POLYCYCLIC AROMATIC HYDROCARBONS, HEAVY METALS, PESTICIDES AND FATTY ACID LEVELS IN FIVE TYPES OF WIDELY CONSUMED DRIED FISH SAMPLES CONSUMED IN OWERRI

BY

OPALEYE, BUKOLA RUTH (B.Sc. Unilorin)

20184138678

A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FEDERAL UNIVERSITY OF TECHNOLOGY OWERRI, IMO STATE

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) IN ENVIRONMENTAL BIOCHEMISTRY

OCTOBER, 2023.

CERTIFICATION

This is to certify that this thesis titled "**POLYCYCLIC AROMATIC HYDROCARBONS, HEAVY METALS, PESTICIDES AND FATTY ACID LEVELS IN FIVE TYPES OF WIDELY CONSUMED DRIED FISH SAMPLES CONSUMED IN OWERRI**" was carried out by OPALEYE BUKOLA RUTH (Reg. No. **20184138678**) of the Department of Biochemistry, Federal University of Technology, Owerri, Imo State.

.....
Prof. C.O. Ujowundu
(Supervisor)

26/03/2024
Date

.....
Dr. (Mrs.) D.I. Ukairo
(Co-Supervisor)

26/03/2024
Date

.....
Prof. L.A. Nwaogu
(Head, Department of Biochemistry)

26-03-24
Date

.....
Prof. C.S. Alisi
(Dean, School of Biological Sciences)

26/08/24
Date

.....
Prof. B.O. Esonu
(Dean, Post Graduate School)

.....
Date

.....
Prof. (Mrs.) I.I. Ijeh
(External Examiner)

19/10/2023
Date

DEDICATION

This work is dedicated to the all sufficient God, my wonderful parents; Mr. S.A. and Mrs. M.M. Opaleye, my spiritual parents; Pastor Jos3333eph and Pastor (Mrs.) Joy Ajibade and my sweetheart, Dennis Chukwudi Ojieh.

ACKNOWLEDGEMENT

My special appreciation goes to God Almighty who never fails for seeing me through this research work.

My unreserved gratitude goes to my amiable supervisors; Prof. Cosmas O. Ujowundu and Dr. (Mrs.) Doris I. Ukairo for their relentless support, contribution and encouragement.

I must also acknowledge the impacts of the Head of Department, Professor L.A. Nwaogu and all postgraduate lecturers; Prof. R.N. Nwaoguikpe, Prof. C.O. Ibegbulem, Prof. A.C. Ene, Prof. K.M.E. Iheanacho, Prof. (Mrs.) A. A. Emejulu, Dr. C.U. Igwe, late Dr. Kalu O. Igwe, Dr. (Mrs.) C.H. Onuoha. I also appreciate Dr. (Mrs.) F.N. Ujowundu for her technical support and contributions in carrying out my statistical analysis.

To my Course mates; Tata Favour, Onyegbula Akudo, Samuel Kalu, Ikunga Chinyere, thank you for being a support system and a source of encouragement towards the success of this program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

ABSTRACT

The anthropogenic pollutants derived from dumping of agrochemicals and industrial pollutants in the aquatic systems and indiscriminate and destructive fish handling practices poses health hazards to the consumers. These activities have necessitated environmental biomonitoring of fish and fish products which serve as a major and available source of protein. This study quantitatively analysed the polycyclic aromatic hydrocarbons (PAHs), heavy metals, pesticides and fatty acid content in five types of widely consumed dried fish samples in Owerri, Imo State. Heavy metal was determined using Atomic Absorption Spectrophotometer, and pesticides, polycyclic aromatic hydrocarbons and fatty acid content were determined by Gas Chromatography fitted with flame ionization detector. Heavy metals contents in Round fish, Stock fish, Cray fish, Cat fish and Bonga fish showed highest values of 0.27±0.24, 0.04±0.01, 0.06±0.01, 0.06±0.03, and 0.02±00 mg/kg of Lead (Pb), Cadmium (Cd), Nickel (Ni), Mercury (Hg) and Arsenic (As) respectively. While the least concentrations of Pb (0.06±0.04 mg/kg) in Round fish, Cd was not detected in Round fish, Stock fish and Cray fish with the least Cd (0.01±00 mg/kg) in Cat fish; Ni was not detected in Round fish and Stock fish with the least concentration (0.004±0.00 mg/kg) in Cray fish; lowest Hg (0.01±0.00 mg/kg) in stock fish; lowest As (0.004±0.00mg/kg) in Round fish and Cat fish. Pesticide residues of Aldrin, Biphenyl, Carbofuran, Dichlorodiphenyltrichloroethane (DDT), DichhloroBiphenyl, Dichlorvos (2,2-dichlorovinyl dimethyl phosphate-DDVP). Endosulfan, g-chlordane, Glyphosphate, and Hexachlorobenzene (HCB), Heptachlor, Isopropylamine, Lindane, p'p'-DDD, Profenofos, t-nonachlor were detected in varying concentrations in the dried fish samples. Concentrations of aldrin residues in the fish samples were in decreasing order; Stock fish (1.082 ±0.001 mg/kg) > Bonga fish (0.679±0.001mg/kg) > Crayfish (0.205±0.001 mg/kg) > Cat fish (0.001±0.000mg/kg). Biphenyl residues were not detected in all the dried fish samples except in Cat fish (0.378±0.006mg/kg). DDT residues were not detected in all the dried fish samples except in Cray fish (0.318±0.007mg/kg). Concentrations of DichhloroBiphenyl were 0.095±0.041mg/kg in Crayfish, 0.068±0.007mg/kg in Bonga fish, 0.036±0.024mg/kg in Catfish, and .802±0.547mg/kg in Stockfish. Sixteen PAHs were detected in all the fish samples studied. Total PAHs concentrations in decreasing order were Cat fish (11.84±10.00 µ/kg)>Round fish (10.32±8.74 µ/kg)>Bonga fish (8.04±3.00 µ/kg) > Cray fish (3.92±0.54 µ/kg)> Stock fish (3.53±0.08 µ/kg). Twelve fatty acids were detected in varying concentrations in the five dried fish samples. Total Fatty acids concentration in decreasing order were Bonga fish (139.42±12.12 µ/kg) > Cray fish (66.15±4.80 µ/kg) > Cat fish (60.68±2.22 µ/kg) > Stock fish (59.13±4.79 µ/kg) > Round fish (58.28±10.09 µ/kg).The results obtained in this study indicates that indiscriminate dumping of agrochemicals and industrial pollutants in the aquatic systems and indiscriminate and unwholesome fish processing and handling practices as source of pesticides, heavy metals and PAHs. Furthermore, processing and handling practices were implicated in the decrease in the polyunsaturated and the essential fatty acid in the smoked dried fish samples (Bonga fish, Cat fish and Round fish) as compared to the sun dried fish samples(Cray fish and stock fish).

Keywords: Heavy metals, Pesticides, Polycyclic Hydrocarbons, Fatty acids, Dried fish.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

The global consumption of fish and derived fish products has generally increased during recent decades (Wim *et al.*, 2007) due to change from animal protein to fish protein with reduced cholesterol levels. It has been predicted that fish consumption in developing countries will increase by 57 percent, from 62.7 million metric tons in 1997 to 98.6 million in 2020 (Delgado *et al.*, 2003). However, growing demand for aquatic products in both developing and developed countries has necessitated the need to maintain the present *per capita* supply of aquatic products in the future. Besides, practices such as environmental perturbations, overexploitation, dumping of agrochemicals and industrial pollutants, indiscriminate and destructive fishing practices in the aquatic systems not only reduces abundance and fish size but also poses health hazards to the consumers.

Massive amounts of domestic wastewater and industrial effluents are transported by rivers and discharged into the sea, contaminating rivers and coastal waters. Such anthropogenic pollutants are the main sources of heavy metal contaminants in the ocean (Lakshmanan *et al.*, 2009). Heavy metals carried down by effluents are major threats for fish consumers and the effects on the contamination of fishing products has become an important issue that must be addressed. The metal contaminants in aquatic systems usually remain either in soluble or suspension form and finally tend to settle down to the bottom or are taken up by the organisms (Reddy *et al.*, 2007). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Vosyliene and Jankaite, 2006). Fishes being one of the main aquatic organisms in the food chain may often accumulate large amounts of certain metals (Mansour and Sidky, 2002). Among animal species, fishes are the inhabitants that cannot

escape from the detrimental effects of these pollutants (Olaifa *et al.*, 2004). Moreover, it is worthy to note that these heavy metals accumulate in various tissues and organs of fish species, which in turn may enter into the human metabolism through consumption causing serious health hazards Metal contamination in aquatic environments has recently received huge concern due to its toxicity, abundance and persistence in the marine environment, and subsequent accumulation in aquatic fauna and flora, which, in turn may enter into the human food chain and result in health problems (Heba, 2004).

On the other hand, it must be well emphasized that the aquaculture industries are also exposed to many chemical, biological and other pollutants such as polycyclic aromatic hydrocarbons (PAHs) and pesticides. Polycyclic aromatic hydrocarbons (PAHs) are wide spread organic pollutant in the environment; well known for their mutagenic and carcinogenic effects and bioaccumulate in animal and human tissue. The sources of PAHs are both from natural and anthropogenic activities mainly from incomplete combustion of organic materials, fossil fuel and petroleum (Liang *et al,* 2007). PAHs can also form in meat cooked or fish smoked at high temperatures. PAHs are also produced during food processing such as grilling, roasting, smoking, and barbecuing (Adeshina *et al.*, 2021). Fish smoking is highly practiced as a means of prolonging shelf life, enhancing flavor, and increasing utilization (El-Lahamy *et al.,* 2019). Nigeria produces 194,000 metric tons of dried fish annually, 61% of which is smoked fish (Silva *et al.*, 2011). Since smoking is a major source of PAH contamination in fish, the health risks associated with the consumption of smoked fish in Nigeria may be high (Taiwo *et al*., 2019). The impact of the smoking techniques on the amount and type of PAHs that are generated, however, varies (Akinrotimi *et al.*, 2013)

The environmental pollutants commonly called Persistent Organic Pollutants (POPs) include compounds previously synthesized for use as pesticides and halogenated industrial compounds. These compounds can resist chemical and microbial degradation and can persist for a long time in the environment. Being lipophilic in nature, they readily

bioaccumulate in the fatty tissues of organisms except for perflourinated contaminants that bind to proteins. These compounds can be transported far beyond the point of use or application. POPs can be categorized into four major groups (WHO, 2020) of which Pesticides is one, (organochlorine pesticides, OCPs): aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene.

Organochlorine pesticides (OCPs) have been widely used by farmers for pest control. They are synthetic, non-polar, toxic, and environmentally persistent dichlorodiphenylethanes, cycodienes or chlorinated benzenes. The Polychorinated dibenzo-p-dioxins (PCDD), polychorinateddibenzofurans (PCDF), and polychlorinated biphenyls (PCBs), are a family of complex chlorinated compounds with similar structures and biological activity of which 29 (7 of the 75 PCDD compounds, 10 of the 135 PCDF compounds, and 12 of the 209 PCB compounds) have been identified as having dioxin-like toxicity (Malisch, 2017). The health challenges associated with these chemicals led to the ban of PCB (Koppe, 2001).however dioxins are unintentional byproducts. Brominated flame retardants, mainly the polybrominateddiphenyl ethers (PBDE (e.g., pentabromodiphenyl ether (PentaBDE). and octabromodiphenyl ethers (OctaBDE) belong to a class of POPs banned in the Stockholm Convention (UNEP, 2020). These were subsequently replaced with novel brominated flame retardants (NBFRs) and organophophorus flame retardants (OPFRs) (Yang *et al.*, 2019). Most POPs (with the exception of those which are by-products, e.g., dioxins) are components of different industrial and commercial appliances as well as additives in pesticides, plasticizers in paints, plastics, rubber products, etc. (Kodavanti, 2014). The challenge in Nigeria, and globally, is the resistance of these compounds to degradation and the leaching from disposal systems and landfills (Weber *et al.*, 2018) In Nigeria, there are efforts both from national regulatory agencies and international intervention to deal with POPs in landfills (UNDP, 2020). Humans are exposed to these compounds through the food chain, inhalation of air (outdoors, indoors and at the workplace). and from occupational and accidental exposure (Athanasiadou *et al.*, 2008).

In water, they are taken up by phytoplankton, then fish (bioaccumulation and bio-magnification processes particularly in oily fish). In Owerri, very few of the foods that are commonly consumed have been subject to any toxicological testing and yet they are generally accepted as being safe to eat. However, all chemicals, including those naturally found in foods, are toxic at some dose. Hence, it is therefore crucial to quantify these toxicants (Heavy metals, polycyclic aromatic hydrocarbons and pesticides) in dried fish samples consumed in Owerri.

1.2 Statement of the Problem

The ubiquitous nature of polycyclic aromatic hydrocarbons and the inevitable vulnerability of the Imo State residents to environmental contaminants such as pesticides and heavy metals make the quantitative analysis of these contaminants in food in particular smoked/dried fish consumed in Imo state is of utmost importance. There is no published data on the quantitative analysis of toxicants in dried fish samples consumed in Imo State. This reason necessitated this research.

1.3 Justification for the Study

Dried fish is a common protein food source ingredient used for local soups, stew and food seasoning with its unique indigenous aroma and taste generally and more specifically, residents of Owerri metropolis, Imo State. Food consumption is one major exposure route of toxicants to humans, but few data exist for Owerri, Imo State. In the process of drying of fish, sun-dried included, the level of toxic elements will become more concentrated and in combination with atmospheric deposition during drying, the probabilistic health effect in consumption of those fish may increase many fold. While a number of studies have been conducted on toxicants in different edible fish species there are only limited studies on dried fish toxicity. As dried fish occupies a special place in the diet and in most societies such as Owerri, Imo state and is consumed frequently, there is a need to determine the level of toxicants in dried fishes, and assess the concomitant risk associated with their

dietary intake. Result from this study may provide some relevant data on their quantitative levels which can be useful for environmental biomonitoring since there is no published data on the quantitative analysis of these toxicants in dried fish samples consumed in Owerri, Imo State

1.4 Aim and Objectives of Study

The aim of this study was to quantitatively analyze the Polycyclic Aromatic hydrocarbons, Heavy metals, Pesticides and Fatty acid levels in five types of widely consumed dried fish samples in Owerri metropolis, Imo State.

The Objectives of this study were;

i. To determine the Polycyclic Aromatic Hydrocarbon level
ii. To determine the concentration of heavy metals (Lead, Cadmium, Nickel, Mercury and Arsenic) present in the dried fish samples
iii. To determine the pesticides levels in dried Cat fish (*Clariasgariepinus*), Round fish (River African Cat fish), Cray fish (*Cambarellusdiminutus*), Stockfish (*Gadusmorhua*) and Bonga fish (*Ethmalosafimbriata*).
iv. To determine the fatty acids concentration in the dried fish samples.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fish Processing

Fish Processing is the processes associated with fish and fish products between the time in which fish are caught or harvested and the time in which the final product is delivered to the customer. Fish is an extremely perishable food item (Agbon *et al*., 2002; Singh and Heldman, 2013; Pigott, 2015). Soon after death, fish begins to spoil. In the healthy live fish, all the complex biochemical reactions are balanced, and the fish flesh is sterile. After death, however, irreversible change that results in fish spoilage begins to occur. The resultant effect is the decomposition of the fish (Akinola *et al*., 2006; Singh and Heldman, 2013; Pigott, 2015). A considerable effort has been directed to extend the shelf life of fish using preservation and processing techniques, such as refrigeration, freezing, canning, smoking, salting and drying (Akinyemi *et al.*, 2011; Okonta and Ekelemu, 2005; Singh and Heldman, 2013; Pigott, 2015). Efficient preparation of fish is important if top quality, maximum yield and highest possible profits are to be achieved (Davies and Davies, 2009). According to Davies *et al.* (2008) the processed fishery products were still stored using traditional processing and storage technologies, respectively. Lack of adequate fish handling, processing techniques and storage facilities contribute significantly to the low supply of fish to poor rural dwellers that form three quarters of the population in developing countries (Ayuba and Omeji, 2006). The long distance of distribution necessitates some processing and storage, as preservation through refrigeration is not readily available in Nigeria (Agbon *et al.*, 2002; Singh and Heldman, 2013; Pigott, 2015). Ayuba and Omeji (2006) reported that insect infestation is the cause of most prominent losses in quality and quantity of stored, dried fish in Nigeria. The need for the development of fish preservation and processing machinery and techniques for effective fish handling, harvesting, processing and storage can never be over-emphasized especially now that

aquaculture production is on the increase in Nigeria (Davies *et al.*, 2008). With continuing growth in population, income and urbanization in Nigeria, consumer theory assures the future demand for good quality fish and other animal products (FAO, 2002). According to Horner (1992) storage life extension of smoked fish can result from a combination of lowered water activity and the uptake by the product of bactericidal and antioxidant components of wood smoke (Singh and Heldman, 2013; Pigott, 2015). With improved technologies, fresh fish can be processed as wanted without any significant loss of quality. Appropriate processing of fish enables maximal use of raw material and production of value-added products which is obviously the basis of processing profitability. Freshwater fish processing, like the processing of the other food raw materials should assure best possible market quality, provide a proper form of semi-processed final product, assure health safety of products, apply the most appropriate processing method and reduce wastes to the barest possible extent (Tawari and Abowei, 2011). Tawari and Abowei (2011), Akinneye *et al.* (2007) and Davies (2005) reported that the development of appropriate fishing machinery and techniques that use effective production, handling, harvesting, processing and storage, cannot be over-emphasized especially in the age when aquaculture development is fast gathering momentum in Nigeria. Presently in Nigeria, the mechanization level of fish processing is low which results from the overall limited production, seasonal availability of fish, poor information dissemination of the available improved technology to processors and lack of inexpensive equipment adaptable for processing (Davies and Davies, 2009). The production system is mainly artisanal, and fish are marketed mostly in five different forms; fresh, smoked, dried, salted and frozen (FAO, 1996). In Nigeria, processing of fish either through smoking or drying is widely used in fish preservation. In the process, moisture content present in the fish is extracted through heating, thus inhibiting the action of microorganisms and prolonging shelf life (Clucas and Ward, 1996; Oyeleye, 2003; Amoo *et al.*, 2007; Singh and Heldman, 2013; Pigott, 2015). Many fish species have very good preservation qualities after salting, sun drying and even

smoking (Madu *et al.*, 1984; Singh and Heldman, 2013; Pigott, 2015). Eves and Brow (1993) reported that the processing of fish by smoking or drying enhances the nutritive value and promotes digestibility of protein reported that food quality and safety associated with aquaculture products will differ from region to region and habitat to habitat and will vary according to the method of production and harvesting process. Some of the traditional fish processing methods are associated with contamination which may be injurious to consumers. Many researchers have worked on traditional fish processing in Nigeria; Davies (2005) suggested the adoption of appropriate processing technologies that give satisfaction to consumers and equally preserve economical balance. Eyo (1997) reported the high level of post-harvest losses in Kainji lake Basin, revealing that about 35 per cent of fish is lost. Olokor (1997) analyzed the various advantages of adopting solar energy as a means of drying fish traditionally. Akinola *et al.* (2006) also reported different types of fish preservation and processing methods. It has been observed that the most prominent fish preservation method in Nigeria is smoke drying. This could be as a result of the fact that most of the coastal communities have no access to electricity to preserve and or process their products. Bolaji (2005) reported that despite the rudimentary nature of traditional processing methods, the lack of control over the drying rate, sometimes results to under- or over-drying and expose fish to wind, dust, dirt, insect infestation and contaminants such as flies. These methods still remain predominant in Nigeria. There are also reports that most of the fish processing communities in Nigeria used traditional techniques that have been in existence for many years (Tawari and Abowei, 2011; Tawari, 2006; Davies and Davies, 2009). To reduce post-harvest losses and improve fish product quality, traditional processing technology must be improved by upgrading traditional fish processing technologies, especially by developing increased control over the production processes. Most available modern drying technologies are expensive and not appropriate for developing countries where prerequisites for these technologies, such as electricity are not available. Therefore, this study was focused on reviewing traditional fish processing

in Nigeria, identifying the needs for improvement in fish processing, the types of processing methods available, the modern methods of fish processing and the quality and safety status of traditional processed fish from Nigeria.

