Nigerian Research Journal of Engineering and Environmental Sciences 11(1) 2026 pp. 330-336
p ISSN: 2635-3342; e ISSN: 2635-3350

Nigerian Research Journal of Engineering and Environmental Sciences
Journal homepage: www.rjees.com

Original Research Article

Investigating the Prevalence of Oil-Based Contaminants Within the Sedimentary Layers and Local Flora/Fauna of Bille Creek

***John, G.N. and Omozuwa, P.O.**

Department of Applied Chemical Science Laboratory Technology, Faculty of Science Laboratory Technology, University of Benin, Benin City, Nigeria.
*godson.john@uniben.edu

http://doi.org/10.5281/zenodo.21381889

ARTICLE INFORMATION

Article history:
Received 06 May 2026
Revised 20 Jun. 2026
Accepted 21 Jun. 2026
Available online 30 Jun. 2026

Keywords:
Crude oil
Heavy metal
Contamination
Biota
Crabs

ABSTRACT

Crude oil exploration and related activities in the Niger Delta region of Nigeria have continued to raise concerns about environmental quality and food safety in coastal communities. The aim of this study was to analyse petroleum hydrocarbon contamination in biota from Bille, Rivers State, Nigeria. Sediment and biota (periwinkles and crab) samples were collected from Bille creek and analyzed for total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAHs), heavy metals, and hydrocarbon-utilizing microorganisms using standard laboratory procedures. Results showed that mean TPH concentrations were 33.80 ± 10.09 mg/kg in sediment, 3.56 ± 0.03 mg/kg in periwinkle, and 240.50 ± 0.20 mg/kg in crab, indicating higher accumulation in crabs. Several PAHs were detected, with naphthalene occurring in sediment (0.02 ± 0.01 mg/kg), periwinkle (0.03 ± 0.00 mg/kg), and crab (0.57 ± 0.00 mg/kg). Higher molecular weight PAHs such as benzo(b) fluoranthene and benzo(k) fluoranthene were detected mainly in crab samples. Heavy metal analysis revealed zinc as the most prominent metal with concentrations of 0.39 ± 0.07 mg/kg in sediment, 0.22 ± 0.00 mg/kg in periwinkle, and 0.85 ± 0.00 mg/kg in crab, while cadmium, chromium, and lead were below detectable limits. Hydrocarbon-utilizing bacteria recorded 0.25 × 10^2 cfu/g, whereas fungal counts were <0.01 × 10^2 cfu/g. The study concluded that petroleum-related contaminants are present in the Bille environment. Hence, it is recommended that continuous environmental monitoring and public awareness on seafood safety be strengthened to reduce potential health risks.

1. INTRODUCTION

Petroleum is a naturally occurring complex mixture composed mainly of carbon and hydrogen, with smaller proportions of heteroatoms such as sulphur, nitrogen, oxygen, and trace metals. These

components originate from the thermal transformation of ancient organic matter subjected to prolonged geological pressure and temperature over millions of years (Dembicki, 2022; Speight, 2020).

The dominance of hydrocarbons within petroleum makes them the most environmentally significant constituents when crude oil or its derivatives are released into natural ecosystems. Hydrocarbons present in petroleum are broadly classified based on their chemical structure into saturated and unsaturated forms. Saturated hydrocarbons, mainly alkanes and cycloalkanes, contain single carbon–carbon bonds, whereas unsaturated hydrocarbons, such as alkenes and alkynes, possess one or more double or triple bonds (Speight, 2017). Aromatic hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs), represent another important group due to their chemical stability, toxicity, and resistance to degradation. These hydrocarbon fractions vary widely in molecular weight, solubility, volatility and environmental persistence, and these properties influence their distribution, environmental fate and ecological effects (Kuppusamy *et al*., 2020).

