



Evaluation of the Total Hydrocarbon content of some Seafood and Water from the Great Kwa River in Cross River State

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Abstract

Hydrocarbon contamination is of great worry because of its widespread effect on all forms of life. Pollution caused by increasing crude oil exploration is of great concern, as well as inadequate refuse disposal problems. Crude oil and petroleum products are among the most common pollutants in the marine environment. This study was aimed at determining the total hydrocarbon content of water, mud, shrimp and periwinkle from the Great Kwa River in Calabar, Cross River State. The water, mud, shrimp and periwinkle were collected from the Great Kwa River in the Calabar metropolis during the rainy season. Total hydrocarbon content was analysed using ultraviolet spectroscopy. From the results, the total hydrocarbon content varied widely across the biomarkers adopted by this study (water 2.28 - 738.31ng/μl, shrimp 46.28 - 60.39ng/μl, periwinkle 0 ng/μl and mud 1.01 - 422.18ng/μl). The three highest concentrations of hydrocarbon components observed in the water are n-pentadecane 738.31ng/μl, n-hexadecane 584.54ng/μl, and n-tridecane 383.00ng/μl. The three least hydrocarbon components observed in the water are n-hexacosane 2.28ng/μl, n-octacosane 4.22ng/μl, and n-dotriacontane 8.91ng/μl. However, n-nonane 60.39ng/μl, and n-decane 46.28ng/μl as observed in shrimps are the only hydrocarbon components that were biotransferable. From this study, it is observed that the hydrocarbon concentrations in the Great Kwa River are quite significant and some of the concentrations are higher than safe levels (both for ingestion and inhalation), hence regular biomonitoring of the River is recommended to check source pollution. In-depth research into their oxidative stress responses, metallothioneins, oxygen affinity and thus, toxicological effects is also suggested.

Keywords: Hydrocarbon, pollution, Great Kwa River, shrimp, periwinkle

Introduction

The aquatic environment is currently facing a lot of threats resulting from land-based activities (Dixit *et al.*, 2015). Hydrocarbon compounds are found naturally in many oil sand regions in association with minerals and sediments, and they originate from fossil fuels (i.e. bitumen), the combustion of organic matter, as well as chemical and biological transformation of organic molecules (Chiesa *et al.*, 2019). Hydrocarbon contamination is of great worry because of its widespread effect on all forms of life. Pollution caused by the increasing use of crude oil is ordinary because of its extensive application and its related transport and dumping problems (Bock, 2019). Crude oil contains a complex mixture of aliphatic, aromatic, and heterocyclic compounds and oil is one of the most common pollutants in the marine environment (Chiesa

et al., 2019). One of the main difficulties in interpreting oil pollution data for the seas, especially in cases of low concentrations, is the natural biogenic origin of many hydrocarbons which are typical for the chemical composition of oil (Bock, 2019). The extraction and usage of petroleum products as energy sources globally have led to widespread pollution of the biosphere. About 6-10 million barrels of crude oil enter the aquatic environment yearly (Horsfall *et al.*, 2012). The control of such pollution problems in the aquatic environment is very difficult because of the large number of input sources and their geographic dispersions. Contrary to popular opinion, evidence is accumulating to buttress the fact that petroleum hydrocarbon mixes with water and penetrates the underlying sediments (Wahua, 2013). The resultant effects of the above are a change in desirable portable water characteristics (Ahmed, 2012);

impaired growth of marine organisms which depend basically on the quantity and quality of the primary production of phytoplankton; fish, crustaceans and molluscs acquire objectionable odour or flavour, thereby causing a reduction in their marketability and acceptance as food (Corredor *et al.*, 2014). Death of the fauna and flora from oil spills is common in the Niger Delta region of Nigeria where the most intensive oil exploration, exploitation and refining occur (Chindah *et al.*, 2013). Cross River State, whose capital city is Calabar, is located in the Niger Delta region of the country. In addition, oil contamination of coastal amenities has adverse effects on tourism, recreation and aesthetics of the impacted area. This effect can be substantial on a community like Calabar whose economy depends on tourism.

