

Polycyclic Aromatic Hydrocarbons Identification and Source Discrimination in Rural Soil of the Northern Persian Gulf Coast

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Abstract

Due to strategic situation of the Persian Gulf, identifying the petroleum pollution level and source is an important issue. Therefore, this paper enhanced fingerprinting method of applying biomarkers Polycyclic Aromatic Hydrocarbons (PAHs) in identifying source and distribution of oil spills of the exposed areas. 10 soil samples collected from the northern coasts of the Persian Gulf along three provinces in the south of Iran. PAH concentrations in the soil ranged from 42.76 to 5596.49 ng.g⁻¹. In the present study the distribution of 3 ringed PAHs was much higher than the other PAHs. Phenanthrene and alkylated derivatives of phenanthrene such as 3-methyl, 2-methyl, 9-methyl and 1-methyl phenanthrene were distinctively higher than the other components. According to the result PAHs concentration can be considered as no or little risk of toxicity for the organisms living in soil except for Fluoranthene, comparing LD₅₀. Applying marker ratios revealed that in most of the sampling sites showed perogenic sources and it emphasizes on the direct impact of oil and petroleum products to the lands due to oil well exploitation and transferring pipelines.

Keywords: Polycyclic Aromatic Hydrocarbons; Persian Gulf; rural soil; marker ratios

1. Introduction

The Persian Gulf, located in southwestern Asia, is a vast expansion of the Indian Ocean situated between Iran and the Arabian Peninsula. The Persian Gulf and its coastal areas are the world's largest particular source of crude oil and related industries govern the region. It is distinguished as a great economic source of oil and gas and two third of the oil resources of the world is to be found here. The oil exploration and exploitation of the seabed, possible eruption of oil wells, tankers clash and sink, discharge of the balanced water of the ship, military attack to oil tankers oil fields and explosion of the oil in the uninterrupted wars of the region, caused to the Persian Gulf, a susceptible environment. Polycyclic aromatic hydrocarbons (PAHs), are almost ubiquitous contaminants in surface soils and sediments worldwide due to the mentioned activities. They are intensely toxic and have carcinogenic properties and are being recognized with rising occurrence as major contaminants in sediments, particularly in urban environments. All of the priority pollutants PAHs are non-alkylated; they contain no carbon side-chains.

Anthropogenic PAHs in the environment were originated principally from two different sources (combusted/pyrolyzed fossil fuel versus spilled petroleum, or pyrogenic versus petrogenic). These two source classes could be simply identified on the basis of their alkyl PAH distributions (Giger and Blumer, 1974; Lee *et al.*, 1977). Since the nature of their formation is similar in physical/chemical properties, groups of petrogenic or pyrogenic PAHs tend to co-exist in the environment. This knowledge help the investigator to differentiate specific PAH accumulation, or fingerprints, as resulted from a specific source (Wang *et al.*, 1998).

Molecular indices based on PAHs physical-chemical behavior have been commonly used to assess the differences between those of pyrolytic and petrogenic origin (Guinan *et al.*, 2001; Baumard *et al.*, 1998a; Budzinski *et al.*, 1997). Based on the research by (Takada and Eganhouse, 1998) to help the source identification of petroleum pollutants some molecular biomarkers are employed. Molecular markers are defined as compounds whose structures are associated to specific origin. Biological markers or molecular biomarkers are molecular remnants of previously living

or organisms. Their unique structures can provide very precise information on the origins of the compounds (Takada and Eganhouse, 1998).

All oils contain four methyl-phenanthrenes (in two pairs of doublet peaks: 3- and 2-, and 9- and 1-MP). Several attempts have been made to reveal the ratios among four methyl phenanthrene isomers have been widely used to relate the thermal history of crude oils and its source strata, and numerous methyl phenanthrene indices have been defined for monitoring the thermal maturities of oils (Radke *et al.*, 1986) and for spill oil source identification (Wang *et al.*, 1999). PAH distributions are the most practical tool in distinguishing pyrogenic from petrogenic hydrocarbons. Pyrogenic materials generally display alkylated PAH homologue distribution patterns in which the parents PAHs are often the most abundant. The ratio of the sum of methyl-phenanthrenes to phenanthrene (MP/P), an index of petrogenic PAHs contribution, was more than unity for most of the sediment samples and it indicated that they are derived from pyrogenic sources.

There was not a comprehensive research on the soil of the Persian Gulf coast. Therefore, the purpose of this study is to apply environmental fingerprinting techniques and to identify the origin of oil spills as soil contaminants. Therefore, this study focused on the polynuclear aromatic hydrocarbons contamination, on the soil of northern Persian Gulf coast in order to study petroleum pollution along three provinces being identified as the sources of oil and gas fields.

