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Full Length Research Paper

Application of correlation analysis in assessment of relationships between mineral hydrocarbon levels and hydrocarbonoclastic bacteria count in tropical mangrove estuarine sediments

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Pearson's Product-Moment correlation analysis of the relationships between total hydrocarbon content (THC) and hydrocarbon utilizing bacteria population (HUB) in mangrove sediments and overlying water of the Qua Iboe Estuary, Nigeria was carried out. The results show that there was in general a close relationship between the two variables, but also a large amount of variation not explained by the analysis. The strongest positive relationships (p = 0.05) were found for THC in epipellic (intertidal) sediment and water (r = 0.65) while the relationship between THC in benthic (subtidal) sediment and water (r = 0.028) was weak, and positively insignificant (p = 0.05). A correlation of HUB densities on THC in benthic sediment was strong and positive (r = 0.91) but characterized by high HUB/HET ratios. That is, the strength of the relationship with respect to the oil degrading potentials of the bacterial was fairly low. In contrast, the relationships between the two variables in epipellic sediment (r = 0.66) was positively significant (p = 0.05) but with a substantial presence of heterotrophic bacteria. This implies that a quick-analysis of hydrocarbon content in epipellic sediment in relation to hydrocarbon utilizing bacteria densities following an oil spill is reliable. However, such analysis on benthic sediment may not be reliable in estuarine environment with chronic exposure to crude oil pollution. This is despite the wide distribution of HUB (82.4% in benthic sediment, 43.1% in epipellic sediment, and 33.3% in surface water) in the ecosystem as revealed by the coefficient of determinations (R^2) values.

Key words: Hydrocarbons, hydrocarbonoclastic bacteria, mangrove sediments, correlation analysis.

INTRODUCTION

The natural environment contains a wide variety of hydrocarbons of biogenic, petrogenic and pyrogenic origin (Boehm, 1981). Hydrocarbons are ubiquitous organic pollutants that contaminate the environment (Ashok et al., 1995). Most hydrocarbons, especially polycyclic aromatic hydrocarbons (PAHs) are genotoxic, mutagenic, carcinogenic and can persist in the environment (O'Clair et al., 1996; Moles and Norcross, 1998). These hydrocarbons are incorporated into the sediments where they can persist for years (Prahl and Carpenter 1983; Babcock et al., 1996; O'Clair et al., 1996). The differential effects of hydrocarbons on the epipsammic and benthic microalgae of estuaries sandy beach have also been reported by Essien and Antai (2005) and Uehlinger et al. (1997).

Although hydrocarbons may undergo phytolysis, chemical oxidation and volatilization, microbial degradation is the major process affecting their fate in the environment (Ashok and Saxena, 1995; Ashok et al., 1995). Despite decades of study of effects of crude oil on fishes and aquatic microbiota, the relationship between hydrocarbon content and oil degrading microorganisms in sediment is shrouded with uncertainty, and little is known about the relationship in estuarine mangrove swamp of the oil impacted Niger Delta region of Nigeria. The Qua Iboe estuary experiences cases of aquatic perturbations arising from anthropogenic inputs such as oil spills. The most

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recent case of oil pollution occurred on 22nd November, 2003 from a leakage in the estuarine environment (Essien and Antai, 2005). The spillage occurred during the period of our investigation on the relationship between total hydrocarbon content (THC) and counts of hydrocarbon utilizing bacteria (HUB) in the pelagic water column, intertidal (epipellic) and subtidal (benthic) sediments of the Qua Iboe Estuary mangrove swamp. This information is needed for optimizing environmental monitoring programs in the Niger Delta of Nigeria.

MATERIALS AND METHODS

Study area

The Qua Iboe Estuary, (Figure 1) a mesotidal estuary is located in the coastal zone of Nigeria. The estuary lies within latitude 4° 30′ to 4° 45′ N and longitude 7° 31′ to 8° 00′E. Although sandy beaches are known to develop in some portions of the estuary, most of them are fringed with tidal mud flats and oligotrophic mangrove swamp (Essien and Ubom, 2003). The estuary constitutes a major inlet into the land and is often utilized by the inhabitants of the oil producing communities of the Niger Delta as the main transport route. It is a multi use resource with fishery as the most dominant. The estuary also serves as the receiving water body for domestic and industrial wastes especially petrochemical wastes.

