

## Monitoring of Petroleum Hydrocarbons in Sediment and Gastropods from Suez Gulf, Red Sea

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**Abstract:** In order to evaluate the petroleum hydrocarbons pollution level in the Suez Gulf, Polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbons (ALHs) were detected and quantified in marine sediments and four gastropod species including; *Cellana rota*, *Nerita sanguinolenta*, *Echinolittorina marsubri* and *Cerithium caeruleum*. The sediment and gastropods samples were collected from three different studying sites from the Suez gulf during autumn 2013.  $\Sigma$ PAHs of the Suez gulf sediment ranged from 772.18 to 1189.3  $\mu\text{g/g}$  (dry weight) with an average of 719  $\mu\text{g/g}$  (dry weight). However, in gastropod species samples;  $\Sigma$ PAHs ranged from 12.36 to 29.01  $\mu\text{g/g}$  with an average of 20.04  $\mu\text{g/g}$  (wet weight), and in which  $\Sigma$ PAHs seems dependents on PAHs levels in the environment more than their ability on the depuration of PAHs, this is due to the correlations between such paired values across different study sites. Unlike the sediments (which contain all 16 identified PAHs), only 13 individual PAHs values were detected in all gathered gastropod samples. The BaA/CHR and Flu/Pyr ratios in sediment PAHs patterns indicating the petrogenic source of PAHs in all sampling sites. Concerning the aliphatic petroleum quantification,  $\Sigma$ ALHs of the Suez gulf sediment ranged from 392.19 to 568.62  $\mu\text{g/g}$  (dry weight) with an average of 505.72  $\mu\text{g/g}$  (dry weight). In gastropod species samples,  $\Sigma$ ALHs ranged from 9.07 to 27.04  $\mu\text{g/g}$  with an average of 12.86  $\mu\text{g/g}$  (wet weight). It seems relatively depending on the ability of gastropod species to metabolize the aliphatic compounds tend to accumulate inside their tissues. Unlike the sediments (which contain all 17 identified ALHs (C14-C30) only 15 individual ALHs values were detected in all gathered gastropod samples. The (LMW/HMW) ratios in the sediment ALHs patterns suggested that ALHs in the Suez Gulf attributed to the contamination from a petroleum source. The results of the  $\Sigma$ PAHs and  $\Sigma$ ALHs levels in different size-groups for each single gastropod species showed a significance regression between gastropod size and their hydrocarbons contents. Individual PAHs concentrations ranged from 3.14 to 187.80  $\mu\text{g/g}$  dry weight and from 0.75 to 14.32  $\mu\text{g/g}$  wet weight (for detected PAHs) in gastropods. While Individual ALHs concentrations ranged from 4.57 to 64.78  $\mu\text{g/g}$  dry weight in sediment and from 0.36 to 10.11  $\mu\text{g/g}$  wet weight (for detected ALHs) in gastropods. [Ahmad M. Azab; Walaa M. Shaban; Mona S. Zaki; Mohammad M. N. Authman and Mostafa F. Abdel Zaher. **Monitoring of Petroleum Hydrocarbons in Sediment and Gastropods from Suez Gulf, Red Sea.** *Life Sci J* 2016;13(7):46-59]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). <http://www.lifesciencesite.com>. 6. doi: [10.7537/marslsj130716.06](https://doi.org/10.7537/marslsj130716.06).

**Key words:** Polycyclic aromatic hydrocarbons (PAHs); and aliphatic hydrocarbons (ALHs); gastropod sediment; and Suez Gulf.

### 1. Introduction

Petroleum Aliphatic and aromatic hydrocarbons are the most significant classes of organic pollution to the water environment, causing a serious effect on the water ecosystem and are being harmful to the health of the living creatures and human body (Neff, 1979; Couch *et al.*, 1985; Maher *et al.*, 1992; Ankley *et al.*, 1994; Wilcock *et al.*, 1995 & 1996; El Nembr *et al.*, 2004 and Zhang & Kang, 2009). The Aliphatic hydrocarbons including n-alkanes generally constitute the major fractions of petroleum, which may be used to detect its presence in the environment. In addition, n-alkanes, particularly, are the major fractions of saturated hydrocarbons, the distribution patterns of n-alkanes characterize by carbon-number (carbon chains) ranges and its predominance is depending on the

nature of the source material and its microbial or geochemical alteration (El Nembr *et al.*, 2004).

Polycyclic aromatic hydrocarbons (PAHs) are widespread and mainly originate from fossil fuel combustion and the release of petroleum and petroleum products (El Nembr *et al.*, 2004; Andres *et al.*, 2010 and Chao *et al.*, 2010). Chemically, PAHs are compounds contain two or more fused aromatic rings in linear, angular or clustered arrangements. They generally possess high chemical stability, which results in high levels in the environment and enhanced bio-accumulation (Toro *et al.*, 2000; Arey and Atkinson, 2003 and El Nembr *et al.*, 2007). Many PAHs have toxic, mutagenic and/or carcinogenic properties. Although the large number of individual PAHs that occurred in the environment, most regulations, analyses, and data reporting focus on only a limited number of PAHs, typically between

14 and 20 individual PAH compounds. Such compounds are given the priority because they are the most PAHs effective as toxic or carcinogenic pollutants (Ruiz *et al.*, 2011; Liu *et al.*, 2012a; Salem *et al.*, 2014 and Abdel-Shafy & Mansour, 2016).

In the marine environment, PAHs occur by atmospheric depositions, riverine inputs and oil spillage (Oros & Ross, 2004; Culotta *et al.*, 2006). The Gulf consider the most important geographical and economical part of the Egyptian Red Sea regions due to its important location as a compulsory part of the Suez Canal shipping route for oil and other products; also, due to the heavy industry activities, including oil refining along the coast of the gulf. Due to these characteristics and the extensive off-shore oil exploration and production activities, probabilities of major oil spills are high. There are regular losses of oil to the sea during routine transfer operations involving loading, deballasting of oil tankers, in preparation of loading during off-shore drilling and from coastal refineries, and other sources (Gladstone *et al.*, 2013 and El-Sheshtawy *et al.*, 2014 ).

PAHs are hydrophobic compounds and become more hydrophobic with increasing of molecular weight. Due to their high hydrophobic properties, PAHs tend to adsorb strongly on particle surfaces and thus can be deposited in sediments (Means *et al.*, 1980; Kim *et al.*, 1999 and Ko *et al.*, 2007). Consequently, marine sediments often contain concentrations of PAHs of higher magnitudes than those in the overlying water (El Nemr *et al.*, 2007). The contamination of the sediment may pose a high toxic threat to the marine fauna, which tend to accumulate (biologically) the organic pollutants. Uptake of PAHs by marine organisms is dependent on the environmental bioavailability of the PAHs as well as the physiology of the organisms (Juhász & Naidu, 2000; Meador *et al.*, 1995). Gastropods molluscs are excellent bioaccumulators of a wide range of pollutants (Simkiss *et al.*, 1982 and Livingstone, 1991). They are filter feeders, herbivores or carnivores and have the potential to bio-concentrate contaminants, which would normally be present in the water or within sediments at concentrations too low for detection by routine monitoring techniques. They are also ideal species

for environmental monitoring, because their sedentary nature does not require consideration of complex migratory factors in the interpretation of the bioaccumulation data (Short & Sharp, 1989 and Livingstone, 1991).