2. Materials and Methods

2.1. Study area

In order to do this purpose soils from northern coasts of Persian Gulf have been selected. Bushehr,

Hormozgan and Khuzestan Provinces are selected as gas and oil producers. Soil samples were selected from rural lands. Relative location of each sampling site was investigated first, using aerial and satellite images.

2.2. Sampling sites

Soils were taken using quadrat which was thrown thrice randomly; final sample was a composite one from 10 cm of top soil with a clean stainless steel spoon. The positions of sampling sites were recorded using GPS (Table 1). All samplings were conducted during June 2011. Map of sampling is shown in (Fig. 1). The soil samples (0-10 cm) were collected in the pre-cleaned containers with dichloromethane and n-hexane with aluminum foil inserts. The samples were stored in the ice box and darkness during transportation to the laboratory of Persian Gulf Research and Studies Center (PGRSC) and kept frozen (-20°C) till being analyzed.

2.3. Analytical procedure

The analytical methods used in this study cover a wide range of compounds from non polar hydrocarbons (e.g., n-alkanes) to semi volatile compounds (e.g., PAHs). Authentic standards of deuterated PAHs (naphthalene-d₈, phenanthrene-d₁₀, p-Terphenyl-d₁₄, chrysene-d₁₂, perylene-d₁₂) and PAHs (dibenzothiophene, phenanthrene, anthracene, methyl anthracene, fluoranthene, pyrene, methylpyrene, chrysene, benzo(a)anthracene, benzo(k)fluoranthene, benzo(e)acephenanthrene, benzo(e)pyrene, benzo(a)pyrene, dibenzo(a,h)anthracene) were purchased from Sigma-Aldrich pure chemical. The soil samples were dried using sodium sulphate anhydrous, spiked with 100 µL of 10 µg.g⁻¹ deuterated surrogates (naphthalene-d₈, phenanthrene-d₁₀, chrysene-d₁₂ and perylene-d₁₂)

Table 1. Locations of soil sampling

Sample Site	Sample Code	DMS (N-E)		Soil Texture
Haleh	S1	N:27.23.36.3	E:52.38.50.1	Sandy loam
Zabareh-Bonood	S2	N:27.21.40.4	E:52.42.49.5	Silt loam
Lavarchahu-Hashniz	S3	N:27.04.35.4	E:53.31.05.1	Sandy loam
87 km to bandar lengeh	S4	N:26.48.36.8	E:54.11.03.6	Sandy loam
Charak and Lengeh	S5	N:26.47.35.2	E:54.18.09.7	Silt loam
Southern Mianloo	S6	N:27.46.48.8	E:52.07.18.1	Sandy loam
Deylam-Amery	S7	N:30.08.59.8	E:50.11.06.00	Silt loam
Deylam-Hendiyan	S8	N:30.06.38.00	E:50.09.11.6	Silty clay loam
Abadan-Emam	S9	N:30.26.13.3	E:48.26.10.9	Sandy loam
Mond	S10	N:26.50.13.1	E:52.57.12.3	Silt loam

DMS (N-E)=Degree Minute Second (North-East) of geographic coordinates of the sampling sites, S=soil

