloticus* and *O. niloticus* (N = 32)

Heavy metal	Minimum level (ppm)	Maximum level (ppm)	Mean (mg/kg)	Std. Deviation	t-value	P-value
Mercury	0	0	0	0		
Lead	0	1.21	0.28	0.38	-0.269	0.790
Cadmium	0	0	0	0		

The laboratory results were categorized based on the recommended level of Pb as a cutoff point to determine the percentage of those which are above or below the recommended level. The result in Table 4 shows that 65.6% of the samples were below the recommended limit of 0.3 mg/kg and 34.4% of the samples analyzed were above the recommended level.

± 0.43 and 0.15 ± 0.28 ppm, respectively. The mean Pb concentration recorded across regions on average were not statistically significantly different (Table 5).

The current study shows that smoked *Lates niloticus* and *Oreochromis niloticus* contained Pb in varying amounts. Lead was detected in some fish samples in a higher concentration but

Table 4: Category of Pb level based on recommended level (0.3 mg/kg)

	Frequency	Percent
Below the recommended level	21	65.6
Above the recommended level	11	34.4

The results show that there is a significant difference in Pb concentrations across species at $P \leq 0.05$. The mean Pb levels recorded for *Lates niloticus* and *Oreochromis niloticus* were 4.40

were not detected in other smoked fish samples. The highest concentration of Pb was found in smoked Lates niloticus with a concentration of 1.21 mg/kg from Mwanza.

Table 5: Mean differences in Pb concentration across species and regions ($P \leq 0.05$)

Variable	Mean (mg/kg) \pm SD	F-ratio	(P-Value)
Specie	<i>L. niloticus</i>	4.40 \pm 0.43	3.918 (0.05)
	<i>O. niloticus</i>	0.15 \pm 0.28	
Region	Mara	0.26 \pm 0.36	0.080 (0.78)
	Mwanza	0.30 \pm 0.41	

Lead, a non-essential metal has been shown to be toxic and there is no known level of exposure that is considered safe (Tchounwou *et al.*, 2012; WHO, 2017). Higher levels of lead in the human body have been linked to the damage of the nervous system, brain and kidney; gastrointestinal diseases and adverse effects in vitamin D metabolism (Ogwuegbu and Muhanga 2005; Tchounwou *et al.*, 2012). The mean Pb concentration detected was 0.28 mg/kg which is slightly lower than 0.3 mg/kg which is the maximum permissible level recommended by WHO (2017). The levels of Pb observed in the smoked fish samples could have come from the firewood is used as a source of heat and smoke, the smoking process or a result of bio-accumulation. It has reported that use of charcoal and materials containing paints in the smoking process produces smoke that contain Pb (Adekunle and Akinyemi, 2004). The Pb contained in the smoke may attach to the fish meat and contaminate it. Also, smoking of fish can result in increase of the concentration of toxic heavy metals (Wangboje and Miller, 2018). The heavy metals can also originate from the concentration of the metals contained in the fresh fish when moisture is removed by the smoking process (Igwegbe *et al.*, 2015; Adekunle and Akinyemi, 2004; Megasari *et al.*, 2019). Pollution of water reservoirs by municipal effluents and industrial activities are other sources of lead contamination in fish. Significant amount of lead may accumulate in fish depending on the degree of water pollution in their habitat, exposure to the pollution and eating habit of the fish (Winiarska-Mieczan *et al.*, 2018). This could attribute to the presence of lead in smoked *Lates niloticus* and *Oreochromis niloticus*.

There was a significant difference in lead levels between smoked *Lates niloticus* and smoked *Oreochromis niloticus* (Table 6). Higher valued was detected on *Lates niloticus* (4.40 ± 0.43 mg/kg) compared with *Oreochromis niloticus* (0.15 ± 0.28 mg/kg). The difference may be due to the size of the two species and the eating habit of the fish. *Lates niloticus* is a predator and may accumulate the metal contained in the fish preyed upon. Also, the large size of *Lates niloticus* compared to

Oreochromis niloticus results to the use of more smoking materials thus allowing deposition of this metal in a great proportion. The findings of Pb concentration levels in smoked *Lates niloticus* and *Oreochromis niloticus* agreed with Igwegbe *et al.* (2015) who reported the increase of Pb levels in smoked fish after the smoking process, recording the highest concentration of Pb to be 0.00363 mg/kg. Also, Essuman (2005) found higher levels (2.8 mg/kg) of Pb in smoked fish. Likewise, the elevated levels of Pb in smoked fish in Nigeria were reported to increase after local smoking process to levels varying from 0.14 ± 0.02 mg/kg to 0.95 ± 0.01 mg/kg (Adekunle & Akinyemi, 2004).