Sampling

Monthly sampling of the intertidal (epipellic) and subtidal (benthic) sediments, and the overlying surface water was carried out during the wet (June 2003 - September, 2003) and dry (November 2003 - February 2004) seasons. A short core sampler was used to retrieve epipellic sediment with undisturbed sediment-water interfaces. Subtidal or benthic sediment samples were obtained with the aid of a Shipek grab sampler. During each sampling, samples were obtained from different locations, homogenized and the subsamples carefully transformed into clean glass containers, and preserved in ice-cooled boxes. All water samples were collected from the surface (10 - 25 cm) in sterile glass bottles. The containers were opened to fill and closed below the water. All containers were rinsed at least three times with the water being sampled before collection.

Both samples were transported to the laboratory for analysis. A total of 72 samples, comprising 24 samples, each of water, epipellic and benthic sediments were collected and analyzed within 12 h of collections.

Determination of total hydrocarbon contents of sediments and water samples

Analysis for total hydrocarbons content (THC) followed a standard procedure (APHA 1998; Radojevic and Bashkin, 1999). Samples for total hydrocarbon analysis were serially extracted with 100 mL methyl isobutyl ketone (MIBK) analar grade and the extracts allowed to settle. Each extract was centrifuged for 5 min and decanted. The volumes of the supernatant were reduced to about 5 ml over a rotary evaporator maintained at 20°C. For the determination of total hydrocarbon content, a Gas Chromatograph was employed, using 1 µL of aliquot of each extract and the total peaks obtained were converted to weight using hydrocarbons standard calibration (FEPA, 2001). Duplicates and method blanks were treated identically using the same reagents to test for the precision, accuracy and reagent purity used in the analytical procedures.

Enumeration of heterotrophic and hydrocarbon utilizing bacteria

The counts of heterotrophic bacteria (HET) and Hydrocarbon utilizing bacteria (HUB) in the sediment and water samples were enumerated by the pour plate and surface spreading techniques respectively (Harrigan and McCance, 1990) using diluents prepared with 25% Ringer's solution and cultured in nutrient agar (Difco) and oil-mineral salt medium (MSM). The media were supplemented with cycloheximide (100 µg/ml and benomyl (50 µg/ml) to prevent fungal growth (Kinkel et al., 1995). The crude oil used was sterilized by millipore (0.45 µ pore size), filtered and stored in sterile bottles. 1 ml quantities of each dilution were prepared and incubated at 28 $\pm$ 2°C for 5 days. At the end of incubation, counts were performed on culture plates, which showed counts between 30 and 300 colonies (Harrigan and McCance, 1990; Amadi and Braide, 2003). This method has previously been adopted by Antai and Mgbomo (1989) Ijah and Ukpe (1992), Itah and Essien (2001), Essien et al. (2003) and Itah and Essien (2005).

Correlation analysis

The relationship in THC between the pelagic column and sedimenttary habitats was determined. Also calculated was the extent to which hydrocarbonoclastic bacteria densities were related to different hydrocarbon measurements. These were evaluated with Pearson's correlation analysis.

Correlation analyses were performed with the Analyze–It + General 1.73 statistical software®. To establish the relationship between total hydrocarbon content and counts of hydrocar-bonoclastic bacteria in sediments, Pearson Product-Moment correlation (r) analyses were done on Log-transformed estimates of densities of hydrocarbon utilizing bacteria (Log CFU/g) at $P<0.05$. The coefficient of determinations (R^2) was also computed to determine the extent of distribution (Pearson and Hartley, 1958) of the hydrocarbon and hydrocarbon utilizing bacteria in sediments. The values are reported in percentages of R^2.