Shrimp (*Caridea*) and periwinkle (*Tympanotonus fuscatus* var. *Radula*) are of economic importance to the natives in the Niger Delta region of Nigeria. Periwinkles are a rich source of protein (21.04%) and are gathered daily for food (Osuji *et al.*, 2013). Like all other intertidal organisms, shrimps and periwinkles are very vulnerable to oil pollution because their habitats are susceptible to coating with oil and maybe smothered in the event of heavy oil drifting ashore (Carbioch *et al.*, 2012). The creek and its environs bearing the flow station facilities affect the well-being of the natives of the area as they ferment cassava into raw 'fufu' (locally made starchy food) from dug-out holes on the mudflats of the area, and fishing operations are being carried out daily (Farrington *et al.*, 2014). Many water bodies flow through the southern part of the country and empty into the Atlantic Ocean. The Great Kwa River is an example of such a water body; it flows through Cross River State, Nigeria, draining the east side of the city of Calabar, the River originates in the Oban Hills, in the Cross River National Park, and flows southwards to the Cross River estuary (Akpan *et al.*, 2011). Human activity in the Great Kwa basin has traditionally been limited to small-scale farming, aquaculture and artisanal fisheries, mainly for shrimp (Eja *et al.*, 2012). However, Calabar is growing, due in part to the Calabar Free Trade Zone, causing growing numbers of houses and factories to be built in the freshwater and mangrove swamps of the River (Akpan *et al.*, 2011). The River ecology is under threat from human activity, Calabar Municipality has no waste treatment facilities, inadequate refuse disposal facilities, and heavy rains wash human and industrial wastes into the River (Eja *et al.*, 2012).

Water is a basic requirement to maintain life, Rivers are a major source of water for rural areas and even some inhabitants of the urban areas. There is a need to check the quality of water consumed daily. Shellfish, periwinkle, fish and other aquatic organisms are suitable for food and are rich sources of biological valuable protein (Howgate, 2013). Shrimp is one of the world's most popular shellfish. Safety of the seafood products and their quality is currently a major problem in the food industry. Civilization and industrialisation have led to an increase in the release of pollutants to the ecosystem.

Some of these pollutants, such as hydrocarbons, are directly discharged by industrial plants and municipal sewage treatment plants; others come from polluted runoff in urban and agricultural areas. Many toxic compounds, when released into the aquatic environment, accumulate in the soil and sediments of water bodies. The lower aquatic organisms absorb and transfer them through the food chain to higher trophic levels.

Seafoods are low in saturated fats and rich in "heart healthy" polyunsaturated fats, including omega-3 fatty acids. They are considered as healthier meat options and their consumption can decrease the risk of heart attack, stroke, obesity and hypertension (Wahua, 2013). Shrimp and periwinkle are consumed by both the rich and poor; they have been used as food condiments by riverine dwellers and are recommended for pregnant women and protein-malnourished children because of their protein and iron content (Horsfall *et al.*, 2012). Hydrocarbons tend to accumulate in the organs of fish and other seafood, which in turn get consumed by humans causing various health hazards. Ingested hydrocarbons (above tolerable levels) cause coughing and choking, which allows the hydrocarbon liquid to enter the airways and irritate the lungs, a serious condition in itself (chemical pneumonitis), and can lead to severe pneumonia. It has been reported that severe poisoning also can affect the brain, heart, bone marrow, and kidneys. The knowledge of the potential contamination of the aquatic environment, which affects the quality of water, and its consequent accumulation in seafood is important for food safety and consumer protection. Figures 1 and 2 show diagrams of aliphatic and aromatic hydrocarbon compounds.

Materials and Methods

Ethics

For this study, a waiver was obtained from the Faculty Animal Research Ethics Committee (FAREC-FBMS), Faculty of Basic Medical Sciences, University of Calabar, Cross River State on the 7th of June, 2022.

