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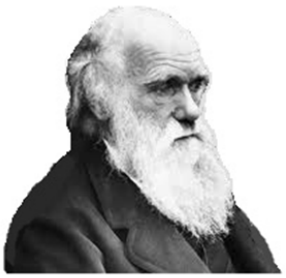

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Polycyclic aromatic hydrocarbon in African Giant Snail (*Archachatina marginata* (Swainson, 1821))

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ABSTRACT

The concentrations of polycyclic aromatic hydrocarbons (PAHs) were examined in African Giant Snail samples collected from five snail farms in Etche Local Government Area, Port Harcourt, Rivers State, Nigeria. With the view to providing information on the health risks to humans from the consumption of these foods. The concentrations of PAHs were measured by means of Gas Chromatography Mass Spectrophotometer (GC-MS). The results obtained from the analysis showed that the concentrations of 16 PAHs in the snails ranged from 0.04 to 4.148 mg/kg. The dominant PAHs compounds in the snail species were 3-, 5- and 7- rings PAHs which are phenanthrene, benzo(b)fluoranthrene and indeno(1,2,3-cd)pyrene which suggests worrisome pollution levels of the soil and environment of Port Harcourt Nigeria. The findings of this study thus point to the need for improved caution in the consumption of snails harvested from the study area. It is expected that this will help to improve public health awareness and practice.

Keywords: African Giant Snail, gas chromatography, mass spectroscopy, phenanthrene, polyaromatic hydrocarbons

1. INTRODUCTION

Terrestrial habitats have largely been adversely affected by humans, industrial and natural activities. These adverse effects on the inhabitant of the terrestrial habitats also translate to effect on the human health because of their significance in our food chain. These effects, being toxic, are mostly because of pollution to the terrestrial region. The basic concern of pollution

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here is the distribution of certain specific compounds called polycyclic aromatic hydrocarbons (PAHs) because of their chemical nature and hence their mode of absorption into the bodies of these terrestrial organisms (Agarwal, 2009). A large portion of the Polycyclic aromatic hydrocarbons (PAHs) enter the atmosphere through natural processes that cannot be controlled by humans, such as bush fires or volcano eruptions (Agbozo et al., 2008). In addition, this group of substances is a natural component of fossil raw materials, specifically coal and petroleum (Kanaly and Harayama, 2000).

Many PAHs are carcinogenic, mutagenic, and toxic for reproduction (Cernigilia, 1992). Some PAHs are at the same time persistent, bio-accumulative, and toxic for humans and other organisms (Ayadinuno et al., 2021). Their persistence implies that they remain in the environment for a long time and can hardly decompose (Igbini, 2020; Ugbena, 2020). Bio-accumulative chemicals accumulate in organisms including the human body (Iwegbue et al., 2015). This increasing concern is aggravated due to their potential toxic effects and ability to bio-accumulate in terrestrial ecosystems. PAHs also known as polynuclear aromatic hydrocarbons (PNAs) and polycyclic organic matter (POM) are compounds composed of hydrogen and carbon arranged in the form of two or more fused benzene rings in linear, angular or cluster substituted groups attached to one or more rings. They are generally produced because of incomplete combustion of organic matter such as wood, coal or oil whose input into the environment has increased intensively in the 20th century (Hobbs and Hale, 2008).

Among PAHs compounds, some have potential for being carcinogenic, mutagen and disturbing human endocrine system (Power et al., 2021; Sharma et al., 2020). Therefore, they are categorized as environmentally high priority contaminants. They are a large group of organic compounds that are included in the European Union and US Environmental Protection Agency (USEPA) priority pollutant list. They are lipophilic compound, that is, they mix easily with oil than with water and consist of 2 to 7 benzene rings; the 2-4 rings are classified as lower molecular weight PAHs are more soluble in water and are acutely tonic to human and living organisms whereas higher molecular weight PAHs are highly soluble in lipid and more carcinogenic, mutagenic with more time effect (Power et al., 2021). The hydrophobic and lipophilic properties of some higher molecular weight PAHs make them relatively insoluble in water and tends to accumulate on surface or in non-polar materials (Iwegbue et al., 2015).

As lipophilic compound, they can easily cross lipids membranes and have the potential to bioaccumulate in aquatic and terrestrial organisms (Grigoriou et al., 2022). Although for most people, snails and seafood represents only a small part of the total diet, the contribution of this food group to the daily intake of PAHs in some individuals may be comparatively important since studies have evaluated the genotoxic and carcinogenic risks associated with the consumption of oil containing food substances that have absorbed PAHs. PAHs are released into the environment via natural and anthropogenic sources. Natural sources include oil seeps, volcanoe, grass fires, chlorophyllous and non-chlorophyllous plants (Abass and Brack, 2006).

