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SUSTAINABLE FISHERIES MANAGEMENT PROJECT (SFMP)

Microbiological (PAHs) Analysis Of Fish From Selected Areas From Central And Western Regions Of Ghana

SEPTEMBER, 2017

THE UNIVERSITY OF RHODE ISLAND GRADUATE SCHOOL OF OCEANOGRAPHY | CRC COASTAL RESOURCES CENTER

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Cover photo: Fish Smoking Activity in Shama (Credit: SNV)

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ACRONYMS

ACN	Acetonitrile
AOR	Agreement Officer's Representative
CRC	Coastal Resource Center
CSIR	Council for Scientific and Industrial Research
DI	De-ionized Water
EU	European Union
FAO	Food and Agricultural Organization
FACP	Fishery and Aquaculture Country profiles
GC/MS	Gas Chromatography/ Mass Spectrometry
GDP	Gross Domestic Product
GSA	Ghana Standard Authority
HPLC	High Performance Liquid Chromatography
ISO	International Standards Organization
NMKL	Nordic Committee on Food Analysis Method
MOFA	Ministry of Food and Agriculture
MSD	Mean Square Displacement
QC	Quality Control
PAHs	Polycyclic Aromatic Hydrocarbons
SFMP	Sustainable Fisheries Management Project
SNV	Netherlands Development Organization
TEF	Toxic equivalency factors
TEQ	Toxic Equivalent Quantity
UCC	University of Cape Coast
USAID	United States Agency for International Development
USEPA	United States Environmental Protection Agency

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SECTION 1 BACKGROUND

1.1 General Context

Ghana is significantly endowed with valuable fish stocks and a strong tradition and culture of fishing comparable to other West African nations. Indeed, the country produces on the average 440,000 tons of fish from its waters each year. Ghana is both an exporter and importer of fish.

Over the years, Ghana has exported large volumes of fish to international markets and as many as 2.2 million people in Ghana are dependent on the sector for their livelihoods, including some 135,000 fisher men and women in the marine sector alone. Revenues from the fisheries sector accounts for about US$1 billion per year, contributing at least 4.5 percent to Ghana's GDP. Ghana's fish also contribute indirectly to regional food self-sufficiency through trade and exports. As a matter of fact, the country is a regular supplier of smoked fish to neighboring countries such as Togo, Benin, Cote d'Ivoire and Nigeria. It is noteworthy that fish supplies naturally augment food availability. Fish availability ensures food security and good nutritional outcomes particularly of poor and rural populations. Particularly, fisheries products supply 60 percent of the animal protein consumed in the country and per capita fish consumption is 27 kg per year – more than double the world average.

Fresh, but more often smoked, dried, or even as powder, fish is a critical source of dietary protein and micronutrients such as iron, iodine, zinc, calcium, vitamin A and vitamin B for many communities in rural areas particularly in the poorest regions of Ghana. Therefore, fish is an important source of animal protein in Ghana with 75 % of the total annual domestic production of fish consumed locally (MOFA, 2017). In spite of this, imports of fish into the country are often made (FACP, 2016), suggesting that the local supply is insufficient for the high demand. Currently, Ghana is the number one fish consumer in the world (FACP, 2016). The traditional methods of fish processing and preservation comprise smoking, drying, frying, salting and fermenting and in some circumstances a combination of these methods. It had been reported that 95% of fish processed in Ghana is smoked, 3 % salted while the remainder is dried, fried or fermented (SMFP, 2015).

Unfortunately, the fish production in the country is confronted with a number of challenges. It is worthy to note that fish is exposed to microbial and chemical contamination right from the catch, through the processing, storage and final display at various local markets. Microorganisms may either be beneficial or pathogenic and the determination of the microbial status of food usually connotes the hygiene conditions under which the fish was handled. Smoking preserves and enhances flavor; however, as the fish gets in contact with the smoke from the firewood, polycyclic aromatic hydrocarbons (PAHs) are formed which are released into the product.

PAHs are a class of high lipophilic compounds that comprise of chemical compounds known to be potent carcinogens (Simko, 2002). Structurally, they consist of one or more aromatic ring and their structure is known to influence their toxicity. They are produced from the incomplete combustion of organic matter and exist in mixtures. So far, more than 600 PAH compounds have been identified.

PAHs formation in smoked foods depend on several variables in the smoking process, including type of smoke generator/stove, type of fuel used, combustion temperature, and degree of smoking (Garcia and Simal, 2005). Other factors include, fat content of fish species and cooking time.

The smoking industry in Ghana is largely unregulated with various oven types, hygienic issues and wood fuels used for smoking; as such the quality of smoked fish varies from place to place. Fish smoked in Ghana is sold in-country with some units exported to other regional markets. Accessing the EU market has however proven quite difficult as the Union demands adherence to stringent regulations and standards regarding the sourcing, handling and processing of smoked fish. Amongst such standards, is the limit of Polycyclic Aromatic Hydrocarbons in smoked fish.

The research focused on quantifying the various PAH levels in different fish species of both smoked and fresh fish on the Ghanaian market to be able to provide scientific information and risk exposure to the ordinary consumer. Further analysis into microbiological profiles of fish both smoked and fresh was carried out by sampling fish from the popular sales points and markets in Ghana to be able to determine their contamination levels.

It is based on these results and existing information (from FAO and others) that SNV in consultation with project partners and national stakeholders aim to develop smoked fish production protocols to reduce contamination levels in smoked fish.

1.2 Project Background

The United States Agency for International Development (USAID) has committed funds to the implementation of a Sustainable Fisheries Management Project (SFMP) in Ghana for five years. The objective is to rebuild marine fisheries stocks and catches through adoption of responsible fishing practices. The project will contribute to the Government of Ghana's fisheries development objectives and USAID's Feed the Future Initiative.

Working closely with the Ministry of Fisheries and Aquaculture Development and the Fisheries Commission, USAID/Ghana SFMP aims to end overfishing of key stocks important to local food security through a multi-pronged approach:

- • Improved legal enabling conditions for co-management, use rights and effort-reduction strategies.
- • Strengthened information systems and science-informed decision-making
- • Increased constituencies that provide the political and public support needed to rebuild fish stocks.
- • Implementation of applied management initiatives for several targeted fisheries ecosystems.

USAID selected the Coastal Resources Center (CRC) at The University of Rhode Island's Graduate School of Oceanography as lead implementer of the SFMP. In leading the project, CRC will work with The Ministry of Fisheries and Aquaculture Development and the Fisheries Commission along with a consortium of international and local partners, including SNV Netherlands Development Organization.

SNV as part of its Year 2 implementing activities worked with UCC to carry out research into the production of smoked fish with low PAH levels. Also with UCC, SNV conducted microbiological profiles on smoked fish to assess contamination levels. This study aimed at identifying the levels of various PAHs and to determine the microbial profiles of various fish samples in Ghana.