The present study aimed to evaluate the concentrations of both aromatic and aliphatic hydrocarbons in surface sediment; as well as in some marine gastropod species (i.e. *Cellana rota*, *Cerithium caeruleum*, *Echinolittorina marsubri* and *Neritidae sanguinolenta*) from the western coast of the Suez Gulf.

## 2. Material and Methods

### Study area

The Suez Gulf (Figure, 1) is located in a continental-type rift system at the northward continuation of the Red Sea, Egypt. It is large semi-closed area, extends for about 250 km south-southeast from the Suez port in the north (Lat. 29° 56') to Shadwan Island in the south (Lat. 27° 36'). Depth increases abruptly to about 250 m at its mouth (Shukri, 1945). The width of the Suez Gulf fluctuates between 20 and 40 km. Its depth throughout its axis is relatively constant with a mean of 45 m and has a total surface area of about 10,510 km<sup>2</sup> (El-Sabh & Beltagy, 1983; El-Moselhy & Gabal, 2004 and Nemr *et al.*, 2005 and Hamed & Emara, 2006).

Three remarkable active oil-industry sites (Lat. & Long. detection of studying sites showed in table, 1) were selected on the western coast of the Suez Gulf with variable degrees of native petroleum pollution supply. These sites included Ras Ghareb (RGh), Ras Shokier (RSh) and Gemsha Bay (GmB) (Figure, 1). Ras Ghareb (Site 1) is a tanker loading facility, operated by the Egyptian General Petroleum Corporation considered as one of the leading centers of petroleum production in Egypt. Ras Shokier (Site 2), however, is offshore oil loading tanker terminal operated by Gulf of Suez Petroleum Company (GUPCO). Finally, Gemsha Bay (Site 3) in which, the petroleum spills occurred in five on-shore/off-shore locations in addition to a leak's petroleum in six ancient wells, this area was previously exposed to continuous petroleum leaks since 2009 until now.

**Table (1): Location (longitude and latitude) of the studied sites at Suez Gulf**

Site No.	Site Name	Longitude	latitude
1	Ras Ghareb (RGh)	28°22'44.20"N	33° 4'12.17"E
2	Ras Shokier (RSh)	28° 8'26.90"N	33°15'58.26"E
3	Gemsha Bay (GmB)	27°42'2.18"N	33°32'47.51"E



Figure (1): Map showing the Studying sites at Suez Gulf.

### Sample collection

In order to establish the hydrocarbons pollution levels in the Suez Gulf, both sediment and gastropod species samples were diurnally collected from the above studying sites; during the autumn season in 2013.

The sediment samples were taken from under-water on-shore surface sediment by clean sampler. The samples were transferred to a clean glass jar and kept in insulated icebox. Upon arriving to the laboratory, the sediment samples were frozen immediately and stored at  $-20\text{ }^{\circ}\text{C}$  prior to analysis.

Four gastropod species (including *Cellana rota* "Patella", *Nerita sanguinolenta*, *Echinolittorina marsubri* and *Cerithium caeruleum*) were collected alive from each studying site along with the sediment samples. Selection of the gastropod species was depending on the availability of these species in all sampling sites in Suez Gulf. Gastropod samples were collected from these oily polluted sites in attempt to assess the potentiality of using them as bio-monitors of aliphatic and polycyclic aromatic hydrocarbons after accumulating considerable amounts in their soft parts. In addition, concentration levels of total polycyclic aromatic hydrocarbons ( $\Sigma\text{PAHs}$ ) and total aliphatic hydrocarbons were assessed against the gastropod size to obtain the regression curve and bioaccumulation ratios of  $\Sigma\text{PAHs}$  and Total aliphatic hydrocarbon in each gastropod species.

Collected gastropod samples after catching were kept in the ice, and transported to the laboratory. In the laboratory, shell length and soft part wet weight for each individual gastropod in each species were taken. The soft parts were labeled, grouped according to their shell lengths, After

grouping process it immediately frozen and kept in the refrigerator under  $-20\text{ }^{\circ}\text{C}$ . Samples of each species were divided according to length into four size groups: A, B, C and D. Grouping were applied for all gastropod species with the same shell length ranges, for convenience and due to their relative closeness in shell length, as the following: Group A: 1.0 – 1.5 cm, Group B: 1.6 – 2.0 cm, Group C: 2.1 – 2.5 cm and Group D: 2.6 – 3.0 cm.

### Extraction of samples and analysis by Gas Chromatography (GC):

The extraction of sediment samples were earned out as follow: 20 g of well-mixed sediment sample was ground with 60 g of anhydrous sodium sulfate to dry. The mixture was stored in a clean glass jar and allowed to stand overnight for complete dehydration. The sediment sample was transferred to cellulose extraction thimble (pre-extracted with n-hexane) and a layer of anhydrous  $\text{Na}_2\text{SO}_4$  has been added over the sample to ensure that the n-hexane extract remains dry. The sample was then exhaustively extracted with 125 ml of n-hexane in a soxhelt apparatus for not less than 8 hours at approximately 20 cycles/h. Once cool, the sample extract is quantitatively transferred to a 100 ml volumetric flask and the volume adjusted to exactly 100 ml, then stored in fridge for further analysis.

Gastropods samples were removed from the refrigerator and allowed to thaw at room temperature for about 5 h. Ten grams of the gastropod soft tissue (wet weight) were treated with 30 g of anhydrous sodium sulfate and the mixture was blended at high speed for 5 min. Then the mixture was extracted with a soxhlet extractor with 200 ml of methanol for 8h (UNEP/IOC/IAEA, 1991). Then, 0.7 M KOH (20

ml) and distilled water (30 ml) were added to the flask and the reflux was continued for 2 h to saponify the lipids. The content of the extraction flask was extracted three times in a separating funnel with 80 ml hexane. Then the extracts were combined, dried with anhydrous sodium sulfate and filtered through glass wool.

After the extraction process of the sediment and gastropods samples, the hexane fraction was concentrated with a rotary evaporator down to about 15 ml at 30 °C, followed by concentration with nitrogen gas stream down to a volume of 1 ml. Clean-up and fractionation was performed by passing the concentrated extract through a silica/aluminum oxide column. The chromatography column was prepared by slurry packing 20 ml (10 g) of silica, followed by 10 ml (10 g) of aluminum oxide and finally 1 g of anhydrous sodium sulfate. The extract (1 ml) was sequentially eluted from the column with 25 ml of hexane for the saturated aliphatic fraction (F1). Then 40 ml of hexane and dichloromethane (90:10) were added for the unsaturated and aromatic hydrocarbons fraction (F2). F1 and F2 were concentrated by using gentle stream of nitrogen for instrumental analysis.

