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Lipophilicity of mercury and genotoxicity of polycyclic aromatic hydrocarbon (PAHs) in selected fishes at Port Harcourt metropolis, Nigeria

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This research evaluates the lipophilicity and toxicity of (methyl) mercury in two fishes namely; Catfish and Bonga fish samples from three different sampling locations in Port Harcourt metropolis. The genotoxicity of PAHs in the fresh and smoked/dried fish was also investigated. The mercury loadings were determined using the cold vapor Atomic Absorption spectrophotometer and PAHs analysis was carried out using a Gas Chromatography-Flame ionization Detector (GC-FID 6890 model). The three sampling market locations are at Alakahia, Rumuokoro and Borokiri (Greek road) all in PortHarcourt Metropolis Rivers state Nigeria. The results obtained for mercury loadings showed that the average mercury in the form of methyl mercury is slightly higher in the Bonga (0.0614mg/kg) fish (fresh and dried/smoked) than in Catfish samples (0.0474mg/kg). This could be attributed to the relatively higher fat content of Bonga fish than that of catfish. However, both are below the WHO/FAO maximum permissible limits of 0.5 to 1.0 mg/kg in seafood. Methyl mercury will bioconcentrate, bioaccumulate and biomagnify more in Bonga fish due to its lipophilicity in higher fat content making continuous consumption of Bonga fish and Catfish as well risky to children and fetuses. There seems to be no threat of genotoxicity of PAHs in all the fishes as their respective loadings in the fish samples are below the detection limits of the instrumentation used i.e. 0.01mg/kg.

Capsule Summary: The potential of lethal lipophilic methyl mercury to bioaccumulate, bioamplify and biomagnify makes continuous consumption of either the fresh or smoked/dried Catfish or Bonga fishes as protein staples, sources of concern because of the toxicity associated with methyl mercury.

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INTRODUCTION

Mercury, a heavy and nonessential metal is extremely toxic and well distributed within the aquatic ecosystems. Lithogenic and anthropogenic activities contribute to the presence of water and fish which poses serious environmental concerns even at low concentrations (Azevedo et al., 2011; Balshaw et al., 2007; Condin et al., 2011). Mercury exists as elemental mercury (Hg), inorganic mercury (Hg, $HgCl_2$, Hg_2Cl_2) and organic mercury (methyl and ethyl mercury). Mercury enters the food chain and causes serious harm to humans. Human exposure occurs mainly through inhalation of elemental mercury vapor or

ingestion of mercury bonded to organic moieties primarily from seafood (WHO, 1991; Richardson, 1996). In water mercury is converted to methylmercury through microbial activities and is oleophilic, accumulating, bioaccumulating and amplifying up the food chain to man (Roberta, 2005). Children suffer from neurological disorders, including low intelligence quotient and a decrease in attention, language, memory and visual-spatial abilities at low mercury concentrations. At high concentrations, Hg has been linked to mental retardation, lung and kidney damage and death. Environmental Pollution Agency EPA has estimated that about 8% of women of childbearing age have blood levels of Hg exceeding 5.8ppb and at this level, children born by these women are at risk of non-neurological adverse health effects. Human effects of Hg exposures are irreversible including fertility issues, heart attacks and coronary diseases in adult men (Roberta, 2005).

Polycyclic aromatic hydrocarbons (PAHs) are a chemical group of compounds that have two or more condensed aromatic rings and are found in air, water, and soil, and are tagged as general environmentally harmful pollutants (Nakata et al., 2014). PAHs are widely detected in the aquatic environment, i.e., water, sediment, fish, benthic invertebrates, sea birds, and sea mammals. Polycyclic aromatic hydrocarbons (PAHs) in the aquatic environment or seafood are mainly considered to be of four types: derived from fuels (petrogenic), derived from an incomplete combustion process (pyrogenic), generated by organic metabolism (biogenic), and generated by the transformation process in sediment (diagenetic). Of these four types of sources, petrogenic and pyrogenic sources are mainly artificial and are important contributors to environmental PAHs pollution in aquatic ecosystems (Pies et al., 2008). Polycyclic aromatic hydrocarbons (PAHs) can also be grouped as organic compounds with more than one aromatic ring fused and are produced mainly as a byproduct of pyrolysis and or incomplete combustion of mostly fossil fuel or organic compounds, hence are naturally present in the environmental matrix. Anthropogenic sources include; tobacco smoking, coke production, processing of crude oil, industrial emissions, incineration, domestic heating and automobile emissions (Hites and Wagrowski, 1997).