1.3 Objectives

The primary objective of the study was to conduct chemical (PAHs) and microbiological profiles on smoked fish to assess contamination levels within the Ghanaian market.

Specific objectives

The objectives of the study were:

- To provide scientific evidence on smoked fish production to guide the promotion of improved processing methods, product quality, packaging, labeling and marketing to significantly increase the value of smoke/dried fish products and shelf life, allowing better penetration to domestic markets, where demand is strong, as well as to neighboring countries.
- To investigate into microbiological profiles of smoked and salted fish from popular sales points and markets in Ghana.
- To analyzes the levels of Aerobic mesophile, yeast and molds, coliform bacteria, *E. Coli*, *Enterococcus* sp., *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringes*, *Vibrio* sp., *Salmonella* sp., *Listeria* sp. and document their impacts of these levels on the health of consumers.
- To discuss causes of the contamination and suggest appropriate remedies to reducing these levels.
- To analyses the levels of Polycyclic Aromatic Hydrocarbons levels in the different species of smoked fish by the different smoking techniques and procedures in Ghana.
- To explain the causes of rise in PAH levels in smoked fish and suggest ways of reducing these levels without causing a significant change in the taste and appearance of the final product (smoked fish) so as not to distort the smoked fish market.

1.4 Expected outcome

A scientific analysis report of PAH levels and microbiological profiles of smoked and salted fish in Ghana.

SECTION 2 MATERIALS AND METHODS

2.1 Study Sites and Sample Collection

The study was carried out in six (6) coastal towns in the Western and Central Regions of Ghana namely Axim, Agona Nkwata, Sekondi, Elmina, Cape Coast and Moree respectively (Figure 1). The economy of these towns is dominated by services including fishing. The various collection sites comprised major landing beaches, and collections from fish processors in some local markets in Axim, Agona Nkwanta, Sekondi, Elmina, Cape Coast and Moree.

Three types of fish namely sardines (*Sardinella aurita*), chub mackerels (*Scomber japonicus*) and anchovies (*Engraulis encrasicolus*) were obtained in August 2016. The fish samples were placed on ice and sent to the laboratory where they were stored at -80°C for laboratory analyses.

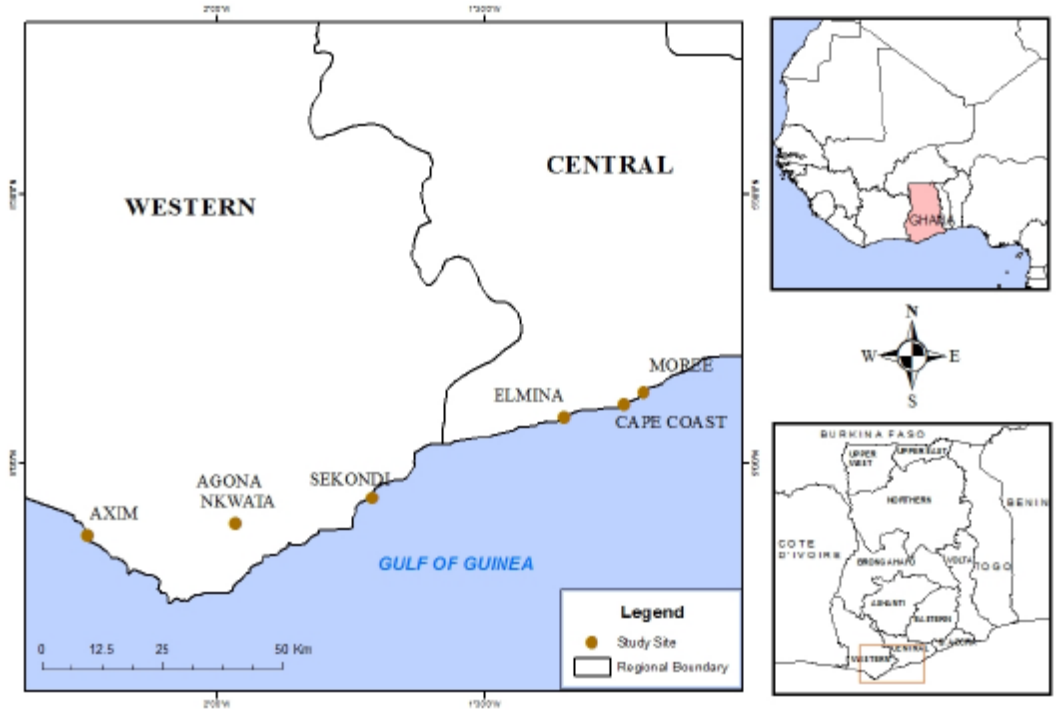

Figure 1 Geographic locations of study sites (Source: Centre for Costal Management)

2.2 Determination of PAHs levels in fish samples

Polyaromatic Hydrocarbon Analysis in Fish by GC/MS

Reagents and Chemicals

All reagents and solvents are HPLC or Ultra-pure grade. Acetonitrile and other reagents were obtained from VWR International (West Chester, PA, USA). The 18-component PAH standard used was obtained from Dr. Ehrenstorfer GmbH, Germany.

Solution and Standards

The PAH stock standard solution (10 µg/ml of 18 polyaromatic hydrocarbons) was diluted in acetonitrile to produce a spiking solution of 1ppm (µg/ml). The spiking solution was used to prepare the 6 points multi-level calibration curve containing concentrations of 5, 10, 20, 50, 100 and 200 ppb.

Sample Preparation

Before analysis, the bones and heads of the fish samples are removed. The samples are then comminuted thoroughly to achieve sample homogeneity, ready for extraction or can be kept in freezer at ≥ -20oC.

Extraction and Purification

The extraction used the QuEChERS method followed by dSPE clean-up technique. Weigh 3g sample (± 0.05g) in 50 ml centrifuge tube.

NB: Quality control (QC) samples are spiked with an appropriate amount of PAH spiking solution to yield QC sample with concentrations of 50 and 100 ng/ml (ppb).

Add 12 ml of de-ionized water (DI) and 15 ml of acetonitrile, then macerate the sample for 1min using Ultra-Turrax homogenizer. Add the QuEChERS extraction salt containing 6g MgSO4 and 1.5g NaCl to the centrifuge tube. Shake the capped tubes vigorously for 1 min on Vortex Mixer possibly at 1500 rpm. Centrifuge at 4000 rpm for 5 min.

Then transfer 6 ml of the acetonitrile (ACN) layer to dSPE clean-up agents consisting of 300 mg PSA, 300 mg C18 and 900 mg MgSO4 in 15ml centrifuge tube.