Blanks of 1000 fold concentration were analyzed by gas chromatography with a flame ionization detector (FID). All samples were analyzed by a Hewlett Packard HP-5890 series II equipped with split/splitless injector and a fused silica capillary HP-1 (30 m, 0.32 mm, 0.17  $\mu$ m) 100% dimethylpolysiloxane. The temperature was programmed from 60–300 °C with rate of 5 °C/min and was then maintained at 290 °C for 25 min. The injector and detector temperatures were set at 280 and 300 °C, respectively. The carrier gas was nitrogen flowing at 1.2 ml/min. All solvents were pesticides grade purchased from Merck.

According to Miles and Delfino (1999) only 16 PAHs compound were suggested by USEPA (United States Environmental Protection Agency) as a priority PAHs pollutants. Consequently, the same compounds were considered for detection and quantification in the present work. Identification and quantification of 16 PAHs were based on matching their retention time with a mixture of PAH standards. The 16 PAH compounds were naphthalene (NAPH), acenaphthylene (ACTHY), acenaphthene (ACE), fluorine (FL), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLTH), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo-[k]fluoranthene (BkF), benzo[a]pyrene (BaP), benzo[ghi]perylene (BghiP), indo- [1,2,3-cd]pyrene (InP), and dibenzo[a,h]anthracene (DBA). Aliphatic Hydrocarbons were identified by comparison of retention times with those of n-alkanes standard

ranging from C14 –C32. These standards were guaranteed by Supelco Inc.

### 3. Results and Discussion

Table (2) and (4) gives the total concentrations of polycyclic aromatic hydrocarbons ( $\Sigma$ PAHs) and the concentrations of individual PAHs constituents, at different study sites, in sediment and gastropod species, respectively. Concerning the sediment samples, Ras Ghareb site were the most contaminated by  $\Sigma$ PAHs with 1189.3  $\mu$ g/g dry weight followed by Ras Shokier site with 772.18  $\mu$ g/g dry weight and then Gemsha site with 195.53  $\mu$ g/g dry weight with an average of 719  $\mu$ g/g dry weight. Similarly, in gastropod samples, Ras Ghareb coast recorded the highest average values of  $\Sigma$ PAHs ranged from 12.36  $\mu$ g/g in *Cellana sp.* at Ras Shokier site to 29.01( $\mu$ g/g) in *Nerita sp.* with an average of 20.04  $\mu$ g/g wet weight.

Range and average  $\Sigma$ PAHs in the sediment of the Gulf of Suez in the present study, measured in 2013, is higher than that measured in the same region by El Nemr *et al.* (2005) with range of 158-10463 ng/g and average of 234 ng/g dry sediments. Due to its huge annual oil inputs and its nature as narrow semi-closed area, the Gulf of Suez extremely loaded by the PAHs pollutants being higher than the other Red Sea regions that recorded in the previous studies. For example El Nemr *et al.* (2004) considered the Red Sea as relatively unpolluted with 5452 ng/g average  $\Sigma$ PAHs in Red Sea mussel and the present findings also higher than the polluted sediment in El-Quseir with 456.91 ng/g (Salem *et al.*, 2014). Moreover,  $\Sigma$ PAHs concentrations in the present study are also have a significance increase on the maximum concentrations found in the close regions at the Mediterranean coast with 1,464 ng/g wet weight in El Deep *et al.* (2007), for both sediments and fauna samples, and with 6338 ng/g dry weight in El Nemr *et al.* (2007) for sediment.

In the present results, by comparison data in both sediments and gastropods a across different site species,  $\Sigma$ PAHs values in the gastropod species seems decedents on the levels of  $\Sigma$ PAHs in the environment more than the ability of this species on the depuration of PAHs. It is not conflict with the findings of Ahmed *et al.* (2014) in different marine species collected from the same current study area and their results showed that the contamination by Petroleum Hydrocarbons is more likely occurred because of their ingesting contaminated material than direct body exposure to oil residues in the water column.

Individual PAH concentrations of all sediment samples in this study was above the limit of detection (Detection limit = 0.1  $\mu$ g/g dry weight). The

highest concentration of the sediment PAHs was Fluoranthene compound recording 187.8  $\mu\text{g/g}$  (dry weight) in Ras Shokier site while the lowest one was Naphthalene compound recording 3.14  $\mu\text{g/g}$  dry weight in Gemsha Bay site (Table 2). Figure (2) summarized the average contents of individual PAHs in the sediment samples and obtained that Fluoranthene, Chrysene and Phenanthrene have been detected in the sediment with very high average contents recording 125.08, 104.08 and 87.99  $\mu\text{g/g}$  dry weight, respectively. However, PAHs with low average contents in the sediment involving Indo-[1,2,3-cd]pyrene (12.89  $\mu\text{g/g}$  dry weight), Naphthalene (14.03  $\mu\text{g/g}$  dry weight) and Anthracene (15.54  $\mu\text{g/g}$  dry weight). The rest PAHs average concentrations ranged from 18.56 to 68.32  $\mu\text{g/g}$  dry weight.

In relation to the origin of PAHs in the environment, scientists detect the source of PAHs by calculation the BaA/CHR ratio. If the ratio was higher than "1" PAHs origin will attributed to pyrolytic activities. However, if that ratio was lower than 1 the petrogenic source will be concluded (Yunker *et al.*, 1996; Budzinski *et al.*, 1997; Yunker *et al.*, 2002 and Lin *et al.*, 2011). In the current results, as shown in Table (2), the ratios of BaA/CHR in all studied sites were lower than 1, and this indicated that the source of PAHs in sediment samples was attributed to petrogenic inputs. Similarly, The Flu/Pyr ratio is used for evaluating the attribution of PAHs pollution in sediments. When the (Flu/Pyr) ratio is become less than 1 that means PAHs will be from a petroleum source (Guinan *et al.*, 2001; Magi *et al.*, 2002; Li *et al.*, 2006). As shown in Table (2), in this study, the ratios of Flu/Pyr in all studied sites were less than 1 indicating the source petrogenic of PAHs.

IARC (1991) restricted the carcinogenic PAH compounds to include BaA, BbF, BkF, BaP, DBA, BghiP and InP and evaluated the PAHs risk assessment by measuring their dominancy. In the current results, total concentration of carcinogenic PAHs (average=214.41  $\mu\text{g/g}$  dry weigh) were detected with higher amounts in the sediment of Suez Gulf. These findings suggests the risky ecological PAHs effects on human health, enter the food chain and concentrated in marine sediments, where bottom-feeding fish and filter-feeding invertebrates are particularly prone to exposure and accumulation of such compounds (Phillips, 1999).

In gastropods samples our results showed that only 13 PAHs are above the detection level and there are 3 PAHs have never been detected in all gastropod samples including Dibenzo[a,h]anthracene, Benzo-[K]Fluoranthene and Benzo[ghi]Perylene. Anthracene was occurred in all gastropod species and

in all study sites except in *Nerita sp.* at Gemsha bay. Naphthalene is only detected in *Nerita Sp.* samples and Indo-[1,2,3-cd]pyrene is only detected in *Cellana sp.* samples (Figure. 4).