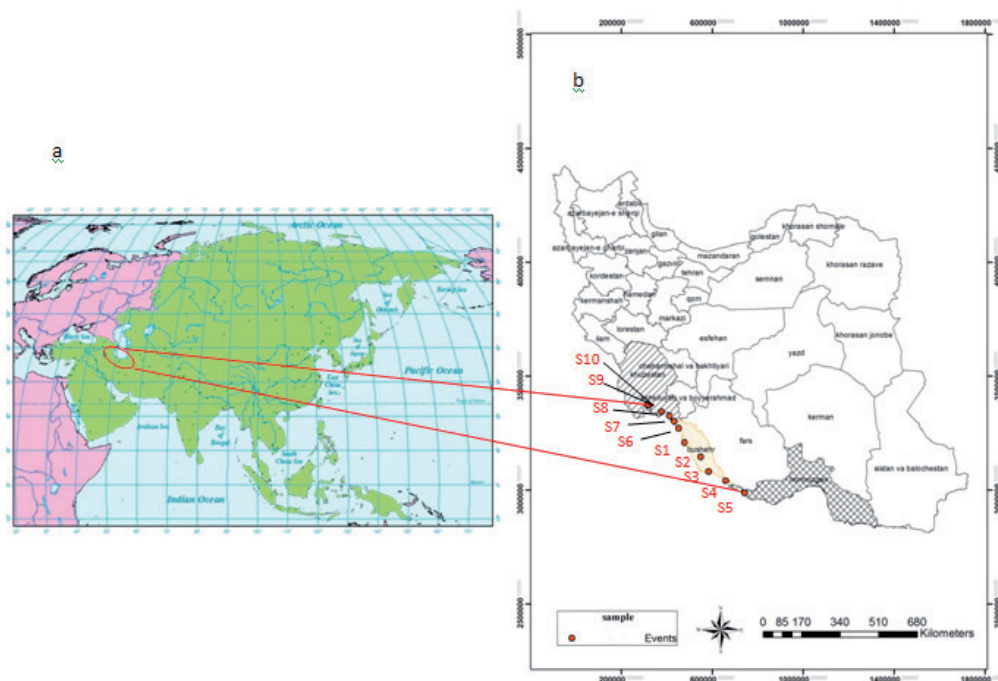


Figure 1. a) Geographical location of Iran in Asia, b) Soil sampling location in the northern coast of the Persian Gulf

and 100 μL of a 10 $\mu\text{g}\cdot\text{g}^{-1}$ internal injection standard (p-terphenyl- d_{14}) (Zakaria *et al.*, 2002). The samples were then subjected to soxhlet extraction with 350 ml dichloromethane for 8 h (Anyakora *et al.*, 2005; Culotta *et al.*, 2006; Nikolaou *et al.*, 2009). Extracted samples were purified and fractionated into an aliphatic and an aromatic fraction through two-step silica gel column chromatography (Boonyatumanond *et al.*, 2006; Yim *et al.*, 2005; Zakaria *et al.*, 2001). Elemental copper (granular) was activated using Hydrochloric Acid 1N and then the samples contained activated copper remained constant overnight. The hydrocarbon fraction was subsequently injected into the GC-MS using the Selected Ion Monitoring (SIM) mode (Zakaria *et al.*, 2000; Zakaria *et al.*, 2001).

2.4. Quality control and quality assurance

Quality control for the PAHs analyses was carried out by monitoring the recovery of surrogate standards (naphthalene- d_8 , phenanthrene- d_{10} , chrysene- d_{12} and perylene- d_{12}) spiked just before extraction. The PAH concentrations were corrected against the spiked surrogates. The recovery of surrogates generally ranged from 70% to 110% of the spiked concentration. Samples with recoveries below 70% were re-analyzed. Reproducibility of analyses was tested by four replicate analyses of sediment extracts; it ranged from 8.5% to 12% of relative standard deviation. Procedural blanks were run with every batch. The quantification limit

for individual PAH species was set at five times the detected amount in the procedural blank. Procedural blanks for PAHs normally gave 0.0625 to 1 $\text{ng}\cdot\text{g}^{-1}$ total PAHs per sample.

Statistical analyses, including the ANOVA, analysis were performed using SPSS 16.0 for Windows. A repeated measures one-way ANOVA procedure was carried out to assess the significant differences of the PAHs dataset. The significance level was set to 0.05.

3. Result and Discussion

3.1. PAHs concentration in soil samples

A total number of 10 samples of soil collected and analyzed for 15 individual PAHs. Total PAHs concentration ranged from 42.76 to 5596.42 $\text{ng}\cdot\text{g}^{-1}$ dry weight (Fig. 2). Total PAHs concentrations are illustrated in (Fig. 2). The highest concentration was in S_9 followed by S_4 as described previously. Fig. 3 shows the PAHs component concentrations of rural soil in the northern coast of the Persian Gulf in the present study. It can be understood that PAHs in these samples are distributed in all molecular weights.

Phenanthrene and alkylated derivatives of phenanthrene such as 3-methyl, 2-methyl, 9-methyl and 1-methyl phenanthrene are distinctively higher than the other components (Fig. 2). This might be attributed to the fresh inputs of PAHs products into the soil. It has been reported (CEN, 2002) that, in many crude oils,