Athropogenic sources includes discharge from routine oil transportation, oil spills, power plants based on fossil fuel consumption, biomass burning, pyrolysis of wood and internal combustion in industrial and vehicle engines. They are present in terrestrial, aquatic and atmospheric environments and have been detected in surface waters, ground water, soil, plants and animals (Edwards, 1983; Teaf, 2008). PAHs are very widespread, partly because they are released from sources that are used every day and, in every community, and partly because they can attach to particles in the air (Effiong *et al*., 2016). Giant African Snail is one of the world's largest land snails. It is a non-host specific and can consume at least 500 different types of

plants, including bread fruit, cassava, cocoa, papaya, peanut and most varieties of beans, peas, cucumber, and melon. The snail is native to coastal East Africa (Kenya and Tanzania) but is not widespread on all countries except Antarctica. It is highly adaptive to a wide range of environmental conditions but are highly affected by polycyclic aromatic hydrocarbon. In west African the most predominant giant land snail species include *Achatina achatina, Achatina fulica and Archachatina marginata*. The African giant land snail (*Archachatina marginata*) is the most accepted and highly cherished snail species in Nigeria. It is used as a protein source (as delicacy) as well as vital ingredient in traditional drugs for many ailments.

The giant African snails are distributed all over the humid tropical zones of African and species that are common in tropical African include *Achatina achatina*, *Achatina fulica*, and *Archachatina marginata* (Iwegbue et al., 2022). The success of African giant snails in terrestrial habitat has been attributed to various structural physiological and behavioural specializations (Riddle 1983). *Archachatina marginata* is the biggest snail specie found in the terrestrial region of tropical West African Countries. It has the highest body and shell parameters when compared to other snail species (Idowu et al., 2008). Giant African land snail is the common name used to describe any of the three snail species native of Africa. *Achatina achatina, Achatina fulica* and *Archachantina marginata*. They are the large terrestrial snails that can reach up to 20 cm (8 inches) in length and 10 cm (4 inches) in maximum diameter. These snails are about the size of an average sized adult fist. The brownish shell with darker brown vertical stripes covers at least half of the length of the snail.

The African giant snails are primarily nocturnal and are generally found under protective cover (for moisture maintenance) only where population are very high, will individuals clion trees and walls (Pradad et al., 2004). The snail lays around 6 clutches of eggs every year, hence laying an average of 200 egg per clutch and hatchling reach their adult size by the time they are 6 months old, and although their growth rate slow at this point, the African giant snails never stop growing. Most African giant snail reach between 5-6 years of age, but some individuals have been known to be more than 10 years.

During periods of extreme drought, the African giant snail goes into aestivation (summer sleep) which it seals itself inside its shell to retain water and it does this three times a year depending on the areas in which they inhabit and hibernate during the winter seasons in higher latitudes 9and altitudes), and came out of aestivation and hibernation as the environment becomes welter and warmer. The hatchlings and adults tend to display homing behavior while the intermediate size snails (immature) tend to be more active and disperse more easily, the hatchlings are primarily detritivorous, but can also feed on some preferred plants. The immediate size snails are phytophagous and are serious pests. While the adult African giant snails are detritivorous, carpophagus and necropagus.

Archachatina marginata is a popular food item in West Africa, a recent study indicates that its meat has more protein and iron and is better flavoured than beef. *Archachatina marginata* have also been recommended for the treatment of anemia asthma, high blood pressure and other related ailments due to their relatively low cholesterol levels. Also, it has been described as palatable, nutritious, and rich in essential amino acids such as lycine, leucine, isoleucine and phenylalamine as well as high iron content.

In recent years, much research has focused on metal concentration in protein sources: beef, goat meat, poultry products as well as fish and other sea foods. In Nigeria, African giant snail is widely consumed by various ethnic groups. Since snail farming is popular in Nigeria the snails are usually collected in forests and transported to nearby urban markets.

Once the PAHs is emitted from its various sources, it is widely dispersed in the air, water and could be retained in the terrestrial soil matrix for a long time after absorption into the soil organic matter. Soils and sediment are often the ultimate repository for polycyclic aromatic hydrocarbon whereas the African giant snails feed on the debris from the terrestrial soil surface thereby contracting the polycyclic aromatic hydrocarbon.

