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Health Risk of Heavy Metal and Genotoxicity of Polycyclic Aromatic Hydrocarbons (PAHs) in Extra-Virgin Olive Oils (EVOO) available in PortHarcourt Metropolis, Nigeria

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Abstract

This research aims at determining the loading of contamination of some popular brands of extra-virgin olive oil (EVOO) in Port Harcourt, Nigeria, with genotoxic PAHs and Heavy Metals to ascertain its safety for consumption. The health risk associated with these heavy metals will be calculated and assessed. Extra virgin olive oil has experienced a surge in its consumption all around the world owing to its high nutritional content. Contamination with genotoxic PAHs and Heavy Metals can cause severe health defects if consumed regularly. PAHs and heavy metals are two distinct groups of ubiquitous contaminants with grave health consequences such as Tumorigenesis, carcinogenesis, organ failures etc. Samples of EVOO were analysed with Gas Chromatography/Mass Spectrophotometer and Flame Atomic Absorption Spectrophotometer (FAAS) for PAHs and heavy metals respectively. After extraction with Dichloromethane and digestion with aqua regia (HCl/HNO_3; 3:1) respectively, the results showed the presence of few genotoxic PAHs such as benzo[a]pyrene, PAH4 and PAH8 with concentrations above regulatory limits of 0.002µg/g, 0.01µg/g and 0.01µg/g respectively. Among the heavy metals, Pb and Cd showed the most alarming levels of contamination of the heavy metals, above regulatory limits of 0.1µg/g and 0.05µg/g, however, analysis with Health Risk Index of consumption showed all samples were safe for consumption.

Keywords: Health Risk Index, Heavy Metal, PAHs, PAH4, PAH8

Introduction

Oils are important part of human diet. Delivering important micro nutrients such as vitamins E and K for proper growth and functioning of living tissues. Olive oil is the oil extracted from the fruit of the *Olea european sativa* tree. Extra-virgin olive oil is extracted mainly mechanically. Sometimes with little heat treatment that does not alter the desired properties of the oil. Treatments can include washing, filtration, decantation, centrifugation, without any further refining either with chemicals or other processes such as re-esterification (United States Standard for grades of Olive oil, 2010). Standard extra-virgin olive oils usually have an acid value of less than 0.8 with a fruity taste. It is believed to have lots of nutritional benefits such as anti-inflammatory properties, rich source of omega-3 fatty acid, improve bone health, protection against heart diseases etc. It has also been alleged to protect against certain cancers such as breast cancer (kiritsakis and Markakis, 1988; Covas, 2007) [28, 8]. This has led to a surge in its consumption all around the world, from the Mediterranean where it is said to have originated. As with other fatty foods EVOO can easily be contaminated with PAHs as PAHs are very soluble in fats and are ubiquitous environmental contaminants easily finding their way into all types of foods. EVOO can also be regularly contaminated significantly with heavy metals found in the environment from a plethora of sources such as metal smelting sites, rock weathering, burning of petroleum, metal wastes found in the environment etc, if correct hygiene and proper standards are not maintained during planting, harvesting and production. Raising concerns of severe health risks instead of benefits.

Polycyclic aromatic hydrocarbons are a group of hydrocarbon compounds with at least two aromatic rings fused together to form a compound without hetero atoms (ATSDR, 1990) [2]. Formed from incomplete combustion or pyrolysis of organic substances such as petrol, wood, firewood, bushes etc, but could also be found naturally in crude oil and coal. Mainly generated from anthropogenic activities, but could also be generated from natural processes such as volcanic eruptions, wildfires etc (Lee *et al*, 1981) [29] Studies have shown that the most common and prevalent source of PAHs contamination in EVOO is due to environmental atmospheric exposure of the olive fruit. (Rodriguez-acuna *et al*, 2008) [41].

