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Evaluation of Hydrocarbons in Tissues of Bivalve Mollusks from the Red Sea Coast

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***Abstract*—**The concentration of polycyclic aromatic hydrocarbons (PAH) in clams (*A. glabrata*) was examined in samples collected from Alseef Beach, 30 km south of Jeddah city. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the 14 PAHs. The concentration of total PAHs was found to range from 11.521 to 40.149 ng/gdw with a mean concentration of 21.857 ng/gdw, which is lower compared to similar studies. The lower molecular weight PAHs with three rings comprised 18.14% of the total PAH concentrations in the clams, while the high molecular weight PAHs with four rings, five rings, and six rings account for 81.86%. Diagnostic ratios for PAH source distinction suggested pyrogenic or anthropogenic sources.

***Keywords*—**Bivalves, biomonitoring, hydrocarbons, PAHs

I. Introduction

THE Saudi Red Sea coast has been exposed to many contaminants during the past few decades, including petroleum hydrocarbons, due to the rapid growth of industrial and urban activities in the Kingdom [1]. Activities such as oil exploration and production, movement of heavy oil tankers with accidental oil spills, industrial and municipal effluent discharges, as well as urbanization, and population growth with an increase in the number of vehicles result in seashores being stressed by oil pollution and the other fossil fuels which could have harmful effects on the marine environment if it is uncontrolled [2], [3]. However, there are little data on the contamination by petroleum hydrocarbons in the coasts of Jeddah.

PAHs are a group of environmental pollutants generated mainly during the incomplete combustion of organic materials and occur naturally in crude oil, and petroleum products [4], [5], PAHs cause harmful effects on organisms and the environment [6], And some PAH compounds have been classified as priority pollutants by the US Environmental Protection Agency (US-EPA) [7]. High-molecular weight of PAHs compounds with four-to seven-rings are mutagenic and carcinogenic, while low molecular weight compounds with two-to three-ring, although less mutagenic, can be extremely toxic [8]. The effects of polycyclic hydrocarbons on organisms include the inhibition of enzymatic activity and severe debility and reducing the immune system [9], [10]. These biological effects may reduce the ability of organisms to survive and be reproductive, thus leading to specific ecological changes, and changes in the structure of community and functions [11]. This disturbance of the ecosystem has severe consequences for the environment and human health, leading to social and economic impacts on communities [12].

Measuring pollutants in marine organisms to monitor water quality is more accurate than direct sampling, as it reflects the effect of the physical and chemical state to which the organisms have been exposed over a period of time because hydrophobic pollutants accumulate in the lipids of the organism, over seasonal or annual time periods [13], while the physical and chemical analyses reflect the water quality only at the time of the sampling, which may imply a higher grade of water quality than reality [14]. Bivalves like clams are considered good sentinel organisms and are widely used to monitor contaminations in marine environments since the sessile nature of bivalves prevents their migration from areas with highly concentrated contaminants thus reflecting PAH exposure at specific sites, and because of their known capacity to accumulate hydrocarbons in their tissues due to their slow metabolism of PAHs [15], [4].

The bioaccumulation of PAHs in bivalves is a complex process that involves many factors such as the lipid contents, bioavailability of the PAHs, depuration/elimination of PAHs and the nutritional capacity of the media (sediment and water) [16]-[19]. Moreover, the exposure of bivalves to PAHs fractions, low molecular weight (LMW) or high molecular weight (HMW) is also influenced by the route of absorption, as the absorption not only occurred by direct filtration through the gills (mostly low molecular weights) but also absorption through the digestive system, which means greater absorption of PAHs molecules of high molecular weight [26].

II. Methodology

A. Field Monitoring

Al Seef Beach (21.1773921, 39.1739681), the sampling area, is located 30 km south of Jeddah city.