In *Cellana sp.* samples, 10 PAHs were detected and their concentrations ranged from 0.75  $\mu\text{g/g}$  wet weight (for Benzo[B]Fluoranthene) to 11.19  $\mu\text{g/g}$  wet weight (for Fluoranthene). In similar, 10 PAHs were also detected in *Echinolittorina sp.*, but Acenaphthylene and Pyrene are added to its PAHs pattern list instead of Fluorine and Indo-[1,2,3-cd]pyrene. In *Cerithium sp.* and *Nerita sp.*, however, only 7 individual PAHs were detected in its individual aromatic patterns and their concentrations ranges were 0.75-14.04 and 1.94-14.32  $\mu\text{g/g}$  wet weight, respectively (Table, 4).

Fluoranthene is the most highest concentration of all PAHs in gastropod samples, which detected with large contents in *Cellana sp.*, *Cerithium sp.* and *Nerita sp.* recording 11.19, 14.04, 14.32  $\mu\text{g/g}$ , respectively (all in Ras Ghareb site) while Anthracene was recorded the highest concentration compound value in *Echinolittorina sp.* with 8.42  $\mu\text{g/g}$  wet weight (at Gemsha Bay site). However; Acenaphthene (0.59  $\mu\text{g/g}$  in Ras Shokier), Benzo[B]Fluoranthene (0.75  $\mu\text{g/g}$  in Ras Shokier ) and Benzo[A]Pyrene (0.75  $\mu\text{g/g}$  in Ras Ghareb) represented the lowest individual PAH concentrations detected in *Echinolittorina sp.*, *Cellana sp.* and in *Cerithium sp.*, respectively.

Tables (3) and (5) show the concentrations of total aliphatic hydrocarbons ( $\Sigma\text{ALHs}$ ) and its ALHs individual concentrations patterns at different study sites in sediment and gastropod species, respectively. In sediment  $\Sigma\text{ALHs}$  highest concentration was recorded at Gemsha site with 568.62  $\mu\text{g/g}$  (dry weight) which slightly decreased in Ras Shokier site to 556.35  $\mu\text{g/g}$  (dry weight), while Ras Ghareb site recorded the lowest  $\Sigma\text{ALHs}$  concentration with 392.19  $\mu\text{g/g}$  (dry weight). Generally,  $\Sigma\text{ALHs}$  in the sediment of Suez Gulf were averaged 505.72  $\mu\text{g/g}$  (dry weight). Range and average of  $\Sigma\text{ALHs}$  pollution level in sediment, in all sites in the current study, are higher than the limit value of the environmental quality guidelines for total petroleum hydrocarbon in sediment (10  $\mu\text{g/g}$ ) that recommended by UNEP (United Nations Environment Program). Consequently, the sediments of Suez Gulf are suggested to be a heavily contaminated area by aliphatic hydrocarbons. In addition, levels of  $\Sigma\text{ALHs}$  in the investigated area were higher than that reported in the same region by Abd El-Ghafar (2003) with 13.2 - 20.2  $\mu\text{g/g}$  range and also higher than that recorded in other Red Sea regions such as Safaga, El-Quseir and Marsa Alam with an average of 174.8 ng/g (Salem *et al.*, 2014).

$\Sigma$ ALHs in the current analysis were also higher than those recorded in the Egyptian Mediterranean Sea (6.0 to 565.2 ng/g dry weight) by **El Deeb et al. (2007)**. However such  $\Sigma$ ALHs level, in this study, was lower than the levels measured in both southwest coast of Puerto Rico (35.1- 1832.8  $\mu$ g/g) (**Klekweski et al., 1994**) and the coast of Safax, Tunisia (198-1589  $\mu$ g/g dry weight) (**Louati et al., 2001**).

However, in gastropod samples, the highest value of  $\Sigma$ ALHs was detected in *Cellana sp.* (27.04  $\mu$ g/g wet weight) at Gemsha site while the lowest value was detected in *Cerithium sp.* (9.07  $\mu$ g/g wet weight) at Ras Shokier site but the close value also detected in *Echinolittorina sp.* (9.32  $\mu$ g/g wet weight) at Gemsha bay. The present data revealed that  $\Sigma$ ALHs values in gastropod species seem relatively depending on their ability to metabolize the aliphatic compounds tend to accumulate inside their tissues. Concentrations range of  $\Sigma$ ALHs, in current investigated gastropods species, were higher than that found in the mussels from the Red Sea (range= 8 - 425 ng/g wet weight and the average = 103 ng/g) (**El Nemr et al., 2004**). In similar, average  $\Sigma$ ALHs concentrations in this study were higher than the average concentrations in fishes and invertebrates species from Egyptian Mediterranean regions including *Solea solea*, *Diplodus vulgaris*, *Peneaus japonicas* and *Donax trunculu* with 21.93 , 319.27, 307.28, 326.15 ng/g wet weight, respectively, (**El Deeb et al. 2007**). However,  $\Sigma$ ALHs maximum concentrations in current gastropods species were lower than even the average of  $\Sigma$ ALHs in fish and invertebrate samples from Port-Said (A commercial harbor on the Egyptian Mediterranean coast located near the Suez Canal and Suez Gulf) including; *Lutjanus lineolatusand*, *Alosa fallax nilotica* and *Penaeus joponicusit* (12700, 110200 and 20400 ng/g, respectively) (**Soliman et al., 2000**). It also were lower than  $\Sigma$ ALHs range recorded in the mussel, *Mytilus galloprovincialis*, from Venice Lagoon, Italy (rang = 8000 ng g-1 to 87000 ng/g wet weight) (**Fossato & Siviero, 1974**) and from Spanish Western Mediterranean Coast (rang = 1800 - 58400 ng/g wet weight) (**Albaiges et al., 1982**).

17 n-alkanes (C14-C30) were positively detected in the sediment samples. The highest concentration among all detected ALHs in all sediment samples was C20 recording 64.78  $\mu$ g/g (dry weight) at Ras Ghareb site while C28 was the lowest one recording 4.57  $\mu$ g/g at Ras Ghareb site also (Table, 3). Figure (3) compare between the mean concentrations of individual aliphatic hydrocarbons in sediment samples. The current findings obtained that the individual ALHs concentrations ranged from 11.44 to 52.29  $\mu$ g/g dry weight and the compounds with carbon numbers C18, C19, C20, and C25 have

been detected in the sediment with very high average contents recording 47.61, 45.75, 52.29 and 43.21  $\mu$ g/g dry weight, respectively. However, the compounds with carbon numbers C27, C29 and C30 have been detected with a relatively low average concentrations recording 13.12, 16.38 and 11.44  $\mu$ g/g (dry weight) respectively, in the sediment samples. The low molecular weight (LMW) and high molecular weight ratio (HLM) are used as a source identifier of aliphatic hydrocarbon in sediment, Values below 1 and above 1 show natural input from marine biogenic sources. Values around 1 is from a petrogenic sources (**Fagbote and Olanipekun, 2013**). In current study, The (LMW/HMW) ratios were ranged from 0.82 to 0.96 and it could be attributed to the contamination from petroleum source.

