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Hydrocarbon Degrading Bacteria as Indicator of Petroleum Pollution in Ismailia Canal, Egypt

[1]M. Youssef, [1]G.E. El-Taweel, [2]A.Y. El-Naggar,
[1]Sh. E. El-Hawary, [3]M.A. El-Meleigy and [3]S.A. Ahmed

[1]Water Pollution Research Department, National Research Centre, Dokki, Giza, Egypt
[2]Analysis and Evaluation Department, Egyptian Petroleum Research Institute, Egypt
[3]Botany and Microbiology Department, Faculty of Science (for Girls), Al-Azhar University, Egypt

Abstract: Water and sediment samples were collected from four sites extending for about 20 Km from the beginning of Ismailia canal. The samples were collected seasonally for the chemical screening of hydrocarbon pollutants via capillary gas chromatography and collected monthly for the bacterial assessment during the period from August 2007 to July 2008. The results demonstrated that all sites contain a mixed source of biogenic and petrogenic hydrocarbons but the biogenic hydrocarbons were stronger than the petrogenic hydrocarbons in the beginning of the canal while in the other three sites especially at petroleum companies, the pollution source was largely of petroleum-derived materials. The average count of total heterotrophic bacteria in water samples was 10^4 CFU/ml, while in sediment samples was 10^5 CFU/ml. The average density of crude oil utilizing bacteria in water samples was 10^3-10^4 MPN/100ml, while in sediment samples was 10^5 MPN/100ml. The average of hydrocarbon degrading bacteria within the average of heterotrophic communities was fluctuated between 0.2% to 2.1%. The highest average percent of degrading bacteria in water and sediment samples was found at petroleum companies. So the percentage of hydrocarbon degrading bacteria was taken as a sensitive index of environmental exposure to hydrocarbon pollutants.

Key words: Biogenic hydrocarbons · Petrogenic hydrocarbons · Ismailia canal · Water · Sediment · Capillary gas chromatography

INTRODUCTION

Petrochemical industries and petroleum refineries generate large amounts of priority pollutants. The major pollutants found in these industries are petroleum hydrocarbons [1]. It is estimated that between 1.7 and 8.8 million metric tons of oil are released into the world's water every year [2, 3], 90% of which is directly related to human activities including deliberate waste disposal [3, 4]. It is also estimated that about 30% of the spilled oil enters freshwater systems [3]. Although Ismailia canal is a branch of River Nile in the north of Cairo the quality of its water is totally different from the River Nile water [5]. The Ismailia canal represents the most distal downstream of the main River Nile. Thus its water contains all the proceeded pollutants discharged into the Nile. In addition to this, the canal is endangered from unwise, direct and indirect activities in the surrounding environments (e.g. collapses of the canal bank due to the seepage effluent, close distribution of farmer's houses to the canal banks, industrial zone located directly on the canal bank). The upstream portion of the Ismailia canal (from Cairo to Abu - Zaabal, western side) includes the largest industrial zones in the region (Shupra El-Kheima, Musturod and Abu – Zaabal industrial zones). The canal acts as an effluent stream along these sites [6]. Also, discharging of petroleum and petroleum derivatives industrial wastewater from petroleum factories to the canal pollutes its water with polynuclear aromatic hydrocarbons (PAHs), alkanes and aromatic hydrocarbons [5].

Pollution of aquatic environments and soil is a worldwide problem that can result in uptake and accumulation of toxic chemicals in food chains and harm to the flora and fauna of affected habitats [7]. When crude oil or crude oil products enter the water environment, they adhere rapidly onto the particles found in water columns [3]. So, surficial sediment of the river or canal is an important medium for petroleum hydrocarbon accumulation [8].Sediments can serve as long-term sinks for oil contaminants if they are not degraded. When this

Corresponding Author: G.E. El-Taweel, Water Pollution Research Department, National Research Centre, Dokki, Giza, Egypt

occurs, the sediments subsequently become the sources of contamination of the water upon release through sediment suspension. Therefore, a cleanup of water column without even cleaning the sediment (if contaminated) is considered a no good job [3].