Humans are exposed to PAHs through many pathways like tobacco smoking, food, breathing etc. Studies have shown that PAHs are toxic to humans and the toxicological effects are countless; haematotoxicity, immunotoxicity, reproductive and developmental defects and causing death. A vast array of PAHs especially benzo (a) pyrene (BaP) is carcinogenic to experimental animals and humans, hence PAHs presence in foodstuffs rings an alarming bell to environmentalists. PAHs, like polychlorinated biphenyl (PCBs) and dioxin are lipophilic compounds and thus do not persist for a long time in the human body. (IARC, 2012).

Bonga and Catfish are the foremost sources of protein from fishes consumed in the Port Harcourt metropolis and as an Environmentalist, there are mounting concerns about the toxicity of Mercury and genotoxic PAHs due to these fishes on the vast population in the metropolis who depend on these fishes as their main source(s) of protein. In the past decades, toxicological data on PAHs were evaluated by the Scientific Committee on food (SCF), the International Programme on Chemical Safety (IPSC) and the joint FAO/WHO Experts Committee on Food Additives (JECFA). The SCF committee concluded that 15 PAHs namely benz(a)anthracene, benzo (b) fluoranthene benzo (j) fluoranthene, benzo (k) fluoranthene, benzo (g,h,i) perylene, benzo (a) pyrene, chrysene, cyclopenta (cd) pyrene , dibenzo (a,h) anthracene, debenzo (a,e) pryrene, debenzo (a,h) pyrene, dibenzo (a,i) pyrene, dibenzo (a,l) pyrene, indeno (1,2,3-cd) pyrene and 5-methylchrysene show clear evidence of mutagenicity/genotoxicity in a somatic cell in experimental animals in vivo. Except for benzo(ghi)perylene, has also indicated clear carcinogenic effects in experimental animals. The SCF recommended that all 15 of these compounds should be regarded as potentially genotoxic and carcinogenic to humans and therefore, there is the risk of long-term adverse health effects following dietary intake of PAHs (FAO/WHO, 2005).

The objective of this research is to determine the loading of mercury using a cold vapor atomic absorption spectrophotometer (Automatic Mercury analyzer) and PAHs using Agilent 6890 Gas chromatography in Bonga and Catfishes from three different locations in Port Harcourt Metropolis, Nigeria. After analyzing the results concerning their mercury toxicity and PAHs genotoxicity, it was correlated the analysis with maximum accepted limits and as well predicted the actual toxicity risk to human health and more so those that depend majorly on these fishes as their staple protein source and relevant government agencies for the information.

MATERIAL AND METHODS

Chemicals/reagents

All the chemicals used were of analytical grade and were obtained from standard commercial supplies. Acetylene, Nitrogen dioxide gas, Methylene chloride, Potassium chloride, Aluminium nitrate solution, Tetraoxosulphate(VI) acid (H_2SO_4), Trioxonitrate(V) acid (HNO_3), Perchloric acid ($HClO_4$)

Sample collection

A total of three fish samples were collected. One each of fresh and dried Catfish and Bonga fish from Obio/Akpor Local Government Area Port Harcourt. The fish samples were placed in an isolated container inside a clean cooler with ice and immediately taken to the laboratory for analysis. Ice was used to minimize tissue decay and to maintain moist conditions during transportation.