In gastropods samples, only 15 aliphatic hydrocarbons compounds were detected in all studying sites ranged from 0.36 to 10.11  $\mu$ g/g (Figure 5). In *Cellana sp.*, samples only 13 aliphatic hydrocarbon compounds were detected and their concentrations ranged from 0.36  $\mu$ g/g wet weight (for C26) to 8.63  $\mu$ g/g wet weight (for C20). On the same way, 12 ALHs were detected in *Cerithium sp.* where C20 and C22 recorded the highest concentration values with 10.11 and 9.16  $\mu$ g/g respectively. However, *Echinolittorina sp.* and *Nerita sp.* have 10 and 12 detected ALHs in low concentrations with ranges of 0.81-2.97 and 0.76- 3.65  $\mu$ g/g, respectively (Table, 5).

The present work aimed also to study the relationship between gastropod species size from one-side and hydrocarbons accumulation levels in such species from the other-side using their PAHs and ALHs contents. This is probably give us the accurate accumulation effectiveness of a certain gastropod age (exposure time) and will be useful in detecting the right conditions that must be follow when choosing the appropriate gastropod size for hydrocarbons monitoring.

$\Sigma$ PAHs and  $\Sigma$ ALHs contents in different size groups in each single gastropod species are given in Table (6). From the results,  $\Sigma$ PAHs and Total aliphatic hydrocarbons are significantly increase with the increasing in the gastropod size, i.e. the first/smallest gastropods size group (A) have almost a non-detected/lowest concentrations of both hydrocarbons types in all species in different sampling sites, while the largest concentrations of such hydrocarbons were recorded in the final/largest size group (D). For example, in *Cellana sp.*  $\Sigma$ PAHs ranged from 1.51 ( $\mu$ g/g) in size group A at Gemsha bay to 37.95 ( $\mu$ g/g) in size group D at Ras Ghareb (Max. Concentration in D group = 25.13 times of Min. Concentration in A group). Statically the R<sup>2</sup>

range of such concentrations (0.947-0.997) indicating a sharp regression against the gastropod size. A strong regression in other gastropod species was also recorded in *Echinolittorina sp.* and *Nerita sp.* with narrow range (0.94-0.99) which slightly decreased in *Cerithium sp.* (0.76-0.98). The maximum accumulation ratio was recorded in *Cerithium sp.* which have widest range from ND to 44.54 ( $\mu\text{g/g}$ ) with approximately 39 times of the smallest detected value in size group B. In similar, total aliphatic hydrocarbons seems strongly dependent on gastropods size with  $R^2$  values ranged from 0.77 in *Echinolittorina sp.* at Ras Ghareb site to 0.98 in *Cellana sp.* at Ras Shokier site. Accordingly, the maximum accumulation duplication 28.95 was estimated for *Cellana sp.* at Gemsha bay site. Such ratios in other gastropods samples are relatively lower than 8.96 and higher than 2.03.

Accumulation of PAHs from the environment occurs in all marine organisms; however, a wide range in tissue concentrations results due to variations in environmental concentrations, time of exposure, and species ability to metabolize these compounds. The *Mytilus edulis* mussel, for example, processes large volumes of sea water (approximately 2 L per hour) and thereby accumulate

organic composites by a factor of 2 to 5 orders of magnitude compared to sea water in their habitat (Lee *et al.*, 1991). In addition, Al Deep *et al.* (2007) revealed that accumulation of total PAHs were more pronounced in the tissues of higher lipids contents, which increase in the large and old individuals, and they found that the bivalve molluscans comes second regarding to their PAHs accumulations ratios after the dwelling fish species. Size of the marine organisms may be not only contribute to change the accumulation factors responsible for the quantities of pollutants such as time exposure and lipids contents but also in the factors responsible for the pollutants selectivity. For example, Maioli *et al.* (2010) demonstrated that bivalve molluscans of greater size accumulated PAHs of low molecular mass (Nap, Acth, Ace, Fluo, Phen and Ant) whereas the smaller mussels had accumulated greater concentrations of high molecular mass PAHs (BaA, Chry, BbF, BkF, BaP, InP, DBA and BghiP). Metabolism may explain this pattern, because it is suspected that PAH of High Molecular Mass (HMW) are more rapidly metabolized than Low Molecular Mass (LMW) due to differences in enzyme affinity (Schnell, *et al.*, 1980).

**Table (2): Concentrations ( $\mu\text{g g}^{-1}$  dry weight) of Polycyclic Aromatic Hydrocarbons (PAHs) in surface sediment collected from different sites of the Suez Gulf, during autumn 2013.**

PAHs	Sites			Average
	RGh	RSh	GmB	
NAPH	29.26	9.68	3.14	14.03
ACTHY	55.2	26.66	4.94	28.93
ACE	84.52	20.4	11.14	38.69
FL	32.23	20.72	12.87	21.94
PHE	172.37	64.41	27.18	87.99
ANT	22.86	13.36	10.39	15.54
FLTH	167.95	187.8	19.50	125.08
PYR	122.75	68.98	13.23	68.32
CHR	157.82	123.2	31.21	104.08
BaA	103.75	75.61	9.62	62.99
BaP	55.91	37.14	12.10	35.05
DBA	24.34	19.94	20.52	21.60
BbF	72.56	35.47	4.26	37.43
BkF	27.87	21.19	6.61	18.56
BghiP	41.27	33.14	3.27	25.89
InP	18.64	14.48	5.55	12.89
$\Sigma$ PAHs	1189.3	772.18	195.53	719.0
BaA/CHR Ratio	0.66	0.61	0.30	
FL/PYR Ratio	0.26	0.30	0.97	

RGh: Ras Ghareb; RSh: Ras Shokier; GmB: Gemsha Bay; NAPH: Naphthalene; ACTHY: Acenaphthylene; ACE: Acenaphthene; FL: Fluorine; PHE: Phenanthrene; ANT: Anthracene; FLTH: Fluoranthene; PYR: Pyrene; CHR: Chrysene; BaA: Benzo[A]Anthracene; BaP: Benzo[A]Pyrene; DBA: Dibenzo[a,h]anthracene; BbF: Benzo[B]Fluoranthene; BkF: Benzo[K]Fluoranthene; BghiP: Benzo[Ghi]Perylene; and InP: Indo-[1,2,3-cd]pyrene.

**Table (3): Concentrations ( $\mu\text{g g}^{-1}$  dry weight) of Aliphatic Hydrocarbons (ALHs) in surface sediment, collected from different sites of the Suez Gulf, during autumn 2013.**

ALHs	Sites			Average
	RGh	RSh	GmB	
C14	24.79	15.12	22.65	20.85
C15	8.93	18.72	33.25	20.30
C16	14.43	27.19	16.95	19.52
C17	27.01	53.44	17.12	32.52
C18	40.51	52.47	49.86	47.61
C19	12.41	60.63	64.22	45.75
C20	64.78	40.26	51.82	52.29
C21	8.89	51.91	34.03	31.61
C22	55.14	22.36	28.79	35.43
C23	22.10	41.82	31.54	31.82
C24	25.19	23.74	32.65	27.19
C25	32.82	54.84	41.96	43.21
C26	17.91	34.93	55.86	36.23
C27	6.94	13.72	18.69	13.12
C28	4.57	24.61	32.12	20.43
C29	15.08	13.49	20.58	16.38
C30	10.69	7.10	16.53	11.44
$\Sigma$ C14 – C30	<b>392.19</b>	<b>556.35</b>	<b>568.62</b>	<b>505.72</b>
LMW ( $\leq$ C20)	192.86	267.83	255.87	
HMW ( $\geq$ C21)	199.33	288.52	312.75	
LMW/ HMW Ratio	0.96	0.92	0.82	