2.1.1 The needs for fish processing in Nigeria

The quality of the freshly caught fish and its usefulness for further utilization in processing is affected by the fish capture method. Unsuitable fishing method does not only cause mechanical damage to the fish but also creates stress and the conditions which accelerate fish deterioration after death. Fish is highly susceptible to deterioration without any preservative or processing measures (Okonta and Ekelemu, 2005). Emokpae (1979) reported that immediately after the fish dies, a number of physiological and microbial deterioration set in and thereby degrade the fish. Fish is a major source of protein and its harvesting, handling, processing and distribution provide livelihood for millions of people as well as providing foreign exchange earning to many countries (Al-Jufaili and Opara, 2006). Appropriate processing of fish enables maximal use of raw material and production of value-added products which is obviously the basis of processing profitability freshwater fish processing, like the processing of the other food raw materials should: assure best possible market quality, provide a proper form of semi-processed final product, assure health safety of products, apply the most appropriate processing method and reduce wastes to a tolerable extent. Al-Jufaili and Opara (2006) reported high incidence of fish losses as a major impediment to the realization of government goal toward increasing the contribution of the sector to the overall national economy. The need to mechanize fish processing techniques has drawn the attention of national agricultural research to devote utmost interest and resources to engineering research in operation, to minimize the drudgery, reduce labor operation and unsanitary and inherent unhygienic handling that are mostly involved in the traditional manual operations. Eyo (1997) and Abowei and Tawari (2011) reported abundant fish catch in the dry season. During dry season, ponds, lakes and

streams experience reduced water level, for easy harvest. Thus, period of fish scarcity is often encountered especially during the flood and rainy seasons, during which fish are in short supply. Thus, it is imperative to process and preserve some of the fish caught in the period of abundance, so as to ensure an all year round supply. This will invariably reduce postharvest losses, increase the shelf life of fish and guarantee a sustainable supply of fish during off season with concomitant increase in the profit of the fisher folks.

2.2 Fish Processing and Preservative Techniques in Nigeria

Several authors (Olokor, 1997; Okonta and Ekelemu, 2005; Bolaji, 2005; Akinola *et al.*, 2006; Tawari, 2006) reported different types of preservation methods: drying, smoking, freezing, chilling and brining. But the most prominent fish preservation in Nigeria is smoke drying. According to Ita (1972) preservation is carried out for the purpose of extending the self-life of fish. The major preservation methods are mentioned below.

Chilling

Chilling involves cooling of fish to low temperatures without necessarily hardening fish. Chilling does not prevent spoilage. However, the colder the fish, the better and the lower are the incidences of microbial or enzymatic spoilage. Bacteria or enzyme action are not completely stopped, but they may be temporarily halted by chilling (Tawari and Abowei, 2011).

Freezing

Freezing is different from chilling of fish. It involves turning majority of the water in the fish to solid at a temperature below 0°C. Freezing can extend the shelf life of products for a long period. Freezing is essential for export purposes (Anthonio, 1970). Fish that have to be preserved by freezing should be cleaned and packed before rigor mortis sets in for easy operation and maximum use of freezing space. Fresh fish have a characteristic sweet flavor, which is due in part to inosinic acid. The breakdown of inosinic acid during autolytic spoilage resulting in the production of hypoxanthine results in the loss of the

sweet flavor to bitter flavor. Sugar is produced by enzymatic action, which in turn reacts with the amino acids to produce the brownish or yellowish color found in frozen fish (Tawari and Abowei, 2011). Pure water freezes at 0°C. Fish contains about 80 per cent water, salts and minerals. As would be expected therefore, fish can be frozen at temperatures lower than 0°C. As the water freezes out, the concentration of salts and chemicals increases thereby lowering the freezing temperature. At about 5°C, up to 20 per cent of water in fish is still unfrozen (Davies, 2005; Tawari and Abowei, 2011).

Drying

Drying is the removal of water by evaporation. When applied to fish, drying is the removal of water by any method as a means of fish preservation to prolong the shelf life. In areas where sun drying is used traditionally, the effects of wind and weather conditions are important. Basically, the drying effect of the sun depends on the emission of heat from the sun. This is transferred to the fish, and it is accompanied by heat transfer within the fish. During drying, the fish shrinks and undergoes irreversible changes. Water is removed from the surface in the following sequence. First, water on the surface of fish evaporates. Water migrates to the surface of the fish from within fish tissues and evaporates. The air surrounding the fish then experiences a drop in temperature. This is accompanied by cooling of the surface of the fish. The energy required to drive the moisture from the surface of the fish can be obtained from a variety of sources including wood smoke, sun drying, solar drier electricity and mechanical driers (Davies *et al.*, 2008; Tawari and Abowei, 2011).

Smoking

Smoking is commonest traditional method of fish preservation in Nigeria. Smoking combines the effect of the destruction of bacteria by compounds in the smoke, such as phenols and the cooking of the fish, as high temperatures will be generated. Smoked fish products have long shelf life, which has been attributed to the drying, antibacterial and antioxidant and cooking effects of smoke. Wood smoke is a mixture of complex chemical

product gases, vapor and volatile substances. The volatile substances are absorbed on the wet surfaces of fish during the smoking and produce the characteristic aroma (FAO/ UN, 1970a; Tawari and Abowei, 2011). As it is frequently seen in fish markets, properly smoked fish products are dark brown in color and are mostly near perfectly dried. This ensures that the shelf life is prolonged, and the products get to the consumer in relatively good state (Tawari and Abowei, 2011).

Salting

Salting involves addition of salt to the fish to be preserved. There are four standard methods for salting fish. These are brine, dry, kench and pickle salting methods. In brine salting, the fish are immersed in a solution of salt in water. Where granular salt is rubbed into the surface of fish, the process is referred to as dry salting. Granular salt is also used in kench salting. In this process, the salt is rubbed into the surface of split fish, and the fish are stored with salt placed between each layer of fish. The liquid formed is not allowed to drain off the fish, which will eventually become covered with the liquid. The liquid is referred to as pickle. In pickle salting, the fish are packed in watertight containers with salt between each layer of fish. If the pickle formed does not cover the fish within 4 h, saturated brine is added to the fish so that it becomes immersed by the pickle. Otherwise, the fish may spoil (Tawari and Abowei, 2011). In dry and kench salting, the fish are packed, surrounded by dry granular salt. The salt dissolves on the fish surface. The liquid, which exudes from the fish, does not cover the fish thereby exposing surface of the fish to air. It is therefore the practice to keep fish in saturated brine until salt has been rubbed into fish. Otherwise, fat oxidation, discoloration of fish flesh and the development of rancidity ensure. During pickle curing of fish, the large quantity of salt used ensures that the salt is available in sufficient quantity to form the prickle in which the fish is eventually immersed (FAO, 2002; Tawari and Abowei, 2011; Abowei and Tawari, 2011).

2.3 Dried fish Species Consumed in Owerri

2.3.1 Cray Fish (*Palaemon hastatus*)

Crayfish are an important component of the stream ecosystem. They are significant links in the complex aquatic and terrestrial food webs in the ecosystem and by their feeding, burrowing and foraging activities, they help to maintain a high level of water quality in our streams which has great benefit to human lives (Reynolds *et al.,* 2013). Most crayfish produced are sold to the food industry, and although some are sold for recreational fish bait, a small portion is marketed to the aquarium trade and to educators, who use it as study specimens (Ogbonna, 2013). Although in most parts of the world, crayfish are sold alive and eaten fresh/boiled but in Nigeria, they are usually smoked and occasionally sun-dried to form an indispensable food item in the diet of the entire Southern States in particular and Nigeria as a whole It is noteworthy that all animals that are fully aquatic and can live only in water and not out of the water are all lawful and permissible to consume without cultural, traditional or religious barriers. These includes fish, sea crab, lobster, crayfish, shrimp, sharks squids etc. (Mufti, 2016). Since animal protein sources such as beef, chicken, snails, and mutton are presently beyond the reach of the average Nigerian household, many people now settle for seafood products such as fish and crayfish as cheap sources of animal protein (Esheya, 2021a). Crayfish is known to have a super combination of nutrients such as vitamin B6 and B12, fat soluble vitamins (vitamins A, D, E & K), phosphorus, zinc, iron, calcium, magnesium, sodium and other macro-nutrients (Udoh, 1997). Sodium and Potassium which are vital nutrients in crayfish play the role of maintaining and balancing the body's fluid, helping the blood vessels to relax and maintain normal pressure. It is a good source of easily digestible protein with low fat. It is a high quality protein source because it contains all nine essential amino acids and also contains fatty acids (omega-3 and omega-12). The presence of omega-3 in crayfish aid in promoting cognitive function and helps in promoting eyesight by decreasing the risks of loss of vision and developing the overall body system. Crayfish is more easily digested than any other

type of meat due to its short muscle fiber (Teitz, 2014). Crayfish, just like fishes are eaten worldwide (Adamu and Esheya, 2022).

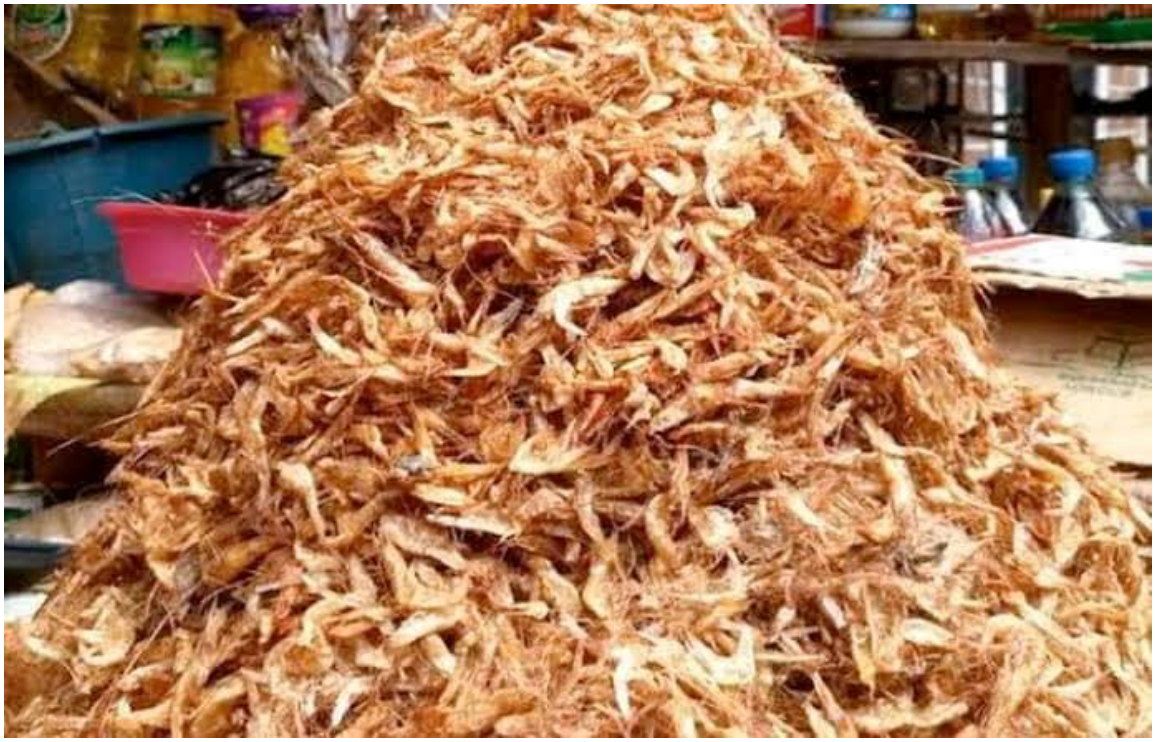

Plate 1: Picture of dried Cray fish (Independent Newspaper, 2019)

2.3.2 Bonga Fish (*Ethmalosa fimbriata*)

Ethmalosa fimbriata is a popular fish consumed in Nigeria both as a source of protein and for taste. It is commonly known as bonga fish in the southern part of Nigeria.

Ethmalosa fimbriata belongs to the family clupeidae and order clupiformes. It is a coastal and estuarine clupeid found on the West African coast. *E. fimbriata* is Pelagic-neritic; catadromous freshwater; brackish; marine, usually 0 - 50 m long, occurs in inshore waters, lagoons and more than 300 km up rivers (e.g. Gambia River). It feeds by filtering phytoplankton, chiefly diatoms and breeds throughout the year in waters of salinities 3.5-38 ppt, but with peaks in at least some areas, spawns in the sea, in estuaries and rivers and marketed fresh, smoked or dried. Apart from being a cheap source of highly nutritive protein, it also contains other essential nutrients required by the body (Sikoki and Otobotekere, 1999).

Plate 2: Picture of dried Bonga Fish (Yeside Adesiyun, 2020)

2.3.3 Stock Fish (*Gadus morhua*)

Stockfish is unsalted fish dried by cold air and wind on wooden racks on the foreshore, called 'hjell' (Kurlansky, 2014). Stockfish commonly referred to as "Okporoko" among the Igbos in Nigeria, is an important source of vitamin and protein which plays essential role in human development. Cod (*Gardus morhua*) is the most common fish used in stockfish production, while others such as Ling (*Molvamolva*). Haddock (*Melaogrammusae glefinus*). Tusk (*Brosmebrosme*) etc. are used to a lesser degree (Koster *et al.,* 2009). The consumption of stockfish has been highly recommended because they are good sources of omega-3 fatty acids and other minerals associated with the health benefits due to their cardio-protective effect (Brawn, 2011).

However, the level of contamination in these stockfish and the poor managerial policies of our aquaculture are of particular interest, because of the potential risk to humans who consumes those (Ukoha *et al.*, 2014). The aquaculture industries are exposed to many chemical, biological and other pollution, mining, drilling and dumping of industrial wastes which have induced high level of polyaromatic hydrocarbons (PAHs) and heavy metals contamination in stockfish which may cause toxicity to humans (Amos-Tautau *et al.*, 2013).