Mercury is one of the heavy metals that can be toxic in food if present in high amounts. Higher levels of mercury in the human body affects the brain and cause impairment of other organs leading to the malfunctioning of nerves, kidneys and muscles (Jaishankar *et al.*, 2014). The recommended concentration level for Hg in smoked fish products is 0.5 mg/kg (WHO, 2017) above which is harmful to health of the consumers. This study observed the concentrations of Hg in smoked *Lates niloticus* and *Oreochromis niloticus* samples to be very low and below the detection limits. The low levels observed might be influenced by low or no accumulation of mercury by the fresh fish which were smoked, absence of mercury in the smoking materials used or degradation of methylmercury by the smoking process. Firewood and charcoal are the major source of heat and smoke in Mara and Mwanza. The absence of mercury in the smoked fish samples indicates that the firewood and charcoal used to smoke fish does not contain mercury. Moreover, it has been shown that mercury may contaminate fish through polluted water from contaminated run-offs, human activities like mining, agriculture and industrial activities (Igwegbe *et al.*, 2015). A study conducted by Mrosso & Werimo (2015) reported a huge and fast-growing human population in both rural and Urban areas surrounding Lake Victoria. These populations especially the urban produces industrial and domestic wastes which are discharged into the lake and become pollutants to the lake, affecting the water quality and organisms living in the Lake. Mercury

may accumulate in fish tissue especially if their source water contains their residues (Sserunjogi, 2009). It has been reported that smoking of fish may degrade methylmercury, a toxic form of mercury (Donkor *et al.*, 2006). This could attribute to the absence of Hg in smoked *Lates niloticus* and *Oreochromis niloticus*. This study agreed with finding of Essuman (2005) in which mercury could not be detected in all smoked fish samples analysed. However, low levels of mercury were observed in a study conducted by Adeyeye *et al.* (2016) in fish smoked using different smoking methods of drum-smoking and convective smoking and the levels observed were below the permissible level set by the World Health Organization of 0.2 ppm (Adeyeye *et al.*, 2016).

The concentrations of Cadmium in smoked *Lates niloticus* and *Oreochromis niloticus* samples observed were very low and below the detection limits. The levels were lower than the set limit for Cd in smoked fish of 0.5 mg/kg. This may be due to low accumulation in fish muscles, size of fish and the smoking materials used. Cadmium is a metal which has no benefits to the human body and it is toxic even at very low concentrations (Kumar & Singh, 2010). The metal may accumulate in fish tissues if fish are exposed to polluted water (Winiarska-Mieczan *et al.*, 2018). The accumulation is greatest in

Moreover, the accumulation of cadmium in fish tissues has been shown to increase with age and size of fish, with small fish accumulating small concentrations and vice versa (Ciardullo *et al.*, 2008; Farkas *et al.*, 2003). This study found out that the fish smokers in Mara and Mwanza prefer small-sized fish which might influence the absence of Cd among the fish samples. Cadmium may also be present in smoked fish if the smoking materials used contain this toxic metal, such as plastics, paints and batteries (WHO, 2017). This study found that plastic materials and wastes were not used to smoke fish in the two regions attributing to the absence of Cd in the two fish species. These findings agree with Fakunle and Effiong (2012) who did not detect cadmium in all smoked fish species in the study. However, increase of the level of Hg and Cd after the smoking process has been reported by Igwegbe *et al.* (2015) contrary to the findings of this study.

Benzo (A) pyrene (BaP) levels in smoked fish *Lates niloticus* and *Oreochromis niloticus*

The results for benzo (a) pyrene levels are presented in Table 6. From the results, BaP ranged from 0.87 µg/kg to 13.7 µg/kg. The mean BaP level was 4.79 µg/kg which is above the acceptable level of 2 µg/kg in smoked fish (EU, 2011).