RGh: Ras Ghareb; RSh: Ras Shokier; GmB: Gemsha Bay; C14: Tetradecane; C15: Pentadecane; C16: Hexadecane; C17: Heptadecane; C18: Octadecane; C19: Nonadecane; C20: Eicosane; C21: Heneicosane; C22: Docosane; C23: Tricosane; C24: Tetracosane; C25: Pentacosane; C26: Hexacosane; C27: Heptacosane; C29: Nonacosane; C30: Triacontane; LMW: Low Molecular Weight and HMW: High Molecular Weight

**Table (4): Concentrations ( $\mu\text{g g}^{-1}$  wet weight) of polycyclic aromatic hydrocarbons (PAHs) in different Gastropod species, collected from different sites of the Suez Gulf, during autumn 2013.**

PAHs	Gastropod species											
	<i>Cellana rota</i>			<i>Cerithium caeruleum</i>			<i>Echinolittorina marsubri</i>			<i>Nerita sanguinolenta</i>		
	RGh	RSh	GmB	RGh	RSh	GmB	RGh	RSh	GmB	RGh	RSh	GmB
NAPH	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<b>2.55</b>
ACTHY	ND	ND	ND	ND	ND	ND	4.10	ND	ND	ND	5.07	ND
ACE	ND	1.0	ND	ND	ND	ND	1.96	0.59	ND	12.75	4.08	ND
FL	2.52	1.20	ND	3.98	4.92	ND	ND	ND	ND	ND	ND	ND
PHE	5.30	ND	1.93	ND	ND	4.46	1.54	0.89	6.85	ND	ND	ND
ANT	2.68	4.95	3.82	2.67	7.35	10.33	4.36	1.47	8.42	1.94	3.89	ND
FLTH	11.19	ND	ND	14.04	ND	ND	5.32	3.10	ND	14.32	2.30	4.35
PYR	ND	ND	ND	ND	ND	ND	2.54	ND	ND	ND	3.79	2.07
CHR	ND	2.67	3.31	ND	5.15	ND	3.46	2.89	5.70	ND	ND	2.0
BaA	ND	1.79	2.86	4.50	7.68	4.16	ND	1.41	ND	ND	ND	2.97
BaP	2.42	ND	ND	0.75	ND	ND	2.87	3.94	ND	ND	ND	ND
DBA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BbF	ND	0.75	1.86	ND	ND	ND	2.10	1.0	ND	ND	ND	ND
BkF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BghiP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
InP	ND	ND	4.65	ND	ND	ND	ND	ND	ND	ND	ND	ND
$\Sigma$ PAHs	<b>24.11</b>	<b>12.36</b>	<b>18.43</b>	<b>25.94</b>	<b>14.1</b>	<b>18.95</b>	<b>28.25</b>	<b>15.29</b>	<b>20.97</b>	<b>29.01</b>	<b>19.13</b>	<b>13.94</b>

N.D.: Not detected; RGh: Ras Ghareb; RSh: Ras Shokier; GmB: Gemsha Bay; NAPH: Naphthalene; ACTHY: Acenaphthylene; ACE: Acenaphthene; FL: Fluorine; PHE: Phenanthrene; ANT: Anthracene; FLTH: Fluoranthene; PYR: Pyrene; CHR: Chrysene; BaA: Benzo[A]Anthracene; BaP: Benzo[A]Pyrene; DBA: Dibenzo[a,h]anthracene; BbF: Benzo[B]Fluoranthene; BkF: Benzo-[K]Fluoranthene; BghiP: Benzo[Ghi]Perylene; and InP: Indo-[1,2,3-cd]pyrene.



**Table (5): Concentrations ( $\mu\text{g g}^{-1}$  wet weight) of Aliphatic Hydrocarbons (ALHs) in different Gastropod species, collected from different sites of Suez Gulf, during autumn 2013.**

ALHs	Gastropod species											
	<i>Cellana rota</i>			<i>Cerithium caeruleum</i>			<i>Echinolittorina marsrubri</i>			<i>Nerita sanguinolenta</i>		
	RGh	RSh	GmB	RGh	RSh	GmB	RGh	RSh	GmB	RGh	RSh	GmB
C14	0.77	ND	ND	0.87	ND	ND	ND	ND	ND	1.39	ND	ND
C15	ND	1.0	ND	2.75	ND	ND	1.31	ND	ND	1.23	ND	1.11
C16	0.85	ND	ND	1.30	ND	3.26	1.76	1.32	ND	2.40	ND	ND
C17	1.50	0.97	ND	1.98	ND	ND	ND	1.47	ND	0.76	ND	ND
C18	0.73	ND	3.98	1.64	3.12	6.50	0.95	ND	2.30	ND	2.03	2.68
C19	ND	2.1	1.57	ND	ND	ND	ND	ND	ND	2.69	ND	ND
C20	1.12	ND	8.63	1.45	10.11	6.67	1.0	2.97	2.30	0.94	2.65	1.70
C21	ND	0.54	2.82	0.95	ND	ND	ND	ND	ND	ND	1.52	2.24
C22	0.48	1.63	1.95	ND	9.16	ND	1.72	1.2	ND	1.32	3.65	1.1
C23	ND	ND	ND	1.71	ND	ND	ND	ND	ND	ND	ND	ND
C24	ND	1.71	4.02	ND	ND	7.16	2.87	1.30	1.32	ND	3.41	1.23
C25	0.88	ND	2.70	1.68	2.55	ND	0.82	ND	ND	2.15	ND	ND
C26	0.36	1.41	ND	0.49	ND	ND	ND	0.81	ND	ND	ND	ND
C27	ND	ND	1.37	ND	ND	ND	ND	ND	ND	ND	ND	1.16
C29	ND	ND	ND	ND	ND	ND	1.47	ND	ND	ND	ND	ND
$\Sigma$ ALHs	11.47	9.36	27.04	14.82	24.94	23.59	11.90	9.07	9.32	12.88	13.26	11.22

N.D.: Not detected; RGh: Ras Ghareb; RSh: Ras Shokier; GmB: Gemsha Bay; C14: Tetradecane; C15: Pentadecane; C16: Hexadecane; C17: Heptadecane; C18: Octadecane; C19: Nonadecane; C20: Eicosane; C21: Heneicosane; C22: Docosane; C23: Tricosane; C24: Tetracosane; C25: Pentacosane; C26: Hexacosane; C27: Heptacosane and C29: Nonacosane

**Table (6): Average concentrations ( $\mu\text{g g}^{-1}$  wet weight) of total Polycyclic Aromatic Hydrocarbons (PAHs) and total Aliphatic Hydrocarbons (ALHs) in different size classes of studied gastropod species, collected from different sites of the Suez Gulf, during autumn 2013.**