B. Sample Collection

A large number of Wedge clams (*Atactodea glabrata*) of similar size (25-30 mm shell length) were collected from the intertidal sandy beach, during the period from May 6 to July 5, 2021. In the laboratory, the clams were washed with water, opened with stainless steel knives, and the soft parts recovered. The wet tissues were placed in the centrifuge tubes and frozen at –20 °C for two days before drying, until the hydrocarbon

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analysis.

C. Analyses of PAH Concentrations in Clam Samples

A sample of 6 grams of dry-weight was analyzed for PAHs following the well-established techniques [20]-[22]. Five samples were extracted with a Soxhlet extractor with 200 ml of methanol for 8 hr. After the extraction, 0.7M KOH (20 ml) and distilled water (30 ml) were added to the flask and the reflux was continued for 2 hr to saponify the lipids. The content of the extraction flask was extracted in a separator funnel with 80 ml of hexane thrice. Then the extracts were combined, dried with anhydrous sodium sulfate and filtered through filter paper. The hexane fraction was concentrated with a rotary evaporator down to about 5 ml at 3 °C followed by concentration with nitrogen gas stream down to a volume of 1 ml. For the fractions: in the first, cotton was transferred into the column followed by 1 g of sodium sulfate, and then 10 g of silica gel, then 10 g of alumina and finally 1 g of sodium sulfate. The extract (1 ml) was sequentially eluted from the column with 40 ml of hexane and dichloromethane (90:10) for the aromatic hydrocarbons fraction. The fractions were concentrated with a rotary evaporator down to about 5 ml at 30 °C followed by concentration with nitrogen gas stream down to a volume of 1 ml.

D. Source Identification of PAHs in Clam Samples

In order to identify the sources of PAHs, the different ring numbers of PAHs and the ratios of Anthracene (Ant), Phenanthrene (Phen), Fluoranthene (Flu) and Pyrene (Py) levels were quantified and plotted as follows: The Ant/(Ant + Phen), Flu/(Flu + Py) [23] and ΣLMW/ΣHMW [24].

E. Statistics

Microsoft Excel was used for basic statistics (Standard deviation, Mean, Average, and Range) and graphs.

F. Assessments of PAH Contamination Levels in Clams

The PAH contamination levels in the clam were also compared with those determined in elsewhere (see Table I).

TABLE I
PAHs in Bivalves from Different Parts of the World

Country	ΣPAH	Dominant PAH	Bivalve species	Reference
Tumaco Port, Colombia	31.0-169.0 ng g−1	HMW	*Anadara tuberculosa*	[25]
Levrier Bay Zone, Atlantic shore of Mauritania	248–257 µg/kg	HMW	Mussel (*Perna perna*)	[26]
Fangcheng Port, China	3.63–12.77 ng/g wet weight	LMW	Clam (*Meretrix meretrix*)	[27]
Cienfuegos Bay, Cuba	47.38–89.30 ng/g wet weight	LMW	Green mussel (*Perna viridis*)	[28]
Beibu Gulf, China	50.9- 80.1 ng/g dw	HMW	Mussel (*Mytilidae* sp.)	[29]

III. Results and Discussion

The concentrations of nine individual PAHs were determined in five clam samples from Alseef Beach. The nine PAHs detected in clam samples were Acenaphthylene, Fluorene, Phenanthrene, Anthracene, Pyrene, Benz[a]anthracene, Benzo[j]fluoranthene, Dibenz[a,h]anthracene, Benzo[ghi]perylene. Among the nine PAHs identified, Benzo[ghi]perylene had the highest mean concentration (ng/gdw) of 5.908, while Pyrene had the lowest (ng/gdw) of .353. Total PAH ranged from 11.521-40.149 ng/gdw, and the mean concentration PAH was 21.857 (ng/gdw). The lower molecular weight PAHs with three rings (Flu, Atr, Acy, Phn) comprised 18.14% of the total PAH concentrations in the clams, while the high molecular weight PAHs with four rings (FIu, Pyr, BaA, and Chr), five rings (BjF, BkF, BeP and DB(ah)), and six rings (InF, BghiP) account for 81.86%.