RESULTS AND DISCUSSION

Results presented in Table 1 revealed a THC range of 1.01 to 8.34 mg/kg in the mangrove swamp water. Higher values of THC were recorded for the epipellic and benthic sediments. This may be ascribed to the physicochemical properties of sediments, which give the hydrocarbons a strong affinity for particulate or organic matter. Therefore, sediments can form a sink for hydrocarbons.

Although hydrocarbons have a tendency to be absorbed on sediments, it is obvious that one important routes of exposure for sediments fauna and flora is via the interstitial water (Law, 2000). This implies that pelagic, marine and brackish water organisms such as fish receive the hydrocarbon exposure direct from the water phase. Therefore, analyzing the THC of the surface water is vital in the evaluation of risk associated with exposure

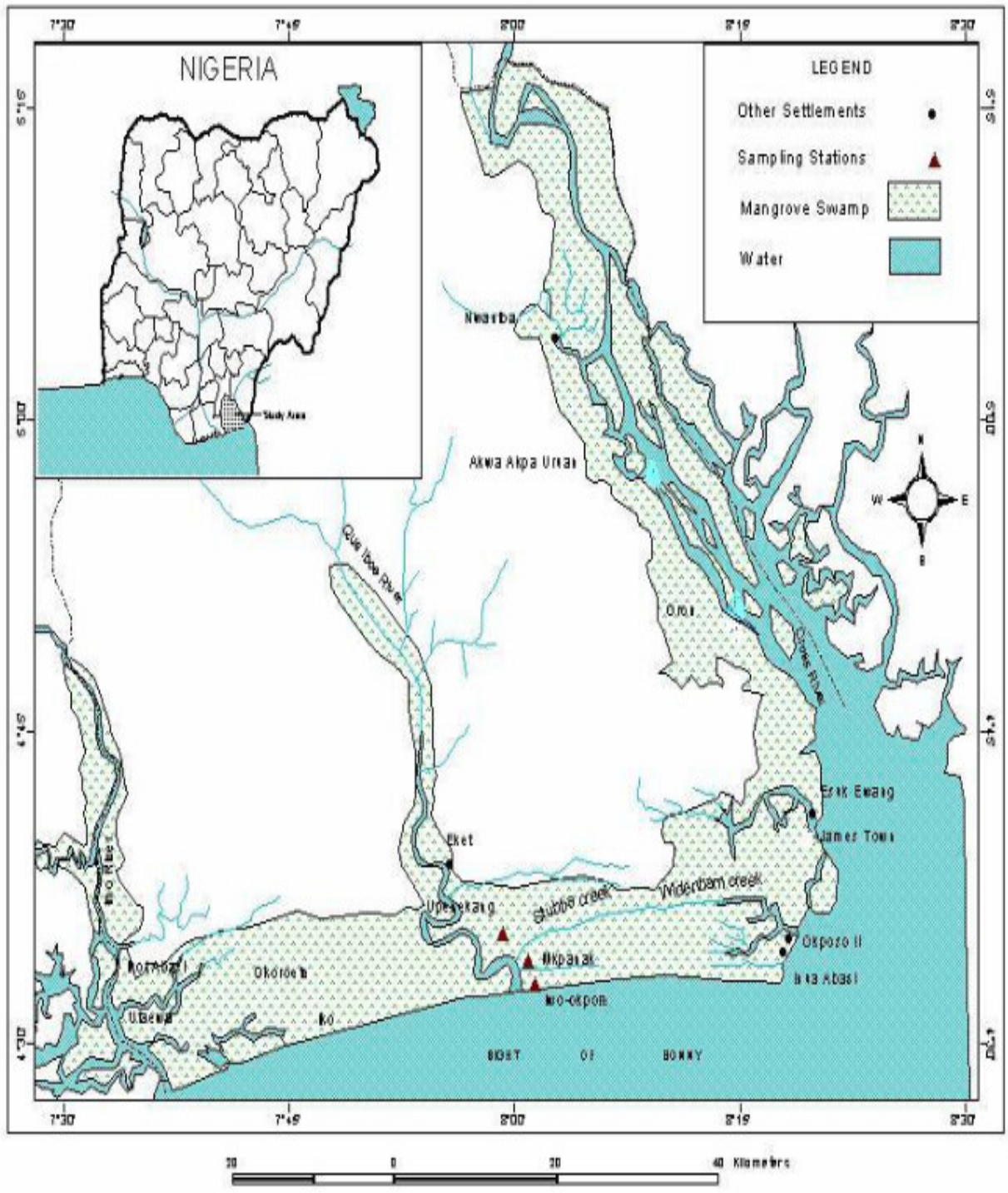