This study therefore aims to determine the concentrations of PAHs in snail samples (African Giant Snails) in Port Harcourt, Nigeria with a view to providing information on the health risks associated with their consumption. A traditional analytical approach (gas chromatography-mass spectroscopy) was deployed to determine the levels of the PAHs in the snail samples obtained from 5 snail farms in the study area. It is expected that the outcome of this study will shine more light into the safety of consuming snails grown in the study area.

2. MATERIALS AND METHOD

All chemicals and reagents used were of analytical grade. Methylene chloride (AR-grade), acetone (AR-grade), silica gel, and anhydrous sodium sulphate were used. All apparatus used were sterilized which include beaker, pipette, round bottom flask using acetone. Gas chromatography mass spectrophotometer (GC-MS) equipment was used in the analysis while the extraction method employed in this analysis is sonication method according to the method employed by Iwegbue et al. (2012).

The snail samples for this research were collected from different sources in Etche Local Government Area Port-Harcourt, Rivers State, Nigeria. 5 samples in all were collected. The samples brought from these locations were identified at the Department of Zoology and Environmental Biology, University of Calabar, Nigeria as *Archachatina marginata* before it was taken to the laboratory for analysis.

A mass of 10 kg of sub sample was extracted using sonication method with 50 ml of acetone and dichloromethane at 70 °C for 10-15 min. After sonication, 10 g of anhydrous sodium sulphate (Na_2SO_4) was added to the sample to develop a clear extract. The extracted solution was placed in a rotary evaporator and the process repeated once more with an additional 50 ml of solvent mix, sonicate, and allow the beaker to settle and decant into the same round bottom flask (rotary evaporator), concentrate the solvent, hexane exchange it and re-concentrate to 1 to 3 ml. At this point, the moisture content was reduced to get a more concentrated solution.

The extract was purified using silica gel cartridge and the elute was transferred into vial bottle with Teflon line spectrum ready for PAH analysis using Gas Chromatography Mass Spectrophotometer (GC-MS).

3. RESULTS AND DISCUSSION

Concentrations of total PAHs in the snail samples from five snail farms in Port Harcourt Nigeria are presented in Tables 1–5. In this study, the concentration of PAHs varies significantly between the snail samples and for different samples of snail. Table 1 shows the result of PAH content (mg/kg) for snail samples collected at one of the snail farms (denoted as Snail Farm A) from Port Harcourt, Nigeria.

Table 1: PAH content for snails in Snail Farm A

Components	Snail Farm A (mg/kg)
Naphthalene	0.000
Acenaphthylene	0.000
Acenaphthene	0.000
Fluorene	0.000
Phenanthrene	0.611
Anthracene	0.159
Fluoranthene	0.000
Pyrene	0.004
Chrysene	0.007
Benz(a)anthracene	0.096
Benzo(b)fluoranthene	0.151
Benzo(k)fluoranthrene	0.000
Benzo(a)pyrene	0.164
Indeno(1,2,3- cd)pyrene	0.587
Dibenz(a,h)anthracene	0.134
Benzo(g,h,i)perylene	0.435
Total	**2.348**

The results in Table 1 reveal that naphthalene, acenaphthylene, acenaphthene, fluorene, fluoranthene, and benzo(k)fluoranthrene are not detected in Snail Farm A. Low levels of anthracene, pyrene, chrysene, benz(a)anthracene, benzo(b)fluoranthene, and dibenz(a,h)anthracene are detected. However, high amounts of phenanthrene, indeno(1,2,3-cd)pyrene, and benzo(g,h,i)perylene are present in the snails. Further explanation to foster understanding of the levels of the PAHs in snails obtained from Snail Farm A can be found in Figure 1.