Table 6: Level of BaP in smoked *Lates niloticus* and *Oreochromis niloticus*

Parameter	Recommended limit (µg kg ⁻¹)	Minimum (µg kg ⁻¹)	Maximum (µg kg ⁻¹)	Mean (µg kg ⁻¹)	Std. Deviation	P-Value
BaP	2	0.87	13.7	4.79	3.48	0.00

the liver and kidney which are important organs for metabolism and detoxification of cadmium in fish. It has been shown that the muscles of fish accumulate negligible concentrations of cadmium (Chowdhury *et al.*, 2004). Prior to smoking, the fish is gutted during which the internal organs such as intestines, liver and kidneys are removed, then cut into pieces depending on the size of the fish (Vidacek & Janci, 2016). These internal organs are the ones which accumulates Cd in great amount compared to the muscles, which may explain the absence of Cd in the fish samples.

One sample t-test was performed on levels of BaP and results indicated that the values are greater than the recommended limits at P<0.05 (P-Value=0.00). The result shows that there is a significant difference in concentrations of BaP between the species (P<0.05) as indicated in Table 7.

Benzo (a) pyrene has been used as a marker for the occurrence, concentration and effects of carcinogenic polycyclic aromatic hydrocarbons (EU, 2005). The presence of higher levels than the recommended levels in foods such as smoked fish poses a health risk to the consumers.

Table 7: Mean differences in BaP concentration across species and regions (P<0.05)

Variable	Mean ± SD	F ratio	(P-Value)
Specie	<i>L. niloticus</i>	6.72 ± 3.71	16.59 (0.00)
	<i>O. niloticus</i>	2.61 ± 3.49	
Region	Mara	4.63 ± 3.96	16.59 (0.79)
	Mwanza	4.96 ± 3.07	

The maximum permissible levels of 0.002 mg/kg (2.0 µg/kg) wet weight for benzo (a) pyrene is recommended by the European Union (EU, 2011). Studies have shown that fish and marine invertebrates may naturally contain small amounts of different PAHs which are absorbed from the environment (Sirkoski & Stolyhwo, 2005). Some PAHs including benzo (a) pyrene are quickly metabolized in fresh fish but do not accumulate in the muscle meat of fish. Levels of benzo (a) pyrene in smoked fish products may greatly come from the materials which are used to smoke the fish. The current study observed levels of benzo (a) pyrene ranging from 0.87 µg/kg to 13.70 µg/kg with the mean concentration of 4.79 µg/kg. This amount is slightly higher than the recommended level of 2.0 µg/kg.

Significant variation (P<0.05) was observed among fish species in the concentrations of the benzo (a) pyrene. The *Lates niloticus* recorded high levels of benzo (a) pyrene of up to 13 µg/kg compared to *Oreochromis niloticus* which was 4.99 µg/kg. This could be attributed to the size of the fish and the time taken in smoking. During smoking fish are exposed to partially burning firewood which is used to generate the smoke. Since firewood is mostly used in fish smoking, the large size of the *Lates niloticus* take a lot of time and firewood to smoke. Some people during fish smoking carry out re-smoking in order to make sure that the fish are completely dry to increase their keeping quality or according to the needs of their customers (Akpambang *et al.*, 2009). This results in deposition of high amounts of benzo (a) pyrene in the fish skin and into the muscle meat of fish. Other reasons for the higher concentration in *Lates niloticus* compared to other species has been explained as differences in bioaccumulation, metabolism kinetics, age and feeding habits of the fish

(Pointet & Milliet, 2000).

According to the EU (2011), the maximum permissible concentration levels of Benzo (a) pyrene in smoked fish products is 2 µg/kg wet weight in muscle meat of fish and smoked fish products. The levels of benzo (a) pyrene observed in this study were similar to other studies. A study on levels of PAHs in smoked and sun-dried *Synodontis victoriae*, *Haplochromis* spp and *Lates niloticus* fish samples from Lake Victoria areas in Mwanza, Tanzania indicated higher concentrations of benzo (a) pyrene in all the smoked fish samples ranging from 0.39 to 1.55 mg/kg. The concentrations of benzo (a) pyrene in *Lates niloticus* ranged from 0.51 to 1.27 mg/kg with a mean of 0.78 mg/kg (Andrew *et al.*, 2018). Likewise, Akpambang and others (2009) reported traditionally smoked and/or grilled fish from Nigerian market highly contaminated with benzo (a) pyrene with levels up to 38 µg/kg, which exceeds by far the limit of 2 µg/kg recommended by the European Commission in 2005. The benzo(a)pyrene concentration levels observed in this study is comparable with other studies (Table 8). This may be due to the type of smoking practices used in Mara and Mwanza in which most of the people use traditional smoking kilns, which use little wood, while others use charcoal. It has been indicated that the use of charcoal does not result in higher levels of benzo (a) pyrene when compared to the use of firewood (Akpambang *et al.*, 2009).