Figure 1. Map of the Niger Delts region of Nigeria showing the Iwo-Okpom, Upenekang and Mkpanak sampling sites within the Qua Iboe Estuary. Inset: Map of Nigeria showing the study area.

exposure via this route (Law et al., 1997).

In line with this attribute, high levels of hydrocarbons were detected in the intertidal and subtidal sediments of the mangrove ecosystem. The levels ranged from 18.01 to 210.23 mg/kg dry w.t. of intertidal sediment, with significantly higher values in samples collected in December /January, suggesting a kind of seasonal effect on total hydrocarbon content in intertidal sediment (Table 2), and similarly from 5.00 to 210 mg/kg of subtidal sediment (Table 3). The various hydrocarbon levels showed distinct variations, with higher values in the epipellic sediment during the rainy season. This may be ascribed to in-

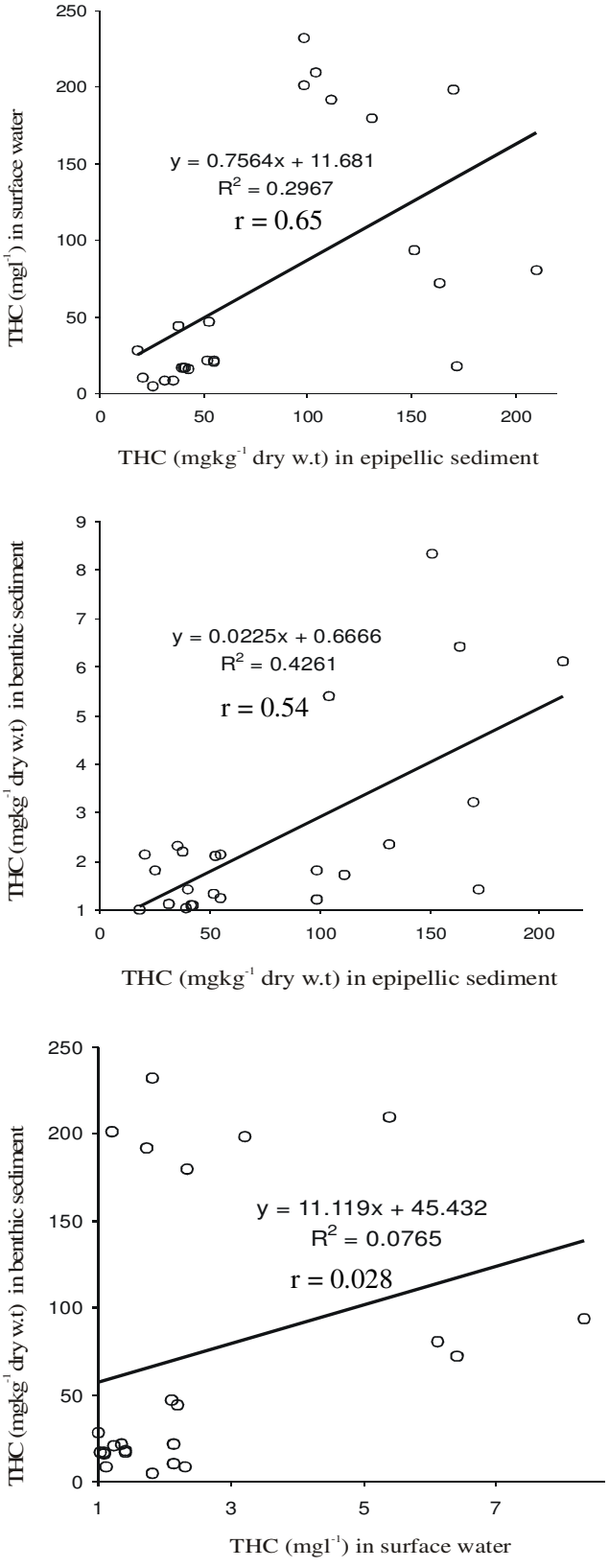

Figure 2. Relationship between THC level in surface water and sediment of the Qua Iboe estuary mangrove swamp.