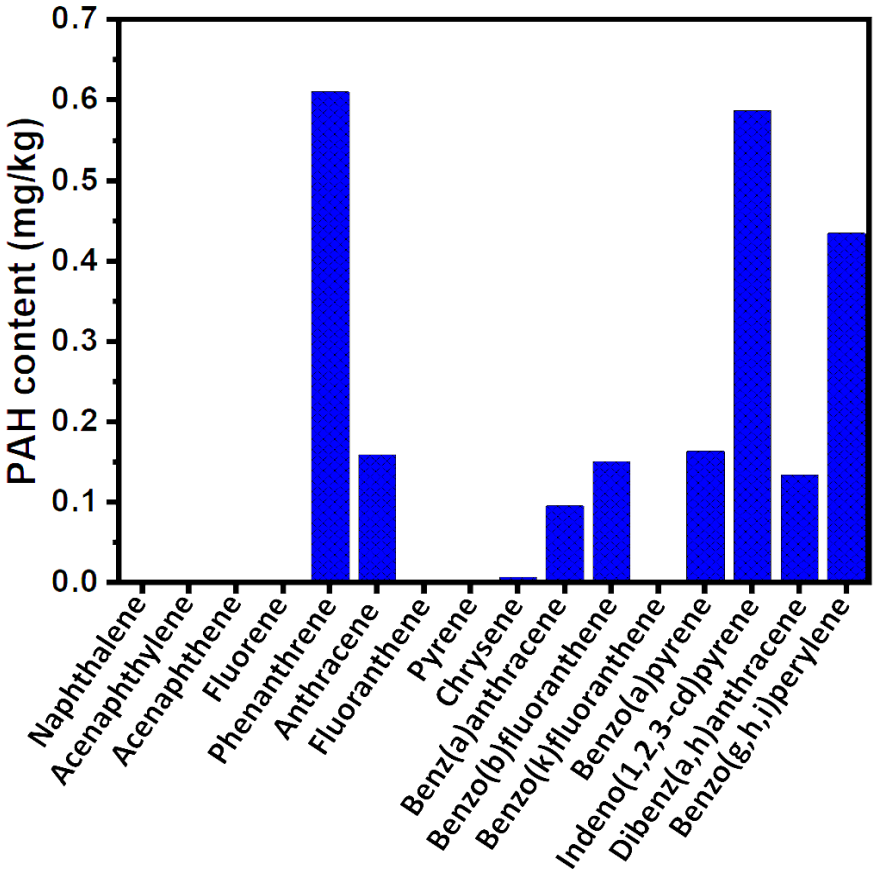

Figure 1. The amounts of PAHs in snails from Snail Farm A.

Snail samples were also obtained from another snail farm (denoted as Snail Farm B) in Etche local government area of Rivers State Nigeria and tested for their PAHs content. The results are presented in Table 2.

Table 2. PAH content for snails in Snail Farm B

Components	Snail Farm B (mg/kg)
Naphthalene	0.000
Acenaphthylene	0.000
Acenaphthene	0.029
Fluorene	0.036
Phenanthrene	0.849
Anthracene	0.851

Fluoranthene	0.000
Pyrene	0.000
Chrysene	0.011
Benz(a)anthracene	0.266
Benzo(b)fluoranthene	0.504
Benzo(k)fluoranthrene	0.086
Benzo(a)pyrene	0.213
Indeno(1,2,3- cd)pyrene	0.479
Dibenz(a,h)anthracene	0.290
Benzo(g,h,i)perylene	0.483
Total	**4.148**

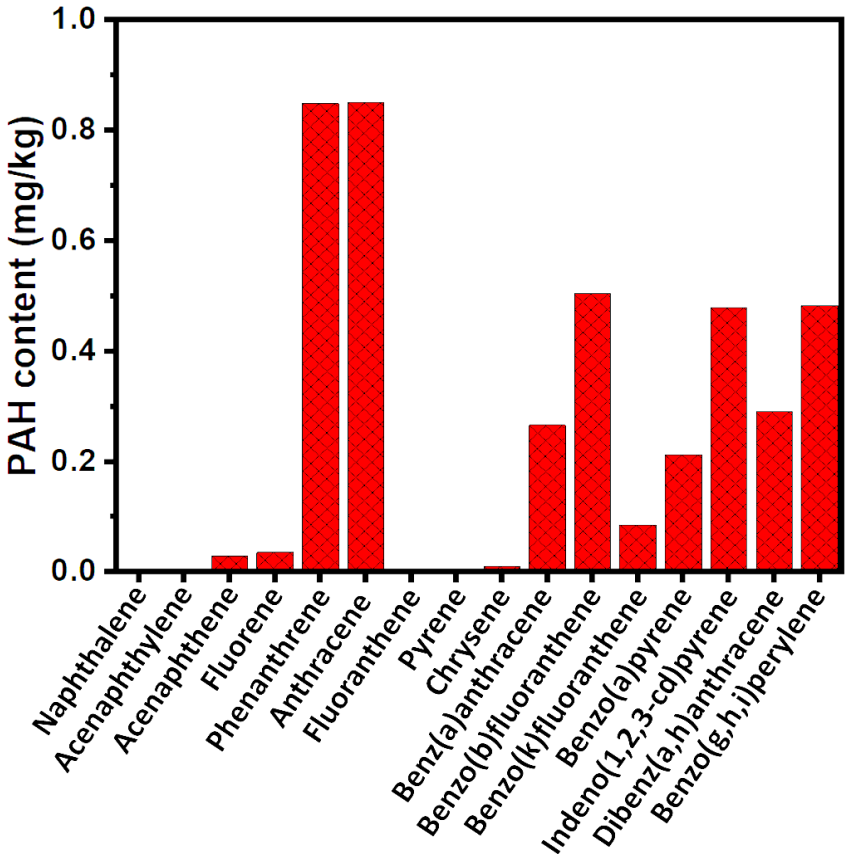

Figure 2. The amounts of PAHs in snails from Snail Farm B.