Hydrocarbons enter the environment through both natural and anthropogenic pathways. Natural sources include the slow decomposition of organic matter and natural oil seeps from subsurface reservoirs. However, in contemporary times, anthropogenic activities constitute the dominant source of petroleum hydrocarbon contamination. These activities include crude oil exploration, drilling, transportation, refining, gas flaring, and the combustion of fossil fuels (Kuppusamy *et al*., 2020). In coastal and estuarine environments, accidental spills and operational discharges are of particular concern because such ecosystems serve as ecological transition zones that support high biological productivity.

Total petroleum hydrocarbons (TPH) is a collective term used to describe the measurable concentration of petroleum-derived hydrocarbons present in environmental media such as water, sediment, and biota. Rather than representing a single compound, TPH encompasses a broad spectrum of hydrocarbon fractions with varying toxicological properties. While some low-molecular-weight hydrocarbons can be degraded relatively quickly by microbial action, heavier aliphatic hydrocarbons and aromatic compounds often persist in sediments and aquatic organisms for extended periods, thereby posing long-term environmental risks (Kuppusamy *et al*., 2020).

One of the major environmental concerns associated with petroleum hydrocarbons is their lipophilic nature. This property enhances their affinity for organic matter and biological tissues, making aquatic organisms particularly vulnerable to accumulation (Kuppusamy *et al*., 2020). When petroleum hydrocarbons are introduced into aquatic environments, they can adsorb onto suspended particles and settle into bottom sediments, where they become available to benthic organisms such as periwinkles and crabs. These organisms are often exposed through direct contact with contaminated sediments, ingestion of polluted food particles, or uptake across permeable membranes (Kuppusamy *et al*., 2020). The ecological effects of petroleum hydrocarbons in aquatic systems range from acute toxicity to chronic sub-lethal impacts. Acute exposure may result in mortality, behavioral changes, and physiological stress, while chronic exposure has been linked to reduced growth, impaired reproduction, genetic damage, and immune suppression in aquatic organisms (Kuppusamy *et al*., 2020).

Certain petroleum hydrocarbon fractions, particularly polycyclic aromatic hydrocarbons, may produce toxic, mutagenic and carcinogenic effects in exposed organisms, depending on the compound, concentration and duration of exposure (Abdel-Shafy and Mansour, 2016). Empirical studies across different aquatic environments have documented elevated levels of TPH in water, sediment, and biota. In Nigeria, Akinola *et al*. (2019) reported petroleum hydrocarbon residues and associated ecological concerns in brackish-water white shrimp collected from the Ayetoro coastal environment in Ondo State. Similarly, TPH contamination has been documented in surface water and sediment from Woji Creek in the Niger Delta, where associated ecological and human-health risks were evaluated (Ihunwo *et al*., 2021). Comparable findings have been reported in other oil-producing regions, such as South Africa, where elevated TPH levels were detected in sediments and coastal waters of Algoa Bay, reflecting sustained anthropogenic pressure (Adeniji *et al*., 2017).

The aim of this study was to determine the concentrations of total petroleum hydrocarbons, polycyclic aromatic hydrocarbons and selected heavy metals in sediment, periwinkle and crab samples from Bille Creek in Rivers State, and to enumerate hydrocarbon-utilising microorganisms in the sediment.

2. MATERIALS AND METHODS

2.1. Sample Collection and Preparation

Sediment, crab, and periwinkle samples were collected from selected locations within Bille community, Rivers State, Nigeria. Standard laboratory procedures were followed during sample preparation. Each sample was individually homogenized using a clean mortar and pestle to ensure uniformity. The homogenized samples intended for heavy metal analysis were stored in sterile, well-labelled containers. Samples designated for polycyclic aromatic hydrocarbon (PAH) analysis were wrapped in aluminium foil to minimize exposure to light and subsequently preserved until analysis.

2.2. Sample Digestion and Heavy Metal Determination

Exactly 2 g of each prepared sediment, crab, and periwinkle sample were weighed using a precision analytical balance and transferred into separate 250 mL beakers. Ten millilitres of aqua regia, prepared by mixing hydrochloric acid (HCl) and nitric acid (HNO_3), were added to each sample. The mixtures were heated on a hot plate until complete digestion was achieved, yielding clear solutions. After cooling, the digested samples were transferred into 50 mL volumetric flasks and made up to volume with distilled water. Concentrations of zinc (Zn), cadmium (Cd), lead (Pb), nickel (Ni), and chromium (Cr) were determined using Atomic Absorption Spectrophotometry (AAS). Blank samples were prepared using the same procedure, excluding the test materials.