Hg analysis

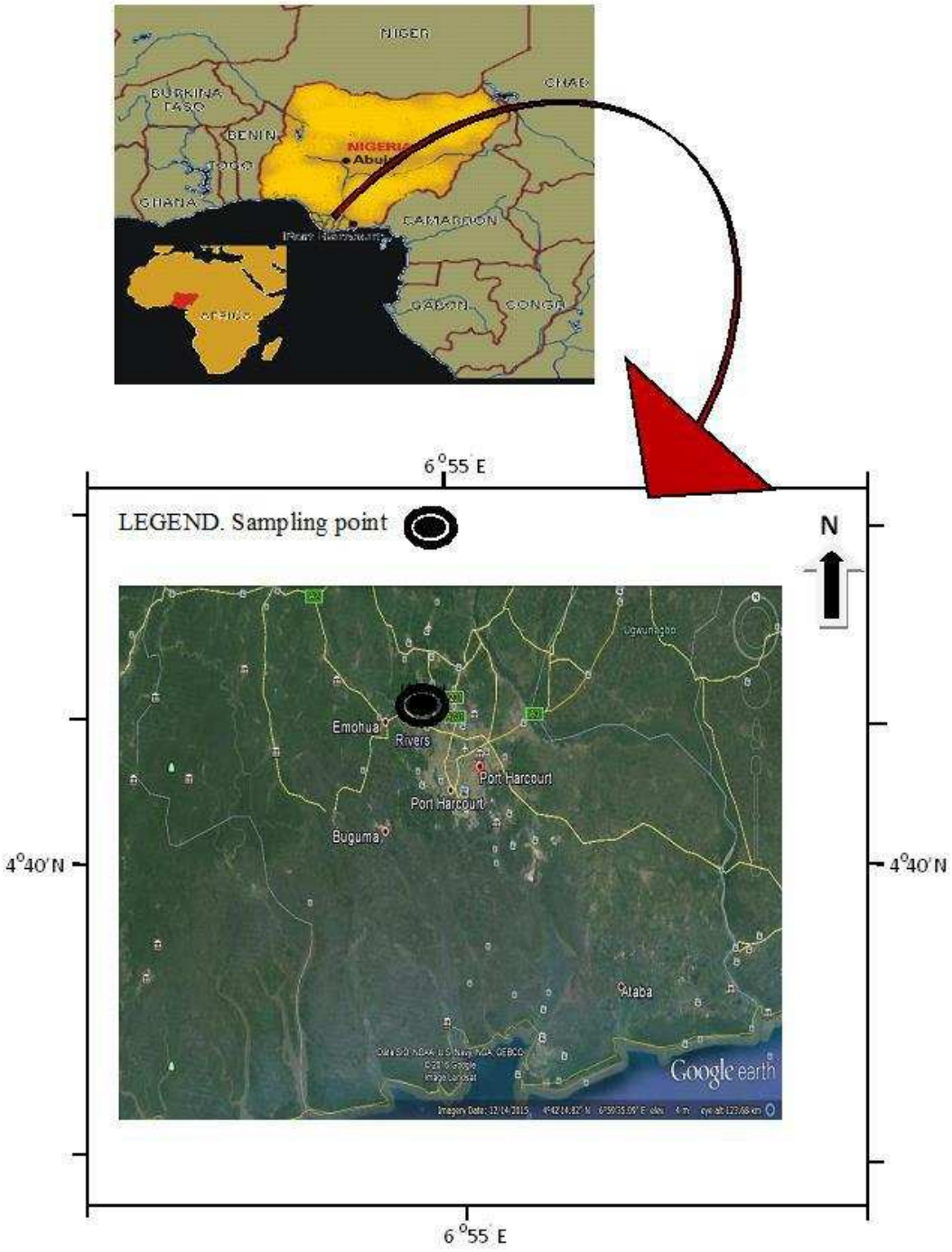

Fig. 1: Map of Port Harcourt Metropolis, Nigeria indicating sampling points (Google Earth, 2023).

AAS, Burner, conical flask, funnel, filter paper, measuring cylinder, weighing balance. All chemicals used were of analytical grade. Deionized water, HNO_3, H_2O_2, Stock metal solution fuel and oxidant, standard metal solution.

Fresh and dried fish samples of catfish and Bonga fishes were collected at three different locations (markets) in the metropolis namely at Rumuokoro, Alakhahia and Borokiri. The fish samples were placed in a cooler filled with ice and were immediately transported to the laboratory for analysis. 1 gram of well-mixed biota was transferred into a conical flask for digestion. 10ml 1:1 of HNO_3 and peroxide was added to the sample in the fume chamber. It was heated to about 95 ^{0}C on the hot plate. Additional 2ml of water and 3ml of hydrogen peroxide covered and heated until effervescence subsides. It was allowed to cool then filtered into a volumetric flask and made up to 100ml. A portion of this solution was used to analyze for mercury.

Table 1: Mercury (Hg) loadings in fresh and dried Catfish and Bonga fish samples across three locations in Port Harcourt (PHC) metropolis

Market Location In PHC metropolis	Average loading of Hg in fresh Catfish (mg/kg)	Average loading of Hg in fresh Bonga (mg/kg)	Average loading of Hg in dried Catfish (mg/kg)	Average loading of Hg in dried Bonga (mg/kg)
Alakahia	<0.001	0.001	0.051	0.061
Rumuokoro	<0.001	<0.001	0.046	0.063
Borokiri	<0.001	<0.001	0.045	0.060
*WHO/FAO	0.5-1	0.5-1	0.5-1	0.5-1

*WHO/FAO (2002)

The digested sample is aspirated into a flame where it becomes atomized and a light beam is directed through the flame into a monochromator and then onto a detector that measures the intensity of light adsorbed. AAS is sensitive in that it depends on the presence of free unexcited atoms, each metallic element has its characteristic absorption wavelength, hence a source lamp of that element screens off other radiation interferences. The Beer Lamberts law is used to calculate the concentration of the mercury present in the fish samples, which is seen in the readout device.