PAHs can be classified based on the number of fused rings: PAHs with fewer than four aromatic rings are classified as low molecular weight PAHs such as naphthene, phenanthrene, fluorene etc, they are to lesser extent associated with disease causation. High molecular weight PAHs are PAHs with at least four fused rings. They are more persistent, have lower vapour pressure, more water insoluble and are important disease causatives (Enviro wiki 2022 [13]; Cerninglia, 1984). Examples include benzo[a]pyrene, chrysene, benzo[a]anthracene, benzo[k]fluoranthene, dibenz[a,h]anthracene etc. It is difficult to ascribe observed health effects in epidemiological studies to specific PAHs because most exposures are due to a mixture of PAHs, however, certain PAHs have been classified as genotoxic (ATSDR, 2009 [3]; Hussain *et al*, 2019). PAHs frequently classifed as genotoxic include benz[a]anthracene, Chrysene, Benzo[e]pyrene, Benzo[b]fluoranthene, Benzo[a]pyrene, Benzo[k]fluoranthene, dibenz[a,h] anthracene, Benzo[ghi]pyrelene, Indeno[1,2,3-cd]pyrene etc (Hussain *et al*, 2018) [16]. Benzo[a]pyrene is the most studied PAH and can be used as a marker for PAHs toxicity (Mafra *et al*, 2010) [31].

Human exposure to PAHs can be through respiration (breathing in), epidemiological contact, and ingested food and water. With the most significant medium of exposure said to be through ingested food for non-smokers (Valavanidis *et al*, 2006 [45]; Rengarajan *et al*, 2005). The presence of PAHs in food has been studied extensive in other to mitigate its negative consequences and provide more information on best practices to eliminate or curb drastically PAHs contamination of foods. Various regulatory parameters such as Margin of Exposure (MoF), Margin of Safety (MoS, where Non Observed Adverse Effect Level, NOAEL, is compared to the human exposure to the xenobiotics) and BenchMark Dose (BMD) have been introduced to help checkmate and provide information on contamination and disease causation of PAHs (Bertoz *et al*, 2021) [5].

PAHs themselves are hydrophobic and chemically inert, however, as with most carcinogens chemical activation is required. This occurs when living systems try to excrete them by metabolizing them into lipophilic (water-soluble) materials such as diols, epoxides and dihydrodiols etc. The initial stage of benzo[a]pyrene metabolism is the formation of several epoxides by microsomal CYP-dependent mono-oxygenase, which can then undergo spontaneous rearrangement to phenols or be hydrolysed by epoxide hydrolase to dihydrodiols or covalent reaction with gluthathione chemically or catalysed by gluthathione-S-transferase. Further oxidation leads to triols, tetraols, and hydroquinones (Kim *et al*, 1998) [26] These metabolised compounds may react with DNA to yield DNA adducts. If not repaired or not properly prepared the adducts may initiate gene mutation and lead to adverse health effects (IARC, 1987) [18]. There are sufficient evidence showing benzo[a] pyrene interactions with mammalian gonads or gem cell DNA to induce such endpoints as unscheduled DNA synthesis, chromosomal aberrations and morphological abnormalities. Benzo[a]pyrene is classified as Group 2A carcinogen (IARC, 1987; Wyrobeck *et al*, 1981) [18, 49]. Alongside its genotoxicity other cases of long term exposure to PAHs include decreased immune function, respiratory diseases, kidney and liver damage etc (Khairy *et al*, 2009) [24].

Heavy metals on their part are a group of high density, persistent elements that are capable of causing diseases in living systems even at very low concentrations. The name heavy metals stems from their high density (at least five times denser than water), their ability to cause diseases in considerably lower concentrations and persistency in the environment (Fergusson 1990; Duffus 2002) [14, 9]. They readily bioaccumulate and biomagnify in living systems leading to causation of all kinds of diseases.