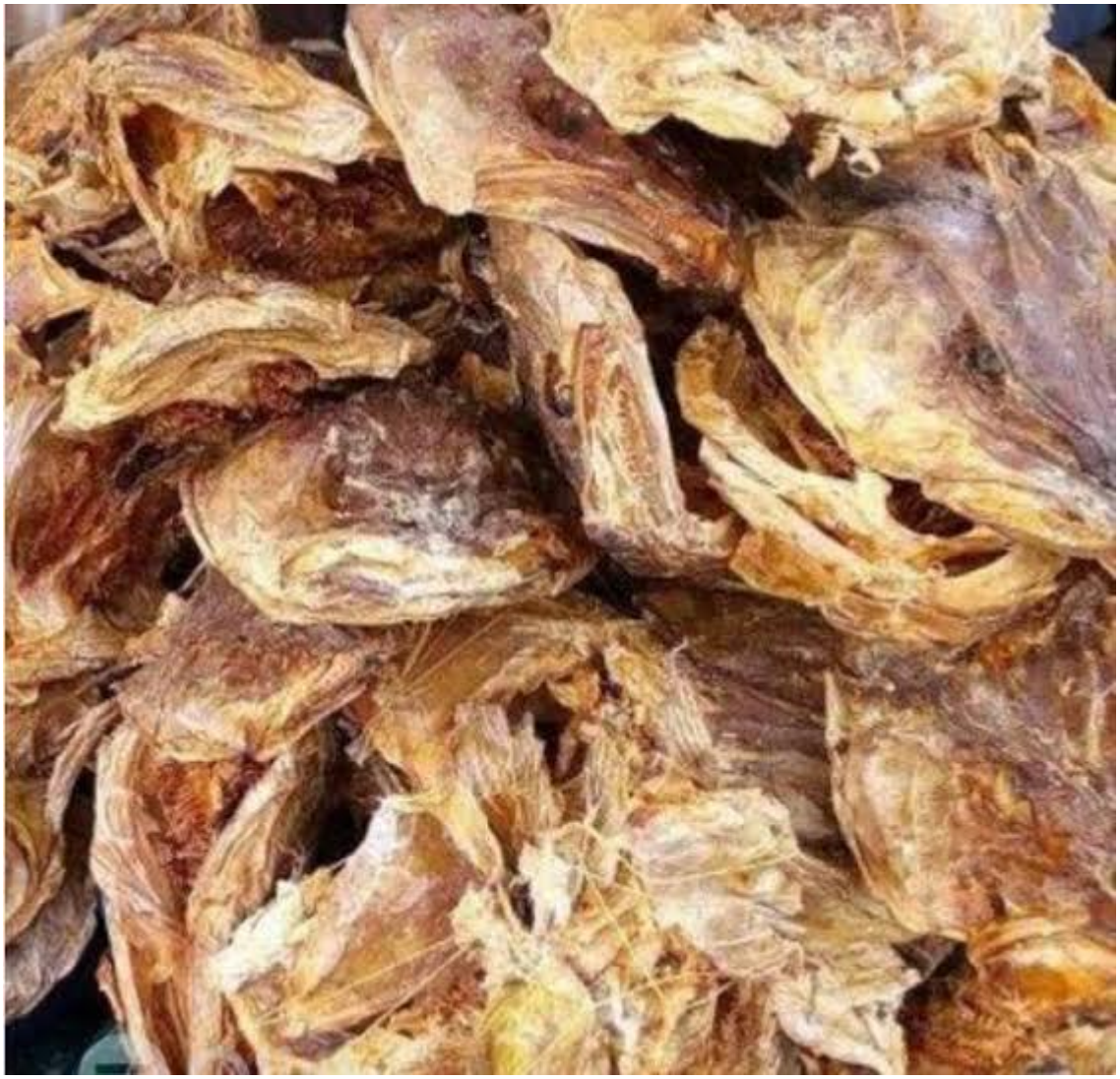

Plate 3: Picture of dried Stock fish (Grace foods, 2020)

2.3.4African Cat Fish (*Clarias gariepinus*)

The African catfish, *Clarias gariepinus* (Family Clariidae) is an economically important food fish; the most cultured fish species in the country, because of its fast growth rate, tolerance to poor water quality and ability to withstand high stocking densities (Saad *et al.,* 2009). They live in freshwater and widely tolerate extreme environmental conditions with pH range of 6.5- 8.0 and depth of 0.8m as well as human madeplaces such as oxidation ponds or even urban sewer system (Skelton, 1993). They possess elongated body, large head and depressed body with small eyes. Narrow and angular occipital process; gills opening wide; air breathing labyrinth organ arising from gill arches; first gill arch with 24-110 gill rakers; cleithrum pointed, narrow with longitudinal ridges and with sharpness (Benech *et al.,* 1993).

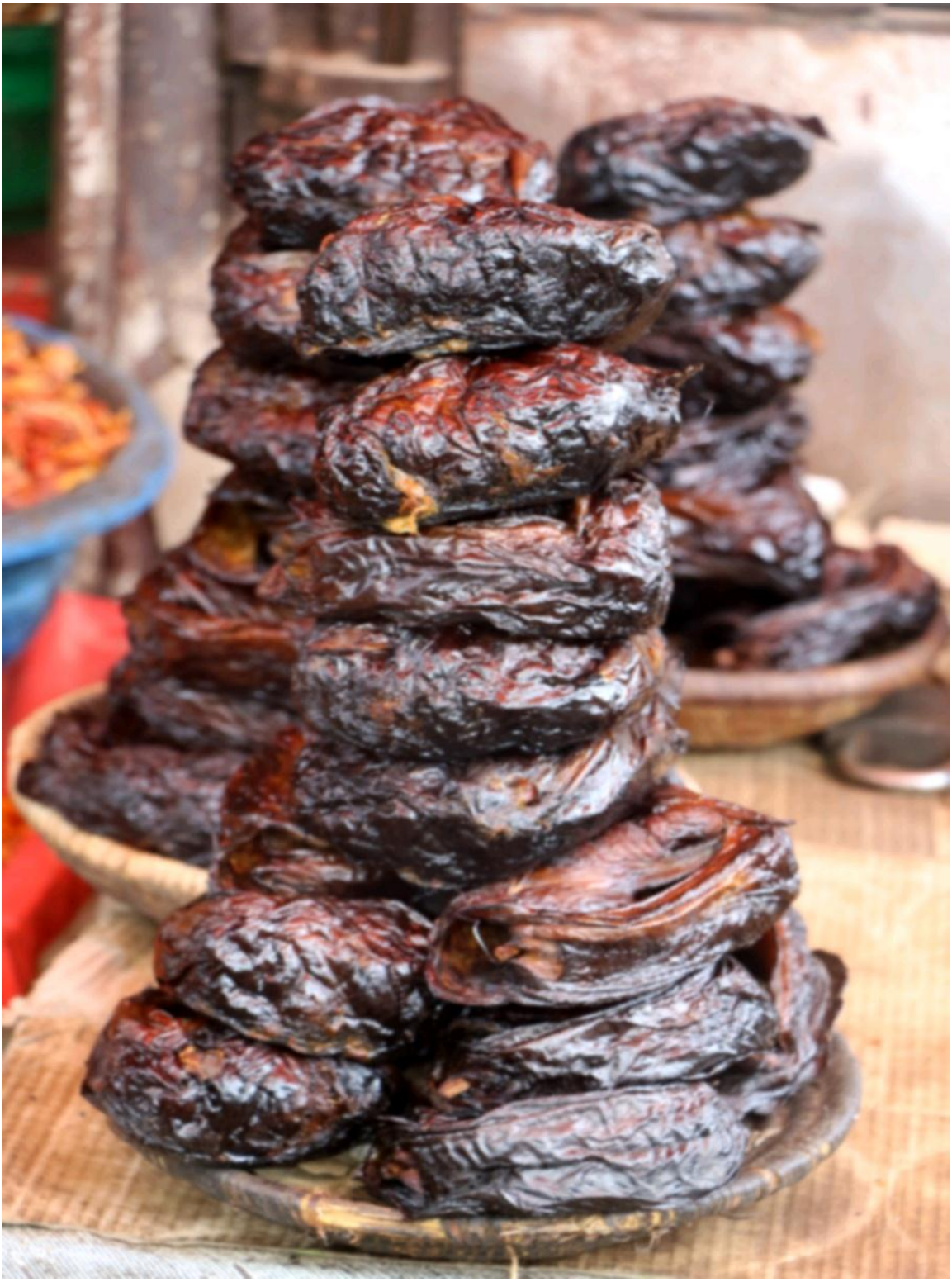

Plate 4: Picture of Dried Cat fish (Godwin Paya, 2017)

2.3.5 Round fish

The round fish considered in this study is also called Mangala fish. It is known to be specie of Cat fish.

Plate 5: Picture of dried Round Fish (Godwin Paya, 2017)

2.4 Toxicants in Dried Fish Samples

2.4.1 Heavy metals

Heavy metals are defined as metallic elements that have a relatively high density compared to water (Fergusson, 1990) With the assumption that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure (Duffus, 2002). In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals. Also, human exposure has risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications (Bradl, 2002). There are reports that sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources (He *et al.*, 2005). Environmental pollution is very prominent in point source areas such as mining, foundries and smelters, and other metal-based industrial operations (He *et al.*, 2005). Although heavy metals are naturally occurring elements that are found throughout the earth's crust, most environmental contamination and human exposure result from anthropogenic activities such as mining and smelting operations, industrial production and use and domestic and agricultural use of metals and metal-containing compounds (He *et al.*, 2005).

Essential heavy metals play biochemical and physiological functions in plants and animals. Several studies have demonstrated that reactive oxygen species (ROS) production and oxidative stress play a key role in the toxicity and carcinogenicity of metals such as arsenic, cadmium, chromium, lead and mercury (Patlolla *et al.*, 2009) and others. Because of their high degree of toxicity, these five elements rank among the priority metals that are of great public health significance. They are all systemic toxicants that are known

to induce multiple organ damage, even at lower levels of exposure. According to the United States Environmental Protection Agency (U.S. EPA) and the International Agency for Research on Cancer (IARC) these metals are also classified as known or probable human carcinogens based on epidemiological and experimental studies showing an association between exposure and cancer incidence in humans and animals.

While it is accordingly appreciated that dried fish represents a popular and nutritious delicacy globally, frequent consumption may just conceivably infer a risk to health, some preservatives containing particular elements that can build up to toxic levels in body stores within the body. Contamination of toxic heavy metals in fish is a common problem worldwide. Trace elements in fish may come from various sources available in the aquatic environment, including within the water body itself, stored in the sediment and/or the terrestrial environment, the dietary habit of the fish, the preservation process and handling etc (Alquezar *et al.*, 2006) Toxic heavy metals are understood to enter the aquatic food chain via both the dietary (direct consumption of water and biota) and non-dietary (uptake through absorbing epithelia in fish) (Olowu *et al.*, 2010) routes, thus aquatic organisms accumulate metal concentration several fold greater than that of the surrounding medium, and become an important media to transfer toxic metal from one trophic level to another.

The Different Heavy Metals Considered in this Study

Arsenic

Arsenic is a ubiquitous element that is detected at low concentrations in virtually all environmental matrices (ATSDR, 2000). The major inorganic forms of arsenic include the trivalent arsenite and the pentavalent arsenate. The organic forms are the methylated metabolites – monomethylarsonicacid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide.

Mechanism of Action

Arsenic exerts its toxic effect through impairment of cellular respiration by the inhibition of various mitochondrial enzymes, and the uncoupling of oxidative phosphorylation. Most toxicity of arsenic results from its ability to interact with sulfhydryl groups of proteins and enzymes, and to substitute phosphorous in a variety of biochemical reactions (Wang *et al.,* 1996). Arsenic *in vitro* reacts with protein sulfhydryl groups to inactivate enzymes, such as dihydrolipoyl dehydrogenase and thiolase, thereby producing inhibited oxidation of pyruvate and betaoxidation of fatty acids (Belton *et al.,* 1985). The major metabolic pathway for inorganic arsenic in humans is methylation. Arsenic trioxide is methylated to two major metabolites via a non-enzymatic process to monomethylarsenic acid (MMA), which is further methylated enzymatically to dimethyl arsenic acid (DMA) before excretion in the urine (Hughes, 2002).

Cadmium

Cadmium is a heavy metal of considerable environmental and occupational concern.

Molecular mechanisms of toxicity and carcinogenicity

Cadmium is a severe pulmonary and gastrointestinal irritant, which can be fatal if inhaled or ingested. After acute ingestion, symptoms such as abdominal pain, burning sensation, nausea, vomiting, salivation, muscle cramps, vertigo, shock, loss of consciousness and convulsions usually appear within 15 to 30min (Baselt, 2000). Acute cadmium ingestion can also cause gastrointestinal tract erosion, pulmonary, hepatic or renal injury and coma, depending on the route of poisoning (Baselt, 2000). Chronic exposure to cadmium has a depressive effect on levels of norepinephrine, serotonin, and acetylcholine (Singhal *et al.*, 1976)

Lead

Lead is a naturally occurring bluish-gray metal present in small amounts in the earth's crust. Although lead occurs naturally in the environment, anthropogenic activities such as fossil fuels burning, mining, and manufacturing contribute to the release of high concentrations.

Adverse Effects of Lead

There are many published studies that have documented the adverse effects of lead in children and the adult population. In children, these studies have shown an association between blood level poisoning and diminished intelligence, lower intelligence quotient-IQ, delayed or impaired neurobehavioral development, decreased hearing acuity, speech and language handicaps, growth retardation, poor attention span, and anti-social and diligent behaviors (Hertz, 2000). In the adult population, reproductive effects, such as decreased sperm count in men and spontaneous abortions in women have been associated with high lead exposure (Hertz, 2000). Acute exposure to lead induces brain damage, kidney damage, and gastrointestinal diseases, while chronic exposure may cause adverse effects on the blood, central nervous system, blood pressure, kidneys, and vitamin D metabolism (U.S EPA, 2002).

Mechanism

One of the major mechanisms by which lead exerts its toxic effect is through biochemical processes that include lead's ability to inhibit or mimic the actions of calcium and to interact with proteins (ATSDR, 1999). Within the skeleton, lead is incorporated into the mineral in place of calcium. Lead binds to biological molecules and thereby interfering with their function by a number of mechanisms. Lead binds to sulfhydryl and amide groups of enzymes, altering their configuration and diminishing their activities. Lead may also compete with essential metallic cations for binding sites, inhibiting enzyme activity, or altering the transport of essential cations such as calcium (Flora *et al.*, 2007).

Mercury

Mercury is heavy metal belonging to the transition element series of the periodic table. It is unique in that it exists or is found in nature in three forms (elemental, inorganic, and organic).with each having its own profile of toxicity (Clarkson, 2003).

Mechanism

The molecular mechanisms of toxicity of mercury are based on its chemical activity and biological features which suggest that oxidative stress is involved in its toxicity (Valko *et al.,* 2006). Through oxidative stress mercury has shown mechanisms of sulfhydryl reactivity. Once in the cell both Hg^{2+} and MeHg form covalent bonds with cysteine residues of proteins and deplete cellular antioxidants. Antioxidants enzymes serve as a line of cellular defense against mercury compounds (Valko *et al.*, 2006). The interaction of mercury compounds suggests the production of oxidative damage through the accumulation of reactive oxygen species which would normally be eliminated by cellular antioxidants.

2.4.2 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that are mostly colorless, white, or pale yellow solids. They are a ubiquitous group of several hundred chemically related compounds, environmentally persistent with various structures and varied toxicity. They have toxic effects on organisms through various actions. Generally, PAHs enter the environment through various routes and are usually found as a mixture containing two or more of these compounds, e.g. soot. Some PAHs are manufactured in the industry. The mechanism of toxicity is considered to be interference with the function of cellular membranes as well as with enzyme systems which are associated with the membrane. It has been proved that PAHs can cause carcinogenic and mutagenic effects and are potent immune suppressants (Hussein and Mona, 2015).

Effects have been documented on immune system development, humoral immunity and on host resistance (Armstrong *et al*., 2009, CCME, 2010) PAHs can be formed both during biological processes and as products of incomplete combustion from either natural combustion sources (forest and brush fires)or man-made combustion sources (automobile emissions and cigarette smoke). Thus, PAHs are commonly detected in air, soil, and water. Therefore, PAHs are considered ubiquitous in the environment (Baklanov *et al*., 2007, Latimer and Zheng, 2003).

Contamination of food by PAHs

Processing procedures, such as smoking and drying, and cooking of food at high temperatures (grilling, roasting, frying) is commonly thought to be the major source of contamination by PAH. Depending on a number of parameters including time, type of fuel, distance from the heat source and drainage of fat, type (grilling, frying, roasting), cooking number of compounds including PAH are formed in the food (Hussein and Mona, 2015)

In dried fish, the practice of using firewood as fuel to preserve or process food by smoking is known to result in high amounts of carcinogenic compounds called polycyclic aromatic hydrocarbons (PAHs) in the food (Stolyhwo and Sikorski, 2005). Studies have linked the occurrence of PAHs in smoked fish in Africa to the practice of smoking fish (Olabemiwo *et al.,* 2011).

Effect on human health

Seventeen (17) PAHs have been identified as being of greatest concern with regard to potential exposure and adverse health effects on humans and are thus considered as a group. Biological monitoring of exposure to PAHs is of primary interest, due to the widespread diffusion of these compounds and to their toxicological relevance. However, the health effects of individual PAHs are not exactly alike. In fact, the International Agency for Research on Cancer (IARC, 2010) classifies some PAHs as known, possibly, or probably carcinogenic to humans (Group 1, 2A or 2B). Among these are benzo[a]pyrene (Group 1), naphthalene, chrysene, benz[a] anthracene, benzo[k]fluoranthene and

benzo[b]fluoranthene (Group 2B) (IARC, 2010). Some PAHs are well known as carcinogens, mutagens, and teratogens and therefore pose a serious threat to the health and the well-being of humans. The most significant health effect to be expected from inhalation exposure to PAHs is an excess risk of lung cancer (Kim *et al.*, 2013)

Metabolism of Polycyclic Aromatic Hydrocarbons

Studies were carried out comparing the metabolism of the PAHs; Phenanthrene (PHE), Flouranthene (FLA) and Benzo(a)pyrene (BAP) in single, binary, and ternary mixtures by monitoring the disappearance of the parent compound. It was observed that PAH metabolism in the single PAH experiment differed from metabolism in both binary and ternary mixtures. Enzyme competition was evident in the metabolism of mixtures, changing significantly the metabolism patterns of individual PAHs. PAH structure was also seen to affect metabolism in mixtures and the possible creation of toxicity effects during mixture metabolism. PAH concentration changed over time with faster change during single PAH metabolism followed by ternary mixture metabolism and finally binary metabolism (Beneditti *et al*., 2007; Tarantini *et al.*, 2011).