Vortex 1 min and then centrifuge at 4000 rpm for 5 min. Transfer 4 ml of the upper ACN layer to pear-shaped flask and then concentrate to dryness using rotary evaporator.

Re-dissolve the dry extract in 1 ml ethyl acetate, and then transfer quantitatively into 2 ml autosampler vials, ready for GC/MS quantitation.

PAH conditions were as follows:

- Injector temperature: 280 ^{0}C, split less mode
- Injection volume: 2 µl
- Column type: HP-5ms (30 m x 0.25 mm x 0.25 µm)
- Column flow – 1.25 ml/min
- Ion source – EI mode
- Source temperature: 300 ^{0}C
- MSD transfer line: 325 ^{0}C
- Column conditions
- 70 ^{0}C (hold, 2 mins) to 150 ^{0}C (at 25 ^{0}C/min) to 200 C (at 3 ^{0}C/min) to 280 ^{0}C (hold, 12.133 mins)
- Solvent delay: 4 mins. Total Time: 44 mins.

2.3 Carcinogenic Risk Assessment Using TEF (TEQ)

Carcinogenic risk for exposure to PAH in fish was assessed following guidelines provided by USEPA (1993). The method uses benzo[a]pyrene as a marker to estimate the effect of PAH in foods using the toxic equivalency factors (TEFs). The cancer potencies of the different PAH compounds are compared to that of benzo[a]pyrene (Nyarko et al., 2011; Essumang et al., 2013). Table 1 shows the PAH and their corresponding TEFs.

Table 1 Toxicity Equivalency Factors (TEFs)

PAH	TEF (USEPA, 1993)
Chrysene	0.001
benz(a)anthracene	0.100
benzo(b)fluoranthene	0.100
benzo(k)fluoranthene	0.010
benzo(a)pyrene	1.000
indeno(1, 2, 3-cd)pyrene	0.100
dibenz(a, h)anthracene	1.000

The concentration of each PAH compound in the sample is multiplied by its corresponding TEF. The values are summed to give the benzo[a]pyrene equivalent concentrations, TEQBaP (AFSSA, 2003). The concentrations of all PAHs in the sample is therefore represented by a single concentration which may reflect the total carcinogenic potential of the PAHs in the sample using the following formula;

$$\mathbf{TEQBaP = \Sigma\ (TEFi\ x\ Ci)}$$

Where Ci is the measured individual PAHs concentrations for the 'ith' compound with the assigned TEFi. (Essumang *et al*., 2013).

2.4 Microbial Analysis

Microbial analysis was performed at the Microbiology laboratory, Food Research Institute, Council for Scientific and Industrial Research (CSIR). The fish samples were subjected to various microbiological tests according to guidelines provided by either the Nordic Committee on Food Analysis Method (NMKL) or the International Standards Organization Method (ISO). The various tests performed and the reference methods adopted have been listed in Table 2.

Table 2 Microbiological tests performed on fish samples

Test Preformed	Reference Method
Bacillus cereus Count	NMKL 67 2010
Staphylococcus aureus count	NMKL 66 2009
Listeria monocytogenes Count	ISO 11290-1 1996
Clostridium perfringens Count	ISO 7937 2004
Vibrio Count	ISO 21872-1 2007
Aeorobic Plate Count	NMKL 86 2013
Coliform Count	NMKL 44 2004
E. coli Count	NMKL 125 2005
Moulds and Yeast Count	ISO 21527-1 1996
Enterococcus Count	NMKL 65 2011

2.5 Statistical Analysis

Microsoft Excel 2011 was used to tabulate all data obtained. Descriptive statistics comprising means, standard deviations and variances were employed to analyze data obtained on PAH levels using the SPSS statistical software version 21.

SECTION 3: RESULTS AND DISCUSSION

3.1 Microbial profile on fish samples

Microbial analyses were performed on 17 fish samples comprising 10 smoked, 2 salted and 5 fresh fish samples (Table 3). Generally, fresh fish recorded the highest microbial load, followed by smoked fish while salted fish recorded the least microbial load. This could be explained by the fact that; the fresh fish provide favorable conditions for successful microbial growth.

Ideally, smoking decreases the total viable count without completely eliminating all microorganisms including bacteria, molds and yeasts (Plahar et al., 1999) as some microorganisms can survive high temperatures. Hence the microorganisms isolated from smoked fish in this study were heat-resistant or resulted from contamination through handling after the smoking process. Salted fish on the contrary was relatively dry with a limited water activity. Hence microorganisms isolated from salted fish were either halophiles or were introduced during the handling processes.

Coliforms were detected in all fish samples. However, the levels recorded for 4 fresh (F14, F15, F16, F17) and one smoked (F3) fish samples were beyond the tolerable limits. This is an indication of fecal contamination suggesting that the fishes were handled and processed under inadequate hygienic conditions. *Enterococcus* sp., *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringens* were detected but their levels were below tolerable limits. *Listeria monocytogenes*, *Salmonella* spp. and *Vibrio* sp. were not detected in any of the fish sampled.

Table 3 Microbial profiles of smoked, salted and fresh fish samples

ND: Not detected

Micro-organism	Microbial load (cfu/g) of fish samples in various states																
	Smoked (n= 10)										Salted (n = 2)		Fresh (n = 5)				
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}	F_{15}	F_{16}	F_{17}
Aerobic Plate Count	$9.2x10^3$	$2.1x10^4$	$2.9x10^4$	$9.1x10^3$	$1.5x10^4$	$1.4x10^4$	$9.5x10^4$	$4.3x10^4$	$1.5x10^4$	$1.4x10^4$	$1.6x10^3$	$1.7x10^3$	$2.4x10^4$	$2.2x10^3$	$2.8x10^3$	$1.7x10^4$	$1.8x10^5$
Coliform Count	<10	<10	176	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	48	20	40	40
E. coli	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Listeria monocytogenes	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Enterococcus sp.	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Bacillus cereus	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Staphylococcus aureus	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Clostridium perferingens	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Vibrio	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Moulds	80	30	<10	<10	<10	60	<10	20	<10	<10	<10	<10	130	<10	20	60	<10
Yeasts	$2.1x10^3$	<10	$3.8x10^3$	<10	<10	$2.1x10^3$	<10	<10	<10	<10	<10	<10	$2.5x10^3$	<10	60	10	<10

3.2 Polycyclic aromatic hydrocarbon (PAH) profile of fish samples

The levels of 16 PAHs in fish samples are presented in Table 4. A total of 20 fishes were analyzed comprising one fresh and 19 smoked fish samples. The fresh fish was a chub mackerel while the 19 smoked fish comprised 9 sardines, 7 chub mackerels and 3 anchovies. Out of the 16 PAHs, naphthalene was not detected in any of the 20 fish samples while the other 15 PAHs were present in various concentrations (Table 3). The concentrations of the 15 PAHs varied from one fish type to the other and within each group of fish of the same type.