Heavy metals usually have variable oxidation states this enable them to mimic various important trace elements in living systems. They interact with biological systems in two ways: Direct interaction which involves binding directly with important macromolecules. For example Pb^{2+} can mimic Zn^{2+} in aminolevulinic acid dehydratase and ferrochelatase and bind to it's active site thereby causing conformational changes in the molecule and as a result alter its biological function (Cangelosi *et al*, 2017) [6], the other pathway is by generating reactive species such as Reactive Oxygen Species (ROS) ad Reactive Nitrogen Species (RNS). These highly reactive species then interact with important biological macromolecules such as the DNA to form DNA adducts, alteration of sulfydryl homeostasis, lipid peroxidation, oxidative stress etc. Interaction can be abstraction or addition reactions by these free radicals leading to Carbon-centered sugar radicals and OH^- or H^- adduct radicals of heterocyclic base (Renner *et al*, 2000; Ravana *et al*, 2000) [40, 38]. The formation of DNA adducts can lead to genotoxicity if not repaired or misrepaired by the DNA. The usual steps in heavy metal toxicity are; Liberation of cation> Reaction with target molecules> cellular dysfunction> Reaction (repair) or (disrepair)> Toxicity development.

Heavy metals frequently encountered as contaminants in extra-virgin olive oils include, Iron (Fe), Copper (Cu), Zinc (Zn), Nickel (Ni), Cadmium (Cd), Lead (Pb) etc. All heavy metals mentioned above have various health effects on living sytems depending on dose. Some of the heavy metals mentioned above can also induce rancidity in the oil due to oxidations reactions such as Fe and Cu. They are readily available in the environment from anthropogenic activities such as mining, metal smelting factories, waste disposal and even burning of hydrocarbon fuels etc. They could also come from natural sources such as volcanic eruptions and weathering of rocks (Wilson and Pyatt, 2007; Ihedioha *et al*, 2017) [46, 17].

Iron (Fe) is the second most abundant metal in the earth crust (Lee, 2009) this tells why it is notoriously present in many food substances to the point of major contamination. It has a density of 7.8g/cm³ and variable oxidation states, with +ll and +lll being the most stable (Lee, 2009). It is obviously the most used metal in the construction industry. Its biological function includes being present in hemoglobin; a component of blood for the transport of oxygen, it supports muscle metaboism and healthy functioning of connective tissues. A healthy human is said to be made up of 3-4g of Fe (Johnson-Wimbley and Graham, 2011) [22]. Despite it's biological importance, higher than normal concentration in living systems can have severe health implications. Mild poison symptoms include vomiting, diarrhea, abdominal pains and drowsiness. In more severe cases symptoms include tachypnea, low blood pressure, seizures and coma, multi organ failure and death (Manoguerra *et al*, 2005) [32].

Lead is a silvery shiny/bluish-gray, dense metal with +ll ad +lV oxidation states with the +lV state being less stable. Pb is used in many engineering and chemical industries such as sulfuric acid plant, Pb batteries, pigments, solder for the electronic industry, previously used as antiknock in petrol etc. (Lee, 2009). All soluble forms of Pb are poisonous, with toxicity increasing with solubility (WHO, 2019) [48]. Pb is a physiological and neurological toxin in humans. Acute Pb poisoning may result in dysfunction in the kidney, reproductive system, nervous system, brain and subsequently death (Odum, 2006; Kazemipour, 2008 [23]). A notably serious effect of Pb poisoning is teratogenicity. Other severe health defects of Pb include anemia, fatigue, gastrointestinal problems, delayed pregnancy, anoxia amongst other health defects. Pb poisoning is mostly severe in children (Needleman, 1988) [35].

Cadmium is a bluish-white rare metal with +ll oxidation state. It was used basically in electroplating steel but recently it has been widely employed in the manufacture of rechargeable batteries. It also finds use as pigments and in electronics (WHO, 2000) [47]. Cd is relatively rare, only about 0.2mg/kg of Cd is found in the earth crust. Cd is regarded as a Group 1 human carcinogen (WHO, 2000) [47].The target organs for Cd toxicity includes liver, placenta, kidney, brain, lungs and bones (Sobha *et al*, 2007) [43]. Exposure to Cd can lead to nausea, vomiting, abdominal cramps, dyspnea, muscular weakness amongs other symptoms depending on the severity of exposure. Severe exposure can cause oedema and death. Subchronic inhalation can lead to renal effects, pulmonary effects-emphysema, bronchiolitis, aveolitis. The itai-itai disease break out in Japan exposed the dangers of Cd to the world. It has also been associated with anosmia, cardiac arrest, cerebrovascular infractions, emphysema, osteoporosis, proteinuria cataract formation in the eye (Deruibe *et al*, 2007) [11].