Study Location

Calabar Metropolis is the capital city of Cross River state (one of nine states in the oil-rich Niger Delta region) of Nigeria with a population of 579,000 as of the year 2020. The city is drained by the Calabar River to the west and the Great Kwa River to the east, both of which discharge into the Cross River Estuary, which subsequently flows into the Atlantic Ocean at the Gulf of Guinea. Both Rivers maintain a network of tributaries and creeks. The Cross River estuary and its systems support a rich mangrove swamp ecosystem with extensive mud flats and swamps, rich in shellfish, including periwinkles.

Samples Collection and Preparation

Freshwater, shrimp, periwinkles and mud were collected from the Great Kwa River within Calabar Metropolis, and transported in sterile polyethylene bags to the Department of Biochemistry Laboratory, University

of Calabar for chemical analysis. Figure 3 shows pictures of shrimps and periwinkles. The four samples: water, mud, shrimps and periwinkles were put into different airtight sample bottles and labelled appropriately before being stored in the fridge pending analysis. The soft tissue (edible part) of the periwinkle and shrimp were extracted, and dried in the oven at 80°C for 72 hours then the weights of the periwinkles and mud from each sampling were measured using an m-medlar weighing balance and the grams of each sample taken were recorded – water 15mls, mud 100g, shrimp (0.2 – 1.09g) and periwinkle (0.23 – 1.00g).

Digestion of samples

After the weight of the above samples was taken, the samples were digested using concentrated Hydrochloric acid (HCl) and nitric acid (HNO₃). Then, 20ml of nitric acid was taken and diluted with 10ml of hydrochloric acid in a beaker containing the sample (periwinkle) and placed on a wire gauze on the cooking gas for digestion to take place. This lasted for 5 to 10 minutes before the digest was taken and filtered using the Whatman filter paper to remove impurities. The same process was also applied to the mud samples. A blank solution of distilled water was used to check the accuracy of the standard solutions. Afterwards, the filtrates of the samples were turned into clean sample bottles and sealed properly.

Experimental Procedure

Determination of Hydrocarbon content

The hydrocarbon content of the various samples of water, mud, shrimp and periwinkles was determined using Ultraviolet Fluorescence (UVF) spectroscopy. An aliquot of the samples was subjected to the removal of contaminants by passing the samples through a silica gel-alumina column. The instrumental analysis of the hydrocarbons was carried out with UV fluorescence, using crude oil standards. Quantification was performed at 310 – 360 nm as excitation and emission wavelengths, respectively.

Data analysis

Laboratory analytical results were compiled and analyzed using a Microsoft Excel 2013 spreadsheet and expressed as mean ± SEM (n = 3). Statistical analyses – Analysis of Variance (ANOVA) and T-tests were used to compare hydrocarbon concentrations in water, periwinkle, shrimp and mud from the Great Kwa River were carried out using Statistical Package for Social Sciences (SPSS version 20.0) and significance was accepted at p < 0.05.

Data availability Statement

The results supporting the findings of this study are published in this article. Any additional information can be made available by the authors, on request.

Results and Discussion

Results

The Hydrocarbon Content of the samples

The hydrocarbon concentration as presented in Table 1 varied widely across the biomarkers adopted by this

study (water 2.28 - 738.31 ng/μl, shrimp 46.28 - 60.39 ng/μl, periwinkle 0 ng/μl and mud 1.01 - 422.18 ng/μl). The three highest concentrations of hydrocarbon components observed in water were n-pentadecane 738.31 ng/μl, n-hexadecane 584.54 ng/μl, and n-tridecane 383.00 ng/μl. The three least hydrocarbon components observed in water were n-hexacosane 2.28 ng/μl, n-octacosane 4.22 ng/μl, and n-dotriacontane 8.91 ng/μl. Furthermore, the three highest concentrations of hydrocarbon components observed in the mud were n-tetratriacontane 422.18 ng/μl, n-hexadecane 163.62 ng/μl, and n-dodecane 136.81 ng/μl. In contrast, the three least hydrocarbon components observed in the mud were n-hexacosane 1.01 ng/μl, n-decane 6.01 ng/μl, and n-octacosane 6.75 ng/μl. However, n-nonane 60.39 ng/μl, and n-decane 46.28 ng/μl as observed in shrimps are the only hydrocarbon components that are biotransferable.