The first line of defense against oil pollution in the environment is the microbial population [9]. With the recent concern for petroleum contamination a consideration has been given to indicators of hydrocarbon levels. Walker and Colwell [10] have demonstrated that the numbers of petroleum degrading microorganisms in waters and sediment of Chesapeake Bay were related to concentration of oil present. Likewise, findings of other workers have supported the concept of using hydrocarbonoclastic microorganisms as indicators of hydrocarbons [11].

Therefore the purpose of this study was, first to determine petroleum hydrocarbon polluted sites in Ismailia canal and second to examine the validity of using hydrocarbon utilizing bacteria as indicator of petroleum level in Ismailia canal.

MATERIALS AND METHODS

Sampling Sites and Collection: Water and sediment samples were collected from four sites extending for about 20 Km from the beginning of Ismailia canal. The sampling sites were: site 1 on the mouth of Ismailia canal (taken as a reference point), site 2 at intake of Musturod drinking water treatment plant before petroleum companies, site 3 at disposal pipe of petroleum companies and site 4 after about 8 km north the disposal pipe. Water and sediment samples were collected from the four sites except at sampling site 1 there was difficulty in the collection of sediment that is because this site was located in the beginning of the canal. The samples were collected seasonally from the four sites for the chemical screening of pollutants and collected monthly for the bacterial assessment during the period from August 2007 to July 2008.

Samples Collected for Chemical Assessment: Water and sediment samples were collected in duplicates in a wide-mouth dark glass bottles that have been washed with soap, rinsed with water and finally rinsed with solvent to remove any residues that might interfere with the analysis. Water samples acidified to pH 2 with 1:1 HCl and sediment samples acidified with 1mL conc. HCl/80 g sample. All samples were preserved in ice box and transferred to the laboratory for hydrocarbon extraction and chromatographic analysis [12].

Samples Collected for Bacterial Assessment: Water and sediment samples were collected in duplicates in a screw capped sterile glass bottles and preserved in ice box and transferred to the laboratory within 1- 2 hours [12].

Screening of the Hydrocarbon Pollutants via Capillary Gas Chromatography: Chemical hydrocarbon pollutants in the water samples were solvent extracted according to the procedure of [13, 14], while the sediment samples were solvent extracted following the EPA 3540C method [15]. The extracted hydrocarbons from water and sediment samples were analyzed via capillary gas chromatography (CGC) [16].

Enumeration of the Total Viable Heterotrophic Bacteria: Total viable heterotrophic bacterial count in the monthly collected water and sediment samples from sites under study was determined at 30°C by spread – plating technique using plate count agar medium. 10 g of each sediment sample was suspended in 90 ml sterile physiological saline (0.90 % NaCl) and vortexing, before being diluted up to 10^{-5} and 10 ml of each water sample was diluted in 90 ml sterile physiological saline. 0.1 ml of the serially diluted water and sediment samples were plated on plate count agar medium supplemented with 50 µg/ml nystatin [17] to suppress the growth of fungi. Duplicate plates were incubated at 30°C for 24 h before the colonies were counted [18].

Enumeration of Hydrocarbon Degrading Bacteria: Crude oil utilizing bacteria in the monthly collected water and sediment samples from sites under study were enumerated by the most probable number (MPN) method. The Egyptian crude oil which used in this study was obtained from Egyptian Petroleum Research Institute (EPRI).

Ten microliters of the crude oil was added to each well of the pre-sterilized 96–well microtiter plates and 200 µl Bushnell – Hass medium (MSM) was added to each well as the growth medium. The formulation per liter of medium is 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 1.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g NH_4NO_3, 0.05 g $FeCl_3$ and the pH was adjusted to 7-7.2 with HCl [19]. Tenfold serial dilution for water and sediment samples, ranging from 10^{-1} to 10^{-5}, was performed and the plates were inoculated by adding 20 µl of each dilution to one of the 12 rows of eight wells (35 wells per sample). One row of wells remained un-inoculated to serve as sterile controls and one row was inoculated with the appropriate sample dilution but without hydrocarbon source to serve as controls. The plates were wrapped in plastic bags and incubated at room temperature (20°C - 22°C) in the dark for 3 weeks then 50 µl INT (Iodonitro tetrazolium chloride 0.3 g/100 ml) was added to each well and the plates were incubated for another 18- 24h. Bacterial growth in each well was judged by turbidity and confirmed by the appearance of a purple color. The color change indicated the reduction of INT by bacteria respiring in this well and that well was judged as a positive well. Positive wells were scored and density of bacteria was calculated by using MPN tables for the five tube format [19, 20].