Nickel is a transition metal with variable oxidation states of (-I), (0), (+ll), (+lll) and (+lV), with (+ll) being its most stable oxidation state. It has a density of 8.9g/cm³. It has application in different industries such as metallurgy, electrical engineering, medicine and even in biological systems such as its role in lipid metabolism and proper functioning of hormones (Lee, 2009). When consumed Ni is widely distributed in an organism; distributed across the blood brain barrier even across placenta membrane in gestating mothers. A higher than normal dose of Ni in the body can have severe health effects such as genotoxicity, hematotoxicity, immunotoxicity etc. (ESFA, 2020) [12]. It also shows strong effects on reproductive systems, and developmental toxicity (EFSA, 2020) [12].

Zinc is a bluish grey to white transition metal with an unusually only (+ll) oxidation state. It finds use in electroplating of iron; galvanised iron, production of batteries, etc. Zn is associated with multiple biological functions, such as being required for the activity of various catalytic enzymes, wound healing, enhancing immune function, protein and DNA synthesis, cell signaling etc., (Lee 2009; McDonald, 2000 [30]; King *et al*, 2014 [27]). It also supports healthy growth and development of a child during pregnancy, childhood and adolescence. Women and men are said to contain about 1.5g and 2.5g of Zn respectively. Zinc is relatively non toxic, lower intakes but higher than recommended dose might induce copper deficiency with symptoms- anemia, neutropenia, impaired immune system function and adverse effects on the ratio of low to high density lipoprotein. Very higher than normal amount of zinc in the body can present symptoms such as nausea, vomiting, epigastric pain, lethargy and fatigue (McDonalds, 2000; King *et al*, 2014) [30, 27].

This research aims at determining the loading of some popular brands of extra-virgin olive oil (EVOO) available in Port Harcourt, Nigeria, with genotoxic PAHs and Heavy Metals to ascertain its toxicity and safety for consumption. The health risk associated with these heavy metals will be calculated and assessed. Contamination with genotoxic PAHs and Heavy Metals can cause severe health defects if consumed. PAHs and heavy metals are two distinct groups of ubiquitous contaminants with grave health consequences such as Tumorigenesis, carcinogenesis, organ failures etc. Samples will be with Gas Chromatography/Mass Spectrophotometer and Flame Atomic Absorption Spectrophotometer (FAAS) for PAHs and heavy metals respectively. Analysis with PAHs indicators like PAH4, PAH8 and BaP as well as Health Risk Index of heavy metals will be done to ascertain safety. The results will be informative enough to make recommendations to the respective and relevant agencies.

Materials And Methods

Sampling:

Samples were bought from reputable retail stores in Rumuokoro, Port Harcourt (4.8703°N, 6.9880°E). Choba, Port Harcourt (4.89°N, 6.9075°E). All samples are pure extra-virgin olive oil without addictives imported from the Mediterranean, NAFDAC and SON certified. All samples were left in their original package and taken to the laboratory.

Polycyclic Aromatic Hydrocarbons analysis

Apparatus

30m × 0.25mm ID fused-silica capillary column, chemically bonded with SE-54(DB-5), 1-µm film thickness. Amber glass bottles to avoid degradation from light sensitive analytes, soxhlet extractor for extraction of PAHs from sample. Mechanical shaker-for improved initial miscibility fume extractor and other basic laboratory equipment.

Reagents

1,1-dimethylchloride, Anhydrous sodium sulphate, methanol for rinsing cartridge, Acetone, Reagent water, HCl 0.05M, Helium-carrier gas for chromatography. All reagents were bought as high purity pesticide quality or equivalent.