crease in anthropogenic sources of pollutants carried in runoff in addition to biogenic hydrocarbons emanating from the waxy coatings of the forest leaves (Boehm, 1981). Much more THC were found in the sediments.

As depicted in the correlation plots, a positive and significant (p = 0.05) relationship was observed between THC in epipellic and surface water (r = 0.65) (Figure 2a) and also between THC in benthic and epipellic sediments (r = 0.54) (Figure 2b). But a positive but or insignificant (p = 0.05) relationship was established between THC in benthic and surface water (r = 0.028) (Figure 2c). It is therefore suggestive that the levels of THC in surface water and benthic sediment in estuarine ecosystem are seriously influenced by its concentration in the intertidal sediment. This confirms that the bottom sediment is a sink of organic pollutants. Such pollutants may be present in the bottom sediment without being detected in the overlying surface water. The highest levels of THC were recorded in December, 2003 and January, 2004 respectively during dry season, for the intertidal and subtidal sediments. These occurred after the November 22, 2003 crude oil spillage from a facility of an oil company located within the estuarine environment. The impact was immediately noticed (within 3 weeks) in the tidal mudflats as reflected by the high HUB/HET ratio recorded in December, 2003, but much later (7 weeks) in the bottom sediment. The bottom depth may influence this and longer time required for the transportation (via water column) and deposition of oil pollutants at the bottom sediment. The high HUB/HET ratio recorded in benthic sediment may be associated with the slow oil degrading activities of the oil degraders despite the presence of crude oil.

Low hydrocarbon utilizing bacteria / heterotrophic bacteria (HUB/HET) ratios were recorded for the epipellic sediment (Table 2). This may be due to proliferation of heterotrophic bacteria. Increase in the activities of the oil degraders in the oxic intertidal sediment would definitely enhance the growth of the non-oil degraders. This implies that low HUB/HET ratio is an indication of high microbial activities and plausibly ‘‘a hasten’’ natural remediation after crude oil pollution. On the other hand the high HUB/HET ratios recorded for the benthic sediment (Table 3) may be associated with low heterotrophic activities of oil degraders in the near “hypoxic” bottom sediment even with the presence of crude oil. It indicates that the bottom sediment harbour high number of culturable bacterial populations with low potential for oil degradation or a high preference for other substrates (Bachoon et al., 2001). Such obvious low density of heterotrophic bacteria signifies stress. Such stress could be attributed to the low dissolved oxygen level and anoxic condition in bottom sediment. This raised the question of the efficiency of microbial degradation of persistent hydrocarbons in subtidal sediments. The efficiency depends on the needs and the ability of microorganisms to utilize the hydrocarbon, the favourable oxidation mechanism involved or the type of