Smoking fish using small quantity of firewood and for a short time may result into low levels of the benzo(a)pyrene. Use of charcoal as it has been observed in the study gives lower levels of benzo(a)pyrene because charcoal is an already pyrolyzed material which produces clean smoke (Akpambang *et al.*, 2009).

Table 8: BaP results from previous work in different countries

Country	Specie of smoked Fish	BaP Concentrations	Study by
Tanzania	<i>L.niloticus, O.niloticus</i>	0.87 to 13.4 µg/kg	This Study
Tanzania	<i>S. victoria, Haplochromis spp and L. niloticus</i>	0.39 to 1.55 mg/kg ww	J.A.M. Mahugija, E. Njale (2018)
Kenya	<i>L. niloticus</i>	7.46 to 18.79µg/kg	Muyela, B (2012) unpublished
Southern Nigeria	<i>Clarias gariepinus, Tilapia zilli, Ethmalosa fimbriata, and Scomber scombrus</i>	max 0.28 mg/kg ww	Tongo <i>et al.</i> (2017)
Poland	<i>Sprats</i>	max 36.5 mg/kg	Zachara <i>et al.</i> (2017)
Nigeria	<i>Tilapia spp., Arius heudeloti</i>	2.4 ± 0.1 to 64.6 ± 0.2 mg/kg	Okenyi <i>et al.</i> (2016)

L=Lates, O=Oreochromis

Conclusion

The results of this study have relived that the smoking practices being carried out in Mara and Mwanza regions which are predominated by young women, uses two kinds of materials as a source of heat and smoke. These materials are firewood which is used in a great extent and charcoal to a lesser extent. The use of other materials like plastics and wastes was not observed. All the fish smokers in Mara and Mwanza were not aware of the harmful effects which may come from the smoke produced by the smoking materials. It was observed that the levels of lead, mercury and cadmium studied were all below the WHO permissible limits. Lead was detected in some fish samples in relatively low amount to high amounts but was not detected in others. On the average concentration, it indicates that lead in smoked fish samples is within the safe level.

It recommended that to carry out evaluation of smoked fish in Mara and Mwanza regions periodically in order to ascertain the use of plastic materials in fish smoking as consumers of smoked fish products may be predisposed to possible health hazards which are associated with the consumption of fish smoked using the plastic materials.

In general, *Lates niloticus* fish species recorded higher values of lead compared to *Oreochromis niloticus*, which might be influenced by its large size and feeding habit, being at the top of a food chain. Mercury and

cadmium were not detected in any of the smoked fish samples suggesting that the firewood and charcoal used in the smoking process did not contain the toxic metals. This implies that the smoked fish products in Mara and Mwanza regions are safe for human consumption due to low levels of lead, mercury and cadmium observed. However, there should be frequent monitoring plans by the central and local government to make sure that the materials used to smoke fish around Mara and Mwanza do not contaminate the fish by harmful chemicals and heavy metals. This study also found out that the mean concentration of benzo(a)pyrene was slightly higher than the recommended level as set by the EU commission. The *Lates niloticus* recorded higher levels compared to *Oreochromis niloticus* which may be related to the size of the *Lates niloticus* which takes longer time to smoke and uses more smoking materials, allowing greater deposition of benzo(a)pyrene to the fish muscle. The levels of benzo(a)pyrene in smoked fish around Mara and Mwanza needs to be monitored frequently since the chemical has carcinogenic, teratogenic and mutagenic effects to the human body.

This involvement of women especially the youths in fish smoking in Mara and Mwanza is a valuable input to the fisheries sector. This calls for the government to formally address the needs and challenges of fish smokers by preparing a policy on fish post-harvest management. This will allow them to access loans and increase

their capital so that they can use improved practices of fish smoking.

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