Extraction of PAHs:

10g of each of the samples were added into a soxhlet extractor, Na_2SO_4 was added and stirred-to remove residual water. 30ml of DCM was added as extracting solvent. Bottle was corked tightly and a mechanical shaker was employed to agitate the mixture for 20 mins after which samples were allowed to settle for 1hr under gravity and then decanted carefully. The filtrate was allowed to concentrate overnight by evaporation in a fume cupboard, before introduction to

GC/MS machine for analysis.

Heavy Metals analysis

Apparatus

Microwave for heating sample to aid digestion. Flame Atomic Absorption Spectrophotometer and other basic laboratory equipment.

Reagents

Aqua regia- concentrated Nitric acid and concentrated hydrochloric acid for digesting metals in sample. Distilled water.

Extraction of Heavy Metals:

20ml of aqua regia-HCl/HNO_3, 3:1 was added to 1g of each of the oil samples in a conical flask. The mixture is digested in a fume cupboard with hot plate, until white fume appears. The solution is then cooled in a 100ml volumetric flask and topped up to mark with distilled water. A portion of the solution is then taken to be analysed with flame atomic absorption spectrometer, to test for the required metals- Fe, Pb, Cd, Ni and Zn.

Results

Table 1: Loadings of various indicator PAHs of different samples

Samples	BaP (µg/g)	PAH4 (µg/g)	PAH8 (µg/g)
Sample A	0.40	0.51	0.8
Sample B	0.35	0.35	0.58
Mean Conc.	0.3725	0.43	0.69
Regulatory limit	0.002	0.01	0.01

Five genotoxic PAHs of the EPA 16 priority PAHs that was tested for was found in both samples from the GC/MS analysis, with 0.80ug/g and 0.58 µg/g for sample A ad B respectively. Although less than half of the PAHs tested for were detected the amount was above the regulatory limits in both samples.

BaP often used as a marker for PAHs contamination in foods was the most prevalent, being found in both samples as opposed to all other PAH8 that were either found in one sample or none of the samples. BaP had concentrations that were very higher than the regulatory limits- 0.002µg/g by JECFA, WHO, IOC and other regulatory agencies in all samples. The values recorded were not far from those of similar researches, values as high as 0.92 µg/g was reported in the range of maximum values by Alorimah *et al* (2010) [4]. While others such as Texira *et al* (2006) reported values of 0.085µg/g, Moret and Conte (2007) 0.009µg/g much more lower values.

PAH4 also showed high concentration, although only two were present. Sample A had a higher amount of PAH4 of 0.51ug/g than sample B with 0.35µg/g although both samples showed concentrations that were far above the regulatory limit of 0.01µg/g by JECFA.

The heavy contamination of samples with genotoxic PAHs can be traced to three major sources uptake from atmospheric exposure; PAHs are ubiquitous and are readily found in the atmosphere generated from human and natural processes such as burning of coal and hydrocarbons for energy, forest fires, volcanic eruption etc. Uptake from soil and endogenous biosynthesis and uptake from surfaces during extraction such as where it is stored, or machine used in processing (Phillips, 1999) [37]. PAHs have been reported in all types of food, Extra-virgin olive oils have limited means of exposure to PAHs during production, however contamination usually remain in the oil whenever they find their way in as further purifications are usually not carried out to enable it remain a "virgin oil". Around 70 different PAHs and related compounds have been identified in food stuffs of which Benzo[a]pyrene and benz[a]anthracene are the most abundant (Smith *et al*, 2001) [42].