RESULTS AND DISCUSSION

Screening of the Hydrocarbon Pollutants via Capillary Gas Chromatography: Capillary gas chromatography (CGC) is a well suited analytical tool for the separation and quantification of minute amounts of components from complex mixtures. The extracted hydrocarbons from water and sediment samples within the area under study were subjected to CGC to demonstrate the composition of pollutants and to identify the sources of these pollutants. The gas chromatogram appears as a number of individual peaks and a hump. These individual peaks represent n-alkanes and iso-alkanes. The hump appears as an elevation of the baseline and represents the unresolved complex mixture (UCM). This UCM is due to a structurally complex mixture of hydrocarbons [21, 22].The GC profiles of recovered hydrocarbons from the collected water and sediment samples show a regular distribution of n-alkanes in the rang n-C_{14} to n-C_{42} in water samples in the four seasons while it was found in the range n-C_{14} to n-C_{46} in sediment samples.

There are three major types of hydrocarbons are generally found in the environment: petrogenic, i.e. crude oil and its refined products; biogenic, i.e. hydrocarbons generated by biological processes and pyrogenic, i.e. compounds generated in combustion processes [23, 24]. The petroleum hydrocarbon pollution sources could be identified by using many parameters of n-alkanes such as carbon preference index (CPI) [8, 25], UCM [21, 22], n-C_{17}/pristane (n-C_{17}/Pr) and n-C_{18}/phytane (n-C_{18}/Ph) [26].

The carbon preference index (CPI) which represents the relative abundance of odd numbered linear alkanes vs. even numbered linear alkanes [27] exhibit a value around one for samples containing petroleum hydrocarbons [26]. Our results (Table, 1) showed that, the average values of CPI (n-C_{14} - n-C_{46}) for the water samples are close to unity in sites 2, 3 and 4 (0.72, 0.75 and 0.72 respectively) but is greater than unity in site 1 (1.16). This indicated that the biogenic hydrocarbons in site 1 are greater than their concentrations in the other three sites and the hydrocarbons in sites 2, 3 and 4 are derived from inputting of petroleum compounds.

The CPI average values in sediment samples in sites 2, 3 and 4 (1.52, 1.75 and 1.61, respectively) are greater than one. This may be due to that river sediments continuously receive fresh organic matter like plant residues, detritus of dead algae and diatoms as reported by Quantin *et al.* [28]. Also, Oudot *et al.* [29] reported that terrestrial, sedimentary, marine invertebrates and plankton biogenic alkanes usually exhibit a CPI >1 [30, 31], where as petroleum alkanes have a CPI close to unity. Mille *et al.* [26] in their study on hydrocarbons sources in the Bay of Forte de France reported that all samples containing petroleum hydrocarbons have a CPI around one.

UCM is another useful parameter to predict the sources of hydrocarbons. Readman *et al.* [21] and Martins *et al.* [22] reported that UCM is normally associated with petroleum residues. Also, Nievas *et al.* [32] and Maioli *et al.* [33] reported that UCM is used as an indicator of petrogenic environmental inputs due to its large persistence after accidental or chronic oil spill.

The results in Table, 1 demonstrated that, the average values of UCM weight percentages for water samples were 20.6, 24.2, 50.3 and 32.0 for sites 1, 2, 3 and 4 respectively, while in sediment were 69.2, 79.3 and 51.9 for sites 2, 3 and 4, respectively. The highest average value was found at site 3 in both water and sediment while the lowest value was found at site 1. These results proved that site 3 (at petroleum companies) comparing to site 1 (at the beginning of the canal) is chronically polluted by petroleum hydrocarbons as documented by Bixiong *et al.* [8], who reported that, the accumulation of UCM in high concentrations provides evidence of long- term petroleum contamination.