Discussion

The quality of water for both industrial and domestic use has become an issue of common concern in countries where crude oil mining activities are present (Ben-Naim *et al.*, 2011). The rising quest for petrochemicals all over the globe has led to the discharge of petroleum-based chemicals into the already overburdened ecosystems. In this study of the total hydrocarbon content (THC) of water, shrimp, mud and periwinkle from the Great Kwa River, the results showed that the THC varied widely across the biomarkers. The concentration of some of the hydrocarbons in water from the river was found to be present at high concentrations. The United States Environmental Protection Agency (EPA) permissible limit for hydrocarbon in water is 418.1 ng/μl (US EPA, 2021). However, n-pentadecane and n-hexadecane were higher than the EPA permissible limit of hydrocarbon in water, n-undecane, n-tridecane, n-tetradecane, n-pentadecane, n-hexadecane, Heptadecane, Pristane, Phytane, n-Nonadecane were higher than the WHO permissible limit (200 ng/μl) for hydrocarbon in water as well (WHO, 2015). Furthermore, n-pentadecane is a hydrocarbon lipid molecule that is very hydrophobic, practically insoluble in water, and relatively neutral. n-Pentadecane may be harmful by inhalation, ingestion, or skin absorption during industrial use, when aspirated into the lungs, is an asphyxiant. n-Pentadecane administered to rats caused significant differences in the rats' mitochondria (Muhammad *et al.*, 2005). A study by Daubert & Danner (2012), found n-Pentadecane to be significantly higher than the concentration reported in this study. This could be a result of differences in methodology, geographical location and level of pollution. Another hydrocarbon of concern is Hexadecane - a colourless liquid. It is used as a solvent, organic intermediate, and ignition standard for diesel fuels. It is also used for the production of detergents. Acute exposure to hexadecane irritates central nervous system (CNS) depression, and gastrointestinal tract irritation (Bingham *et al.*, 2011).

In shrimps, only n-nonane and n-decane were found, hence these are the only two hydrocarbon components

that are biotransferable. However, no hydrocarbons were found in the periwinkles, indicating that the hydrocarbons did not get into the soft tissues of these aquatic animals. This may be attributable to the very hard shells which hide and protect the periwinkles and the operculum which is the 'small lid' which seals the open end of the periwinkle shell. The World Health Organization set 0.2 µg/L as the maximum permissible limit for total hydrocarbon in drinking water while that of benzo(a)pyrene is 0.1 µg/L (WHO, 2015). Pollution of water bodies by chemical toxicants as well as organic by-products, has been in the public domain recently because human beings can be exposed to toxic chemicals which bioaccumulate in aquatic organisms harvested from contaminated waters (Ofosu *et al.*, 2015). It has become of great importance to prevent agricultural and industrial contamination of water resources (Mzoughi & Chouba, 2012). The concentrations of total hydrocarbon content in tissues of shrimp and periwinkle showed variation with previous studies done in petroleum-contaminated water by Edema *et al.* (2011) and Ideriah *et al.* (2012) at another polluted river. Both studies found higher levels of hydrocarbons in shrimps and periwinkles from the Warri River. This may be because oil exploration activities are far more in Warri, Delta state than in Cross River state, hence the higher level of aquatic pollution which was reported. Also, Jack *et al.* (2015), noted that hydrocarbons take a longer time to sink into the river bed and that marine organisms accumulate hydrocarbons due to their sedentary and bottom-feeding habit.

Globally, the human health risk due to consumption of food from aquatic ecosystems contaminated with hazardous chemicals including hydrocarbons has increased (Kalia *et al.*, 2022). There is a possibility that the level of these chemicals in seafood may not have had significant adverse effects on an individual organism but may still have devastating effects on the organisms that consume them due to bioaccumulation and biomagnification arising from the food chain as a result of the trophic transfer factor (Gall *et al.*, 2015). According to Ideriah *et al.* (2012), levels of pollution observed suggest that in addition to tidal actions, domestic wastes containing discharges of heavy metals and THC may have contributed to the level of pollution in aquatic organisms.