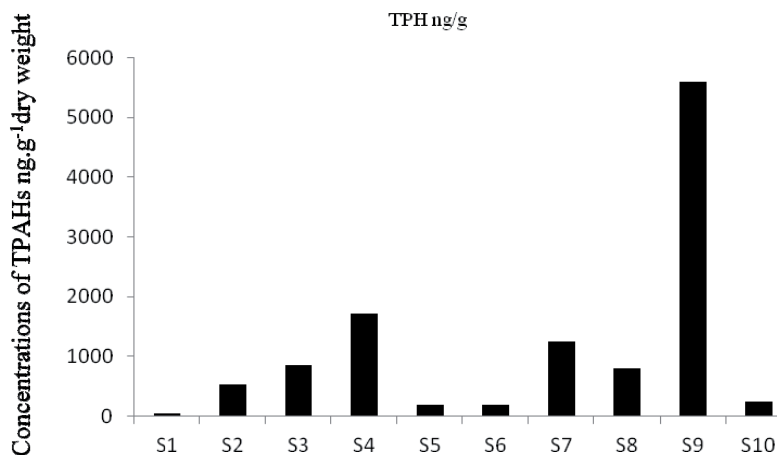


Figure 2. Total PAHs concentrations in rural soil

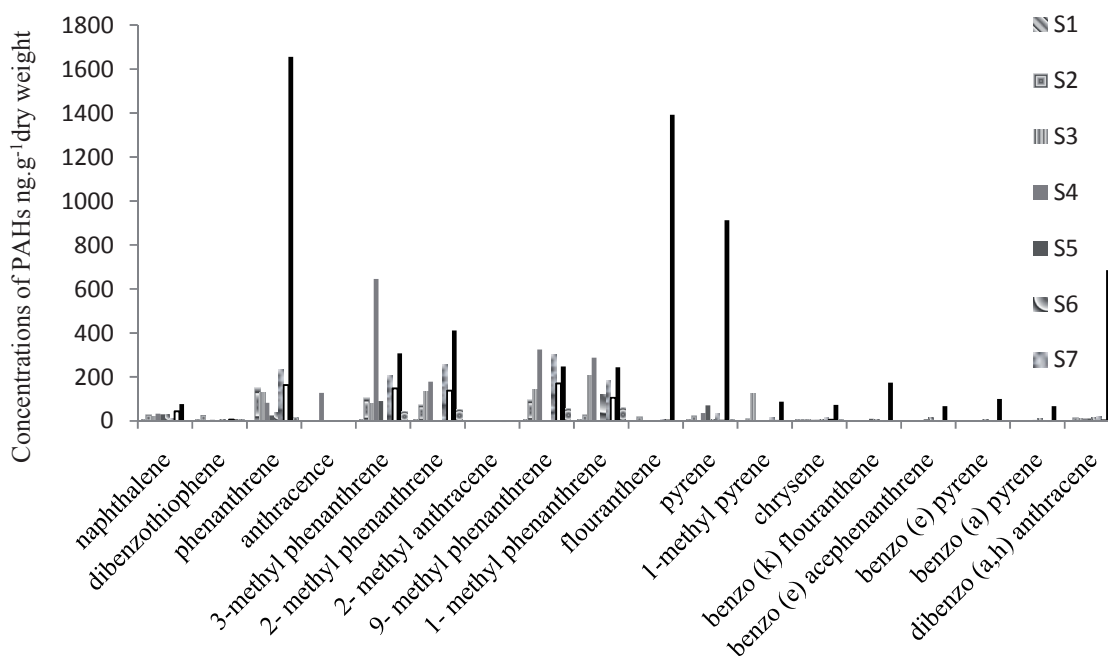


Figure 3. PAHs components concentration in rural soil of the Persian Gulf

the first doublet peak is smaller than the second doublet peak, and the methyl-anthracene (the peak between the two doublet peaks) is often very small or insignificant. Comparing the result to the literature revealed that for methyl anthracene it was in consistent with the literature and it was not detected or it showed a small amount. Lower molecular weight such as naphthalene and dibenzothiophene in most of the samples ranged from low to very low, especially for dibenzothiophene. The highest amount of Naphthalene is in S₉ (76.20 ng.g⁻¹) related to Abadan rural area as a result of oil transferring pipelines along the region.

The amounts of higher molecular weight were low. However, the presence of higher molecular weight PAHs is indicative of high persistence of the PAHs - as its nature - to evaporation and biodegradation in the soil. The most striking result to emerge from the data was in S₉, which is located around Abadan city, one of the most important places of oil production and refineries. It was revealed that almost all 2 and 3-rings parent and alkylated PAHs were greater in this station. As a result, it could be explained by direct impact of crude oil within the area, meanwhile the amount of lower molecular weight showed it was not recent spill oil into the region.