The PAHs concentrations of all 19 smoked fish sampled in this study exceeded the maximum acceptable limit set by the European Commission for 4 carcinogenic PAHs (Pyrene, Benzo (a) anthracene, Chrysene and Benzo(a)Pyrene). These results partly agree with findings of studies conducted in Ghana and Nigeria which reported the presence of PAHs in smoked fish (Nyarko et al., 2011; Essumang et al., 2012; Tongo et al., 2017). It must be noted however that the concentrations recorded in this study are far above those reported in earlier studies (Nyarko et al., 2011; Essumang et al., 2012; Tongo et al., 2017). This observation could be attributed to a number of factors including type of firewood, the type of stove used in smoking, the type of fish, the quality of the water body, the state of the fishing net and many more.

Smoked fish processors in the past had a number of assorted firewood to choose from in order to smoke their fish. With the advent of climate change and other related challenges, present day fish processors are confronted with the scarcity of preferred wood species for smoking such as sugarcane bagasse and some mangroves. They are therefore compelled to use hard wood such as acacia, which have higher lignin content resulting in higher levels of PAHs the smoke produced when wood is subjected to very high temperatures (Kawamoto et al., 2007). It therefore becomes evident that the levels of PAHs in smoked fish are likely to continue to rise if measures are not put in place to educate fish processors on the right type of firewood and stove to use when smoking their fish.

The fresh fish sampled in this study was found to be devoid of any of the 16 PAHs. This confirms results of studies, which reported that fresh fish might naturally contain very minute levels of PAHs absorbed from the external environment (Stolyhwo and Sikorski, 2005; Essumang et al., 2012).

Table 4 Polycyclic Aromatic Hydrocarbon (PAH) levels in smoked and fresh fish samples

PAH	PAH concentrations (µg/Kg) in various fish samples																				Mean	St Dev	Var
	Sardines (n = 9)									Chub Mackerels (n = 8)								Anchovies (n = 3)					
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	*Q	R	S	T			
NAP	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
ACA	143.8	155.8	271.5	405.8	315.2	440.1	461.6	159.7	244.8	210.9	362.1	354.1	306.2	478.6	204.2	157.1	Nd	150.3	319.6	165.1	279.3	114.2	13055.6
ACE	12.4	19.0	37.8	52.8	47.1	38.5	82.5	21.1	26.8	26.5	37.1	29.2	34.6	51.9	3.2	22.0	Nd	17.4	41.7	25.8	33.0	17.8	317.1
FLU	5.9	11.5	233.5	300.1	208.2	214.2	386.4	105.8	140.8	150.1	197.1	186.2	183.5	305.2	21.5	20.1	Nd	143.3	206.1	185.1	168.7	104.0	10822.9
PHE	395.4	512.2	1012.2	1290.4	1198.1	613.2	1728.2	529.3	819.1	759.6	1109.9	547.1	1048.2	1201.5	652.2	499.5	Nd	682.8	1104.2	1101.1	884.4	351.9	123260.9
ANT	288.5	420.1	727.3	917.1	848.5	443.2	1219.9	372.5	84.5	110.5	129.2	396.9	748.6	864.5	468.1	362.4	Nd	480.4	786.2	783.1	550.1	310.7	96545.6
FLT	56.2	88.5	143	116.2	132.1	82.5	141.3	26.3	66.7	100.7	311.4	62.1	114.5	105.2	66.2	57.2	Nd	92.3	135.2	109.1	105.6	59.6	3548.1
PYR	56.8	89.3	142.8	115.8	132.1	81.2	141.1	23.2	66.8	100.6	311.1	61.4	114.4	105.8	67.1	58.2	Nd	90.1	134.1	109.2	105.3	59.6	3559.3
BAA	47.4	82.9	123.1	71.1	54.3	47.8	107.7	8.7	12.5	111.7	156.8	14.7	44.9	87.8	69.1	84.2	Nd	100.3	141.6	139.3	79.3	44.4	1967.9
CHR	49.3	85.1	141.7	72.1	142.8	50.8	49.5	5.7	105.0	108.3	149.2	16.7	51.9	94.1	71.8	93.7	Nd	107.7	152.1	61.8	84.7	43.1	1857.0
BBF	40.9	3.1	30.1	1.5	53.2	26.7	37.1	25.6	40.7	57.1	73.0	16.2	1.4	1.9	1.6	3.1	Nd	50.4	3.6	2.6	24.7	23.1	535.3
BKF	27.5	29.6	27.3	18.5	45.1	21.5	32.4	23.6	41.7	45.6	74.4	10.4	34.2	35.0	18.8	28.6	Nd	40.1	61.6	40.2	34.5	15.3	234.1
BAP	28.0	30.4	27.7	18.5	45.8	21.9	32.8	24.0	42.9	46.3	72.4	10.6	34.3	35.4	19.0	30.8	Nd	40.7	62.9	41.0	35.0	15.1	228.6
IND	1.3	1.1	1.1	1.1	11.9	1.7	6.1	3.1	7.6	8.4	15.2	1.9	1.4	1.5	Nd	1.1	Nd	2.6	4.6	2.1	3.9	4.1	17.3
DAA	1.8	1.6	1.5	1.2	16.4	2.3	8.3	4.2	10.4	11.5	20.7	2.6	2.0	2.1	Nd	1.6	Nd	3.5	6.3	2.9	5.3	5.7	32.3
BGP	1.4	1.1	1.1	1.1	16.1	1.9	8.5	4.3	10.5	11.7	21.1	2.1	1.6	1.5	Nd	1.1	Nd	3.6	5.9	2.5	5.1	5.8	34.4
Total	1156.6	1531.3	2921.7	3383.3	3266.9	2087.5	4443.4	1337.1	1720.8	1859.5	3040.7	1712.2	2721.7	3372.0	1662.8	1420.7	Nd	2005.5	3165.7	2770.9	2398.9	6905.6	820078.0

*: The only fresh fish sample; Nd: Not detected (below the detection limit of 1.0 µg/Kg)

NAP = Naphthalene; ACA = Acenaphthalene; ACE = Acenaphthene; FLU = Fluorene, PHE = Phenanthrene; ANT = Anthracene, FLT = Fluoranthene; PYR = Pyrene; BAA = Benzo(a)anthracene; CHR = Chrysene; BBF = Benzo(b)Fluoranthene; BKF = Benzo(k)Fluoranthene; BAP = Benzo(a)Pyrene; IND = Indeno(1,2,3-c,d)Pyrene; DAA = Dibenzo(a,h)anthracene; BGP = Benzo(g,h,i)perylene