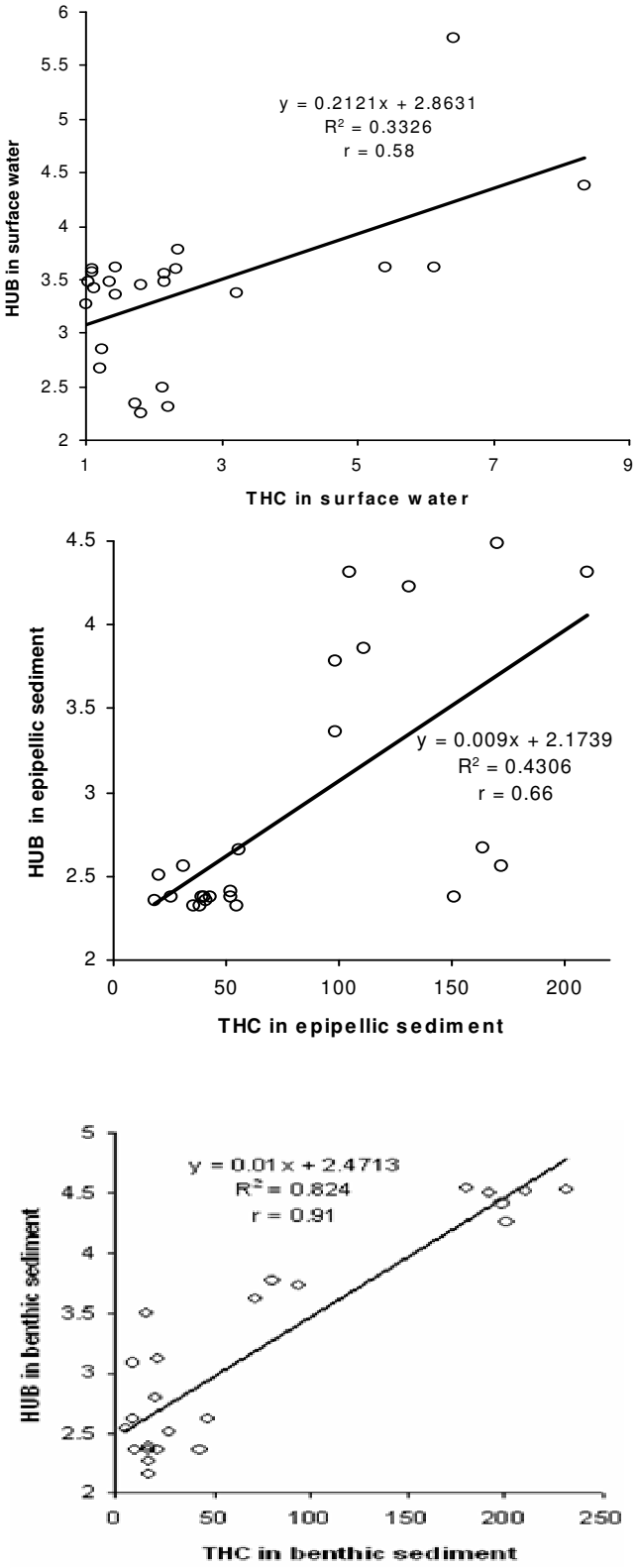

Figure 3. Relationships between total hydrocarbon content (THC) and hydrocarbon utilizing bacteria (HUB) in surface water, epipellic and benthic sediments of the Qua Iboe estuary mangrove swamp.

organism present that can utilize the hydrocarbons (Ibiebele and Braide, 1987).

The hydrocarbon utilizing bacteria (HUB) counts did not exhibit a linear relationship with THC concentrations in mangrove sediments and water samples (Figure 3). This non-linearity between HUB and THC has been reported by Atlas (1981), Amadi and Braide (2003) and Gruttner and Jensen (1983). However, the high concentrations of hydrocarbons recorded in sediments during the dry season (between December, 2003 and February, 2004) and high counts of hydrocarbon utilizing bacteria suggest a response of the microbial population in the environment to seasonal variation. The relationships were positively very significant ($r = 0.91$, $p < 0.05$) in benthic sediment. A positive significant relationship was also recorded between THC and HUB in epipellic sediment ($r = 0.66$), and surface water ($r = 0.58$). The latter may be attributed to tidal influence. The coefficient of the determinations (R^2) also revealed that the distribution of oil degraders in the mangrove ecosystem was more extensive in the benthic sediment (82.40%) than in the epipellic sediment (43.06%) and surface water (33.30%).

Conclusion

Intertidal (epipellic) and subtidal (benthic) sediments in riverine lacustrine and palustrine biotopes are long-term repositories of the residues which petroleum hydrocarbon release to the environment.