2.3. Determination of the Polycyclic Aromatic Hydrocarbon Content

2.3.1. Sample extraction

Ten grams (10 g) of each sample were transferred into amber glass bottles, followed by the addition of 10 g of anhydrous sodium sulphate (Na_2SO_4) to remove residual moisture. The contents were thoroughly mixed. A surrogate standard (1-chlorooctadecane) at a concentration of 300 µg/mL was added to each soil sample to monitor extraction efficiency. Subsequently, 30 mL of dichloromethane (DCM) was introduced as the extraction solvent. The bottles were tightly sealed and placed on a mechanical shaker, where they were agitated continuously for 5–6 hours at room temperature. After agitation, the samples were allowed to settle for one hour and then filtered through 110 mm filter paper into clean beakers. The resulting filtrates were concentrated by allowing them to evaporate overnight in a fume cupboard until a final volume of approximately 1 mL was obtained.

2.3.2. Sample clean – up

Sample clean-up was conducted using a glass column packed with silica gel. A slurry was prepared by dissolving 10 g of silica gel in 50 mL of DCM and carefully transferring it into the column. An additional 10 g of anhydrous sodium sulphate was layered on top to remove residual moisture. Pentane was used to condition the column. The concentrated extract was mixed with 20 mL of cyclohexane before being loaded into the column. Separation was achieved by eluting the column with 30 mL of pentane, followed by an additional 20 mL to enhance elution efficiency. The column was finally rinsed with 20 mL of DCM. The eluate was collected and allowed to evaporate overnight in a fume chamber at room temperature.

2.3.3. Separation and detection

PAH separation and quantification were carried out using an Agilent 6890N Gas Chromatograph equipped with a Flame Ionization Detector (FID). Three microlitres (3 µL) of the cleaned extract were injected into the GC system. Before sample injection, the microsyringe was rinsed repeatedly with DCM and sterilized with the sample extract to prevent contamination. The PAH compounds were separated

within the column and detected using the FID. Quantification was achieved by comparing chromatographic peaks with standard references, and results were expressed in milligrams per kilogram (mg/kg).

2.4. Enumeration of Hydrocarbon Utilizing Microorganisms

Mills *et al*. (1978) described an early most-probable-number method for enumerating petroleum-degrading microorganisms. The vapour-phase transfer and mineral-salts spread-plate procedure used in the present study followed the configuration reported by Ogbonna *et al*. (2020). The approach utilized sterile filter paper discs, saturated with 2 ml of sterilized crude oil, which were aseptically positioned on the inner cover of inverted Petri dishes before to incubation. One milliliter (1 ml) of the composite soil samples underwent serial dilution from 10^{-1} to 10^{-7}. Aliquots of 0.1 milliliters from 10^{-2} dilutions were inoculated into the medium in duplicate. To enumerate hydrocarbon-degrading bacteria, the medium was augmented with Miconazole Nitrate to inhibit fungal contamination. The mineral salts medium, augmented with streptomycin to suppress bacterial contamination, was employed to facilitate the enumeration of oil-degrading fungi. The plates were incubated at ambient temperature for five days before to enumeration.

2.5. Quality Control and Assurance

Quality assurance procedures were strictly adhered to throughout the study. Standard Operating Procedures (SOPs) were established for sample preparation, instrument calibration, data collection, and analytical processes. All equipment was calibrated in accordance with manufacturer specifications and standard laboratory protocols. Samples were carefully handled to prevent contamination, while proper procedures were followed for labelling, storage, and traceability.

2.6. Data Analysis

Data generated from the study were carefully entered, coded, and organized using Microsoft Excel 2019. Statistical analyses were performed using IBM SPSS version 21. One-way analysis of variance (ANOVA) was applied to test for significant differences among means at a 5% level of significance. Where significant differences were observed, Least Significant Difference (LSD) and Tukey's post-hoc tests were employed for multiple comparisons.