3.3 Human Health Risk Assessment

The data generally suggest that there are just too high carcinogenic risks (See Appendix 1 for details on this Section). The total PAH levels in the smoked sardine samples in this work ranged from 1155.7 to 4443.4 µg/kg. The maximum level of total PAH in smoked sardines recorded in this work is quite elevated as compared to that reported in literature. This elevated level is quite alarming and may render the smoked fish sample unwholesome for human consumption. The individual PAH levels recorded in this work ranged from 1.1 to 1728.2 µg/kg (Appendix 1). Naphthalene levels recorded for smoked sardine samples were all below detection limits (ND) used for analysis of the samples. Phenanthrene recorded the highest level in all samples analyzed. In this work, B[a] P a definite carcinogen and biomarker used in controlling levels of PAHs in foods recorded levels ranging from 18.5 to 45.8 µg/kg. These levels are comparable to maximum levels recorded by Akpambang et al. (2009), Wretling et al. (2010) and Essumang et al. (2012). Inferring from the statement of Stolyhwo and Sikorski, (2005) and Kant.laboratorium (2005), it may be said that the sardine samples were heavily smoked using traditional kiln with wood fuel.

Unfortunately, all the B[a] P levels recorded for this work, far exceeded the EU's acceptable set value of 2.0 µg/kg B[a] P in smoked fish. These elevated levels of B[a] P recorded indicate that the samples herein are highly contaminated with PAH and may have dire implications on the health of consumers of such sardine samples. The total PAH levels in smoked *Chub* Mackerel samples from the Ghanaian market recorded values ranging from 1420.7 to 3372 µg/kg. These elevated and alarming levels are comparable with results obtained by Silva et al. (2011). Again naphthalene levels recorded were all below the detection limit (1.0 µg/kg). The individual PAH levels recorded maximum value of 1201.5 µg/kg again for phenanthrene (see Appendix I). The elevated PAH levels recorded in Chub Mackerel may imply that the fish samples were heavily smoked. Essumang et al. (2012; 2013) and Wretling et al. (2010) asserted that heavily smoked fatty fish samples such as mackerel usually tend to accumulate high levels of PAH.

Benzo[a]pyrene levels recorded in smoked mackerel ranged from 10.6 to 72.4 µg/kg (Table 2). These values, except for the minimum level, far exceed the limits of 2 µg/kg in smoked fish set by EU and the Turkish codex. These elevated levels of BaP and total PAHs recorded suggest a serious contamination of smoked *Chub Mackerel* samples on the Ghanaian market. These may pose a significant health risk to consumers and may also taunt Ghana's reputation on the international market. This needs urgent attention. The total PAH levels in *Engraulis encrasicolus* sampled from Ghana market ranged from 2005.5 to 3165.7 µg/kg. The individual PAH recorded levels between below detection limit (0.10 µg/kg) and a maximum of 1104.2 µg/kg. The elevated PAH levels recorded in the samples may be an indication that, the fish samples were heavily smoked, perhaps accumulated more PAH during the smoking. These levels are well elevated when compared with those reported in literature.

The B[a] P level recorded in *Engraulis encrasicolus* sample ranged from 41.0 to 62.9 µg/kg. These elevated levels recorded, which are well above all the permissible levels set by the international communities, may have a dire implication on the health of consumers of such smoked fish product. It is thus, recommended that, such smoked fish product be remove from the Ghanaian market and the smoking process be investigated further to ascertain the source of the contamination. The Benzo[a]pyrene equivalence dose (BaPeq) ranged from 16.76 to 39.05 µg/kg/day-1 for smoked sardine samples from Axim CS and Elmina CS1 respectively. These correspond to carcinogenic risk of 1.04E-04 to 2.85E-04 respectively (Appendix 1). These suggest that about 1 person out of 10,000 adults and about 3 persons out 10,000 adults respectively are likely to suffer from cancer in their life time when the smoked sardine

sample is consumed. These values are quite alarming since they are well above USEPA's acceptable risk values of 10-5 (upper boundary) and 10-6 (lower boundary). These may suggest that the smoked sardine samples collected are unwholesome for human consumption.

The BaPeq dose ranged from 13.8 to 86.7 μg/kg/day-1 for smoked mackerel samples from Sekondi and Axim respectively. These correspond to carcinogenic risk value of 6.3E-05 and 4.5E-04 respectively. Suggesting the about 6 out of 100,000 adults and about 5 out 10,000 adults respectively are likely to suffer from cancer in their life time. Again, in this Mackerel the values far exceed acceptable cancer risk values of 10-6 and 10-5. Inferring from Table 6, cancer risk values obtained suggest high carcinogenic risk upon consuming smoked mackerel on the Ghanaian. This may render the smoked mackerel sample unwholesome for consumption. This needs urgent attention to help reverse the situation, since it may cast aspersion on the fish processing industry in Ghana.

The BaPeq dose calculated for smoked *Engraulis encrasicolus* ranged from 30.70 to 44.39 μg/kg/day-1. These correspond to a cancer risk values of 2.24E-04 and 3.24E-04 respectively for the consumption of smoked *Engraulis encrasicolus* on the Ghanaian market. These values suggest that 2 persons out 10,000 adults and 3 persons out 10,000 adults respectively are likely to suffer from cancer in their life time upon consumption of this smoked *Engraulis encrasicolus* samples.

SECTION 4 CONCLUSIONS

Relatively high concentrations of PAHs were recorded for the fish samples that were analyzed. The data generally suggests that smoked fish had extremely high public health risks due to their carcinogenic content. For instance, all the B[a]P levels recorded in this work, far exceeded the EC's acceptable set value of 5.0 μg/kg B[a]P for smoked fish. Indeed, the elevated levels of B[a]P recorded indicate that the samples herein are highly contaminated with PAH and may have dire implications on the health of consumers. For instance, the total PAH levels in smoked *Chub* Mackerel samples from the Ghanaian market recorded values ranging from 1420.7 to 3372 μg/kg which renders its consumption unwholesome.

Also, smoked, salted and fresh fish samples were found to carry various loads of microorganisms including bacteria, molds and yeasts. This study reiterates the need to intensify education on hygienic and best processing practices of fish.

4.1 Limitations of the study

It was not possible to perform a source assessment for PAH level and microbial profiles due to the nature of the experimental design adopted. A number of inconsistencies were observed in the number of fish samples based on the following: sites of collection, type of fish sampled, type of stove or wood used for smoking and the state of fish samples (fresh, smoked or salted) at the time of sampling.

4.2 Recommendations

It is therefore recommended that further systematic scientific study be carried out. Preferably scaled up country-wide to assess the extent of the problem nation-wide and address these issues. For policy advice, such broad-based assessment would be necessary. In addition, more education should be given on dangers associated with PAHs and fish processors must be sensitized on best practices on handling fish. The advantage of using soft wood as firewood and the right type of stove for smoking of fish as a way to reduce the levels of PAHs in smoked fish is highly emphasized.