Table 2: Loadings of various Heavy Metals of different samples

samples	Fe (µg/g)	Pb (µg/g)	Cd (µg/g)	Ni (µg/g)	Zn (µg/g)
Sample A	4.281	0.136	0.363	0.198	0.507
Sample B	2.031	1.102	0.121	0.095	0.336
Mean Conc.	3.156	0.619	0.242	0.146	0.422
Regulatory limit in oils (WHO, FAO, IOC).	3.00	0.1	0.05	0.2	0.05

All five heavy metals showed heavy presence in all samples, with Fe having the highest concentration and also showing a mean concentration that was above the maximum allowable regulatory limit. Concentration was in the range of 2.031µg/g-4.281µg/g. Others such as Kabaran *et al* (2006) reported concentrations of the range of 0.079µg/g - 4.00 µg/g. Possible sources of Fe contamination in the oils analysed could be from fertilizers, pesticides, or even metal surfaces during extraction (Kabaran *et al*, (2006). Fe is also found in ambient air; 1.6 µg/m³ and abundant in the earth crust.

Pb had a mean concentration of 0.619µg/g that was above the regulatory limit by WHO/FAO. Sample A had a concentration that was a little above the regulatory limit while sample B had an outrageously high and alarming concentration of 1.102µg/g, such that can cause significant poisoning. Other researchers reported 0.0277 µg/g to 0.1263µg/g, Karaban *et al* (2020). Pb is a major environmental pollutant; water, land and air pollutant. Sources of Pb pollution include Pb smelters, industrial waste water discharge, run offs from dumpsites, pesticides etc.

Cd also showed heavy metal load in both oils exceeding the regulatory maximum limit by regulatory authorities. Mean concentration 0.242µg/g and the concentration in both samples exceeded the regulatory limits stated in the table above. Gubec (2013) [15] reported concentrations of 0.000088 - 0.00036µg/g in some Italian EVOO, which is way lower and below regulatory limits of relevant authorities. Dugo (2010) [10] reported values of 0.0021µg/g in oil samples near a mining site which are all much lower than seen in this samples. Contamination can come from elemental Cd present in ambient air (WHO), plants can also take up Cd directly from the soil which can lead to contamination. Other sources can include factory discharges, dump site leachates etc.

Nickel which is found more abundantly in the earth crust than Pb and Cd unexpectedly showed a lower mean concentration of 0.146µg/g, 0.198µg/g and 0.095µg/g for sample A and B respectively. Mean concentration and individual samples concentration was below regulatory limit. Nickel is said to constitute 0.008% of the earth crust, enriched in coal and crude oil up to 300mg/kg and can be found in ambient air at about 124ng/m³ (WHO, 2000) [47].

Zn had a considerably lower level of contamination compared to Fe and even Pb, with a mean concentration of 0.442ug/g. Zn is also to a lesser extent associated with causation of diseases and may require a very high amount to induce its negative effects. Therefore, the samples can be

said to be free from any serious Zn contamination.
Health Risk Index (HRI) of Heavy metals in Samples.
HRI=DIM/RfD
DIM=(X_{oil}xquantity)/bodyweight.
DIM:daily intake of metal.
X_{oil}:concentrationofmetalinoil(µg/g)
RfD: Reference daily intake or. safe maximum provided by (FAO/WHO,US-EPA).
bw:bodyweight.. HRI > 1: unsafe for consumption. HRI < 1: safe for consumption.

Table 3: Health Risk Index (HRI) of heavy metals

Heavy Metal	Reference Daily Intake (RfD)(mg/kg.bodyweightperday)	Health Risk Index (HRI)
Ni	0.02	2.8 x10^{-3}
Pb	0.025	9.30 x 10^{-3}
Cd	0.001	2.50 x 10^{-2}
Fe	0.8	1.52 x 10^{-3}
Zn	0.3	5.4 x10^{-4}

Analysis with HRI show that all samples are safe for consumption with regards to the heavy metals tested for.

Conclusion

After extraction with Dichloromethane and digestion with aqua regia (HCl/HNO_3; 3:1) respectively, the results showed the presence of few genotoxic PAHs such as benzo[a]pyrene, PAH4 and PAH8 with concentrations above their respective regulatory limits. Lead (Pb) showed the most alarming level of contamination of the heavy metals above regulatory limits, however, analysis with Health Risk Index of consumption showed all samples were safe for consumption and no significant threat of heavy metal toxicity.

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