3. RESULTS AND DISCUSSION

3.1. Total Petroleum Hydrocarbons

The mean TPH concentrations recorded in sediment, periwinkle and crab are presented in Table 1. The concentration was highest in crab, followed by sediment and periwinkle. Crab contained a markedly higher TPH concentration (240.50 ± 0.20 mg/kg) than sediment (33.80 ± 10.09 mg/kg) and periwinkle (3.56 ± 0.03 mg/kg). The sediment value falls within the 16.6545–50.5040 mg/kg range reported for sediments from the Orashi River in Rivers State (Edori and Edori, 2021), indicating that the Bille result is comparable with concentrations documented in another petroleum-influenced river system in the Niger Delta. The contrast among matrices may reflect differences in exposure pathway, feeding behaviour, tissue composition, metabolic processing and contaminant retention. TPH has also been reported in brackish-water shrimp from the Nigerian coast, supporting the capacity of crustaceans to accumulate measurable petroleum hydrocarbon residues (Akinola *et al*., 2019). Nevertheless, the present data establish comparative tissue burdens rather than a bioaccumulation factor because concentrations in environmental media and tissues were not normalised to exposure or lipid content.

Table 1: Mean total petroleum hydrocarbon in sediment and biota from Bille in Rivers State

Parameter	Concentration (mg/kg)		
	Sediment	Periwinkle	Crab
TPH	33.80 ± 10.09	3.56 ± 0.03	240.50 ± 0.20

3.2. Polycyclic Aromatic Hydrocarbons

The individual PAH concentrations are shown in Table 2. The sum of detected PAHs was 0.20 mg/kg in sediment, 0.05 mg/kg in periwinkle, and 0.85 mg/kg in crab. Naphthalene occurred in all three matrices, while several other PAHs were detected only in crab. Naphthalene dominated the crab PAH profile at 0.57 ± 0.00 mg/kg. This concentration is close to the 0.517 ± 0.011 mg/kg reported in crab muscle from Woji Creek by Ihunwo and Ibezim-Ezeani (2021), although the total PAH concentration in the Bille crab (0.85 mg/kg) was lower than the 1.720 ± 0.320 mg/kg reported for crab muscle in that study. Pyrene was the principal PAH detected in sediment (0.16 ± 0.05 mg/kg), which is consistent with the tendency of more hydrophobic PAHs to associate with particulate matter and sediment organic phases (Abdel-Shafy and Mansour, 2016). The detection of benzo(a)pyrene in periwinkle and crab and of benzo(b)fluoranthene and benzo(k)fluoranthene in crab warrants continued monitoring because these compounds are among PAHs of toxicological concern. However, the measured concentrations alone do not constitute a dietary-risk assessment; such an assessment would require consumption rates, body-weight assumptions, toxicological reference values and analytical detection limits. The occurrence of PAHs in edible fish from the Bodo/Bonny River provides additional regional evidence that aquatic food resources can carry petroleum-derived PAHs (Ogbonna and Origbe, 2021).

Table 2: Mean concentration of PAHs in sediment and biota from Bille in Rivers State

Parameter	Concentration (mg/kg)		
	Sediment	Periwinkle	Crab
Naphthalene	0.02 ± 0.01	0.03 ± 0.00	0.57 ± 0.00
Acenaphthylene	BDL	BDL	0.02 ± 0.00
Acenaphthene	BDL	BDL	0.02 ± 0.01
Fluorene	BDL	BDL	0.01 ± 0.00
Anthracene	BDL	BDL	0.04 ± 0.01
Phenanthrene	BDL	BDL	0.05 ± 0.00
Fluoranthene	0.01 ± 0.00	BDL	BDL
Pyrene	0.16 ± 0.05	0.01 ± 0.00	0.05 ± 0.01
Benzo (a) anthracene	0.01 ± 0.00	BDL	BDL
Chrysene	BDL	BDL	BDL
Benzo (b) fluoranthene	BDL	BDL	0.03 ± 0.01
Benzo (k) fluoranthene	BDL	BDL	0.03 ± 0.00
Benzo (a) pyrene	BDL	0.01 ± 0.00	0.01 ± 0.00
Dibenz(a,h)anthracene	BDL	BDL	BDL
Indeno(1,2,3-cd) pyrene	BDL	BDL	BDL
Benzo (g,h,i) perylene	BDL	BDL	0.02 ± 0.00