It can be seen from Table 2 that naphthalene, acenaphthylene, fluoranthene, and pyrene are not detected in the snail samples. Acenaphthene, fluorene, and chrysene are detected in low amounts, while the amount of benzo(k)fluoranthrene is moderate. Benz(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, and benzo(g,h,i)perylene are detected in relatively high amounts. The amounts of phenanthrene and anthracene are the highest in Snail Farm B. A clear representation of the levels of the PAHs in Snail Farm B is shown in Figure 2.

Another snail farm in Etche local government area of Rivers State Nigeria (denoted as Snail Farm C) was tested to determine the PAHs contents of the snails therein. Corresponding results of GC-MS analysis of the PAHs are presented in Table 3.

Table 3. PAH content for snails in Snail Farm C

Components	**Snail Farm C (mg/kg)**
Naphthalene	0.000
Acenaphthylene	0.000
Acenaphthene	0.000
Fluorene	0.000
Phenanthrene	0.503
Anthracene	0.292
Fluoranthene	0.000
Pyrene	0.034
Chrysene	0.087
Benz(a)anthracene	0.056
Benzo(b)fluoranthene	0.052
Benzo(k)fluoranthrene	0.062
Benzo(a)pyrene	0.300
Indeno(1,2,3- cd)pyrene	0.919
Dibenz(a,h)anthracene	0.139
Benzo(g,h,i)perylene	0.357
Total	**2.800**

The data presented in Table 3 show that naphthalene, acenaphthylene, acenaphthene, fluorene, and fluoranthene are absent in the snails obtained from Snail Farm C. Low levels of pyrene, chrysene, benz(a)anthracene, benzo(b)fluoranthene, and benzo(k)fluoranthrene are detected. Relatively high amounts of anthracene, benzo(a)pyrene, dibenz(a,h)anthracene, and benzo(g,h,i)perylene are detected in Snail Farm C. Additionally, high amounts of phenanthrene and indeno(1,2,3-cd)pyrene are present in the snails. Further explanation to foster understanding of the levels of the PAHs in snails obtained from Snail Farm C can be found in Figure 3.