PAHs analysis

Agilent Gas chromatography (Agilent 6890 Gas Chromatograph with a flame ionization detector), Autosampler vials, Vial crimper and decrimper, 10 microliter autosampler syringe. A non-polar capillary column, Laboratory fume hood, Analytical balance

Determination of PAHs in seafood sample

Polycyclic aromatic hydrocarbon (PAHs) concentrations in seafood (shellfish) using gas chromatography and HPLC, following the modified method of determination of PAHs in seafood (shellfish). The principle of gas chromatography involves the separation of components of the sample under test due to the partition between the gaseous mobile phase and stationary liquid phase. The organic compounds are separated due to differences in their partitioning behaviour between the mobile gas phase and stationary phase in the column.

A 50 mL of the sample was measured into a bottle seal via a separatory funnel. Then, 50 mL of methylene chloride was added to the bottle seal containing the sample (fish sample) and it was shaken for 30 seconds to rinse the surface. The mixture was allowed to stand and the organic layer was separated from the water phase for a minimum of 10 minutes. 10 mL of the methylene chloride was delivered into a 250 mL flask. A second 60 mL of the methylene chloride was again added to the sample (seafood) and both the sample and the separatory funnel were rinsed with 20 mL of the solvent into the extract. This procedure was then repeated a second time with both the sample and solvent combined in a flask. The combined extract was then poured into a dried column containing packed cotton wool. The extraction was performed again the third time in the same manner. The combined extract was then poured into a drying column containing sodium sulphate and silica packed with cotton wool which collected the extract into the vial and concentrated it by boiling it down with 1.0 mL nitrogen steam. The remaining extract was then mixed with 1.0 mL of the solvent and 1.0 µL of the mixture was injected into a flame ionization detector gas chromatograph for the analysis of PAHs.

RESULTS AND DISCUSSION

The various concentrations of total mercury in mg/kg in both fresh and dried Bonga and Catfishes are shown in table 1.0 and the results appears to be harmless when correlated with the accepted maximum permissible limit of between 0.5-1.0 mg/kg as set by WHO/FAO (2002). Apart from mercury concentrations of 0.001 mg/kg in fresh Bonga fish at Alakahia market location, the general loading in other fresh fish samples are below the detection limits of the instrumentation i.e. 0.001mg/kg and are above this detection limit in the dried fish samples. This could be due to the oleophilic nature of mercury once ingested by the fish and its bioaccumulation in the form of methylmercury, which is known to be a lethal and toxic form of mercury. Methyl mercury unfortunately is not safe even at parts per billion (ppb) levels and most specifically at above 5.8 ppb loading in fish, it could trigger non-neurological adverse health effects in children and could be unhealthy for fetuses. The dried Bonga and Catfishes have higher loadings of mercury probably because the watery composition of the tissues to which mercury is insoluble has been driving out. Overall, the loading of mercury in the various fish samples appears safe for adult males but the continuous consumption of these fishes by children and women of childbearing age (aggravated by bioaccumulation biomagnification and bioamplification) elevates the risk of non-neurological symptoms. The concentrations in mg/kg of mercury in dried Bonga fish samples across the three market locations (0.061, 0.063, and 0.060) respectively of Alakahia, Rumuokoro and Borokiri (Greek road) in the metropolis are correspondingly higher than that of its loadings in the dried catfishes' samples across the three market locations (0.051, 0.046, and 0.045) respectively of Alakahia, Rumuokoro and Borokiri (Greek road) in the

metropolis. The relatively higher loading of mercury in dried Bonga fish compared to dried catfish has to do with its higher fat content 10-25% compared with catfish which has a relatively lower percentage. Mercury in the form of methyl mercury is fat-soluble or lipophilic or oleophilic and tends to bioconcentrate, bioaccumulate and biomagnify more in Bonga fish. The greater lipophilicity of mercury in the form of methyl mercury in Bonga fish is a game changer here concerning its toxicity potential in the fish samples. Its ability to bioconcentrate, bioaccumulate and biomagnify more in Bonga fish than in Catfish makes long consumption of Bonga fish for women of childbearing age and children more susceptible to methyl mercury toxicity like teratogenic effects and nonurological diseases, especially in children respectively. The results in mg/kg obtained in this research are similar and comparable to those obtained in other species of fish like Sardine (0.013) and Tuna (0.071) conducted by the FDA between 1990 and 2012.