Due to the high lipophilicity of PAHs, their bioavailability after ingestion and inhalation is significant. Scientific investigations have shown that detectable levels of PAH occur in almost all internal organs, particularly in organs that are rich in adipose tissue. These organs can serve as storage depots from which the hydrocarbons can be gradually released. Once the PAHs enter the organism they require a multistep metabolic activation by specific enzymes. The enzyme system that is primarily responsible for PAH metabolism is the mixed-function oxidase system. The first reaction is an epoxidation. PAH epoxides can then be conjugated with glutathione and this is regarded as a true detoxification reaction. The epoxides that are not conjugated with glutathione are converted into phenols and diols. Such PAH metabolites, nevertheless, are sometimes not sufficiently polar to be excreted.

Therefore, they have to be conjugated with glucuronic or sulfuric acids to enable excretion. Most metabolites of PAH are excreted in feaces and urine (Campo *et al.*, 2010)

2.4.3 Pesticides

Pesticides are substances used to control pests, including insects, aquatic weeds, plant diseases, and Aquatic snails that carry the cause of schistosomiasis. Pesticides have been found to be highly toxic not only to fish but also to the other organisms, which constitute the food chain. Pesticides in general, are used very extensively in agriculture, forestry, public health and in veterinary practices. Pesticides are categorized according to their target use. The three major pesticides are herbicides (weed control), insecticides (insect control) and fungicides (Mycotic control), but the more acute toxicity are insecticides. Since fishes are important sources of proteins and lipids for humans and domestic animals, so health of fishes is very important for human beings. Insecticides are the chemicals used to control insects by killing or preventing them from engaging in unwanted behaviors or destructive. The contamination of surface waters by insecticides is known to have ill effects on the growth, survival and reproduction of aquatic animals. Different concentrations of insecticides are present in many types of waste water and numerous studies have found them to be toxic to aquatic organisms, especially fish species. Application of insecticides used for control a wide variety of insectivorous and herbaceous pests which would otherwise diminish the quantity and quality of food production (Farid, 2015).

A Pesticides capacity to harm fish and aquatic animals is largely a function of its toxicity, exposure time, dose rate, and persistence in the environment. A lethal dose is the amount of pesticide necessary to cause death because not all animals of a species die at the same dose, a standard toxicity dose measurement, called a lethal concentration 50 (LC50), is used. This concentration of pesticide that kills 50% of a test population of fish within a set period of time is usually determined after 24 to 96 hours.

Exposure of fish and other aquatic animals to pesticides depends on its biological availability (Bioavailability), bioconcentration, biomagnifications, and persistence in the environment. Bioavailability refers to the amount of pesticide in the environment available to fish and wildlife (Farid, 2015). Some pesticides are rapidly broken down after application. Some bind tightly to soil particles suspended in the water column or to stream bottoms, thereby reducing their availability. Some are quickly diluted in water or rapidly volatize into the air and are less available to aquatic life. Bio-magnification is the accumulation of pesticides at each successive levels of the food chain.

Generally, fish is a perishable commodity and there has been large scale deterioration and losses in the quality of processed fish due to the combined effects of insect infestation and other biological agents that flourish under the tropics hot and humid conditions (Mohammed and Yusuf, 2001). Control measures against insect infestation of dried and smoked fish include the injudicious use of harmful chemical insecticides such as dichlorvos, DDT and heptachlor to keep away insects and other pests (Bhuiyan *et al.*, 2008). These pesticides have induced the development of pests resistance (Mohammed and Yusuf, 2001), leading to the applications of higher pesticides doses (UNEP, 2002).

2.5 Fatty acid profile

For many centuries, fish has been one of the main foods for humans and constitutes an important part of diet in many countries. They are good sources of important nutrients and constitute desirable components of healthy diet. The high nutritional value and easy digestibility are the advantages of fish as food. Fishes are rich source of omega-3 (*n*-3) long chain polyunsaturated fatty acids (PUFAs). These are essential for maintaining the integrity of membrane of all living cells. PUFAs serve as precursors for prostaglandins which regulate inflammation and blood clotting. The fish also contains eicosapentaenoicacid (EPA) and docosahexaenoic acid (DHA) which are important in the reduction of some risk factors associated with arteriosclerosis and heart disease. (Calder

2004). The *n*-3 PUFAs are very important because they play a vital role in the development and functioning of nervous system (brain), photoreception (vision), and reproductive system (Bourre, 2007). Nowadays it is known that the essential fatty acid (EFAs) of the omega-6 series, especially linoleic acid (LA). C18:2n-6 and arachidonic acid (AA). C20:4n-6, and the omega-3 series especially linolenic acid (LNA). C18:3n-3, eicosapentaenoic acid (EPA). C20:5n-3 and docohexaenoic acid (DHA). C22:6n-3 are essential for development and growth, and they play a key role in the prevention and management of coronary heart diseases, hypertension, diabetes, arthritis, cancer and other inflammatory and autoimmune conditions (Simpoulos, 2009). The fatty acid composition of fish may vary from one fish species to another and between freshwater fish to marine water fish, so it is important for human health, to increase the consumption of fish (Sergeant, 1997). Fish being an important source of animal proteins, play a significant role in the diet of many people in developing countries. Their amino acids are nutritionally superior to that of cereals grains. Fish protein can therefore be used to ameliorate protein quality of mixed diet (UNFAO, 2004). As a way of extending storage life and increasing its economic market value, fresh fish in Far North of Cameroon is processed through sun drying, smoking, and freezing (Tull, 1997). The preservation effect of smoking and sun drying are mainly due to the decrease in water activity and thus prevention of growth of many spoilage microorganisms (Doe, 1998).

Several authors pointed out that the processing can affect the proximate composition, the lipid composition of fish, especially the fatty acids content, by changing the nutritional value of processed products in relation of raw samples. Moreover, Tenyang *et al*. (2013) reported that heat treatment can lead to undesirable changes, such as loss of essential fatty acids, reducing nutritional value of fish, mainly due to lipids oxidation. However, there is a great variability in changes concerning individual fatty acids in response to different processing methods (Tenyang *et al.*, 2013) Despite the various studies

focusing on the effect of processing methods on nutritive value, lipid oxidation and fatty acids profile of different fish species (Akinwumi, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1Equipment and instruments

The following equipment were used in this study: Buck 530 Gas Chromatograph equipped with an on-Column, automatic injector, electron Capture detector, HP 88 Capillary Column CA, USA, and Mass Spectroscopy, Florisil Column, Agilent FS240AA Atomic Absorption Spectrophotometer, Rotary Vacuum Evaporator, Evaporating flask, test tubes, pipettes, beaker, desiccators and volumetric flask and Weighing Balance.

3.1.2 Chemicals and reagents

i. Anhydrous Sodium Sulphate
ii. n-Hexane
iii. Activated Florisil (Magnesium Silicate)
iv. Chloroform
v. Benzene
vi. Methanol
vii. Acid mixture (Conc. HNO_3, Perchloric acid and Conc. H_2SO_4)
viii. Metal free distilled water

3.1.3 Collection of samples

The samples of dried Bonga fish (*Ethmalosa fimbriata*), Cray fish (*Palaemon hastatus*), Stock fish (*Gadus morhua*), Cat fish (*Clarias gariepinus*) and River African Cat fish (known as Round fish) sold and consumed in Owerri Imo State Nigeria, were purchased from two popular markets Ekeonunwa market and Relief Market. Five fish (dried) samples of each was purchased from five different outlets in Ekeonunwa Market and pooled together as representative sample for each fish type. Same collection process was used in

Relief Market. The samples were labeled accordingly in a sterile polythene bag and transported to the laboratory. The samples were identified at the Department of Aquaculture and Fishery in the School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State Nigeria. At the laboratory, the samples were dried at 70° C for 1 hour and homogenized using a stainless steel micro hammer mill. The milled samples were stored in a glass vial for digestion and further analysis.

3.2 Methods

3.2.1 Determination of heavy metal concentration in fish

Fish samples were digested for heavy metals determination according to the method of Adrian, (1973). Wet digestion of dried fish sample was performed with the acid mixture: Nitric acid (650 ml conc. HNO_3), Perchloric acid (80 ml $HCIO_4$) and Sulphuric acid (20 ml H_2SO_4). Two grams (2 g) of the dried fish sample was weighed into a digestion flask and 20 ml of the acid mixture was added. The mixture was heated until a clear digest was obtained. The digest was diluted with distilled water to the 50 ml mark in the flask. Heavy metal concentrations in fish samples were conducted using Agilient FS240AA Atomic Absorption Spectrophotometer (AAS) according to the method of APHA (1995). The AAS was calibrated using standard solutions for each metal analysed. Afterward, the digested sample was introduced to the Agilient FS240AA AAS to determine the concentrations of the respective metals of interest. A series of standard metal solutions in the optimum concentration range were prepared, the reference solutions were prepared by diluting the single stock element solutions with water containing 1.5 ml concentrated nitric acid/litre. A calibration blank was also prepared using all the reagents except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

3.2.2 Determination of Pesticide Residues by Gas Chromatography (GC) Analysis

Ten grams of the homogenized fish sample was mixed with 60g of anhydrous sodium sulphate in agate mortar to enable moisture absorption. The homogenate was added into a calibrated 500 ml flask and extraction done with 300ml of n-hexane for 24 h. The crude extract was dried with a rotary evaporator at 40 ^{o}C. Then, 1.0 ml of the filtered residue was dissolved in 50 ml chloroform. Pesticide residues were extracted by n-hexane, benzene and methanol as described by AOAC (1990) and Pesticide analytical manual (1991). Aliquots of 1.0 µl of extracts of pesticide residues were injected into a Buck 530 gas chromatograph equipped with an on – column, automatic injector, Electron capture detector, HP 88 capillary column (100m x 0.25µm film thickness) CA, USA. The Buck 530 gas chromatograph was run under the following GC-instrument conditions: Detector Temperature A: 280^{o}C, Column temperature 210^{o}C, Injector temperature 250^{o}C, and Integrator chart speed: 2cm/min. The oven temperature of GC was fixed at 180^{o}C and was allowed to warm up. While warming, the following temperature conditions were set thus: The initial oven temperature was 120^{o}C, for 4 min and ramp for 10 min to final temperature of 180^{o}C. The injection and detector temperatures were 180^{o}C and 300^{o}C, respectively. Furthermore, 10 µl of accu standard was injected in the chromatography and the retention time compared with retention time of standard. Concentrations of pesticide residues in the fish samples were calculated.

3.2.3 Determination of Polycyclic aromatic hydrocarbons

Dried fish samples content of PAHs was determined using Gas Chromatography as described by AOAC (1990). The fat content was extracted by Soxhlet extraction method using 20g of the homogenized sample mixed with 60g anhydrous sodium sulphate in agate mortar to absorb moisture. The homogenate was placed in 500ml beaker and extracted with 300 ml of n-hexane and the crude extract dried at 40^{o} C with rotary vacuum evaporator.

One gram (1 g) of the n-hexane extract was dissolved in 50ml of chloroform, transferred to a 100ml volumetric flask and dilute to the mark. After evaporation of most of the chloroform at room temperature, 1 ml of a mixture of 20 vol% benzene and 55 vol% methanol was added. The flask was sealed and placed in a water bath for 30 min at 40^{o}C. Afterwards, the organic phase was extracted with hexane and water, to a final mixture of reagent, hexane and water in proportion of 1:1:1 (i.e., add 1ml each of hexane and water to the reaction mixture). The mixture was agitated vigorously for 2 min. Finally, about half of the top hexane phase was transferred to a small test tube for injection into the GC column. The GC-FID instrument used was a Buck 530 gas chromatograph equipped with an on – column, automatic injector, Electron capture detector, HP 88 capillary column (100m x 0.25µm film thickness,) CA, USA, with the following parameters: Detector Temperature A:280 ^{0}C; Column temperature 210 ^{0}C; Injector temperature 250 ^{0}C; Integrator chart speed: 2cm/min. The GC oven temperature was set at 180^{0}C and allowed to warm up after which 1 µl n-hexane extract was injected onto column A of GC using proper injection technique.

3.2.4 Determination of Fatty acid profile

Sample Preparation

The homogenized fish samples (20 g) of each sample were soaked in 300 ml of n-hexane for 48 hrs for oil extraction. Afterwards, the setup was filtered into a round bottom- flask using Whatman filter paper. After filtration, the oil was separated from the sample with the aid of soxhlet apparatus, and the oil was collected using a beaker. Finally, the extracted oil was concentrated at 40^{o} C with rotary vacuum evaporator.

Fatty acid profile was determined by Buck 530 Gas Chromatography as described by AOAC (1990). Some quantity of cotton wool was placed in a separating funnel, then a measurement of 1 g and 0.5 g of sodium sulphate and magnesium silicate was taken

respectively and added into the separating funnel. The mixture was made wet by using 5 ml of n-hexane after which the extracted oil was introduced into the mixture and allowed to stand for 20 mins. The tap of the separating funnel was unlocked and solvent was allowed to go into the gas chromatography sample container. With the aid of a syringe 2 ml of the solvent was taken and injected into the GC. The 530 gas chromatography was equipped with an on- column, automatic injector, electron capture detector, HP 88 capillary column (100m × 0.25µm film thickness). CA, USA. The oven temperatures was programmed from an initial temperature of 180 ^{0}C (4 min hold) rising to 200 ^{0}C in 10 min. Identification was made by comparison of retention time to those of authenticated standards for 45 min per parameter after which the result was printed out.

Statistical Analysis

At the end of the study data obtained were subjected to analysis of variance (ANOVA) and least of significant difference (LSD) at $P<0.05$. Data were analyzed using SPSS 20.0 software and results were presented as mean values ± standard deviation (SD).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

Table 4.1.1: Concentrations (mg/kg) of heavy metals in dried fish samples consumed in Owerri, Imo State Nigeria

Heavy metals	Round Fish	Stock Fish	Cray Fish	Cat Fish	Bonga Fish
Lead	0.06 ± 0.04^{a}	0.09 ± 0.09^{a}	0.16 ± 0.13^{a}	0.17 ± 0.07^{a}	0.27 ± 0.24^{a}
Cadmium	ND	ND	ND	0.01 ± 00^{a}	0.04 ± 0.01^{b}
Nickel	ND	ND	0.004 ± 0.00^{a}	0.005 ± 0.00^{a}	0.06 ± 0.01^{b}
Mercury	0.02 ± 0.00^{ab}	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}	0.02 ± 0.005^{a}	0.06 ± 0.03^{b}
Arsenic	0.004 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 00^{ab}	0.004 ± 00^{ab}	0.02 ± 00^{c}

Rows represent mean ± standard deviation of duplicate determinations. Values bearing different superscripts per row are significantly ($p<0.05$) different

In this study, five heavy metals in dried fish samples which include lead (Pb), cadmium (Cd), Nickel (Ni), mercury (Hg) and Arsenic (As) were evaluated. The result showed the presence of Pb, Hg and As in all the fish samples evaluated whereas in Cray fish, Round fish and Stock fish, Cd was not detected and Ni was not detected in Round fish and Stock fish. Furthermore, Pb showed the highest concentration in all the fish samples with Pb concentration of 0.27±0.24 mg/kg in Bonga fish and Pb concentration of 0.17±0.07 mg/kg Cat fish presenting the highest amount.

Residual Pb concentration presented no significant difference ($P \leq 0.05$) amongst the fish samples studied. Residual Pb concentration ranged from 0.06±0.04mg/kg in Round fish to 0.27±0.24 mg/kg in Bonga fish and these concentrations were below 1.0 mg/kg maximum permissible limits (MRL). Residual Cd was significantly ($P \leq 0.05$) higher in Bonga fish (0.04±0.01 mg/kg) compared to Cat fish (0.01±00 mg/kg) and these Cd concentrations were below 0.3 mg/kg maximum permissible limits for Cd. Nickel residues were 0.004±0.00 mg/kg in Crayfish to 0.06±0.01 mg/kg in Bonga fish and these were below the 0.10 mg/kg maximum permissible limits for Ni. Mercury and Arsenic residues were detected in all the fish samples but their concentrations were below 0.5 mg/kg and 1.4 mg/kg maximum permissible limits for Hg and As respectively. Furthermore, this study recorded heavy metal concentration of selected fish in the following descending order in Bonga Fish: Pb, 0.27±0.24 mg/kg > Hg, 0.06±0.03 mg/kg > Ni, 0.06±0.01 mg/kg > Cd, 0.04±0.01 mg/kg > As, 0.02±00 mg/kg. Cat Fish: Pb, 0.17±0.07 mg/kg > Hg, 0.02±0.005 mg/kg > Cd, 0.01±00 mg/kg > Ni, 0.005±0.00 mg/kg > As, 0.004±00 mg/kg

Table 4.1.2: Concentrations (mg/kg) of Pesticides in dried fish samples consumed in Owerri, Imo State Nigeria

Pesticides (mg/kg)	Round fish	Stock fish	Cray fish	Catfish	Bonga fish
Aldrin	ND	1.082 ±0.001^{d}	0.205±0.001^{b}	0.001±0.000^{a}	0.679±0.001^{c}
Biphenyl	ND	ND	ND	0.378±0.006^{a}	ND
Carbofuran	0.281±0.007^{b}	ND	ND	ND	0.207±0.007^{a}
DDT	ND	ND	0.318±0.007^{a}	ND	ND
DichhloroBiphnyl	ND	ND	0.095±0.041^{c}	0.036±0.024^{ab}	0.068±0.007^{bc}
Dichlorvos	0.505±0.077^{c}	1.119±0.353^{e}	0.668±0.071^{d}	0.146±0.014^{a}	0.282±.007^{b}
Endosulfan	0.421±0.092^{a}	1.430±0.021^{b}	0.5950±0.160^{a}	0.388±0.007^{a}	0.663±0.369^{a}
g-chlordane	0.257±0.141^{ab}	0.745±0.187^{c}	0.544±0.050^{bc}	ND	0.373±0.256^{abc}
Glyphosphate	0.332±0.014^{a}	ND	ND	0.420±0.083^{a}	ND
HCB	0.291±0.120^{a}	1.802±0.547^{b}	0.339±0.014^{a}	0.001±0.00^{a}	ND
Heptachlor	0.528±0.287^{a}	1.096±1.007^{a}	0.737±0.014^{a}	ND	ND
Isopropylamine	0.550±0.137^{a}	ND	0.485±0.099^{a}	0.397±0.128^{a}	0.362±0.115^{a}
Lindane	0.174±0.014^{b}	1.515±0.141^{c}	ND	0.039±0.014^{ab}	ND
p'p'-DDD	0.175±.014^{b}	0.601±0.007^{c}	0.174±0.014^{b}	0.097±0.138^{ab}	ND
Profenofos	ND	0.169±0.021^{a}	0.503±0.007^{b}	ND	ND
t-nonachlor	0.270±0.046^{a}	0.506±0.339^{a}	0.350±0.071^{a}	0.187±0.014^{a}	0.347±0.490^{a}

Rows represent mean ± standard deviation of duplicate determinations. Values bearing different superscripts per row are significantly ($p<0.05$) different. ND = Not detected

In this study sixteen pesticides; consumed in Owerri Imo State Nigeria. The result shows that all the dried fish samples studied contain one or more of these pesticide residues.