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APPENDIX 1 ANALYTICAL TEST REPORT ON PAHS LEVELS IN FISH SAMPLES

Table 5 PAH levels (µg/kg) in smoke sardines on the Ghanaian market

PAH	Elmina, MS	Elmina CS1	Elmina, CS2	Elmina , CS/SM	Agona Nkwanta	Cape Coast	Moree	Sekondi	Axim, CS
Naphthalene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acenaphthale	244.8	315.2	461.6	159.7	143.8	155.8	271.5	405.8	440.1
Acenaphthene	26.8	47.1	82.5	21.1	12.4	19	37.8	52.8	38.5
Fluorene	140.8	208.2	386.4	105.8	5.9	11.5	233.5	300.1	214.2
Phenanthrene	819.1	1198.1	1728.2	529.3	395.4	512.2	1012.2	1290.4	613.2
Anthracene	84.53	848.5	1219.9	372.5	288.5	420.1	727.3	917.1	443.2
Fluoranthene	66.7	132.1	141.3	26.3	56.2	88.5	143	116.2	82.5
Pyrene	66.8	132.5	141.1	23.2	56.8	89.3	142.8	115.8	81.2
Benzo(a)anthracene	12.5	54.3	107.7	8.7	47.4	82.9	123.1	71.1	47.8
Chrysene	105	142.8	49.5	5.7	49.3	85.1	141.7	72.1	50.8
Benzo(b)fluoranthene	40.7	53.2	37.1	25.6	40	3.1	30.1	1.5	26.7
Benzo(k)fluoranthene	41.7	45.1	32.4	23.6	27.5	29.6	27.3	18.5	21.5
Benzo(a)pyrene	42.9	45.8	32.8	24	28	30.4	27.7	18.5	21.9
Indeno(1,2,3-c,d)pyrene	7.6	11.9	6.1	3.1	1.3	1.1	1.1	1.1	1.7
Dibenzo(a,h)anthracene	10.4	16.4	8.3	4.2	1.8	1.6	1.5	1.2	2.3
Benzo(g,h,i)perylene	10.5	16.1	8.5	4.3	1.4	1.1	1.1	1.1	1.9
Total PAHs	1720.83	3267.3	4443.4	1337.1	1155.7	1531.3	2921.7	3383.3	2087.5

Table 6 PAH levels (µg/kg) in smoked Chub Mackerel samples from the Ghanaian market

PAH	Cape Coast	Sekondi	Agona	Moree, CS	Axim	Elmina,MS/SM	Elmina CS/SM
Naphthalene	ND	ND	ND	ND	ND	ND	ND
Acenaphthale	157.1	204.2	478.6	306.5	354.1	362.1	210.9
Acenaphthene	22	3.2	51.9	34.6	29.2	37.1	26.5
Fluorene	20.1	21.5	305.2	183.5	186.2	197.1	150.1
Phenanthrene	499.5	652.2	1201.5	1048.2	547.1	1109.9	759.6
Anthracene	362.4	468.1	864.5	748.6	396.9	129.2	110.5
Fluoranthene	57.2	66.2	105.2	114.5	62.1	311.4	100.7
Pyrene	58.2	67.1	105.8	114.4	61.4	311.1	100.6
Benzo(a)anthracene	84.2	69.1	87.8	44.9	14.7	156.8	111.7
Chrysene	93.7	71.8	94.1	51.9	16.7	149.2	108.3
Benzo(b)fluoranthene	3.1	1.6	1.9	1.4	16.2	73	57.1
Benzo(k)fluoranthene	28.6	18.8	35	34.2	10.4	74.4	45.6
Benzo(a)pyrene	30.8	19	35.4	34.3	10.6	72.4	46.3
Indeno(1,2,3-c,d)pyrene	1.1	ND	1.5	1.4	1.9	15.2	8.4
Dibenzo(a,h)anthracene	1.6	ND	2.1	2	2.6	20.7	11.5
Benzo(g,h,i)perylene	1.1	ND	1.5	1.6	2.1	21.1	11.7
Total PAHs	1420.7	1662.8	3372	2722	1712.2	3040.7	1859.5

Table 7 Levels of PAH (µg/kg) in smoked Engraulis encrasicolus

PAH	Elmina, CS	Sekondi	Agona
Naphthalene	ND	ND	ND
Acenaphthale	319.6	150.3	165.1
Acenaphthene	41.7	17.4	25.8
Fluorene	206.1	143.3	185.1
Phenanthrene	1104.2	682.8	1101.1
Anthracene	786.2	480.4	783.1
Fluoranthene	135.2	92.3	109.1
Pyrene	134.1	90.1	109.2
Benzo(a)anthracene	141.6	100.3	139.3
Chrysene	152.1	107.7	61.8
Benzo(b)fluoranthene	3.6	50.4	2.6
Benzo(k)fluoranthene	61.6	40.1	40.2
Benzo(a)pyrene	62.9	40.7	41
Indeno(1,2,3-c,d)pyrene	4.6	2.6	2.1
Dibenzo(a,h)anthracene	6.3	3.5	2.9
Benzo(g,h,i)perylene	5.9	3.6	2.5
Total PAHs	3165.7	2005.5	2770.9

Table 8 Benzo A pyrene equivalence dose

PAH	Elmina, MS	Elmina CS1	Elmina, CS2	Elmina , CS/SM	Agona Nkwanta	Cape Coast	Moree	Sekondi	Axim, CS
Benzo(a)anthracene	1.25	5.43	10.77	0.87	4.74	8.29	12.31	7.11	4.78
Chrysene	0.105	0.1428	0.0495	0.0057	0.0493	0.0851	0.1417	0.0721	0.0508
Benzo(b)fluoranthene	4.07	5.32	3.71	2.56	4	0.31	3.01	0.15	2.67
Benzo(k)fluoranthene	0.417	0.451	0.324	0.236	0.275	0.296	0.273	0.185	0.215
Benzo(a)pyrene	42.9	45.8	32.8	24	28	30.4	27.7	18.5	21.9
Indeno(1,2,3-c,d)pyrene	0.76	1.19	0.61	0.31	0.13	0.11	0.11	0.11	0.17
Dibenzo(a,h)anthracene	10.4	16.4	8.3	4.2	1.8	1.6	1.5	1.2	2.3
Sum of TEQ	59.90	74.73	56.56	32.18	38.99	41.09	45.04	27.33	32.09
BaPeq daily Dose, $(\mu g/kg)day^{-1}$	31.30	39.05	29.55	16.82	20.37	21.47	23.54	14.28	16.76
Cancer Risk	2.28E-04	2.85E-04	2.16E-04	1.23E-04	1.49E-04	1.57E-04	1.72E-04	1.04E-04	1.22E-04