Hydrocarbons	Size classes	Gastropod species											
		<i>Cellana rota</i>			<i>Cerithium caeruleum</i>			<i>Echinolittorina marsrubri</i>			<i>Nerita sanguinolenta</i>		
		RGh	RSh	GmB	RGh	RSh	GmB	RGh	RSh	GmB	RGh	RSh	GmB
PAHs	A	6.78	1.91	1.51	4.97	ND	ND	2.49	ND	ND	8.66	4.18	1.65
	B	9.98	3.18	3.91	11.46	4.16	1.14	6.41	2.36	1.14	13.83	7.83	3.23
	C	26.12	7.51	5.89	12.67	8.29	1.96	7.89	4.71	1.96	15.01	11.36	4.2
	D	37.95	11.3	8.54	44.54	10.85	2.59	10.6	6.32	2.59	18.76	16.53	6.4
ALHs	A	3.28	ND	ND	5.89	ND	3.23	1.76	1.1	4.16	3.55	4.1	3.15
	B	5.58	2.1	3.23	6.28	5.86	11.1	1.93	1.32	6.86	4.25	6.75	7.89
	C	6.59	4.18	17.76	8.92	7.12	24.41	2.14	2.54	8.23	6.49	10.07	10.94
	D	10.24	5.16	28.95	9.88	8.66	28.95	3.58	4.58	11.57	11.19	15.67	18.03

N.D.: Not detected; RGh: Ras Ghareb; RSh: Ras Shokier; GmB: Gemsha Bay;

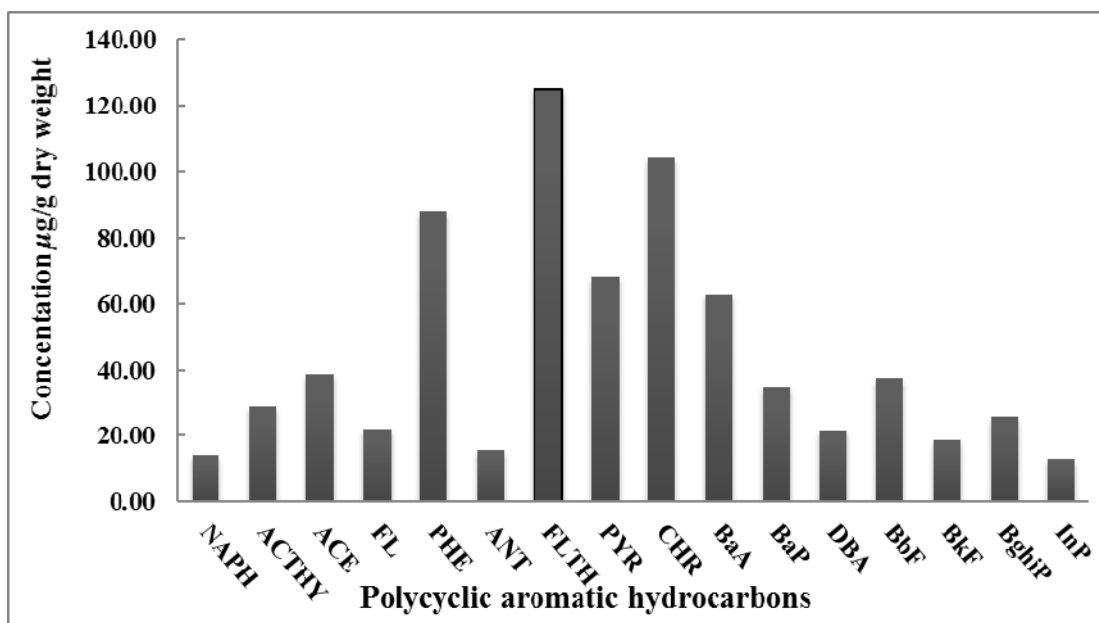


Figure (2): Average concentrations of polycyclic aromatic hydrocarbons (PAHs) in the surface sediment from the Suez Gulf.