TABLE II
Average Concentrations of PAHs in Clams PAH (ng/g dw)

Stdeva	Median	Range	Average	Compounds
1.082998	0.81	2.836	1.049	Acenaphthylene
1.489463	0.848	3.39	1.299	Fluorene
0.374375	0.46	0.852	0.657	Phenanthrene
0.376099	0.38	0.917	0.532	Anthracene
0.275561	0.313	0.77	0.353	Pyrene
1.684766	1.75	4.219	2.338	Benz[a]anthracene
0	0	0	0	Chrysene
6.900209	0	12.598	5.039	Benzo[j]fluoranthene
0	0	0	0	Benzo[k]fluoranthene
0	0	0	0	Benzo[e]pyrene
0	0	0	0	Indeno[1,2,3-cd]fluoranthene
6.560841	2.676	16.06	4.678	Dibenz[a,h]anthracene
6.687644	5.371	16.115	5.908	Benzo[ghi]perylene
12.52961	18.619	29.731	21.857	Σ PAH

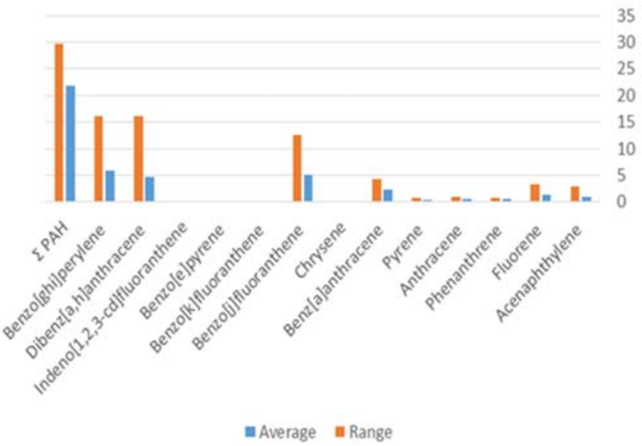

Fig. 1 Concentrations of PAHs, average and range

The LMW-PAH/HMW-PAH ratios in clams were < 1. Ant/(Ant + Phen) ratio was .45, Flu/(Flu + Py) ratio was .78. The ratio of Ant/Ant+Phe of > 0.1 refers to the dominance of heavy fuel combustion [29]. Thus, high molecular weight PAHs were predominant over low molecular weight PAHs, indicating that PAH contamination in the coastal waters of Alseef Beach is mainly from pyrogenic or anthropogenic sources. Possible sources of HMW PAH in this region are through vehicle engines that are transported to the sea by direct precipitation from the atmosphere and rainwater runoff. The high content of HMW fractions in the results may be due to the lower volatility, lower water solubility, and higher stability of HMW compared to LMW in the aquatic environment. Moreover, the PAH uptake pathway may have a role in the dominance of HMW. Through the clam's reliance on absorption through the digestive system from sediments as it is the main source of HMW PAHs, rather than filtration from the water column.

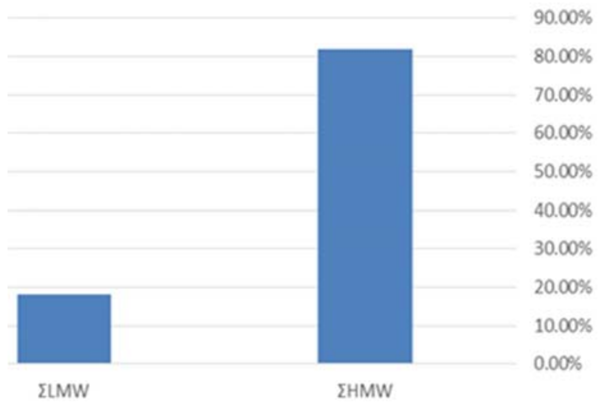

Fig. 2 Relative percentages (%) of LMW and HMW

IV. Conclusion

The average PAH concentrations in this study were very low when compared to most reports worldwide. Therefore, pollution levels are not expected to cause any effects on benthic organisms in the intertidal zone.

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