The average value of UCM weight percentage in site 2 water (before petroleum companies) nearly close to its value in site 1 while in site 4 water (after nearly about 8 km from site 3) the value is lower than site 3, this may be due to the dilution factor. Also, in sediment the average value of UCM weight percentage in site 4 is lower than their values in site 3 and in site 2.These results coincide with Tarek and Ali [5], they reported that, the concentrations of n-alkanes, aromatic hydrocarbons, PAHs and chlorinated hydrocarbons increased after oil companies and this due to drainage of the oil and industrial waste of other companies into the canal water and the concentrations decreased after that due to dilution.

Table 1: The average values of; UCM weight percentage, CPI, n-C_{17} / Pr and n-C_{18} / Ph for the collected water and sediment samples

Sampling sites		UCM	CPI	n-C_{17}/Pr	n-C_{18}/Ph
Site 1	Water	20.6	1.16	0.44	0.37
Site 2	Water	24.2	0.72	0.45	0.86
	Sediment	69.2	1.52	0.51	0.65
Site 3	Water	50.3	0.75	0.38	0.35
	Sediment	79.3	1.75	0.71	0.77
Site 4	Water	32.0	0.72	0.72	0.42
	Sediment	51.9	1.61	0.63	0.57

Table 2: Total heterotrophic bacterial count (CFU /ml water and /g sediment) at 30°C in the monthly collected water and sediment samples from the four sites in Ismailia Canal

	Total viable bacterial count (CFU / 1 ml water and / 1 g sediment)						
	Site 1	Site 2		Site 3		Site 4	
Months	water	water	sediment	Water	sediment	water	sediment
Aug.2007	8.8x10^3	8.2x10^3	2.3x10^5	1.2x10^5	7.6x10^5	1.0x10^4	4.8x10^5
Sep. 2007	3.7x10^5	1.9x10^5	7.9x10^5	1.2x10^5	1.7x10^6	1.8x10^5	6.2x10^5
Oct. 2007	3.0x10^3	1.3x10^4	3.9x10^4	7.0x10^3	1.4x10^5	3.5x10^3	1.9x10^5
Nov.2007	1.1x10^5	2.0x10^4	2.9x10^4	3.3x10^4	6.7x10^4	1.4x10^4	5.1x10^4
Des.2007	2.5x10^3	4.3x10^3	9.0x10^5	1.9x10^4	9.5x10^5	5.3x10^3	5.8x10^4
Jan.2008	8.6x10^2	1.2x10^3	1.5x10^4	7.8x10^4	2.2x10^4	2.0x10^3	1.1x10^4
Feb.2008	1.2x10^3	5.0x10^2	3.9x10^5	3.1x10^4	2.8x10^4	3.1x10^3	5.0x10^3
Mar.2008	3.0x10^2	7.9x10^2	4.7x10^4	5.8x10^4	1.3x10^4	4.5x10^2	6.0x10^3
Apr.2008	1.0x10^3	2.1x10^3	3.0x10^5	2.8x10^4	4.3x10^4	1.1x10^3	2.2x10^3
May2008	9.8x10^3	1.9x10^3	4.6x10^5	3.1x10^4	5.3x10^4	9.7x10^2	2.8x10^4
June2008	8.9x10^2	8.8x10^2	6.9x10^5	8.7x10^3	2.9x10^4	1.0x10^3	9.1x10^3
July 2008	3.7x10^2	2.2x10^2	8.6x10^5	1.2x10^4	7.2x10^4	5.8x10^2	4.9x10^4
Maximum	3.7x10^5	1.9x10^5	9.0x10^5	1.2x10^5	1.7x10^6	1.8x10^5	6.2x10^5
Minimum	3.0x10^2	2.2x10^2	1.5x10^4	7.0x10^3	1.3x10^4	4.5x10^2	2.2x10^3
Average	4.2x10^4	2.0x10^4	3.9x10^5	4.6x10^4	3.2x10^5	1.9x10^4	1.3x10^5

The average values of n-C_{17}/Pr and n-C_{18}/Ph in water and sediment samples in the four sites were less than one (Table, 1). This indicates that all sites contain petroleum hydrocarbons and this coincides with Mille *et al.* [26], who reported that, n-C_{17}/Pr and n-C_{18}/Ph ratios were less than one in sites contaminated by petroleum hydrocarbons and are close to one or greater than one in other sites.