Table 9 Cancer risk assessment using TEF/TEQ for smoked Mackerel product on the Ghanaian Market

PAH	Cape Coast	Sekondi	Agona	Moree, CS	Axim	Elmina,MS/SM	Elmina CS/SM
benz(a)anthracene	8.42	6.91	8.78	4.49	1.47	15.68	11.17
Chrysene	0.0937	0.0718	0.0941	0.0519	0.0167	0.1492	0.1083
benzo(b)fluoranthene	0.31	0.16	0.19	0.14	1.62	7.3	5.71
benzo(k)fluoranthene	0.286	0.188	0.35	0.342	0.104	0.744	0.456
benzo(a)pyrene	30.8	19	35.4	34.3	10.6	72.4	46.3
indeno(1,2,3-cd)pyrene	0.11	0	0.15	0.14	0.19	1.52	0.84
dibenz(a,h)anthracene	1.6	0	2.1	2	2.6	20.7	11.5
Sum of TEQ	41.62	26.33	47.06	41.46	16.60	118.49	76.08
BaPeq daily Dose, $(\mu g/kg)day^{-1}$	21	13.8	24.6	21.7	86.7	61.9	39.8
Cancer Risk	1.59E-04	1.00E-04	1.80E-04	1.58E-04	6.33E-05	4.52E-04	2.90E-04

Table 10 Carcinogenic risk assessment using TEF/TEQ for smoked Engraulis encrasicolus on the Ghanaian market

PAH	Elmina, CS	Sekondi	Agona
benz(a)anthracene	14.16	10.03	13.93
Chrysene	0.15	0.11	0.06
benzo(b)fluoranthene	0.36	5.04	0.26
benzo(k)fluoranthene	0.62	0.40	0.40
benzo(a)pyrene	62.90	40.70	41.00
indeno(1,2,3-cd)pyrene	0.46	0.26	0.21
dibenz(a,h)anthracene	6.30	3.50	2.90
TEQ	84.95	60.04	58.76
BaPeq daily Dose, (µg/kg)day-1	44.39	31.37	30.70
Cancer Risk	3.24E-04	2.29E-04	2.24E-04

APPENDIX 2 TEST REPORT ON MICROBIAL PROFILES OF FISH SAMPLES

Council for Scientific and Industrial Research
Food Research Institute

Microbiology Div.
P. O. Box M.20
Accra, Ghana.

30th August, 2016

Source of Sample: **ELIZABETH AHADZI**
Address: **DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CAPE COAST**
Description of Sample: **FRESH FISH, SMOKED FISH AND SALTED FISH**
Date Received: 03-06-16
Date Examined: 04-08-16
Sample No: FRI MS 16/1697 – 16/1705
Report No: FRI MR 16/1697 – 16/1705

Sample No. FRI MS	Sample Code/Description	Aerobic Plate Count @ 30°C/72h cfu/g NMKL 86 2013	Coliform Count cfu/g NMKL 44 2004	*E. coli* Count cfu/g NMKL 125 2005	Mould & Yeast Count cfu/g ISO 21527-1: 2008		*Listeria monocytogenes* cfu/25g ISO 11290-1 1996	Enterococcus Count cfu/g NMKL 65 2011
					Yeasts	Moulds		
16/1697	SMOKED SARDINA QURITA - MOREE MARKET	9.1×10^3	<10	<10	<10	<10	Not Detected	<10
16/1698	SMOKED CHUB MACKEREL (ELIMINA)	1.5×10^4	<10	<10	<10	<10	Not Detected	<10
16/1699	SMOKED CHUB MACKEREL (CAPE COAST)	1.4×10^4	<10	<10	2.1×10^3	60	Not Detected	<10
16/1700	SMOKED SARDINA QURITA (CAPE COAST)	2.9×10^4	176	<10	3.8×10^3	<10	Not Detected	<10
16/1701	SMOKED CHUB MACKEREL (AGONA NKWANTA MARKET)	9.5×10^4	<10	<10	<10	<10	Not Detected	<10
16/1702	SMOKED SARDINA QURITA (SEKONDI MARKET)	2.1×10^4	<10	<10	<10	30	Not Detected	<10
16/1703	SMOKED CHUB MACKEREL (SEKONDI MARKET)	4.3×10^4	<10	<10	<10	20	Not Detected	<10
16/1704	SMOKED SARDINA QURITA (ELMINA)	9.2×10^3	<10	<10	2.1×10^3	80	Not Detected	<10
16/1705	SMOKED ENGRAULIS ENERASICOLUS (SEKONDI MARKET)	1.5×10^4	<10	<10	<10	<10	Not Detected	<10

Note: 1. NMKL - Nordic Committee on Food Analysis Method
2. ISO - International Standards Organization Method
3. CFU - Colony Forming Unit

Supervised By: **AMOO-GYASI MICHAEL**
HEAD, ANALYTICAL SERVICES/TECHNOLOGIST-IN-CHARGE
Signature:----------

Signed:----------
AMY ATTER (MRS.)
HD.MICROBIOLOGY/ RESEARCH LAB

TO: ELIZABETH AHADZI DEPARTMENT OF CHEMISTRY, U.C.C, CAPE COAST

1. The results relate only to the sample(s) examined.
2. No part of this report may be reproduced or utilized in any other form without written permission from the Food Research Institute (CSIR).

Council for Scientific and Industrial Research
Food Research Institute

Microbiology Div.
P. O. Box M.20
Accra, Ghana.

30th August, 2016

Source of Sample: **ELIZABETH AHADZI**
Address: **DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CAPE COAST**
Description of Sample: **FRESH FISH, SMOKED FISH AND SALTED FISH**
Date Received: 03-06-16
Date Examined: 04-08-16
Sample No: FRI MS 16/1706 – 16/1713
Report No: FRI MR 16/1706 – 16/1713

Sample No. FRI MS	Sample Code/Description	Aerobic Plate Count @ 30°C/72h cfu/g NMKL 86 2013	Coliform Count cfu/g NMKL 44 2004	*E. coli* Count cfu/g NMKL 125 2005	Mould & Yeast Count cfu/g ISO 21527-1: 2008		*Listeria monocytogenes* cfu/25g ISO 11290-1 1996	Enterococcus Count cfu/g NMKL 65 2011
					Yeasts	Moulds		
16/1706	SMOKED ENGRAULIS ENERASICOLUS (ELMINA)	1.4×10^4	<10	<10	<10	<10	Not Detected	<10
16/1707	SALTED CHUB MACKEREL (AGONA NKWANTA MARKET)	1.6×10^3	<10	<10	<10	<10	Not Detected	<10
16/1708	SALTED CHUB MACKEREL (ELMINA)	1.7×10^3	<10	<10	<10	<10	Not Detected	<10
16/1709	FRESH CHUB MACKEREL (AXIM)	2.4×10^4	<10	<10	2.5×10^3	130	Not Detected	<10
16/1710	FRESH CHUB MACKEREL (ELMINA)	2.2×10^3	48	<10	<10	10	Not Detected	<10
16/1711	FRESH CHUB MACKEREL (SEKONDI)	2.8×10^3	20	<10	60	20	Not Detected	<10
16/1712	FRESH SARDINA QURITA (SEKONDI)	1.7×10^4	40	<10	10	60	Not Detected	<10
16/1713	FRESH SARDINA QURITA (ELMINA)	1.8×10^5	40	<10	<10	<10	Not Detected	<10