Table 2 shows the PAHs present in the fish samples analyzed and at various concentrations. Some were present above the detection limits of the instrumentation used while others were below its detection limits of 0.01mg/kg. Analysis for PAHs in the fresh and smoked/dried Catfish samples showed the presence of naphthalene, acenaphthylene, fluorene, anthracene, phenanthrene while fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo (b)fluoranthene, benzo (k) fluoranthene, benzo (a)pyrene, Dibenzo (a,h)anthracene, indeno (1,2,3-cd)pyrene, and benzo (ghi)perylene were below detection limits of the instrumentation. Analysis for PAHs in fresh Bonga fish samples showed the presence of naphthalene, acenaphthylene, fluorene, anthracene, phenanthrene, fluoranthene while pyrene, benz (a)anthracene, chrysene, benzo(b)fluoranthene, benzo (k) fluoranthene, benzo(a)pyrene, Dibenzo (a,h)anthracene, indeno (1,2,3-cd)pyrene, and benzo (ghi) perylene were below detecton limits of the instrumentation. Analysis for PAHs levels in smoked dried bonga fish showed the presence of naphthalene, acenaphthyleene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benz(a)anthracene while chrysene, benzo (b)fluoranthene, benzo (k) fluoranthene, benzo (a)pyrene, Dibenzo (a,h)anthracene, indeno (1,2,3-cd)pyrene, and benzo (ghi)perylene were below detection limits of the instrumentation. In all PAH analyses in the samples, 2-methyl naphthalene was below the detection limits of the instrumentation.

The genotoxic PAHs present in all samples of fishes include chrysene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a)pyrene, dibenzo (a,h)anthracene, indeno (1,2,3-cd)pyrene, and benzo (ghi)perylene and fortunately, all were below detection limits of the instrumentation i.e. <0.01mg/kg. The implication that both fresh and smoked/dried Bonga and Catfish have a low risk of damaging or destroying DNA suggests that the levels of PAHs present in these fishes are below thresholds considered harmful to human health. This information would indeed be valuable to the population in the respective area, especially for those who rely on these fishes as a significant part of their diet. However, it is important to note that the safety of consuming fish depends

Table 2: PAHs load detected in fresh and dried Catfish and Bonga fish samples across three (Alakahia, Rumuokoro and Borokiri) locations in Port Harcourt (PHC) metropolis

Names of PAHs	Average loading of PAHs in fresh Catfish (mg/kg)	Average loading of PAHs in fresh Bonga(mg/kg)	Average loading of PAHs in dried Catfish(mg/kg)	Average loading of PAHs in dried Bonga(mg/kg)
Naphthalene	0.01	0.01	0.01	0.01
2-Methyl Naphthalene	<0.01	<0.01	<0.01	<0.01
Acenaphthalene	0.01	0.01	0.01	0.01
Acenaphthene	0.01	0.01	0.01	0.01
Fluorene	0.01	0.01	0.01	0.01
Phananthrene	0.01	0.01	0.01	0.01
Anthracene	0.01	0.01	0.01	0.01
Fluoranthene	<0.01	0.01	<0.01	0.01
Pyrene	<0.01	<0.01	<0.01	0.01
Benzo (a) Anthracene	<0.01	0.01	<0.01	0.01
Chrysene	<0.01	<0.01	<0.01	<0.01
Benzo (b) Fluoranthene	<0.01	<0.01	<0.01	0.01
Benzo(k)Fluoranthene	<0.01	<0.01	<0.01	<0.01
Benzo (a) pyrene	<0.01	<0.01	<0.01	<0.01
Benzo (g,h,i) Perylene	<0.01	<0.01	<0.01	<0.01
Dibenzo (a,h) Anthracene	<0.01	<0.01	<0.01	<0.01
Indeno (1,2,3-cd) Pyrene	<0.01	<0.01	<0.01	<0.01
Total PAHs	0.06	0.08	0.06	0.10

on various factors, including the methods of catching, processing, and cooking, as well as the overall dietary habits of individuals. Regular monitoring and regulation of PAH levels in fish products are essential to ensure continued consumer safety. Additionally, it is always a good idea for consumers to vary their diet and not rely heavily on any single type of food to mitigate potential risks associated with any specific contaminants.

CONCLUSIONS

The ability of mercury in the form of lipophilic methyl mercury that is lethal even at low concentrations on ingestion to bioconcentrate, bioaccumulate and biomagnify makes regular and continuous consumption of either the fresh or smoked/dried Catfish and Bonga fishes as protein staples, sources of concern concerning toxicity associated with methyl mercury, i.e., teratogenic effect in women of childbearing age and non-neurological diseases in children. There is little or no risk associated with regular consumption of these fishes concerning genotoxicity associated with PAHs.

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