From these results, it is clear that all sites contain a mixed sources of biogenic and petrogenic hydrocarbons but the biogenic hydrocarbons was higher than the petrogenic hydrocarbons in site 1 while in the other three sites especially site 3, the pollution source was mainly of petroleum-derived materials with a lesser contribution from algal and vascular plant origins.

Enumeration of Total Heterotrophic Bacteria: The total aerobic heterotrophic bacterial count (TBC) at 30°C (Table, 2) show that the bacterial community in water samples fluctuated between 3.0×10^2 to 3.7×10^5, 2.2×10^2 to 1.9×10^5, 7.0×10^3 to 1.2×10^5 and 4.5×10^2 to 1.8×10^5 CFU/ml for sites 1, 2, 3 and 4 respectively while in sediment samples fluctuated between 1.5×10^4 to 9.0×10^5, 1.3×10^4 to 1.7×10^6 and 2.2×10^3 to 6.2×10^5 CFU/g for sites 2, 3 and 4, respectively. It is clear that all sites show high load of bacterial count even at site 1 (the beginning of the canal). This may be due to that Ismailia canal represent the most distal downstream of the main River Nile. Thus, its water contains all the proceeded pollutants discharged into the Nile, as reported by Geriesh *et al.* [6]; these pollutants may act as nutritive substances for bacteria. Also, we observed that there was a relatively high bacterial load at site 3 water (at petroleum companies). This may be due to the disposal of wastes from petroleum companies and other companies at this site, these pollutants represent a valuable source of carbon and energy for bacteria. This agree with Hood *et al.* [11], who reported that, the levels of hydrocarbons present in the oil field represent a nutrient enrichment where less-recalcitrant organic carbon may be limiting. Also, Venosa *et al.* [34] reported that, rivers become nutrient-rich downstream after receiving industrial and domestic effluents and agricultural runoff.

Table 3: The density of hydrocarbon degrading bacteria (MPN /100ml water and /100g sediment) in water and sediment samples from the four sites in Ismailia Canal during one year (Aug. 2007- July 2008)

	Most Probable Number Index (MPN) / 100 ml water and / 100 g sediment						
	Site 1	Site 2		Site 3		Site 4	
Months	water	water	sediment	Water	sediment	water	sediment
Aug.2007	$2.2x10^4$	$1.1x10^4$	$4.7x10^4$	$1.7x10^3$	$1.7x10^5$	$3.9x10^3$	$1.7x10^5$
Sep. 2007	$1.4x10^4$	$1.4x10^4$	$5.8x10^4$	$1.7x10^3$	$7.0x10^4$	$3.5x10^4$	$1.7x10^5$
Oct. 2007	$1.7x10^3$	$3.4x10^3$	$5.4x10^4$	$2.2x10^3$	$1.6x10^6$	$3.3x10^3$	$3.4x10^4$
Nov.2007	$7.8x10^2$	$1.3x10^3$	$2.4x10^4$	$3.5x10^4$	$2.4x10^4$	$3.4x10^3$	$5.4x10^4$
Des.2007	$2.2x10^4$	$1.1x10^3$	$1.6x10^6$	$1.7x10^4$	$3.5x10^4$	$2.4x10^4$	$3.3x10^4$
Jan.2008	$3.9x10^3$	$2.4x10^3$	$9.4x10^4$	$5.4x10^5$	$2.1x10^4$	$3.5x10^5$	$1.6x10^4$
Feb.2008	$1.1x10^4$	$2.6x10^3$	$3.5x10^5$	$9.4x10^4$	$5.4x10^5$	$7.9x10^3$	$1.1x10^5$
Mar.2008	$1.4x10^3$	$1.7x10^3$	$2.8x10^5$	$1.4x10^4$	$2.4x10^5$	$9.2x10^3$	$3.5x10^5$
Apr.2008	$6.1x10^2$	$1.3x10^3$	$1.6x10^6$	$3.1x10^4$	$1.6x10^6$	$7.9x10^3$	$5.4x10^5$
May2008	$6.8x10^2$	$2.4x10^3$	$1.7x10^4$	$4.6x10^4$	$1.7x10^4$	$7.8x10^2$	$7.9x10^3$
June2008	$5.0x10^2$	$2.0x10^3$	$7.9x10^5$	$3.3x10^4$	$4.0x10^5$	$1.7x10^3$	$1.2x10^4$
July 2008	$4.0x10^2$	$1.4x10^3$	$1.6x10^6$	$3.5x10^4$	$1.6x10^6$	$3.3x10^3$	$2.8x10^5$
Maximum	$2.2x10^4$	$1.4x10^4$	$1.6x10^6$	$5.4x10^5$	$1.6x10^6$	$3.5x10^5$	$5.4x10^5$
Minimum	$4.0x10^2$	$1.1x10^3$	$1.7x10^4$	$1.7x10^3$	$1.7x10^4$	$7.8x10^2$	$7.9x10^3$
Average	$6.6x10^3$	$3.7x10^3$	$5.4x10^5$	$7.1x10^4$	$5.3x10^5$	$3.8x10^4$	$1.5x10^5$