Aldrin residues in these samples; Stock fish (1.082 ±0.001 mg/kg) > Bonga fish (0.679±0.001mg/kg) > Crayfish (0.205±0.001 mg/kg) were above the maximum permissible limits (MPL) of 0.2 mg/kg for Aldrin as reported by reputable international regulators and agencies for the detected pesticide residues (ATSDR 2002). Biphenyl was not detected in all the fish samples except in Cat fish (0.378±0.006 mg/kg). Carbofuran was only detected in Round fish (0.281±0.007 mg/kg) and Bonga fish (0.207±0.007 mg/kg) samples. DDT residues were not detected in all the dried fish samples except in Cray fish which presented 0.318±0.007mg/kg and it is above MPL of 0.01 mg/kg for DDT in foodstuffs. DichhloroBiphenyl was present in concentrations of 0.095±0.041mg/kg in Crayfish, 0.068±0.007mg/kg in Bonga fish and 0.036±0.024mg/kg in Catfish and these values were below the MPL of 0.2 mg/kg set for DichhloroBiphenyl.

Dichlorvos (DDVP) concentrations of 1.119±0.353mg/kg in Stockfish>0.668±0.071mg/kg in Crayfish > 0.505±0.077 mg/kg in Roundfish> 0.282±.007mg/kg in Bonga fish > 0.146±0.014mg/kg in Catfish were all above the 0.1 mg/kg MPL of pesticides residues in foodstuffs. Endosulfan residues were detected in all the fish samples with the highest amount of 1.430±0.021mg/kg in Stockfish> 0.663±0.369mg/kg in Bonga fish > 0.5950±0.160mg/kg in Crayfish and are above the 0.5 mg/kg MPL of Endosufan. For g-chlordane all the fish samples (except Catfish) presented concentrations of pesticides residues above maximum permissible limits0.1 mg/kg. Glyphosphate residues were detected at concentrations of 0.332±0.014mg/kg in Round fish and 0.420±0.083mg/kg in Bonga fish. Hexachlorobenzene in the fish sample ranged from 0.001±0.00mg/kg in Catfish to 1.802±0.547mg/kg in Stockfish sample, with the Stockfish presenting HCB residue above 0.5 mg/kg MPL. Heptachlor residues were 1.096±1.007 mg/kg in Stockfish> 0.737±0.014 mg/kg in Crayfish > 0.528±0.287 mg/kg Round fish and all are above the MPL of 0.01 mg/kg. Isopropylamine residues were

0.362±0.115mg/kg in Bonga fish to 0.550±0.137mg/kg in Round fish and all above MPL of 0.1 mg/kg. The residues of Lindane of 1.515±0.141 mg/kg in Stockfish, 0.174±0.014mg/kg in Round fish and 0.039±0.014mg/kg in Catfish were significantly higher than the 0.02 mg/kg MPL. p'p'-DDD. Profenofos residues were 0.169±0.021mg/kg in Stockfish and 0.503±0.007mg/kg in Crayfish and within the MPL of 0.5 mg/kg. The residual concentration of t-nonachlor which ranged from 0.187±0.014mg/kg in Catfish to 0.506±0.339 mg/kg in Stockfish were all above the 0.04 mg/kg MPL.

Table 4.1.3: Concentrations (µg/kg) of polycyclic aromatic hydrocarbons in dried fish samples consumed in Owerri, Imo State, Nigeria

PAHs µg/kg	Bonga fish	Cat fish	Cray fish	Round fish	Stock fish
Naphthalene	1.49±1.52[a]	0.39±0.48[a]	0.02±0.00[a]	0.07±0.09[a]	0.01±0.01[a]
2-methyl naphthalene	0.08±0.02[a]	0.46±0.49[a]	0.05±0.03[a]	0.08±0.09[a]	0.04±0.03[a]
Acenaphthylene	1.66±0.14[a]	1.12±1.03[a]	0.16±0.15[a]	0.87±1.00[a]	0.26±0.04[a]
Fluorene	0.69±0.24[b]	0.27±0.33[ab]	0.01±0.00[a]	0.04±0.03[a]	0.01±0.00[a]
Acenaphthene	0.03±0.03[a]	0.07±0.09[a]	0.03±0.01[a]	0.04±0.05[a]	0.01±0.00[a]
Phenanthrene	0.05±0.06[a]	0.15±0.16[a]	0.01±0.00[a]	0.48±0.65[a]	0.07±0.00[a]
Anthracene	0.06±0.05[a]	0.90±1.26[a]	0.00±0.003[a]	0.16±0.21[a]	0.02±0.01[a]
Fluoranthene	0.05±0.03[a]	0.26±0.37[a]	0.00±0.002[a]	0.11±0.14[a]	0.03±0.01[a]
Pyrene	0.02±0.01[a]	1.02±0.32[a]	0.65±0.30[a]	2.65±0.85[b]	0.02±0.02[a]
Benzo (a) anthracene	0.04±0.04[a]	0.02±0.00[a]	0.05±0.03[a]	0.68±0.68[a]	0.10±0.06[a]
Chrysene	0.77±1.06[a]	0.06±0.02[a]	0.05±0.04[a]	0.67±0.81[a]	0.18±0.03[a]
Benzo (b) fluoranthene	0.02±0.00[a]	0.27±0.37[a]	0.00±0.00[a]	0.22±0.29[a]	0.05±0.01[a]
Benzo (k) fluoranthene	0.68±0.08[b]	0.16±0.14[a]	0.05±0.02[a]	0.31±0.21[a]	0.07±0.04[a]
Benzo (a) pyrene	0.01±0.00[a]	0.11±0.16[a]	0.08±0.02[a]	0.22±0.30[a]	0.02±0.02[a]
Dibenz (a, h) anthracene	0.03±0.03[a]	0.44±0.41[a]	0.16±0.08[a]	1.48±1.92[a]	1.12±0.56[a]
Indeno (1,2,3-cd) pyrene	2.06±0.04[a]	5.09±3.80[a]	1.71±0.53[a]	1.25±0.33[a]	0.59±0.29[a]
Benzo (g, h, i) perylene	0.29±0.04[a]	1.04±1.21[a]	0.89±0.64[a]	1.01±1.09[a]	0.95±0.89[a]
Total	8.04±3.00[b]	11.84±10.00[b]	3.92±0.54[a]	10.32±8.74[b]	3.53±0.08[a]

Rows represent mean ± standard deviation of duplicate determinations. Values bearing different superscripts per row are significantly ($p<0.05$) different

The results of Table 4.1.3 shows that total PAHs concentration in the dried fish samples ranged thus 11.84±10.00 µ/kg> 10.32±8.74 µ/kg> 8.04±3.00 µ/kg> 3.92±0.54 µ/kg> 3.53±0.08 µ/kg. Cat fish, Round fish, Bonga fish, Cray fish and Stock fish respectively. The concentrations of BaP (Benzo (a) pyrene) ranged thus: 0.22±0.30 µ/kg>0.11±0.16 µ/kg>0.08±0.02 µ/kg> 0.02±0.02 µ/kg> 0.01±0.00 µ/kg in Round fish, Cat fish, Cray fish, Stock fish and Bonga fish respectively. Also, other important PAHs; benz(a)anthracene, benzo(b)fluoranthene, and chrysene were also detected in appreciable amount.

Table 4.1.4: Concentrations (µg/kg) of Fatty acids in dried fish samples consumed in Owerri, Imo State Nigeria

Sample (mg/kg)	Bonga Fish	Cat Fish	Cray Fish	Round Fish	Stock Fish
Lauric acid (C 12)	9.74±0.00^{c}	2.70±0.01^{a}	ND	ND	3.58±0.04^{b}
Myristic acid (C 14)	0.05±0.05^{a}	3.43±0.04^{a}	ND	2.49±0.12^{a}	6.27±5.67^{a}
Palmitic acid (C 16)	ND	5.86±0.04^{b}	4.98±0.23^{b}	3.62±1.69^{ab}	2.44±0.90^{a}
Stearic acid (C 18)	18.16±2.28^{c}	ND	7.36±0.00^{b}	3.24±1.57^{a}	1.02±0.02^{a}
Oleic acid (C 18:1)	81.93±9.62^{c}	33.66±2.54^{b}	30.39±4.61^{ab}	23.65±4.87^{ab}	17.89±1.54^{a}
Linoleic acid (C 18:2)	6.38±0.63^{a}	ND	6.43±0.21^{a}	6.53±0.07^{a}	11.82±2.15^{b}
alpha-Linolenic acid (C 18:3)	4.12±0.00^{b}	ND	1.67±0.021^{a}	1.19±0.47^{a}	3.82±0.04^{b}
Icosadienoic acid (C 20:2)	ND	9.33±0.72^{b}	ND	3.51±0.06^{a}	3.52±0.07^{a}
Icosatrienoicacid (C20:3)	13.57±0.35^{c}	ND	ND	0.07±0.02^{a}	6.05±0.01^{b}
Arachidonic acid (C 20:4)	ND	ND	3.84±0.26^{a}	2.90±1.1.58^{a}	2.71±0.65^{a}
Tetracosanolpentaenoic acid (C 24:5)	1.06±0.01^{b}	1.18±0.14^{b}	1.59±0.21^{c}	1.64±0.0.14^{c}	0.01±0.00^{a}
Docosahexaenoic acid (C 22:6)	4.36±0.00^{b}	4.52±0.28^{b}	9.90±0.43^{c}	9.44±0.36^{c}	0.01±0.00^{a}
Total	139.42±12.12^{b}	60.68±2.22^{a}	66.15±4.80^{a}	58.28±10.09^{a}	59.13±4.79^{a}

Rows represent mean ± standard deviation of duplicate determinations. Values bearing different superscripts per row are significantly ($p<0.05$) different

In Table 4.1.4 Bonga fish showed significantly high amount of Lauric acid (C 12) (9.74±0.00 mg/kg) when compared to Stock fish and Cat fish. Lauric acid was not detected in Cray fish and Round fish. Myristic acid (C 14) concentration showed no significant difference ($P<0.05$) among the fish samples but the highest amount of 6.27±5.67 mg/kg was observed in stock fish. Palmitic acid (C 16) was significantly high in Cat fish (5.86±0.04 mg/kg) and Cray fish (4.98±0.23 mg/kg) compared to other fish samples and was not detected in Bonga fish. Stearic acid (C 18) at 18.16±2.28 mg/kg in Bonga fish was significantly high compared to 7.36±0.00 mg/kg in Cray fish 3.24±1.57 mg/kg in round fish and 1.02±0.02 mg/kg in Stock fish. Oleic acid concentration (C 18:1) was 81.93±9.62 mg/kg in Bonga fish > 33.66±2.54mg/kg in Cat fish > 30.39±4.61 mg/kg in Cray fish > 23.65±4.87 mg/kg in round fish > 17.89±1.54 mg/kg in Stock fish. Furthermore, linoleic acid (C 18:2) at 11.82±2.15 mg/kg and alpha-Linolenic acid (C 18:3) at 4.12±0.00 mg/kg were the highest in Stock fish and Bonga fish respectively. Also, Icosadienoic acid (C 20:2) at 9.33±0.72 mg/kg in Cat fish and Icosatrienoic acid (C20:3) at 13.57±0.35 mg/kg in Bonga fish showed significantly high amount compared to other fish. Arachidonic acid (C 20:4).Tetracosanolpentaenoic acid (C 24:5) and Docosahexaenoic acid (C 22:6) were detected at varying concentrations. Generally, total fatty acid concentrations in the fish samples were 139.42±12.12 mg/kg in Bonga fish > 66.15±4.80 mg/kg in Cray fish > 60.68±2.22 mg/kg in Cat fish > 59.13±4.79 mg/kg in Stock fish > 58.28±10.09 mg/kg in Round fish.

4.2 DISCUSSION

Heavy metals

Fish contamination by heavy metal is an important and severe threat to humans and aquatic animals and the poorly managed fish processing and handling methods. To evaluate fish contamination, the determination of its heavy metal concentration is important. It is important to note that concentrations of heavy metals- lead (Pb), cadmium (Cd) nickel (Ni), mercury (Hg) and arsenic (As) detected in samples of dried Bonga fish (*Ethmalosa fimbriata*), Cray fish (*Palaemon hastatus*), Stock fish (*Gadusmorhua*), Cat fish (*Clariasgariepinus*) and River African Cat (Round fish) sold and consumed in Owerri Imo State Nigeria were all below the established permissible limits set by the international authorities (FAO/WHO, 2006; FNB, 2004; USEPA, 1988). Furthermore, residual cadmium was not detected in Round fish, Stock fish and Cray fish while residual Nickel was not detected in Round fish and Stock fish samples. It is important to note that the presence of residual Pb concentration in all the fish samples calls for toxicity concern. Lead (Pb) concentration was below the 0.30mg/kg set by EU (2008) but above the tolerable limit of 0.12 mg/kg set by IAEA (International Atomic Energy Agency, 2003). The highest residual Pb of 0.27±0.24 mg/kg detected in Bonga fish was less than the 0.91mg/kg reported by (Adebiyi *et al.,* 2020). Since, Pb is a cumulative metal it becomes a major hazard for human health during chronic ingestion as it can damage the central nervous system, kidney, liver and the reproductive system (Ujowundu *et al*., 2014). Similarly, Nickel dusts are believed to be carcinogenic, and various other nickel compounds may be as well, indicating the presence of residual Nickel in the studied samples a cause for concern (Kasprzak *et al*., 2003; Ujowundu *et al.,* 2014). One of the major cause of aquatic organism contamination include gas flaring and petroleum oil spillage which bioaccumulate in aquatic organisms (Ujowundu *et al*., 2014). Furthermore, it is important to emphasize the toxicological implication of detecting residual Arsenic concentration in

all the fish samples because Arsenic and its compounds are used in pesticides, insecticides, herbicides thus increasing the risk of human exposure. Arsenic is one of WHO's 10 chemicals of major public health concern. The IARC has classified arsenic and arsenic compounds as carcinogenic to humans and arsenic is associated with developmental defects, diabetes, pulmonary disease and cardiovascular disease. Arsenic-induced myocardial infarction in particular can be a significant cause of excess mortality (WHO, 2022). Numerous studies have demonstrated negative impacts of arsenic exposure on cognitive development, intelligence and memory (Tolins *et al*., 2014). Residual mercury was also detected in all the fish samples, but below the maximum permissible limits. Stock fish Hg and As were within the maximum acceptable limit of 0.5 mg/kg set for Stockfish by FAO (1983). Humans may be exposed to mercury in any of its forms under different circumstances. However, exposure mainly occurs through consumption of fish and shellfish contaminated with methylmercury after ingestion of mercury compounds some neurological and behavioural disorders observed include tremors, insomnia, memory loss, neuromuscular effects, headaches and cognitive and motor dysfunction. Effects on kidney after mercury exposure have also been reported, ranging from increased protein in the urine to kidney failure (WHO, 2017).

Pesticides

The detection of residual aldrin in three dried fish samples (Stock fish, Bonga fish, Crayfish) above the maximum permissible limits (MRL) of 0.2 mg/kg for Aldrin (ATSDR, 2002) may cause human health hazard. Aldrin is an organochlorine and a persistent organic pollutant (Robert, 2002) and when it enters the human body or the environment it is rapidly converted to dieldrin (ATSDR, 2022). Human exposure to aldrin and dieldrin occurs on consumption of foods contaminated with either chemical. Chronic exposure of aldrin or dieldrin leads to bioaccumulation causing headaches, dizziness, irritability, vomiting, or

uncontrollable muscle movements. Based on studies in animals, the EPA has determined that aldrin and dieldrin are probable human carcinogens (ATSDR, 2002). Biphenyl was not detected in round fish, stock fish, Cray fish and Bonga fish but was detected at 0.378±0.006 mg/kg in Cat fish. The concentration of biphenly in Cat fish sample was higher than the Reference Dose (RfD) of 0.05 mg/kg body weight per day (CEA, 1999). Biphenyl is used as a food preservative because it prevents the growth of molds and fungi, however its use has been banned as a food additive in the European Union. Furthermore, biphenly prsents toxic effects on the liver, kidneys, central and peripheral nervous systems. Biphenly is not classified as human carcinogen (USEPA, 1984). Round fish showed the highest amount of Carbofuran residues at 0.281±0.007 mg/kg. Carbofuran is a carbamate pesticide, widely used around the world to control insects on a wide variety of field crops, including potatoes, corn and soybeans. The presence of carbofuran in these fish samples may be attributed to indiscriminate and lack of knowledge in the use of pesticides to food preservation. Dichlorodiphenyltrichloroethane (DDT) residues were detected only Crayfish and at concentration above MRL is worrisome. DDT is classified as "moderately toxic" by the U.S. National Toxicology Program (NTP) and "moderately hazardous" by WHO, based on the rat oral LD50 of 113 mg/kg (WHO, 2005). Indirect exposure is considered relatively non-toxic for humans (Agarwal *et al*., 2012). However, DDT metabolite *p,p'*-dichlorodiphenyldichloroethane (*p,p'*-DDD) was detected in all fish samples except Bonga fish. *p,p'*-DDD is a para arene substitution of DDT formed during the synthesis of DDT. DDT, DDE and DDD are lipophilic, can bioaccumulate, and are toxic to a wide range of living organisms, including marine animals such as crayfish, daphnids, sea shrimp and many species of fish magnify through the food chain.