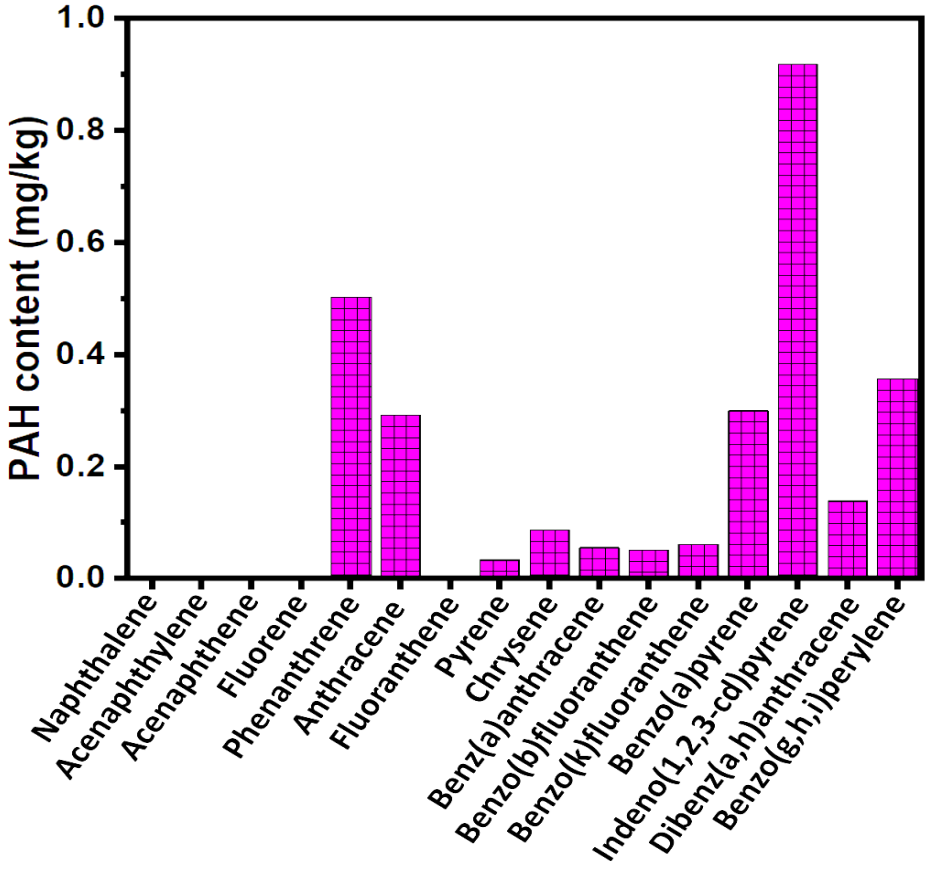

Figure 3. The amounts of PAHs in snails from Snail Farm C.

Snail samples were also obtained from another snail farm (denoted as Snail Farm D) in Etche local government area of Rivers State Nigeria and tested for their PAHs content. The results are presented in Table 4.

The information on Table 4 show that most of the PAHs are not detected in snails obtained from Snail Farm D. Among the undetected PAHs are naphthalene, acenaphthylene, fluoranthene, anthracene, Fluoranthene, Pyrene, Chrysene, Benz(a)anthracene, Benzo(k)fluoranthrene, Benzo(a)pyrene, Dibenz(a,h) anthracene, and Benzo(g,h,i) perylene. The amounts of phenanthrene and indeno(1,2,3-cd)pyrene are quite low. The most prominent PAH in Snail Farm D snails is Benzo(b)fluoranthene but its concentration is still low in

comparison with concentrations of PAHs in other snail farms. To aid more understanding of the PAH contents of the snails in Snail Farm D, the bar chats in Figure 4 are provided.

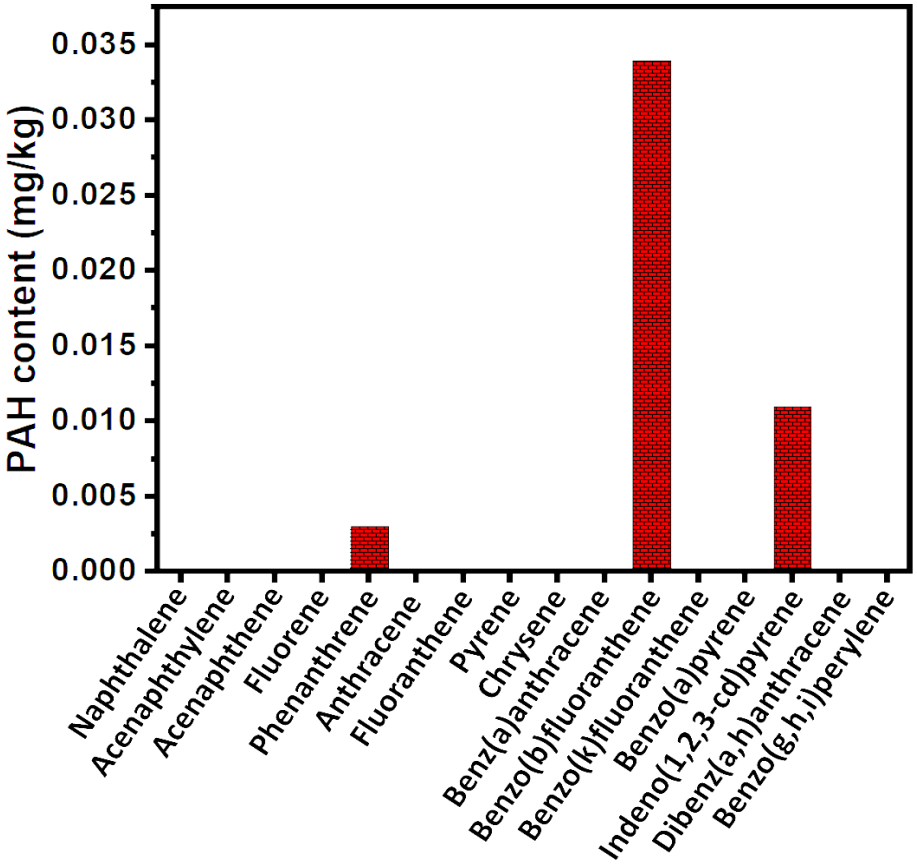

Figure 4. The amounts of PAHs in snails from Snail Farm D.