The results show that the bacterial count in sediment was higher than that the count in the water at the same sites. This may be due to the accumulation of bacteria which tend to attach to the surface of sediment as reported by Xia *et al.* [19]. Also, we noted that there is a great variation in the bacterial count between the months in water samples than in sediment samples. This may be due to that the water is greatly affected by the change in the surrounding conditions than the sediment which reflect stable picture about the real count in the studied sites and this agree with APHA[12] which demonstrated that sediment may provide a stable index of the general quality of the overlying water, particularly where there is great variability in its bacteriological quality.

Enumeration of Hydrocarbon Degrading Bacteria: The enumeration of petroleum degrading microorganisms is important both to determine the potential for removal of oil via microbial degradation and to assess the amount of oil pollution that has occurred, if the populations of petroleum- degrading microorganisms prove to be related to the concentration of polluting oil present [10].

The density of hydrocarbon degrading bacteria (HDB) by most probable number (MPN) method was demonstrated in Table 3 which shows that the density in water samples fluctuated between 4.0×10^2 to 2.2×10^4, 1.1×10^3 to 1.4×10^4, 1.7×10^3 to 5.4×10^5 and 7.8×10^2 to 3.5×10^5 MPN /100ml for sites 1, 2, 3 and 4 respectively, while in sediment samples fluctuated between 1.7×10^4 to 1.6×10^6, 1.7×10^4 to 1.6×10^6 and 7.9×10^3 to 5.4×10^5 MPN /100g for sites 2, 3 and 4, respectively. The average of the density over the period of one year for water samples were 6.6×10^3, 3.7×10^3, 7.1×10^4 and 3.8×10^4 MPN /100ml for sites 1, 2, 3 and 4, respectively, while in sediment samples were 5.4×10^5, 5.3×10^5 and 1.5×10^5 MPN /100g for sites 2, 3 and 4, respectively.

From the results it was observed that all sites contain hydrocarbon bacterial degrader even site 1 (the beginning of the canal). This coincide with Šyvokienë and Michënienë [35], they reported that almost all natural aquatic ecosystems contain populations of bacteria that can metabolize some oil components and related compounds even if those systems have not ever been exposed to oil or oil products.

The results of water samples show that, site 3 (at the petroleum companies) had the highest average density of HDB followed by site 4 (after about 8 Km from site 3) while the lowest average density was found at sites 1 and 2 (before petroleum companies). These results agree with Šyvokienë and Michënienë [35] and Piehler *et al.* [36], who reported that petroleum polluted sites have greater bacterial abundance and a large proportion of bacteria capable of hydrocarbon degradation than the non polluted areas.