DichhloroBiphenyl residues were present at concentration below the MRL. DichhloroBiphnyl is a Polychlorinated biphenyls (PCBs) banned internationally by the Stockholm Convention on Persistent Organic Pollutants in 2001 (Porta and Zumeta, 2002;

Robertson and Hansen 2004). The International Agency for Research on Cancer (IARC) has rendered PCBs as definite carcinogens in humans, while the U.S. Environmental Protection Agency (EPA), stated that PCBs cause cancer in animals and are probable human carcinogens (USEPA, 2016). PCB's toxic effects include endocrine disruption such as blocking of thyroid system functioning and neurotoxicity are known (Boas, 2006).

Dichlorvos, also known as DDVP (2,2-dichlorovinyl dimethyl phosphate is an organophosphate insecticide cum pesticide (USEPA, 2007; Okoroiwu, 2018). All the fish samples showed presence of Dichlorvos (DDVP) with Stockfish presenting the significantly highest concentrations of 1.119±0.353mg/kg and all were above the 0.1 mg/kg MRL of pesticides residues in foodstuffs. The significant concentrations of endosulfan residues in all the fish samples compared to MRL of 0.5 mg/kg is of public health concern and with the highest amount of 1.430±0.021mg/kg in Stockfish. Endosulfan is an off-patent organochlorine insecticide phased out globally due to its acute toxicity, potential for bioaccumulation, and role as an endocrine disruptor (Mathew, 2011). Endosulfan is alleged to be responsible for many fatal pesticide poisoning incidents around the world and can act as an endocrine disruptor (xenoestrogen) (Raun *et al*., 2002; Pesticide Action Network North America, 2006). *In vitro* assays have shown that endosulfan can promote proliferation of human breast cancer cells (Grunfeld *et al*., 2004; Ibarluzea *et al*., 2004; Soto *et al*., 1994) and another study showed endosulfan can induce reactive oxygen species (ROS) (Sebastian, 2016). Gamma-Chlordane (g-chlordane) presented Residual concentrations above maximum permissible limits 0.1 mg/kg. Gamma-Chlordane is a highly poisonous organochlorine insecticide. The EPA has cancelled registrations of pesticides containing this compound with the exception of its use through subsurface ground insertion for termite control and the dipping of roots or tops of non-food plants. Exposure to chlordane and/or its metabolites have been reported as risk factors for many biochemical and physiological conditions such as type-2 diabetes (Evangelou *et al*., 2016), for lymphoma (Luo *et al*., 2016), for prostate cancer (Lim *et al*. 2015), for

obesity (Tang-Peronard *et al*. 2011), for testicular cancer (Cook *et al*., 2011) and for breast cancer (Khanjani *et al*., (2007).

Stockfish sample with 1.802±0.547mg/kg hexachlorobenzene (HCB) residues above 0.5 mg/kg MRL is a public health concern. HCB is a persistent chemical and bioaccumulates in aquatic and terrestrial food chains (ATSDR, 2002). HCB has been used primarily as a fungicide or biocide and this can account for the presence in these fish samples. Some fish handlers can use this pesticide to inhibit growth of fungi and other microbial on the fish samples. The HCB's are seen most prominent on fish samples (stock fish 1.802±0.547 mg/kg and cray fish 0.339±0.014 mg/kg) not processed by heat application (Ujowundu*et al*., 2014). Acute exposure to hexachlorobenzene adversely affects the nervous system causing weakness, tremors, and convulsions. HCB exposure may also induce skin, liver and thyroid lesions, hepatic dysfunction and reproductive system and developmental defects. Heptachlor residues were significantly higher in Stockfish (1.096±1.007 mg/kg) and in Crayfish 0.737±0.014 mg/kg and present in other fish samples at concentration above MRL. Heptachlor is a cyclodiene and an organochlorine insecticide, highly stable and can persist in the environment for decades. The amount that can be present in different foods is regulated (Robert, 2002). Heptachlor is lipophilic and tends to accumulate in the body fat of humans and animals. Human exposure to heptachlor arises from drinking water, foods, and breast milk (ATSDR, 2007). Animals exposed to heptachlor epoxide during gestation and infancy are found to have changes in nervous system and immune function. Exposure to higher doses of heptachlor in newborn animals leads to decreased body weight and death (California Environmental Protection Agency, 1999)

Isopropylamine residues in all the fish samples are above MRL. Isopropylamine is on the hazardous substance list because it is regulated by OSHA and cited by ACGIH, DOT, DEP and NFPA. Isopropylamine is a building block for the preparation of many herbicides and pesticides including atrazine, bentazon, glyphosate, imazapyr, ametryne, desmetryn,

prometryn, pramitol, dipropetryn, propazine, fenamiphos, and iprodione (Karsten *et al*., 2005). Similarly, Stockfish with a 1.515±0.141 mg/kg residual lindane showed significantly higher amount compared to other fish samples and were above the 0.02 mg/kg MRL. Residual Trans-nonachlor (t-nonachlor) in the fish samples were above MRL. Trans-nonachlor is an organochlorine compound and a bioaccumulating component of chlordane (Bondy *et al*., 2000). Chlordane is toxic and persistent mixture with an environmental half-life of 10 to 20 years with use causing accumulation of its components in the food chain.

Polycyclic Aromatic Hydrocarbons

In vitro and *in vivo* studies have implicated a number of PAHs as mutagenic and carcinogenic in experimental animals. Furthermore PAHs have presented adverse haematological, reproductive and developmental and immunological effects in experimental animals (IARC, 1973; Ujowundu *et al*., 2014). Some PAHs such as benzo(a)pyrene is among the most potent and best documented carcinogen and it is classified as carcinogenic to humans (1A) while others such as benz[a]anthracene and dibenz[a,h]anthracene are classified as probably carcinogenic to humans (Group 2A) and others as possibly carcinogenic to humans (Group 2B) or not classifiable as to its carcinogenicity to humans (IARC, 2010; Ujowundu *et al.*, 2014).

The total PAHs concentrations in the dried fish samples processed by smoking and/or by heat as observed in PAHs of Cat fish (11.84±10.00 µ/kg). Round fish (10.32±8.74 µ/kg). and Bonga fish (8.04±3.00 µ/kg) were significantly higher compared to 3.92±0.54 µ/kg in Cray fish and 3.53±0.08 µ/kg in Stock fish processed by sun drying or freeze drying. This implicates the method of processing as the major source PAHs as the exposure of the fish samples to smoke generated by the firewood can cause considerable contamination with various PAHs because the process is usually poorly controlled (SCF, 2002; Ujowundu *et al*., 2014). High concentration of the PAHs in food samples processed by smoke/heat can

also be attributed to its fat content, and pyrolysis resulting from increased amount of melted fat dropped on heat source (Knize *et al*., 1999; SCF, 2002).

The result of this study showed presence of 16 PAHs in all the fish samples studied. However, toxicological attention should be focused on the presence of high molecular weight PAHs which are largely carcinogenic such as benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene and indeno[*1,2,3-cd*]pyrene, benzo[g,h,i]perylene (Ujowundu *et al*., 2014).

The presence of PAHs classified as probable human carcinogens (Group 2A) such as benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene should be a public health concern to consumers. Benzo[a]pyrene is the most potent PAHs and metabolism in human system activates its toxicity (Sims, 1980). On ingestion, the living system attempts to degrade Benzo[*a*]pyrene, however this metabolic process activates the formation reactive metabolites benzo[*a*]pyrene 7,8-dihydrodiol, oxidized to 7,8- diol-9,10-epoxide and this bind to DNA generating disruption normal genetic activities cell and invariable inducing a genotoxic effect (Cavalieri and Rogan, 1995; Wogan 2004; Ujowundu *et al*., 2014).

The highest concentrations of 0.22±0.30 µ/kg benzo[*a*]pyrene and 0.11±0.16 µ/kg benzo[*a*]pyrene were obtained in Round fish and Cat fish respectively. These were lesser than 1.757 µ/kg obtained in freshly processed pork but more than 0.05 µ/kg obtained in long processed fish by Ujowundu *et al.* (2014). Furthermore, the concentration of dibenz[a,h]anthracene a probable human carcinogen at 1.48±1.92 µ/kg in Round fish and 1.12±0.56 µ/kg in Stock fish were higher than the concentrations reported by Ujowundu *et al* (2014). There was also significant presence of Indeno (1,2,3-cd) pyrene in all the samples. It is important to note that the concentrations of benzo[*a*]pyrene which ranged from 0.01±0.00 µ/kg in Bonga fish to 0.22±0.30 µ/kg in stock fish was less than 2 µg/kg and 5µg/kg Maximum permissible levels of benzo[a]pyrene in food, by European Union (EU) 2008 maximum permissible specified in Commission Regulation (EC) 208/2005)

Fatty Acids

Fatty acids are naturally occurring compounds fats formed by the combination of fats with glycerol. Fatty acids, are important compound in metabolism, they are involved in providing metabolic fuel (storage and transport of energy), as essential components of all membranes, and as gene regulators (Wu *et al.*, 2010; Young *et al.*, 2005). In this study twelve fatty acids (Table 4.1.4) were detected in varying concentrations in five dried fish samples of Bonga fish (*Ethmalosa fimbriata*), Cray fish (*Palaemon hastatus*), Stock fish (*Gadus morhua*), Cat fish (*Clarias gariepinus*) and River African Cat (known as Round fish) sold and consumed in Owerri, Imo State, Nigeria.

Appreciable amount of lauric acid were observed in three fish samples with Bonga fish showing the highest value at 9.74±0.00 mg/kg. Lauric acid are easily digestible, and a good source of direct energy. The chemical structure of lauric acid allows the body to absorb them whole (Eyres *et al*., 2016). Lauric acid increases total serum lipoproteins more than many other fatty acids, but mostly high-density lipoprotein (HDL). Therefore, lauric acid is regarded as having a more favorable effect on total HDL than other fatty acids (Mensink *et al.*, 2003). Generally, a lower total/HDL serum lipoprotein ratio correlates with a decrease in atherosclerotic incidence (Thijssen and Mensink, 2005). Myristic acid was detected in appreciable amount in all the fish samples except cray fish with stock fish presenting the highest value of 6.27±5.67 mg/kg. Myristic acid is a saturated fatty acid, involved in the stabilization many different proteins, such as proteins of the immune system and some antitumour proteins (Carta *et al*., 2017). This function is called myristoylation; it occurs when myristic acid is attached to the protein in a specific position where it functions usefully. This highlights the importance of dietary myristic acid to enable anti-cancer and immune system optimal function (Buaud, 2020)

Stearic acid at 18.16±2.28 mg/kg in Bonga fish was the most abundant fatty acid after Oleic acid at 81.93±9.62 mg/kg in Bonga fish in all the fish samples evaluated. Stearic acid is a saturated fatty acid and has been implicated in making absorption of essential nutrient by the digestive system difficult. It can also cause damage to the immune system. However, investigations showed that stearic acid does not raise serum cholestrol (hyercholestrolemic) and may not have the capacity to raise LDL-cholesterol (Kris-Ethertone*t al.,* 2005).

Oleic acid at concentrations of 81.93±9.62 mg/kg in Bonga fish to 17.89±1.54 mg/kg in Stock fish presented the highest values of fatty acids in all the fish samples studied. Oleic acid is a monounsaturated fatty acid and presents beneficial effects such as reduction of inflammation and may have beneficial effects on genes linked to cancer. Oleic acid other functions such as improving insulin sensitivity, lowering blood pressure, lowers cholesterol levels, decrease of chronic nerve pain, slowing of ageing process (Farvid *et al.*, 2014)

Linoleic acid at 11.82±2.15 mg/kg and alpha-linolenic acid at 4.12±0.00 mg/kg showed highest concentration in Stock fish and Bonga fish respectively but both was not detected in Cat fish. Linoleic acid and Linolenic acid are polyunsaturated fatty acids (PUFA). They are involved in lowering serum cholesterol levels, slowing the development of atherosclerosis, and formation of arachidonic acid (Naughton *et al*., 2016). These PUFA have modulatory potentials and are important in blood pressure reduction (Hoshi *et al*., 2013).

The other PUFAs such as Eicosadienoicacid, Eicosatrienoic acid, Arachidonic acid, Tetracosanolpentaenoic acid and Docosahexaenoic acid are regarded as essential fatty acids and important for brain development and function and might be vital for facilitating the transmission of signals between neurons (Leaf, 2008). Many studies have positively correlated essential fatty acids with reduction of cardiovascular morbidity and mortality,

infant development, cancer prevention, optimal brain and vision functioning, arthritis, hypertension, diabetes mellitus and neurological/neuropsychiatric disorders (Nesheim and Yaktine, 2007; Mozaffarian, 2008).

4.3 CONCLUSION

In conclusion, the results obtained in this study implicated the method of processing (heat application such as smoking) as the probable source of PAHs, decrease in the polyunsaturated and decrease in the essential fatty acid content in the smoked dried fish samples (Bonga fish, Cat fish and Round fish) as compared to the sun dried fish samples (Cray fish and stock fish), the smoked dried fish samples also recorded higher heavy metals concentrations while the sun drying method was implicated as the probable source of pesticides in the dried fish samples. Sun dried fish samples showed to have lesser concentrations of polycyclic aromatic hydrocarbons as compared to the smoked dried fish samples. However, there is need for effective legislation and regulation of the use of pesticides as preservatives. This research result is relevant for environmental biomonitoring and can be subjected to further studies.

Contribution to Knowledge

1. The findings of this research project highlight potential food safety risks associated with consuming dried fish. By quantifying elevated levels of pesticides, heavy metals, and PAHs in these samples, the project sheds light on previously unrecognized hazards in the food supply chain.

2. The project provides critical insights into the levels of contaminants present in dried fish consumed by residents of Owerri. This assessment is essential for understanding the extent of human exposure to harmful substances through dietary intake and evaluating associated health risks, including potential long-term health effects.

3. By identifying increased levels of pesticides, heavy metals, and PAHs in some of the dried fish samples, the project contributes to our understanding of environmental contamination sources. This information can inform efforts to

address pollution sources, such as agricultural runoff, industrial discharges, or atmospheric deposition that may contribute to contaminant accumulation in aquatic ecosystems. It also informs on the improving on fish processing and handling practices.

4. The project findings inform the development of mitigation strategies aimed at reducing exposure to contaminants in dried fish. This may include implementing measures to reduce pesticide use, controlling industrial emissions, improving waste management practices, or establishing guidelines for safe food processing and storage to minimize contamination levels.

5. This research results may serve as a basis for risk communication efforts to raise awareness among consumers, policymakers, and stakeholders about the potential health risks associated with consuming contaminated dried fish. Effective communication of these findings can empower individuals to make informed dietary choices and advocate for measures to improve food safety standards.

6. The findings from this research may provide scientific evidence to support the development of food safety regulations and standards aimed at protecting consumer health. This may include setting maximum residue limits for pesticides, establishing thresholds for heavy metal contamination, or implementing monitoring programs to ensure compliance with safety standards in the food industry.

REFERENCES

Adamu, B.D., & Esheya, S.E. (2022). Factors Influencing Participation of Irish Potato Farmers of the Women in Agriculture and Youth Empowerment Programme on Livelihood of Irish Potato Farmers in Plateau State. *Jigawa Journal of Multi-Disciplinary Studies*, 5 (1):130 -152.

Adeyeye, S.A.O., Oyewole, O.B., Obadina, A.O. & Omemu, A.M. (2015). Influence of smoking method on quality of traditional smoked Bonga shad (Ethmalosa frimbriata) fish from Lagos State, Nigeria. *African Journal of Food Science*, 9 (4) 200-207.

Adrian, W.J. (1973). A comparison of a wet pressure digestion method with other commonly used wet and dry- ashing methods. *Analyst*. 98: 213 – 216

Agarwal, A., Aponte-Mellado, A., Premkumar, B.J., Shaman, A. & Gupta, S. (2012). "The effects of oxidative stress on female reproduction: a review". *Reproductive Biology and Endocrinology*. 10 (1): 49. doi:10.1186/1477-7827-10-49. PMC 3527168.

Agbon, A.O., Ezeri, G.N.O., Ikenweiwe, B.N., Alegbeleye, W.O., Oke, O. & Akomolede D.T.(2002). A comparative study of different storage methods on the shelf-life of smoke-cured fish. *Journal of Aquatic Science,* 17: 134-136.

Agency for Toxic Substances and Disease Registry (ATSDR) (2002). Toxicological profile for aldrin and dieldrin. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Available at URL: https://www.atsdr.cdc.gov/toxprofiles/tp1.pdf.

Agency for Toxic Substances and Disease Registry (ATSDR) (2002) Toxicological profile for hexachlorobenzene update [online]. September 2002. Available at URL: https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=627&tid=115. 12/28/12

Akinola, O. A., Akinyemi, A. A. & Bolaji, B. O. (2006). Evaluation of Traditional and solar drying systems towards enhancing fish storage and preservation in Nigeria (Abeokuta Local Government as a case study) *Journal of Fisheries International* 1, (2-4): 44-49.