The level of hydrocarbon concentration was found in mud to vary widely; in this study, the hydrocarbon content of the water was significantly higher than that of the mud. The mining and application of petroleum sources of energy all over the sphere have given rise to extensive pollution of the aquatic environment. Millions of barrels of crude oil go into the water bodies annually (Ge *et al.*, 2020). It has been a bit challenging to control and manage pollution complications associated with discharges of petroleum in a water environment. This is due to the enormous quantity of input sources and their geographical spread (Li *et al.* 2020). The effect of petroleum products on the environment has attracted worldwide interest among researchers because of the perceived health effects which are either carcinogenic,

mutagenic or toxic (Li *et al.*, 2020). Elevated levels of hydrocarbons prevailing in soiled sites may pose a health threat to both flora and fauna within the surroundings. The hazard to several ecologies and their resources has been amplified because of increased environmental ruin. It has been documented that the different activities of man are the major causes of the deterioration of the environment and in a few cases may be the result of natural occurrence (Ge *et al.*, 2020). Nigeria is the sixth largest producer of crude oil in the world. The levels of hydrocarbon in sea organisms' tissues could have been induced by uptake from the burrow pit sediments.

Previous reports have suggested that activities from industry, domestic and wastewater, such as the burning of fossil fuels, land run-off, blow, oil spills, gas leaks and indiscriminate dumping of water materials which characterize the study area may have been responsible for the obtained results. The findings of this study are similar to those reported by Isibor *et al.* (2016). Awareness should be created of the magnitude and impact of aquatic pollution on the health of consumers, then steps must be taken to implement certain remedial actions that can solve the problem.

Limitation of the Study: The main limitation of this study was insufficient funds for further analysis of the heavy metal content of the samples but this will be carried out in subsequent research.

Conclusion

Hydrocarbon compounds are produced in various industrial processes – especially crude oil exploration and can contaminate water bodies through leaks in underground pipes, spills, improper disposal or leaching from landfills. These organic compounds pose environmental contamination risks and human health risks if exposed through drinking water, or ingestion of seafood such as shrimps and periwinkles. The findings of this study show significant concentrations of a lot of hydrocarbons in the water and mud samples but none was found in the periwinkles. The results obtained from the study showed that the total hydrocarbon content in water exceeds the EPA set standard. Only two hydrocarbons n-nonane and n-decane were biotransferable and found in significant quantities in shrimps, whereas there were no transferrable hydrocarbons seen in periwinkle. Considering the enormous commercial, nutritional and industrial importance of shrimps and periwinkles, awareness of possible pollution in some areas and of some seafood must be created. Excessive consumption of certain seafood from areas with serious water pollution should be discouraged until remedial actions are taken for the safety of consumers. Furthermore, steps should be implemented to control the petrochemical industry and waste management operations.

Conflict of interests: The author hereby declares that no conflict of interests exists.

Funding: No funding was received for this research.

Acknowledgement: The author hereby acknowledges Anand Kumar for his assistance in data collection and laboratory analysis.

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Name	Molecular Formula	Condensed Formula	Structural Formula
Methane	CH ₄	CH ₄	
Ethane	C ₂ H ₆	H ₃ CCH ₃	
Propane	C ₃ H ₈	H ₃ CCH ₂ CH ₃	
Butane	C ₄ H ₁₀	H ₃ C(CH ₂) ₂ CH ₃	
Pentane	C ₅ H ₁₂	H ₃ C(CH ₂) ₃ CH ₃	
Hexane	C ₆ H ₁₄	H ₃ C(CH ₂) ₄ CH ₃	
Heptane	C ₇ H ₁₆	H ₃ C(CH ₂) ₅ CH ₃	
Octane	C ₈ H ₁₈	H ₃ C(CH ₂) ₆ CH ₃	
Nonane	C ₉ H ₂₀	H ₃ C(CH ₂) ₇ CH ₃	
Decane	C ₁₀ H ₂₂	H ₃ C(CH ₂) ₈ CH ₃	