3.3. Heavy Metals

The concentrations of cadmium, chromium, lead, nickel and zinc are presented in Table 3. Cadmium, chromium and lead were below detection limits in all matrices. Nickel was detected only in sediment, while zinc occurred in sediment, periwinkle and crab. The non-detection of cadmium, chromium and lead indicates that their concentrations were lower than the analytical detection limits used in this study; it should not be interpreted as proof of absolute absence because the limits were not reported. Zinc was highest in crab (0.85 ± 0.00 mg/kg), followed by sediment (0.39 ± 0.07 mg/kg) and periwinkle (0.22 ± 0.00 mg/kg). Crustaceans regulate and retain essential trace metals differently from molluscs and sediments, so matrix-specific differences are expected (Rainbow, 2007). Heavy-metal accumulation in sediment and aquatic organisms has been documented in the Bonny/New Calabar River estuary (Onojake *et al*., 2015) and in periwinkle from Elechi Creek (Davies *et al*., 2006). The comparatively low values in the present study may reflect differences in local inputs, sampling period, sediment chemistry, species, tissue analysed and analytical sensitivity. Nickel occurred only in sediment (0.15 ± 0.00 mg/kg), suggesting either limited transfer to the sampled tissues or concentrations below the method detection limit in the organisms.

Table 3: Mean concentration of heavy metals in sediment and biota from Bille in Rivers State

Parameter	Concentration (mg/kg)		
	Sediment	Periwinkle	Crab
Cadmium (Cd)	BDL	BDL	BDL
Chromium (Cr)	BDL	BDL	BDL
Lead (Pb)	BDL	BDL	BDL
Nickel (Ni)	0.15 ± 0.00	BDL	BDL
Zinc (Zn)	0.39 ± 0.07	0.22 ± 0.00	0.85 ± 0.00

3.4. Hydrocarbon-Utilising Microorganisms

The culturable populations of hydrocarbon-utilising bacteria and fungi in sediment are presented in Table 4. Bacterial counts were measurable, whereas fungal counts were below 0.01×10^2 CFU/g. The recovery of hydrocarbon-utilising bacteria at 0.25×10^2 CFU/g demonstrates the presence of culturable bacteria capable of growing under the hydrocarbon-selection conditions used. The fungal count was below 0.01×10^2 CFU/g. These counts are lower than populations reported in visibly crude-oil-contaminated soils by Ogbonna *et al*. (2020), although direct numerical comparison is constrained by differences in sample matrix, contamination history, dilution, medium composition and incubation conditions. The result supports the presence of a hydrocarbon-utilising microbial component in the sediment but, by itself, does not prove chronic contamination or quantify in situ natural attenuation.

Table 4: Mean microbial population in sediment from Bille in Rivers State

Parameter	Sediment
Hydrocarbon-utilising bacteria (cfu/g)	0.25×10^2
Hydrocarbon-utilising fungi (cfu/g)	$<0.01 \times 10^2$

4. CONCLUSION

The findings demonstrate the occurrence of petroleum-related contaminants in sediment, periwinkle and crab samples from Bille Creek. Crab contained the highest measured TPH and PAH concentrations, while zinc occurred in all three matrices. Cadmium, chromium and lead were below the analytical detection limits, and culturable hydrocarbon-utilising bacteria were recovered from the sediment. However, the findings do not independently establish bioaccumulation factors, chronic exposure, natural attenuation or quantitative dietary-health risk. Continued environmental monitoring, reporting of analytical detection limits and a formal seafood-consumption risk assessment are therefore recommended.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

REFERENCES

Abdel-Shafy, H. I., and Mansour, M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, 25(1), pp. 107–123.