Akinrotimi, O.A., Edun, O.M, Uka A. & Owhonda, O.N. (2013). Public perception of mudskipper consumption in some fishing communities of River State, Nigeria. *J. Fish. Aquat. Sci.* 8, 208.

Akinwumi, F.O. (2014). Profile for Arsenic TP-92/09. Center for Disease Control, Atlanta, Georgiaand Health Effects. Pergamon Press. Oxford*J. Agric. Sci.* 6, 143

Al-Jufaili, M.S. & Opara, L. U. (2006). Status of fisheries Postharvest Industry in the Sultanate of Oman: Part1 Handling and Marketing System of Fresh Fish. *Journal of Fisheries International* 1, (2-4): 144-149.b

Alquezar, R., Markich, S.J. & Booth D.J. (2006). Effects of metals on condition and reproductive output of the smooth toadfish in Sydney estuaries, south-eastern Australia. *Environmental Pollution* 142(1):116-22.

Alvarez-Pedrerol, M., Ribas-Fitó, N., Torrent, M., Carrizo, D., Garcia-Esteban, R., Grimalt, J.O. & Sunyer, J. (2008). "Thyroid disruption at birth due to prenatal exposure to beta-hexachlorocyclohexane". *Environment International*. 34 (6): 737–740. doi:10.1016/j.envint.2007.12.001.

Amoo, I.A., Akinneye. J.O. & Arannilewa, S.T. (2007). Effect of drying methods on the nutritional composition of three species of fish (*Bonga sp. Sardinella sp*. and *Heterotisniloticus*). *J. Fish. Int*., 2: 99-103.

Amos-Tautua, B.M., Inengite, A.K.,Abasi, C.Y & Amirize, G.C. (2013). "Evaluation of Polycyclic aromatic Hydrocarbons and some Heavy Metals in roasted food snacks in Amassoma, Niger Delta, Nigeria", *African Journal of Environmental Science and Technology*, 70(10): 961-966.

Andersen, H.R., Vinggaard, A.M., Rasmussen, T.H., Gjermandsen, I.M. &Bonefeld-Jørgensen, E.C. (2002). Effects of Currently Used Pesticides in Assays for Estrogenicity, Androgenicity, and Aromatase Activity in Vitro. *Toxicology and Applied Pharmacology*. 179 (1): 1–12.

AOAC (1990). Official Methods of Analysis of the Association of Analytical Chemists, Washington D.C.

Arce, M., Reis, R.E., Geneva, A.J. & Pérez, M.H.S. (2013). Molecular phylogeny of thorny catfishes (Siluriformes: Doradidae). *Mol. Phylogenet. Evol*. 67, 560–577.

Armstrong, B.G., Hutchinson, E., Unwin, J. & Fletcher, T.(2004). Lung Cancer risk after Exposure to Polycyclic Aromatic Hydrocarbons: A review and Meta-Analysis.*Environ Health Perspect* 112 (9) 970–978.

Ashraj, W. (2005). Accumulation of heavy metals in kidney and heart tissues of Epinephelusmicrodon fish from the Arabian Gulf. *Environ. Monit.Assess.*, 101 (1-3): 311-316.

Athanasiadou, M., Cuadra, S.N., Marsh, G., Bergman, A. & Jakobsson, K. (2008). Polybrominateddiphenyl ethers (PBDEs) and bioaccumulative hydroxylated PBDE metabolites in young humans from Managua, Nicaragua. *Environ. Health Perspect*. 2008, 116, 400–408.

Ayuba, V.O. & Omeji, N.O. (2006). Effect of insect infestation on shelf life of smoked dried fish. Proceedings of the 21st Annual Conference of the Fisheries Society of Nigeria, November 13-17, 2006, Calabar, Nigeria, pp: 357-359.

Azeza, N. I (1976). Fish handling, processing and marketing in Lake Chad Basin (N/M shores) In: proceeding of the conference on the handling Processing and marketing of tropical fish. Tropical product institute London, 251.

Baklanov, A. , Ha¨nninen, O., Slørdal, L.H., Kukkonen, J., Bjergene, N., Fay, B.,Finardi, S., Hoe, S.C., Jantunem, M., Karpinnen, A., Rasmussen, A., Skouloudis, A., Sokhi, R.S., Sorensen, J.H & Odegaard, V.(2007). Integrated systems for Forecasting Urban Meteorology, Air pollution and population Exposure. *Atmos Chem Phys* 7 855–874.

Baselt, R.C. (2000). *Disposition of Toxic Drugs and Chemicals in Man*, 5th Ed. Chemical Toxicology Institute. Foster City, CA.

Belton, J.C., Benson, N.C., Hanna, M.L. & Taylor, R.T. (1985). Growth inhibition and cytotoxic effects of three arsenic compounds on cultured Chinese hamster ovary cells. *J. Environ Sci. Health*, 20A: 37-72.

Benech, V., Teugels, G.G. & Gourene, G. (1993). Criterepractiquepor distinguisher deuxpoissonchatsafricains, *Clariasanguillaris*, *C. gariepinus* (siluriforme: claridae). *Journal of Fisheries andAquatic Science*, 17(1): 83-85.

Benedetti, M., Martuccio, G., Fattorini, D., Canapa, A., Barucca, M., Nigro, M. & Regoli, F. (2007). Oxidative and modulatory effects of trace metals on metabolism of polycyclic aromatic hydrocarbons in the Antartic fish *Trematomus Aquat. Toxicol.* 85:167–175.

Boas, M., Feldt-Rasmussen, U., Skakkebaek, N.E. & Main, K.M. (2006). Environmental chemicals and thyroid function. *European Journal of Endocrinology*. 154 (5): 599–611. doi:10.1530/eje.1.02128. PMID 16645005.

Bolaji, B.O. (2005). Performance evaluation of a simple solar dryer for food preservation, Book of Proceedings of 6th Annual Engineering Technology, Federal University of Technology, Minna, Nigeria, pp. 8-13.

Bourre, J.M. (2007). Review dietary omega-3 fatty acids for women. *Biomed. Pharmacother*, Feb-Apr; *61(2-3)*:105-12.

Bradl, H. (eds.) (2002). Heavy Metals in the Environment: Origin, Interaction and Remediation Volume 6. *Academic Press*. London Remediation Volume 6. Academic Press. London

Brandenberger, H. & Maes, R.A. (1997). Analytical toxicology: for clinical, forensic, and pharmaceutical chemists. Berlin: Walter de Gruyter. p. 243. ISBN 978-3-11-010731-9. Retrieved 2009-05-10.

Brawn, V.M. (2011). "Reproductive Behaviour of the Cod (Gadusmorhua)", Behaviour, 18: 177-197

Buaud, B. (2020). How fats we eat modulate our immunity? *Lipids and health* 27 (22) 10, DOI: https://doi.org/10.1051/ocl/2020009

Calder, P.C. (2004). Long chain n-3 fatty acids and Cardiovascular disease: further evidence and insights, *Nutr. Res. 24*, 761.

California Environmental Protection Agency (1999). Public Health Goal for Heptachlor and Heptachlor Epoxide In Drinking Water - Office of Environment Health Hazard Assessment, California Environmental Protection Agency. "Archived copy" (PDF). Archived from the original (PDF) on 2009-05-04. Retrieved 2013-12-19. (retrieved on April 04, 2009).

Campo, L., Rossella, F., Pavanello, S., Mielzynska, D., Siwinska, E., Kapka, L., Bertazzi, P.A. & Fustinoni S. (2010). Urinary profiles to assess polycyclic aromatic hydrocarbons exposure in coke-oven workers. *Toxicol Lett* 192 (1) 72–78.

Carta, G., Murru, E., Banni, S. & Manca, C. (2017). Palmitic Acid: Physiological Role, Metabolism and Nutritional Implications. *Front. Physiol*. 8:902. doi: 10.3389/fphys.2017.00902

Cavalieri, E.L & Rogan, E.G.(1995). Central role of radical cations in the metabolic activation of polycyclic aromatic hydrocarbons. *Xenobiotica*. 25:677-688.

CCME (Canadian Council of Ministers of the Environment) (2010). Canadian soil quality guidelines for potentially carcinogenic and other PAHs: scientific criteria document. CCME: Winnipeg.

Center for Environmental Assessment (CEA 1999). Office of Research and Development, Washington, D.C.

Chappell, B. (2020). Bayer To Pay More Than $10 Billion To Resolve Cancer Lawsuits Over Weed killer Roundup. NPR. Retrieved 26 June 2020.

Clarkson, T.W., Magos, L. & Myers, G.J. (2003). The toxicology of mercury-current exposures and clinical manifestations. *N. Engl. J. Med.* 349: 1731– 1737

Clucas, I.J & Ward, A.R. (1996). Post-Harvest Fisheries Development: A Guide to Handling, Preservation, Processing and Quality. National Research Institute, UK., ISBN-13: 9780859544412, Pages: 443.

Commission for Environmental Cooperation (2013). The North American Regional Action Plan (NARAP) on Lindane and Other Hexachlorocyclohexane (HCH) Isomers (PDF). Retrieved 2020-02-28.

Davies, R.M. (2005). Development of appropriate technology of fish processing in Nigeria. Proceedings of the Workshop on Intensive Fish Farming, February 24th, 2005, Nigeria.

Davies, R. M. & Davies, O. A.(2009). Traditional and improved fish processing technologies in Bayelsa State, Nigeria. *European Journal of Scientific Research* ISSN 1450-216X 26, (4): 539-548.

Davies, R.M., Davies, O.A., Inko-Tariah, M.B. & Bekibele, D.O. (2008). Mechanization of fish farms in Rivers State, Nigeria, *World Applied Sciences Journal*, 3 (6): 926-929

Davies, R. M. (2005). Development of Appropriate Technology of fish processing in Nigeria. A paper presented at a one-day workshop on intensive fish farming on Thursday, 24th February 2005.

Doe, P. E. (1998). *Fish Drying and Smoking. Production and Quality*, Technomic Publishing Co. Inc., Lancaster: pp. 89–115.

Eliane, E. (2009). UN: Treaty expanded by 9 more dangerous chemicals, Associated Press 2009-05-09.

Emokpae, A.O. (1979). Organoleptic assessment of the quality of fresh fish, Nigerian Institute of Oceanography and Marine Research (NIOMR) Occasional Paper No 12, pp. 1-4.

Esheya, S. E. (2021a). Economics of Partnership Fishing Among Fishermen in Riverine Areas of Ebonyi North Agricultural Zone of Ebonyi State, Nigeria. *Journal of Agripreneurship and Sustainable Development*, 4 (3): 76-83.

Evangelou E, Ntritsos G, Chondrogiorgi M, Kavvoura F.K, Hernandez A.F, Ntzani E.E and Tzoulaki I (2016). Exposure to pesticides and diabetes: A systematic review and meta-analysis. *Environment International*.91:60–68. doi:10.1016/j.envint.2016.02.013.

Eyres, L., Eyres, M.F, Chisholm, A., Brown, R.C. (2016). "Coconut oil consumption and cardiovascular risk factors in humans". Nutrition Reviews. 74 (4): 267–280. doi:10.1093/nutrit/nuw002. PMC 4892314. PMID 26946252.

FAO, (1996). Fisheries and aquaculture in Sub-Saharan Africa: Situation and outlook in 1996. FAO Fisheries Circular No. 922, FAO, Rome, Italy, pp: 1-44.

Farvid, M. S., Ding, M., Pan, A., Sun, Q., Chiuve, S. E., Steffen, L. M., Willett, W. C., & Hu, F. B. (2014). Dietary linoleic acid and risk of coronary heart disease. A systematic review and meta-analysis of prospective cohort studies. *Circulation.*130: 1568- 1578.

Ferraris, Jr., C.J. & Vari, R.P. (2012). Review of the African catfish genus Andersonia (Teleostei:Siluriformes). *Zootaxa* 3210, 39–49.

Ferreira I., Venâncio C., Lopes I. & Oliveira M (2019). Nanoplastics and marine organisms: What has been studied?. *Environmental Toxicology and Pharmacology*. 67(8). Doi:10.1016/j.etap.2019.01.006.

Food and Agriculture Organization (FAO) (2012).State of world aquaculture 2012. FAO Fisheries Technical Paper No. 500, FAO, Rome, Italy pp. 1-134

Food and Agriculture Organization/World Health Organization(2006). Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Certain Additives and Contaminants, 2006, 67th Joint FAO/WHO Expert Committee on Food Additives; WHO Technical Report Series; FAO/WHO: Rome, Italy.

Food and Nutrition Board (2004). Dietary Reference Intakes [DRIs]: Recommended Intakes for Individuals; National Academy of Sciences: Washington, DC, USA.

Godwin, P. (2017). Picture of smoked dried Cat fish (mobile upload) July 15,2017.

Grunfeld, H.T. & Bonefeld-Jorgensen E.C. (2004). Effect of in vitro estrogenic pesticides on human oestrogen receptor alpha and beta mRNA levels, *Toxicol. Lett*., 151(3):467–80.

He, Z.L., Yang, X.E. &Stoffella, P.J. (2005). Trace elements in agroecosystems and impacts on the environment. *J Trace Elem Med Biol* 19(2-3):125-140

Hoshi, T., Wissuwa, B., Tian, Y., Tajima, N., Xu, R., Bauer, M., Heinemann, S. H., & Hou, S. (2013). Omega-3 fatty acids lower blood pressure by directly activating

large-conductance Ca^{2+} -dependent K^{+} channels. Proceedings of the National Academy of Sciences of the United States of America, 110(12). 4816–4821.

Hussein, I.A.& Mona, S.M.(2016). A review on polycyclic aromatic hydrocarbons:Source, environmental impact, effect on humanhealth and remediation *Egyptian Journal of Petroleum*25:107-123.

IARC (International Agency for Research on Cancer) (2010). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures, *Monogr Eval Carcinog Risks Hum* 92:765–771.

IARC (International Agency for Research on Cancer) (1973). Benzo(a)pyrene. In: IARC Monographs on the Evaluation of Carcinogenic Risk of the Chemical to Man. Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds, Vol. 3. World Health Organization, Lyon, France. 91-136.

Ibarluzea, J.M.J., Fernandez, M.F., Santa-Marina L., Olea-Serrano, M.F., Rivas, A.M., Aurrekoetxea, J.J., Exposito, J., Lorenzo, M., Torne, P., Villalobos, M., Pedraza,V., Sasco, A.J. & Olea, N. (2004). Breast cancer risk and the combined effect of environmental estrogens, Cancer Causes Control,15(6):591–600.

Igbeka, J.C. (1986). Evaluation of three types of solar dryers for Nigeria crops, Proceedings of a Workshop on Solar Drying in Africa, Dakar, Senegal, pp. 162-174.

Independent Newspaper (2019).Price of Crayfish soars by 50% in Enugu July 15, 2019.

Ita, E.O. (1972). "Approaches to the evaluation and management of the fish stock in Kainji Lake, Nigeria", paper presented at the CIFA Conference, Fort Lamy, Chad, 29 November-6 December, Rome, FAO, CIFA/72/S, 14, p. 18.

Karsten, E., Erhard, H., Roland, R. & Hartmut, H. (2005). Amines, Aliphatic" in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim. Doi:10.1002/14356007.a02_001

Kasprzak, J., Sunderman, Jr. F.W.&Salinikow, K. (2003). Nickel carcinogenesis. Mutation research. 533(1-2): 67- 97

Khanjani, N., Hoving, J.L., Forbes, A.B. & Sim, M.R.(2007). "Systematic review and meta-analysis of cylodiene insecticides and breast cancer". *Journal of Environmental Science and Health*, Part C. 25 (1): 23–52.

Kim, K., Jahan, S.A., Kabir, E. & Brown, R.J.C., (2013). A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environ Int.* 60, 71–80.

Kodavanti, P.R.S. (2014) Polychlorinated Biphenyls (PCBs). In Encyclopedia of the Neurological Sciences, 2nd ed.; Daroff, R., Aminoff, M.J., Eds.; *Elsevier*: Waltham, MA, USA, pp. 917–921.

Koppe, J.G. & Keys J. (2001). PCBs, and the precautionary principles. In European Environment Agency—Late Lessons from Early Warnings: The Precautionary

Principle 1896–2000; Environmental Issue Report No 22; Office for Official Publications of the European Communities: Luxembourg; p. 210

Koster, F.W., Mollmann, C., Nevenfeldt, S., John, M.A., Plikshs, M. & Voss, R. (2009). "Developing Baltic Cod Recruitment Models", *Canadian Journal of Fisheries and Aquatic Sciences,* 58(8):15-29.

Kris-Etherton, P. M., Griel, A. E., Psota, T. L., Gebauer, S. K., Zhang, J.&Etherton, T. D. (2005). Dietary stearic acid and risk of cardiovascular disease: intake, sources, digestion and absorption. *Lipids.*40: 193- 200.

Kurlansky, M. (2014). Cod: Abiography of the fish that changed the world, Basilicata Cultural Society of Canada, 3:42-56

Lakshmanan, R., Kesavan, K., Vijayanand, P., Rajaram, V. & Rajagopal, S (2009). Heavy metals accumulation in five commercially important fishes of Parangipettai, Southeast Coast of India. *Advance Journal of Food Science and Technology*, 1 (1): 63-65.

Latimer, J. & Zheng J. (2003). The sources, transport, and fate of PAH in the marine environment, in: P.E.T. Douben (Ed.). PAHs: an ecotoxicological perspective, John Wiley and Sons Ltd, New York.

Leaf, A. (2008).Historical overview of n-3 fatty acids and coronary heart disease, *The American Journal of Clinical Nutrition*, 87: 1978S-1980S.

Loomis, D., Guyton, K., Grosse, Y., El Ghissasi, F., Bouvard, V., Benbrahim-Tallaa, L., Guha, N., Mattock, H. & Straif, K. (2015). Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid. *The Oncology*. 16 (8): 891–2. doi:10.1016/S1470-2045(15)00081-9.