Note:
1. NMKL - Nordic Committee on Food Analysis Method
2. ISO - International Standards Organization Method
3. CFU - Colony Forming Unit

Supervised By: **AMOO-GYASI MICHAEL**
HEAD, ANALYTICAL SERVICES/TECHNOLOGIST-IN-CHARGE

Signature:----------
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TO: **ELIZABETH AHADZI DEPARTMENT OF CHEMISTRY, U.C.C, CAPE COAST**

1. The results relate only to the sample(s) examined.
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Council for Scientific and Industrial Research
Food Research Institute

Microbiology Div.
P. O. Box M.20
Accra, Ghana.

30th August, 2016

Source of Sample: **ELIZABETH AHADZI**
Address: **DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CAPE COAST**
Description of Sample: **FRESH FISH, SMOKED FISH AND SALTED FISH**
Date Received: 03-06-16
Date Examined: 04-08-16
Sample No: FRI MS 16/1697 – 16/1705
Report No: FRI MR 16/1697 – 16/1705

Sample No. FRI MS	Sample Code/Description	*B. cereus* Count cfu/g NMKL 67 2010	*Staph. aureus* Count cfu/g NMKL 66 2009	*Listeria monocytogenes* cfu/25g ISO 11290-1 1996	*Salmonella* spp./25g NMKL 71 1999	*Cl. perfringens* Count cfu/g ISO 7937 2004	*Vibrio* Count cfu/g ISO 21872-1 2007
16/1697	SMOKED SARDINA QURITA - MOREE MARKET	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1698	SMOKED CHUB MACKEREL (ELIMINA)	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1699	SMOKED CHUB MACKEREL (CAPE COAST)	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1700	SMOKED SARDINA QURITA (CAPE COAST)	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1701	SMOKED CHUB MACKEREL (AGONA NKWANTA MARKET)	<10	<10	Not Detected	Not Detected	<10	Not Dected
16/1702	SMOKED SARDINA QURITA (SEKONDI MARKET)	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1703	SMOKED CHUB MACKEREL (SEKONDI MARKET)	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1704	SMOKED SARDINA QURITA (ELMINA)	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1705	SMOKED ENGRAULIS ENERASICOLUS (SEKONDI MARKET)	<10	<10	Not Detected	Not Detected	<10	Not Detected

Note: 1. NMKL - Nordic Committee on Food Analysis Method
2. ISO - International Standards Organization Method
3. CFU - Colony Forming Unit

Supervised By: **AMOO-GYASI MICHAEL**
HEAD, ANALYTICAL SERVICES/TECHNOLOGIST-IN-CHARGE

Signature:
Signed:
AMY ATTER (MRS.)
HD.MICROBIOLOGY/ RESEARCH LAB

TO: **ELIZABETH AHADZI DEPARTMENT OF CHEMISTRY, U.C.C, CAPE COAST**

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Microbiology Div.
P. O. Box M.20
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30th August, 2016

Source of Sample: **U.C.C DEPARTMENT OF CHEMISTRY**
Address: U.C.C
Description of Sample: **FRESH FISH, SMOKED FISH AND SALTED FISH**
Date Received: 03-06-16
Date Examined: 04-08-16
Sample No: FRI MS 16/1706 – 16/1713
Report No: FRI MR 16/1706 – 16/1713

Sample No. FRI MS	Sample Code/Description	*B. cereus* Count cfu/g NMKL 67 2010	*Staph. aureus* Count cfu/g NMKL 66 2009	*Listeria monocytogenes* cfu/25g ISO 11290-1 1996	*Salmonella* spp./25g NMKL 71 1999	*Cl. perfringens* Count cfu/g ISO 7937 2004	*Vibrio* Count cfu/g ISO 21872-1 2007
16/1706	SMOKED ENGRAULIS ENERASICOLUS (ELMINA)	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1707	SALTED CHUB MACKEREL (AGONA NKWANTA MARKET)	<10	<10	Not Detected	Not Detected	<10	Detected
16/1708	SALTED CHUB MACKEREL (ELMINA)	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1709	FRESH CHUB MACKEREL (AXIM)	<10	<10	Not Detected	Not Detected	<10	Detected
16/1710	FRESH CHUB MACKEREL (ELMINA)	<10	<10	Not Detected	Not Detected	<10	Detected
16/1711	FRESH CHUB MACKEREL (SEKONDI)	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1712	FRESH SARDINA QURITA (SEKONDI)	<10	<10	Not Detected	Not Detected	<10	Not Detected
16/1713	FRESH SARDINA QURITA (ELMINA)	<10	<10	Not Detected	Not Detected	<10	Not Detected

Note: 1. NMKL - Nordic Committee on Food Analysis Method
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APPENDIX 3 STATISTICAL ANALYSIS ON PAHS LEVELS IN FISH SAMPLES

Table 11 Statistical Analysis on PAHs Levels in Fish Samples

	N	Mean		Std. Deviation	Variance
	Statistic	Statistic	Std. Error	Statistic	Statistic
ACA	19	279.2895	26.21335	114.26136	13055.659
ACE	19	33.0211	4.08534	17.80760	317.111
FLU	19	168.6632	23.86683	104.03312	10822.889
PHE	19	884.4316	80.54449	351.08531	123260.895
ANT	19	550.0789	71.28358	310.71791	96545.618
FLT	19	105.6158	13.66532	59.56576	3548.079
PYR	19	105.3211	13.68692	59.65988	3559.302
BAA	19	79.2579	10.17705	44.36073	1967.875
CHR	19	84.7000	9.88635	43.09358	1857.057
BBF	19	24.7263	5.30781	23.13621	535.284
BKF	19	34.5316	3.50988	15.29920	234.066
BAP	19	35.0211	3.46882	15.12024	228.622
IND	19	3.8842	.95315	4.15468	17.261
DAA	19	5.3105	1.30386	5.68340	32.301
BGP	19	5.1105	1.34612	5.86760	34.429
Total	19	2398.96	207.755	905.582	820077.968
Valid N (listwise)	19				