Statistical analyses of results obtained from the present study have revealed a positive and significant relationship between the densities of hydrocarbon utilizing bacteria and hydrocarbon contents in intertidal sediments. The relationship although significantly positive, was characterized with high HUB/HET ratios in subtidal sediment. Previous or cumulative loads of slow-degrading hydrocarbons would certainly hamper the relationship. This raises doubts on the reliability of this attribute as indicator of crude oil impact on benthic sediment in a tropical estuarine ecosystem repeatedly exposed to crude oil spillages. The concept may be quite useful in a quick and short-term assessment of impact on intertidal sediment; however any delay in the monitoring exercise may affect the quality of results. Similarly, the values of THC, HUB, HET and ratio of HUB/ HET are influenced by seasonal variations and period of sample collection. This is because THC-HUB relationship may be affected by other factors (evaporation, dissolution and photo-oxidation) responsible for the removal of oil in a natural ecosystem. But in subtidal or benthic sediment the THC-HUB relationship may not be reliable in ecosystems frequently contaminated with crude oil.

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Table 1. Total hydrocarbon contents and hydrocarbonoclastic bacteria counts in surface water samples from the Qua Iboe Estuary mangrove swamp.

Time/No. of samples	THC	HUB	HET	HUB/HET-ratio x100
June 2003 – THC 1	2.14	$3.1x10^3$	$1.4x10^4$	22.10
– THC 2	1.82	$2.8x10^3$	$2.4x10^4$	11.70
– THC 3	1.01	$1.9x10^3$	$3.1x10^4$	6.10
July 2003 – THC 4	1.42	$2.3x10^3$	$2.4x10^5$	0.90
– THC 5	2.31	$4.1x10^3$	$3.4x10^5$	1.20
– THC 6	2.14	$3.6x10^3$	$4.2x10^5$	0.90
Aug. 2003 – THC 7	1.09	$4.1x10^3$	$4.4x10^5$	0.90
– THC 8	1.02	$3.1x10^3$	$4.6x10^5$	0.70
– THC 9	1.42	$4.2x10^3$	$4.4x10^5$	1.00
Sept. 2003 – THC 10	1.10	$3.8x10^3$	$4.3x10^5$	0.90
– THC 11	1.12	$2.7x10^3$	$6.3x10^5$	0.40
– THC 12	1.34	$3.1x10^3$	$3.5x10^5$	0.90
Nov. 2003 – THC 13	1.24	$7.1x10^2$	$1.7x10^4$	4.20
– THC 14	2.11	$3.12x10^2$	$2.1x10^4$	1.50
– THC 15	2.20	$2.10x10^2$	$1.9x10^4$	1.10
Dec. 2003 – THC 16	8.34	$2.4x10^4$	$6.2x10^4$	0.40
– THC 17	6.42	$5.7x10^3$	$2.7x10^4$	21.10
– THC 18	6.11	$4.2x10^3$	$5.7x10^4$	7.40
Jan. 2004 – THC 19	5.40	$4.2x10^3$	$1.08x10^4$	38.90
– THC 20	2.34	$6.0x10^3$	$2.31x10^4$	26.80
– THC 21	3.21	$2.4x10^3$	$2.2x10^4$	10.90
Feb. 2004 – THC 22	1.82	$1.8x10^2$	$7.21x10^4$	2.00
– THC 23	1.72	$2.2x10^2$	$5.6x10^4$	0.40
– THC 24	1.21	$4.7x10^2$	$7.2x10^4$	0.60

Table 2. Total hydrocarbon contents and hydrocarbonoclastic bacteria counts in epipellic sediment samples from the Qua Iboe Estuary mangrove swamp.