Figure 1: Aliphatic hydrocarbons (Horsfall, 2012)

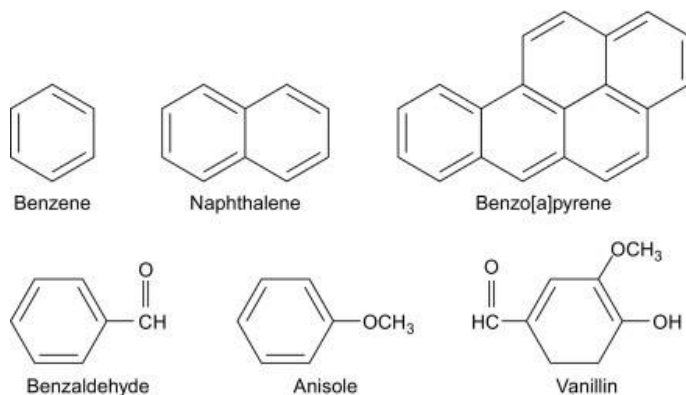


Figure 2: Aromatic hydrocarbon (Horsfall, 2012)



Fig. 3a: Shrimps (Gracia, 2013)



Fig. 3b: Periwinkle in shell (Dambo, 2013)



Fig. 3c: Periwinkle without shell (Dambo, 2013)

Figure 3: Pictures of some seafood

Table 1: Hydrocarbon content of water, shrimp, mud and periwinkle from Great Kwa River

S/N	Hydrocarbon compounds	Water (ng/μl)	Shrimp (ng/μl)	Mud (ng/μl)	Periwinkle (ng/μl)
1	n-Nonane	184.90 ± 0.83	60.39 ± 0.31	-	-
2	n-Decane	-	46.28 ± 0.22	6.07 ± 0.07	-
3	n-Undecane	310.22 ± 1.21	-	-	-
4	n-Dodecane	-	-	136.81 ± 0.91	-
5	n-Tridecane	383.00 ± 1.24	-	51.77 ± 0.42	-
6	n-Tetradecane	300.85 ± 1.09	-	40.61 ± 0.20	-
7	n-Pentadecane	738.31 ± 2.61	-	35.84 ± 0.11	-
8	n-Hexadecane	584.54 ± 2.01	-	163.62 ± 0.77	-
9	n-Heptadecane	256.91 ± 1.03	-	16.92 ± 0.05	-
10	Pristane	282.34 ± 1.11	-	64.41 ± 0.29	-
11	n-Octadecane	63.90 ± 0.54	-	-	-
12	Phytane	365.45 ± 1.07	-	-	-
13	n-Nonadecane	290.21 ± 1.10	-	21.86 ± 0.07	-
14	n-Eicosane	144.42 ± 0.59	-	-	-
15	n-Heneicosane	27.15 ± 0.08	-	-	-
16	n-Hexacosane	2.28 ± 0.01	-	1.01 ± 0.01	-
17	n-Heptacosane	14.03 ± 0.21	-	72.94 ± 0.51	-
18	n-Octacosane	4.22 ± 0.03	-	6.75 ± 0.12	-
19	n-Nonacosane	16.83 ± 0.02	-	25.07 ± 0.23	-
20	n-Tricontane	10.56 ± 0.01	-	23.33 ± 0.20	-
21	n-Hextriacontane	10.25 ± 0.01	-	-	-
22	n-Dotriacontane	8.91 ± 0.01	-	18.79 ± 0.15	-
23	n-Tritriacontane	-	-	24.32 ± 0.21	-
24	n-Tetratriacontane	79.26 ± 0.41	-	422.18 ± 1.51	-
25	n-Pentatriacontane	59.23 ± 0.24	-	-	-
26	n-Heptatriacontane	28.72 ± 0.06	-	-	-

**All the values varied significantly at $p < 0.05$ both within and across the groups*