Table 4. PAH content for snails in Snail Farm D

Components	Snail Farm D (kg/mg)
Naphthalene	0.000
Acenaphthylene	0.000
Acenaphthene	0.000
Fluorene	0.000
Phenanthrene	0.003
Anthracene	0.000
Fluoranthene	0.000

Pyrene	0.000
Chrysene	0.000
Benz(a)anthracene	0.000
Benzo(b)fluoranthene	0.034
Benzo(k)fluoranthrene	0.000
Benzo(a)pyrene	0.000
Indeno(1,2,3- cd)pyrene	0.011
Dibenz(a,h)anthracene	0.000
Benzo(g,h,i)perylene	0.000
Total	**0.048**

The last snail farm in Etche local government area of Rivers State Nigeria (denoted as Snail Farm E) was tested to determine the PAHs contents of the snails therein. Corresponding results of GC-MS analysis of the PAHs are presented in Table 5.

Table 5. PAH content for snails in Snail Farm E

Components	**Snail Farm E (mg/kg)**
Naphthalene	0.000
Acenaphthylene	0.000
Acenaphthene	0.000
Fluorene	0.000
Phenanthrene	0.027
Anthracene	0.000
Fluoranthene	0.044
Pyrene	0.000
Chrysene	0.000
Benz(a)anthracene	0.000
Benzo(b)fluoranthene	0.009

Benzo(k)fluoranthrene	0.000
Benzo(a)pyrene	0.000
Indeno(1,2,3- cd)pyrene	0.016
Dibenz(a,h)anthracene	0.000
Benzo(g,h,i)perylene	0.000
Total	**0.096**

Table 5 shows naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, pyrene, chrysene, benz(a)anthracene, benzo(k)fluoranthrene, benzo(a)pyrene, dibenz(a,h)anthracene, and benzo(g,h,i)perylene are absent in the snails obtained from Snail Farm E. Low levels of phenanthrene, fluoranthene, benzo(b)fluoranthene, and indeno(1,2,3-cd)pyrene are detected. Further explanation to foster understanding of the levels of the PAHs in snails obtained from Snail Farm C can be found in Figure 5.

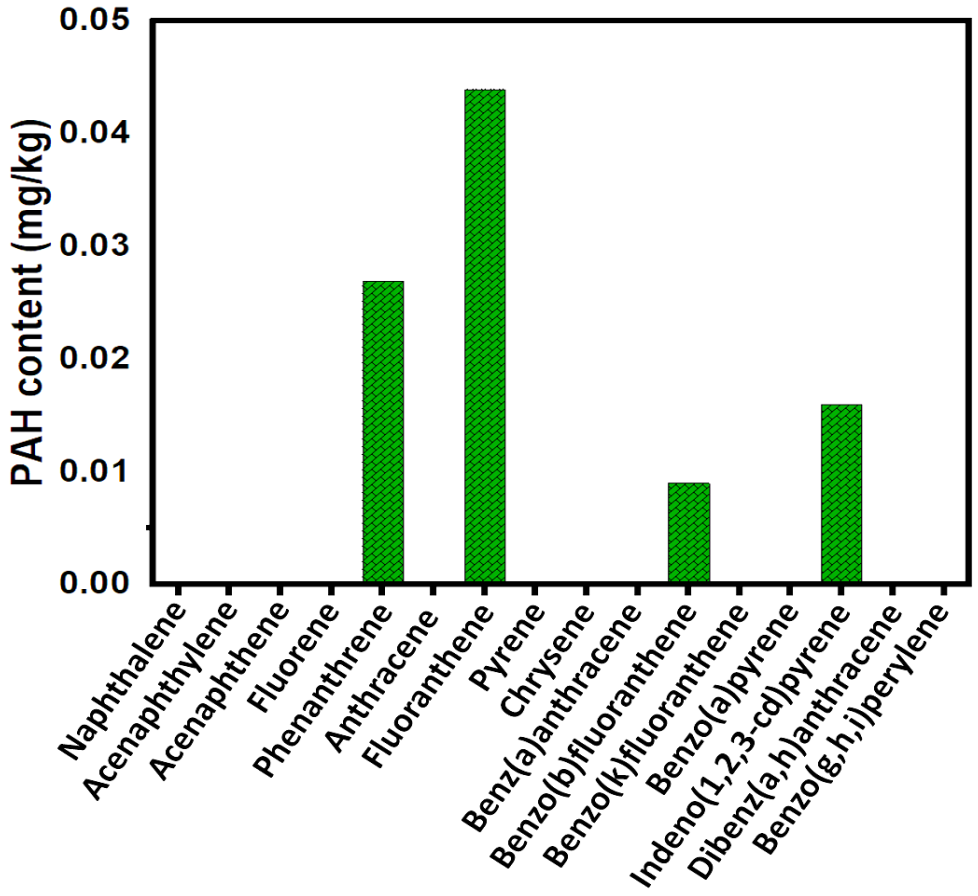

Figure 5. The amounts of PAHs in snails from Snail Farm E.