Time/No. of samples	THC	HUB	HET	HUB/HET-ratio x100
June 2003 – THC 1	20.21	$3.2x10^2$	$3.4x10^5$	0.094
– THC 2	25.11	$2.4x10^2$	$4.1x10^5$	0.059
– THC 3	18.01	$2.3x10^2$	$2.7x10^5$	0.085
July 2003 – THC 4	40.00	$2.4x10^2$	$4.4x10^5$	0.055
– THC 5	35.00	$2.1x10^2$	$2.6x10^5$	0.081
– THC 6	55.00	$4.6x10^2$	$5.1x10^5$	0.090
Aug 2003 – THC 7	41.21	$2.3x10^2$	$3.5x10^6$	0.006
– THC 8	39.21	$2.4x10^2$	$3.3x10^6$	0.007
– THC 9	172.00	$3.6x10^2$	$3.4x10^6$	0.011
Sept. 2003 – THC 10	42.34	$2.4x10^2$	$3.7x10^6$	0.006
– THC 11	31.24	$2.1x10^2$	$2.3x10^6$	0.009
– THC 12	51.82	$2.6x10^2$	$2.7x10^6$	0.009
Nov. 2003 – THC 13	54.71	$2.1x10^2$	$3.4x10^5$	0.062
– THC 14	52.11	$2.4x10^2$	$2.4x10^5$	0.000
– THC 15	37.74	$4.7x10^2$	$3.4x10^5$	0.038
Dec. 2003 – THC 16	151.24	$2.1x10^4$	$2.7x10^5$	7.778
– THC 17	163.24	$1.7x10^4$	$3.1x10^5$	5.484
– THC 18	210.23	$2.1x10^4$	$2.4x10^5$	8.750
Jan. 2004 – THC 19	104.21	$2.1x10^4$	$2.4x10^4$	87.5
– THC 20	131.21	$1.7x10^4$	$3.7x10^4$	45.9

Table 2. Contd.

– THC 21	170.21	$3.1x10^3$	$3.1x10^4$	10.0
Feb. 2004 – THC 22	98.31	$2.3x10^3$	$4.2x10^4$	5.476
– THC 23	111.02	$7.2x10^3$	$7.1x10^4$	10.14
– THC 24	98.31	$6.2x10^3$	$2.4x10^4$	25.83

Table 3. Total hydrocarbon contents and hydrocarbonoclastic bacteria counts in benthic sediment samples from the Qua Iboe Estuary mangrove swamp.

Time/No. of samples	THC	HUB	HET	HUB/HET-ratio x100
June 2003 – THC 1	10.00	$2.3x10^2$	$3.1x10^3$	7.40
– THC 2	5.00	$3.4x10^2$	$4.2x10^3$	8.10
– THC 3	22.00	$3.2x10^2$	$3.8x10^3$	8.40
July 2003 – THC 4	17.12	$1.8x10^2$	$4.2x10^3$	4.30
– THC 5	8.52	$4.2x10^2$	$5.2x10^3$	8.10
– THC 6	21.33	$2.3x10^2$	$6.3x10^3$	3.60
Aug.2003 – THC 7	16.82	$2.4x10^2$	$2.4x10^4$	10.00
– THC 8	17.32	$1.4x10^2$	$3.3x10^4$	0.40
– THC 9	17.33	$2.3x10^2$	$7.2x10^4$	0.30
Sept. 2003 – THC 10	16.12	$3.1x10^3$	$2.8x10^6$	0.10
– THC 11	8.32	$1.2x10^3$	$4.2x10^6$	0.10
– THC 12	21.33	$1.3x10^3$	$3.5x10^6$	0.10
Nov. 2003 – THC 13	20.42	$6.2x10^2$	$3.8x10^4$	1.60
– THC 14	47.01	$4.1x10^2$	$3.2x10^4$	1.30
– THC 15	43.71	$2.3x10^2$	$4.2x10^4$	0.50
Dec. 2003 – THC 16	93.24	$5.2x10^3$	$4.1x10^5$	1.30
– THC 17	72.36	$4.2x10^3$	$2.2x10^5$	1.90
– THC 18	80.44	$5.2x10^3$	$3.9x10^5$	1.30
Jan. 2004 – THC 19	210.11	$3.2x10^4$	$6.6x10^6$	0.50
– THC 20	180.11	$3.4x10^4$	$3.4x10^6$	1.00
– THC 21	198.21	$2.6x10^4$	$3.3x10^6$	0.80
Feb. 2004 – THC 22	232.00	$3.3x10^4$	$4.7x10^6$	0.70
– THC 23	192.11	$7.1x10^4$	$3.2x10^6$	0.90
– THC 24	201.11	$1.8x10^4$	$4.2x10^6$	0.40

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