In sediment samples, also site 3 shows a higher average density of HDB than site 4. This may be due to the presence of high amount of petroleum hydrocarbons at this site. Analogous results were reported by Gunkel [37] who reported that populations of hydrocarbon utilizers were elevated in sediments affected by

Table 4: Average values of total bacterial count (TBC) and hydrocarbon degrading bacteria (HDB) and their proportions within TBC in the collected water and sediment samples from the four sites in Ismailia canal

Sampling sites		Average of total bacterial count (TBC) CFU / ml or /g	Average of (HDB) MPN / ml or /g	Percentage of average of (HDB)
Site 1	Water	$4.24x10^4$	6.57x10	0.28%
Site 2	Water	$2.03x10^4$	3.72x10	0.20%
	Sediment	$3.96x10^5$	$5.56x10^3$	1.40%
Site 3	Water	$4.55x10^4$	$9.71x10^2$	2.10%
	Sediment	$3.23x10^5$	$5.26x10^3$	1.60%
Site 4	Water	$1.85x10^4$	$3.75x10^2$	2.00%
	Sediment	$1.26x10^5$	$1.48x10^3$	1.20%

the Torrey Canyon Spill [38]. Also Stewart and Marks [39] found higher numbers of hydrocarbon utilizers in sediment affected by the Arrow Spill in Chedabucto Bay [38]. Similarly, Šyvokienë and Michënienë [35] and El-Tarabily [40], reported that, the total number of aerobic and anaerobic hydrocarbon utilizing bacteria in sediment polluted with crude oil was significantly higher than in non polluted ones.

In general we observed that, sediment show high average density of HDB than the water at the same sites, this may be due to that bacteria tend to attach and grow on the surface of sediment as reported by Xia *et al.* [19] and Hwang and Cutright [41]. Also Poeton *et al.* [42] reported that, the humic substances and other elements in sediment phase could serve as nutrients for bacteria, stimulating the growth of bacteria.

It is clear from a number of studies that the distribution of hydrocarbon utilizing microorganisms reflects the historical exposure of the environment to hydrocarbons [38]. If correlation are to be made between presence of petroleum degraders and concentration of oil in the sample, percentage of petroleum degraders in the total microbial population of the sample should be used, not the total numbers [10].

Also, Hood *et al.* [11] reported that the ratio of hydrocarbonoclastic bacteria to total bacteria appears to be a more consistent and valid indicator than the absolute number of hydrocarbonoclasts. This was parallel with Walker and Colwell [10]; they reported that petroleum degrading microorganisms should be expressed as a percentage of the total population whenever possible.

In our study the frequency of occurrence of the average of HDB relative to the average of total heterotrophs was given in Table 4. From the results, it was observed that, the proportions of the average of HDB within the average of heterotrophic communities fluctuated between 0.2 to 2.1%. The percentage of average of degrading bacteria in water samples were 0.2%, 0.2%, 2.1% and 2.0% for sites 1, 2, 3 and 4, respectively, while in sediment samples were 1.4%, 1.6% and 1.2% for sites 2, 3 and 4, respectively. The highest percentage of average of hydrocarbon degrading bacteria in water samples was found at site 3 followed by site 4 and the lowest percentages of average were found at sites 1 and 2. While in sediment samples, the highest percentage of average of hydrocarbon degrading bacteria also was found at site 3 followed by sites 2 and 4.These high values at site 3 (water and sediment) and at site 4 may be attributed to the high amount of petroleum hydrocarbons at site 3 and these petroleum pollutants was moved with water to site 4 as the GC profiles proved that. Analogous results were reported by Oppenheimer *et al.* [43]; they found a tendency toward higher ratios of hydrocarbon utilizing bacteria to total viable heterotrophs in the active Ekofisk oil field of the North Sea, probably due to the occurrence of hydrocarbons in the sediment of this region [38]. Also, our results coincide with Atlas [44] who reported that populations of hydrocarbon-degraders normally constitute less than 1% of the total microbial communities but when oil pollutants are present this hydrocarbon degrading populations increase, typically to 10% of the community.

From our results we observed that, the percentage of petroleum degrading bacteria in the total viable heterotrophic bacterial count increase with the increasing the amount of petroleum hydrocarbons at the sites under study. So we can rely on the percentage of HDB as a sensitive index of environmental exposure to hydrocarbons.

This conclusion agree with a large number of reports, reviewed by Atlas [38]; Colwell and Walker [45]; Bossert and Bartha [46]; Floodgate and Leahy [47] and Colwell [48], who reported that, the numbers of hydrocarbon utilizing microorganisms and their proportion in the heterotrophic community increase upon exposure to petroleum or other hydrocarbon pollutants and that the levels of hydrocarbon utilizing microorganisms generally reflect the degree of contamination of the ecosystem.

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