Luo, D., Zhou, T., Tao, Y., Feng, Y., Shen, X. & Mei, S.(2005). Exposure to organochlorine pesticides and non-Hodgkin lymphoma: a meta-analysis of observational studies. Scientific Reports. 6: 25768. doi:10.1038/srep25768. PMC 4869027.

Madu, C.T., Okoye F.E., Sado E.K., Omorinkoba W.S., Bankole N.O. & Ita, E.O. (1984). A preliminary report of induced breeding trials with the mud fish (*Clariasanguillaris*). KLRI Annual Report, Kainji Lake Research Institute, pp: 144-159.

Malisch, R. (2017). Incidents with dioxins and PCBs in food and feed-investigative work, risk management and economic consequences. *J. Environ. Prot*. 8, 744–785.

Mansour, S.A. & Sidky, M.M. (2002). Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. *Food Chem.*, 78: 15–22.

Mathew, R. (2011) Stockholm Convention approves recommendation for ban on endosulfan. *The Hindu, April 30, 2011,*

Mensink, R.P., Zock, P.L., Kester, A.D. & Katan, M.B. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials.

American Journal of Clinical Nutrition. 77(5):1146–1155. doi:10.1093/ajcn/77.5.1146. ISSN 0002-9165.

Metcalf, R.L. (2002) Insect Control" in Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH, Weinheim, 2002. doi:10.1002/14356007.a14_263

Mohammed, A.A.K.& Yusuf, S.A.K. (2001). Insect infestation and preventive measures in dry fish storage of Chittagong, Bangladesh, *J. Biol. Sci*., 1:963-965.

Mozafarian, D. & Rimm, E. (2006).Fish Intake, Contaminants, and Human Health: Evaluating the Risks and the Benefits, *Journal of the American Medical Association*. 296: 1885-1899.

Mufti, W. K. (2016). Is Crawfish Halal in Islam? (Crayfish). Accessed from: https://imanupdate.com/ on 2ndApril, 2023.

Naughton, S. S., Mathai, M. L., Hryaiw, D. H. & McAinah, A. J. (2016). Linoleic acid and the pathogenesis of obesity. *Prostaglandins and otherLipid Mediat.*125: 90-99.

Nesheim, M.C. & Yaktine, A.L. (eds.) (2007). Seafood Choices: Balancing Benefits and Risks. National Academy of Sciences, Committee on Nutrient Relationships in Seafood: Selections to Balance Benefits and Risks Food and Nutrition Board, M.C. National Academies Press. Washington, DC

Nriagu, J.O. (1989). A global assessment of natural sources of atmospheric trace metals. *Nature*, 338, 47-49.

Ogbonna, P. A. (2013). A Review of Crayfish Fishery of Nigeria with Special Reference to the Cross River State. In 4th Annual Conference of the Fisheries Society of Nigeria (FISON). 26–29 November 1985, Port Harcourt, Nigeria. Pp. 42–46

Okonta, A.A.&EkelemuJ.K. (2005). A prelimnary study of micro-organisms associated with fish spoilage in Asaba, Southern Nigeria. Proceedings of the 20th Annual Conference of the Fisheries Society of Nigeria, November 14-18, 2005, Port Harcourt, pp: 557-560.

Okoroiwu, H. U. & Iwara, I. A. (2018). Dichlorvos toxicity: A public health perspective. *Interdisciplinary toxicology*, *11*(2). 129–137.

Olabemiwo, O.M., Alade, A.O., Tella, A.C. & Adediran, G.O. (2011) Assessment of polycyclic aromatic hydrocarbons content in smoked *C. gariepinnus*and *T. guineensis* fish species available in Western Nigeria. *Int. J. Basic Appl. Sci.* 11 (02):135-150.

Olokor, I.O. (1997). Solar tent an adoptable fish preservation technology in Kainji Lake Basin. Nigerian-German (GTZ) Kainji Lake Fisheries Promotion Project, New Bussa, Nigeria.

Oyeleye, O. (2003). Export potentials of smoked fish trade in Nigeria, The Guardian, Guardian Newspaper Publication, Lagos, Vol. 19 No. 8916, p. 27.

Patlolla, A., Barnes, C., Field, J.,Hackett, D., Tchounwou, P.B. (2009). Potassium dichromate-induced cytotoxicity, genotoxicity and oxidative stress in human liver carcinoma ($HepG_2$) cells. Int *J Environ Res Public Health.* 6: 643-653

Persistent Organic Pollutant Review Committee (POPRC) (2007). Draft risk management evaluation for lindane. May, 2007. Archived from the original (PDF) on 2007-09-27. Retrieved 2007-09-05.

Pigott, G.M. (2015). Fish Processing, A Book by Singh, R.P. *Encyclopaedia Britania*, Last Updated 1-2-2015, Retrieved 14 January, 2016.

Porta, M. &Zumeta E. (2002). Implementing the Stockholm Treaty on Persistent Organic Pollutants". Occupational and Environmental Medicine. 59 (10): 651–652. doi:10.1136/oem.59.10.651.

Purdue University; EPA (n.d.) (2015). Exploring the Great Lakes. Bioaccumulation/Biomagnification Effects (PDF). Chicago, IL: EPA. p. 2. Archived from the original (PDF) on 17 October 2015. Retrieved 3 October.

Quansah, R., Armah, F.A., Essumang, D.K., Luginaah, I., Clarke, E., Marfoh, K., Cobbina, S.J., Nketiah-Amponsah, E., Namujju, B., Obiri, S.&Dzodzomenyo, M. (2015) Association of arsenic with adverse pregnancy outcomes/infant mortality: a systematic review and meta-analysis.*Environ Health Perspect*. 2015;123(5):412-421.

Reynolds, J., Souty-Grosset, C. & Richardson, A. (2013). Ecological Roles of Crayfish in Freshwater and Terrestrial Habitats. *Freshwater Crayfish*, 19 (2): 197–218.

Saad, Y. M., Shaden Hanafi,M., Essa, M.A., Guerges, A.A. & Fawzia Ali, S. (2009).Genetics signatures of some Egyptian *Clarias gariepinus* populations. *Global Veterinaria*, 3(6): 503-508.

Schmidt, C., Krauth, T. & Wagner, S. (2017). Export of Plastic Debris by Rivers into the Sea" (PDF). Environmental Science & Technology. 51 (21):12246–12253. doi:10.1021/acs.est.7b02368. PMID 29019247.

Scientific Committee on Foods (SCF) Opinion of the Scientific Committee on Food in the risk to human health of PAHs in food. Brussels: SCF; 2002.

Sebastian,R. & RaghavanS.C. (2016). Induction of DNA damage and erroneous repair can explain genomic instability caused by endosulfan. *Carcinogenesis*. 37 (10): 929–940. doi:10.1093/carcin/bgw081.

Sergeant, J.R. (1997). Fish oils and human diet. *Br. J. Nutr*., 78: S5–S13. Doi:10.1079/bjn19970131.

Sikoki,F.D. & Otobotekere, A.J.T. (1999). Fisheries. In:E.C.,Alagoa, (Ed.).,The land people of Bayelsa State Central Niger Delta, *Onyoma Research Publications*, Port Harcourt, pp: 301-319.

Silva, B.O., Adetunde,O.T., Oluseyi, T.O., Olayinka, K.O. &Alo, B.I.(2011) Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria. *Afr. J. Food Sci.* 5, 384–391.

Simko, P. (2002). Determination of Polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *Journal of Chromatography*, B770(1-2):3-18

Simopoulos, A.P. (2009).Omega-6/omega-3 essential fatty acids: biological effects. *World Rev Nutr Diet*. 99:1-16.

Sims, P. (1980). The metabolic activation of chemical carcinogens. *Brit. Med. Bull*. 36(1):pp11-18.

Singh, R.P. & Heldman D.R. (2013). Introduction to Food Engineering, 5th ed., *Academic Press.* ISBN: 9780123985309

Skelton, P. (1993). A complete guide to the fishes of southern Africa. *Southern Book Publishers*, Harare, Zimbabwe. pp 91.

Soto, A.M., Chung, K.L. & Sonnenschein, C. (1994). The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogensensitive cells Archived September 27, 2007, at the Wayback Machine, *Environ. Health Perspect*., 102(4):380–383.

Stołyhwo, A. & Sikorski, Z.E. (2005). "Polycyclic aromatic hydrocarbons in smoked fish – a critical review", *Food Chemistry*, Vol. 91 No. 2, pp. 303-311.

Taiwo, A.M., Ihedioha, E.C., Nwosu, S.C., Oyelakin, O.A., Efubesi, P.C., Shitta, J.S. & Osinubi, T.O.(2019). Levels and health risk assessment of polycyclic aromatic hydrocarbons in protein foods from Lagos and Abeokuta, Southwestern Nigeria. *J. Food Compos. Anal.* 2019, 79, 28–38.

Tang-Peronard, J. L, Anderson, H.R., Jensen, T.K. & Heitmann, B.L. (2011). Endocrine-disrupting chemicals and obesity development in humans: a review. *Obesity Reviews.* 12 (8): 622–636.

Tarantini, A., Maıˆtre A., Lefe`bvre, E., Marques, M., Rajhi, A. & Douki, T. (2011) Polycyclic aromatic hydrocarbons in binary mixtures modulate the efficiency of benzo[a] pyrene to form DNA adducts in human cells. *Toxicology* 279 (1-3): 36–44.Doi:10.1016/j.tox.2010.09.002.

Tawari, C.C. &Abowei, J.F.N. (2011). Traditional fish handling and preservation in Nigeria, *Asian Journal of Agricultural Sciences*, Vol. 3 No. 6, pp. 437-452

Tawari, C.C., (2006). *Effectiveness of agricultural agencies in fisheries management and production in the Niger Delta.*(Ph.D. Thesis), Rivers State University of Science and Technology, Port Harcourt, Nigeria.

Teitze, U. (2014). Economic Performance and fishing Efficiency of Marine Capture Fisheries. *FAO Fisheries Technical Paper*. Food and Agriculture Organization. Pp. 482.

Tenyang, N., Womeni, H.M., Tiencheu, B., Takugan, F.N.H., Tchouanguep, M.F., Villeneuve, P. & Linder, M. (2013). Lipid oxidation of Catfish (*Arius*

maculatus) after cooking and smoking by different methods applied in Cameroon. *Food Nutr. Sci*, 4(1), 176.

The International Agency for Research on Cancer (IARC) (2015). IARC Monographs evaluate DDT, lindane, and 2,4-D (PDF) (Press release). Retrieved 2015-11-19.

Thijssen, M.A. & Mensink, R.P. (2005). Fatty Acids and Atherosclerotic Risk. In Arnold von Eckardstein (Ed.) Atherosclerosis: Diet and Drugs. *Springer*. pp. 171–172. ISBN 978-3-540-22569-0.

Tolins, M., Ruchirawat, M. & Landrigan, P. (2014). The developmental neurotoxicity of arsenic: cognitive and behavioral consequences of early life exposure. *Ann Glob Health.* 80(4):303-14.

Tull, A., (1997) Food and Nutrition, 2nd ed., *Oxford University Press*, Oxford, UK. pp. 104–109.

U.S. Environmental Protection Agency (1984). Health and Environmental Effects Profile for 1,1-Biphenyl. EPA/600/x-84/147. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, Cincinnati, OH.

U.S. Environmental Protection Agency (1998). Toxicological Review of Trivalent Chromium; CAS No. 16065-83-1; U.S. Environmental Protection Agency: Washington, DC, USA, p. 51.

U.S. EPA (U.S. Environmental Protection Agency) (1991). Drinking Water Criteria for Polycyclic Aromatic Hydrocarbons (PAHs). Prepared by the Environmental Criteria and Assessment, Office, Office of Health and Environmental Assessment, Cincinnati, OH, for the Office of Water, Washington, DC. ECAO-CIN-D010.

Udoh N.A. (1997). Analysis of Crayfish in Calabar South Local Government area of Cross River State. A Bachelor of Agriculture Degree Dissertation Submitted to the Faculty of Agriculture, University of Calabar. Unpublished.

Ujowundu, C.O., Ogbede, J.U., Igwe, K.O., Okwu, G.N., Agha, N.C. & Okechukwu, R.I. (2014). Quantitative Assessment of Polycyclic Aromatic Hydrocarbons and Heavy Metals in Fish Roasted with Firewood, Waste Tyres and Polyethylene Materials. *Biochem Anal Biochem* 4: 162. doi: 10.4172/2161-1009.1000162

Ujowundu**,** C. O., Ajoku, C.O.,Nwaogu, L. A., Belonwu, D. C.,&Igwe, K.O. (2014). Toxicological Impact of Gas flaring and Other Petroleum Production Activities in the Niger Delta Area of Nigeria Journal: *Journal of Advances in Chemistry*. 10 (2): 2297-2304. ISSN 2321-807X

Ujowundu, C.O., Ihekweazu, K.L, Alisi, C.S., Ujowundu, F.N. & Igwe, C.U. (2014). Procarcinogens: Polycyclic Aromatic Hydrocarbons and Heavy Metal Content in Some Locally Processed Foods in South Eastern Nigeria. *Current Journal of Applied Science and Technology*, *4*(1). 249-260

Ukoha, P.O., Ekere, N.R., Udeogu, U.V. & Agbazue, V.E. (2014) Potential Health Risk Assessment of Heavy metals (Cd, Cu and Fe) Concentrations in some imported frozen fish species consumed in Nigeria. *Int J Chem Sci* 12(2):366-374.

UNDP (2017). Environmentally Sound Management and Disposal of PCBs in Nigeria. Available online: https://www.ng.undp.org/content/nigeria/en/home/projects/Sound-environmental-management-and-disposal-of-PCBs-in-Nigeria.html (accessed on 2 September 2020).

UNEP (2002). United nations environment program chemicals. Indian ocean regional report. UNEP Chemicals is a part of UNEP's technology, industry and economics division, pp 15-67.

UNEP Stockholm Convention.The New POPs under the Stockholm Convention. Available online:http://www.pops.int/TheConvention/The POPs/The New POPs/tabid/2511/Default.aspx (accessed on 14 April 2020).

United States Environmental Protection Agency (U.S. EPA) (2002). *Lead Compounds. Technology Transfer Network- Air Toxics Website*. Online at: *http://www.epa.gov/cgi-bin/epaprintonly.cgi*.

USEPA (2016). Health Effects of PCBs. Washington, D.C.: U.S. Environmental Protection Agency (EPA). 2016-09-15.

USEPA (United States Environmental Protection Agency) (2016). United States Environmental Protection Agency (USEPA) Reference Dose (RfD): Description and Use in Health Risk Assessments Background Document 1A-March 15.

USEPA: United States Environmental Protection Agency (2007). Dichlorvos TEACH Chemical summary U.S EPA. Toxicity and exposure assessment for children; pp. 1–13.

Valko, M., Rhodes, C.J., Monocol, J. & Izakovic-Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions,* 160: 1-40.

Vosyliene, M.Z. & Jankaite, A. (2006). Effect of heavy metal model mixture on rainbow trout biological parameters. *Ekologija,* 4: 12-17.

Wang, Z. & Rossman, T.G. (1996). In: *The Toxicology of Metals*. Cheng, L.W (Ed.).,*Vol. 1*. CRC Press, Boca Raton, FL. 221-243.

Weber, R., Herold, C., Hollert, H., Kamphues, J., Blepp, M. & Ballschmiter, K. (2018). Reviewing the relevance of dioxin and PCB sources for food from animal origin and the need for their inventory, control and management. *Environ. Sci. Eur*. 30, 42.

WHO (2017)/ Mercury and health https://www.who.int/news-room/fact-sheets/detail/mercury-and-health? Access June 8, 2023

WHO (2020). Food Safety, Persistent Organic Pollutants. Available online: https://www.who.int/foodsafety/areas_work/chemical-risks/pops/en/ (accessed on 15 April 2020).

WHO/FAO/IAEA (1996). *Trace Elements in Human Nutrition and Health*. World Health Organization. Geneva, Switzerland.

Wim, V., Issabelle, S.K., Stefan, D.H. & John,V.C. (2007. Consumer perception versus scientific evidence of farmed and wild fish: exploratory insights for Belgium. *Aquaculture International*, 15: 121-136.

Wogan, G.N., Hecht, S.S., Felton, J.S., Conney, A.H. & Loeb, L.A. (2004). Environmental and chemical carcinogenesis. *Semin. Cancer Biol*. 14(6):473-486.

World Health Organization (2022). Arsenic - World Health Organization fact sheet https://www.who.int/news-room/fact-sheets/detail/arsenic 7 December 2022. Access June 8, 2023

World Health Organization (2005). The WHO Recommended Classification of Pesticides by Hazard. *British Journal of Psychiatrist*, 187,583-584.

Wu, X., Zhou, B., Cheng, Y., Zeng, C. & Wang, C. (2010). Comparison of gender differences in biochemical composition and nutritional value of various edible parts of the blue swimmer crab. *J Food Comp Anal* 23: 154-159.

Yang, J., Zhao, Y., Li, M., Du, M., Li, X., Li, Y. (2019). A Review of a Class of Emerging Contaminants: The Classification, Distribution, Intensity of Consumption, Synthesis Routes, Environmental Effects and Expectation of Pollution Abatement to Organophosphate Flame Retardants (OPFRs). *Int. J. Mol. Sci.* 20(12); 2874.

Yeside A. (2020). Fish of West Africa; Bonga fish BeHealthyAfrica.com June 22, 2020.

Young, G. S., Conquer, J.A. & Thomas, R. (2005). Effect of randomized supplementation with high dose olive, flax or fish oil on serum phospholipid fatty acid levels in adults with attention deficit hyperactivity disorder, *Reproduction, Nutrition, Development,* 45(5); 549-558.