Adeniji, A. O., Okoh, O. O., and Okoh, A. I. (2017). Petroleum hydrocarbon profiles of water and sediment of Algoa Bay, Eastern Cape, South Africa. *International Journal of Environmental Research and Public Health*, 14(10), Article 1263.

Akinola, J. O., Olawusi-Peters, O. O., and Akpambang, V. O. E. (2019). Ecological hazards of total petroleum hydrocarbon in brackish-water white shrimp, *Nematopalaemon hastatus* (Aurivillus, 1898). *Egyptian Journal of Aquatic Research*, 45(3), pp. 205–210.

Davies, O. A., Allison, M. E., and Uyi, H. S. (2006). Bioaccumulation of heavy metals in water, sediment and periwinkle (*Tympanotonus fuscatus* var. *radula*) from the Elechi Creek, Niger Delta. *African Journal of Biotechnology*, 5(10), pp. 968–973.

Dembicki, H. (2022). *Practical Petroleum Geochemistry for Exploration and Production* (2nd ed.). *Elsevier*.

Edori, E. S., and Edori, O. S. (2021). Temporal (monthly) distribution and variation of total petroleum hydrocarbons content in the water and sediments from Orashi River, Engenni, Rivers State, Nigeria. *Academic Journal of Chemistry*, 6(2), pp. 28–36.

Ihunwo, O. C., Ibezim-Ezeani, M. U. (2021). Distribution, source appropriation, and human health risk assessment of polycyclic aromatic hydrocarbons due to consumption of *Callinectes amnicola* from Woji Creek in Sambreiro River. *Turkish Journal of Fisheries and Aquatic Sciences*, 21(5), pp. 245–253.

Ihunwo, O. C., Onyema, M. O., Wekpe, V. O., Okocha, C., Shahabinia, A. R., Emmanuel, L., Okwe, V. N., Lawson, C. B., Mmom, P. C., Dibofori-Orji, A. N., and Bonnail, E. (2021). Ecological and human health risk assessment of total petroleum hydrocarbons in surface water and sediment from Woji Creek in the Niger Delta Estuary of Rivers State, Nigeria. *Heliyon*, 7(8), Article e07689.

Kuppusamy, S., Maddela, N. R., Megharaj, M., and Venkateswarlu, K. (2020). *Total Petroleum Hydrocarbons: Environmental Fate, Toxicity, and Remediation*. *Springer*.

Mills, A. L., Breuil, C., and Colwell, R. R. (1978). Enumeration of petroleum-degrading marine and estuarine microorganisms by the most probable number method. *Canadian Journal of Microbiology*, 24(5), pp. 552–557.

Ogbonna, D. N., Douglas, S. I., and Awari, V. G. (2020). Characterization of hydrocarbon-utilizing bacteria and fungi associated with crude-oil-contaminated soil. *Microbiology Research Journal International*, 30(5), pp. 54–69.

Ogbonna, D. N., and Origbe, M. E. (2021). Distribution of polycyclic aromatic hydrocarbons in surface water and fishes in Bodo/Bonny River, Nigeria. *International Journal of Environment and Climate Change*, 11(6), pp. 90–99.

Onojake, M. C., Sikoki, F. D., Babatunde, B. B., Akpiri, R. U., Akpuloma, D., and Omokheyeke, O. (2015). Bioaccumulation of heavy metals in two matrices of the Bonny/New Calabar River Estuary in Niger Delta, Nigeria. *Ocean Science Journal*, 50(2), pp. 203–208.

Rainbow, P. S. (2007). Trace metal bioaccumulation: Models, metabolic availability and toxicity. *Environment International*, 33(4), pp. 576–582.

Speight, J. G. (2017). *Rules of Thumb for Petroleum Engineers*. *John Wiley and Sons*.

Speight, J. G. (2020). Petroleum and oil sand exploration and production. In R. Malhotra (Ed.), *Fossil Energy* (2nd ed., pp. 17–45). *Springer*.