The differences in the concentrations of PAHs may be due to differences in their origin and environmental contamination. The concentrations of 16 PAHs in the snail samples varies between 0.048 and 4.148 mg/kg. Higher concentrations of 16 PAHs were found in Snail Farm C than other snail samples examined. Lower concentrations of 16 PAHs were observed in Snail Farm D. In all species examined, higher molecular weight PAHs compounds showed dominance over lower PAHs compounds.

The concentration of PAHs on this snail samples as shown in Tables 1–5 ranged from not detected (ND) for naphthalene, not detected to 0.051 mg/kg for acenaphthylene, not detected (ND) to 0.029 mg/kg for acenaphthene, not detected to 0.036 mg/kg for fluorene, 0.003 to 0.849 mg/kg for phenanthrene, not detected to 0.851 mg/kg for anthracene, not detected to 0.044 mg/kg for fluoranthene, not detected to 0.034 mg/kg for pyrene, not detected to 0.87 mg/kg chrysene, not detected to 0.2666 mg/kg for benz(a)anthracene, 0.009 to 0.504 mg/kg for benzo(b)fluoranthene, not detected to 0.086 mg/kg for benzo(k)fluoranthene, not detected to 0.290 mg/kg for dibenz(a,h) anthracene, not detected to 0.483 mg/kg for benzo(g,h,i) perylene. phenanthrene, benzo(b)fluoranthene and indeno(1,2,3-cd)pyrene are dominant pahs compounds in these snail species and occurred in snails from Snail Farms A–E.

The two rings' PAHs (Naphthalene) were not detected in all sample. The four ring PAHs (fluoranthene, pyrene, benz(a) anthracene and chrysene) were detected at concentrations ranging from 0.00 to 0.266 mg/kg. The 5 rings PAHs (Benz(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene and Dibenz(a,h)anthracene) were detected at concentration ranging from 0.00mg/kg to 0.504mg/kg. The 6 rings PAHs (Benzo(g,h,i)perylene, Indeno (1,2,3,)pyrene were detected at concentration ranging from 0.00mg/kg to 0.919mg/kg. In this study, Indeno(1,2,3-cd)pyrene is the dominant 6-ringed PAHs and occurred at concentration of 0.011mg/kg – 0.919 mg/kg.

From Tables 1–5 and corresponding Figures 1–5, it can be seen that phenanthrene, benzo(b)fluoranthrene and indeno(1,2,3,-cd)pyrene had higher concentrations of PAHs relative to others PAHs while napthalene, acenaphthylene, acenaphthene, fluorene and fluoranthene had the lowest concentration of PAHs. This means that the lower molecular weight PAHs is more resistant to PAHs accumulation than the higher molecular weight PAHs found in this study.

4. CONCLUSIONS

The results indicated that concentration of PAHs in African Giant snail from Etche Local Government Area, Rivers State, Nigeria were high compared to those in previous studies. It also indicated that the majority of these snail samples consumed in Port Harcourt are heavily contaminated with Benzo(b) fluoranthene, Indeno(1,2,3-cd)pyrene and phenanthrene. The high concentration of PAHs in African Giant Snail (*Archachatina marginata)* indicates that the soil and environment of Port Harcourt is highly polluted and excessive intake of these snail samples or specie could result into higher health risk which indicates a potential concern for consumers' health. From the studies carried out above, there is a high tendency that the growing concern for the pollution of terrestrial environments would continue hence further research should be carried out to discover more sources of this PAHs and their means of distribution to the terrestrial organisms. This demonstrates the need for the Federal Environment Agency of Nigeria to plan on further steps aimed at limiting the risks for humans and the environment. Also, the establishment of legal limits for PAHs in snails in Nigeria and possible risk

management. Finally, companies should be urged to develop strategies aimed at minimizing PAHs content in Industrial exhaust gases and products that exceed legal requirement.

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