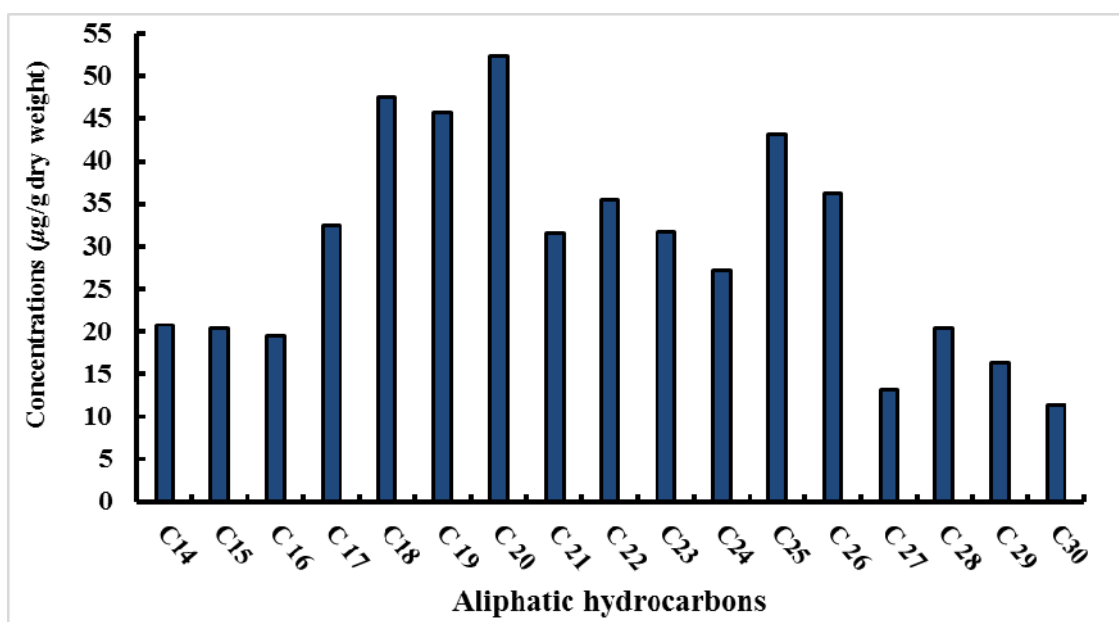
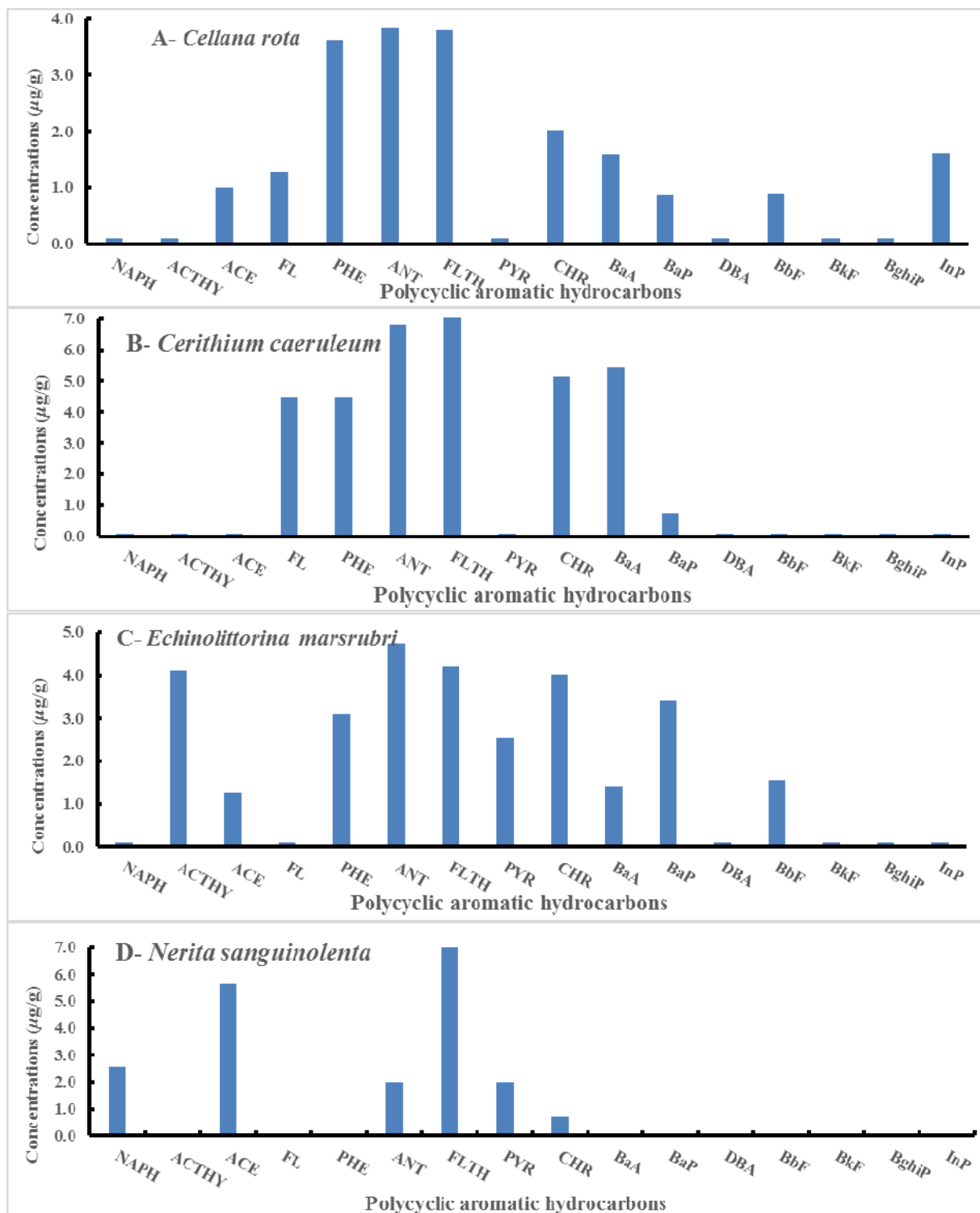
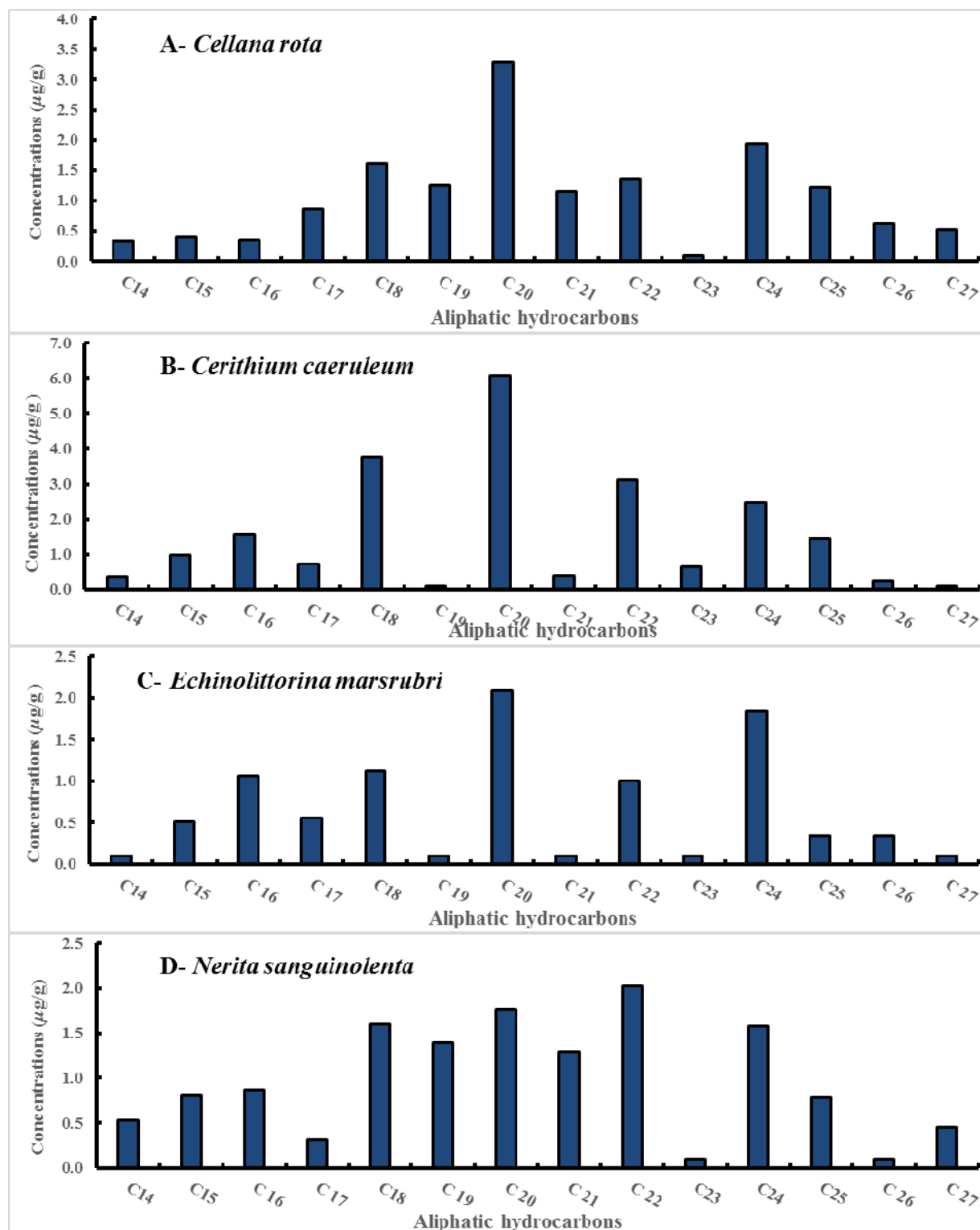


Figure (3): Average concentrations of aliphatic hydrocarbons (ALHs) in the surface sediment from the Suez Gulf.



**Figure (4):** Average concentration of polycyclic aromatic hydrocarbons (PAHs) in soft tissue of studied gastropods from all studied sites in the Suez Gulf.



**Figure (5):** Mean of composition pattern of Aliphatic hydrocarbons (ALHs) in the soft tissue of studied gastropods from all studied sites in the Suez Gulf.

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