According to (Fig. 3) the concentration of alkylated phenanthrene, phenanthrene and anthracene were higher in S4 which is located near Bandarlengeh port relative to other stations. It showed a dramatic effect of crude oil and petroleum products in Hormozgan Province as well. The concentrations for chrysene, Benzo (K) fluoranthene, Benzo (e) acephenanthrene, Benzo (e) pyrene, and Benzo (a) pyrene were low in comparison to the alkylated phenanthrenes. In a combusted related hydrocarbon, the unsubstituted (non-alkylated parent) compounds are much more abundant than alkylated members of any homologous series. This is often most striking for the phenanthrene series. Alkylated phenanthrene members are often the most abundant aromatic constituents of weathered oils (Wang *et al.*, 1999). Contrary to expectations, this study did not find a significant concentration of anthracene in most of the sampling sites. As it can be seen from (Fig. 3), anthracene exists merely in samples S₄ which is related to a rural area near to Bandar lengeh port and it showed an influence of combustion and pyrogenic source of PAHs.

3.2. Source differentiation using marker ratios

PAHs isomer pair's ratios were calculated to differentiate possible sources of PAHs. In contrast to petrogenic sources, pyrogenic sources are characterized by low ratios of methylphenanthrenes/phenanthrene (MP/P<1) in association with higher ratios of fluoranthene/(fluoranthene+pyrene) (Flu/Flu+Pyr>0.4) (Budzinski *et al.*, 1997; Yunker *et al.*, 2002).

Based on the PAH isomer pair ratio measurements compiled by (Yunker *et al.*, 2002), Flu/Flu+Pyr <0.4 implies petroleum, 0.4-0.5 implies petroleum combustion, and >0.5 implies combustion of coal, grass and wood. The Flu/Flu+Pyr ratios calculated for most of the samples were less than 0.4 (from 0.03 to 0.4) (Fig. 4). These ratios show that petrogenic input at most stations (Flu/Flu+Pyr<0.4). Two out of ten samples showed values 0.6-0.65 (Stations S₉ and S₈), suggesting pyrogenic inputs at these two stations. The ratio Flu/Flu+Pyr revealed multiple PAHs sources (petrogenic and pyrogenic).

Methylphenanthrene/phenanthrene (MP/P) ratio which is used for discrimination between petrogenic and pyrogenic sources of PAHs was highly greater than 1 while it should be <1 in combustion mixture and between 2-6 in unburned fossil fuels (Garrigues *et al.*, 1995; Youngblood and Blumer, 1975). In other word, it confirms the petrogenic inputs of the PAHs except for station S9 which shows 0.73 (Fig. 5). It confirms the existence of pyrogenic inputs as well.

One striking result which was different in comparison to the world data on PAHs contamination found for the total PAHs concentration in rural area. Total concentration was significantly high. It was as a result of direct petroleum inputs to the land, since they are located near the oil wells or on the way of oil transferring pipelines. Therefore, they showed dramatically, higher concentration of PAHs. In less urbanized areas additional input of petrogenic sources will results in abundance of petrogenic PAHs above the background levels, which explains the abundance of petrogenic PAHs in almost all the stations.

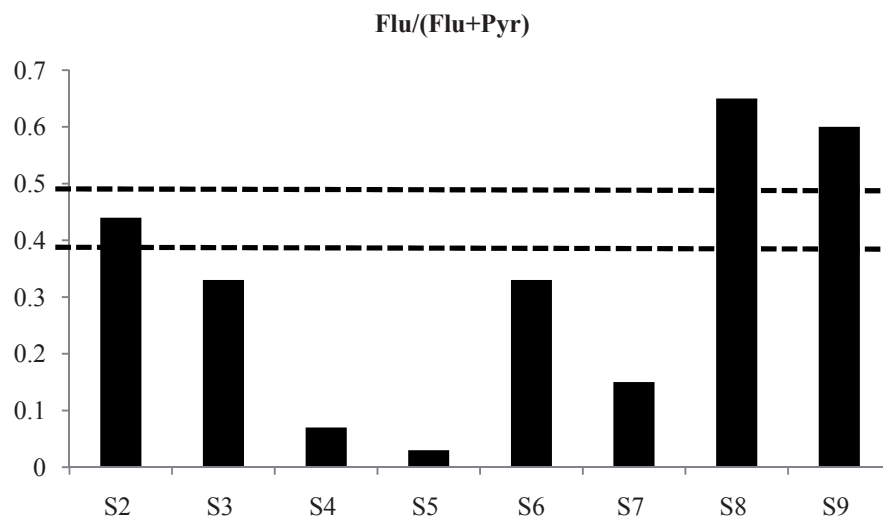


Figure 4. PAHs ratio of Flu/Flu+Pyr in rural soil

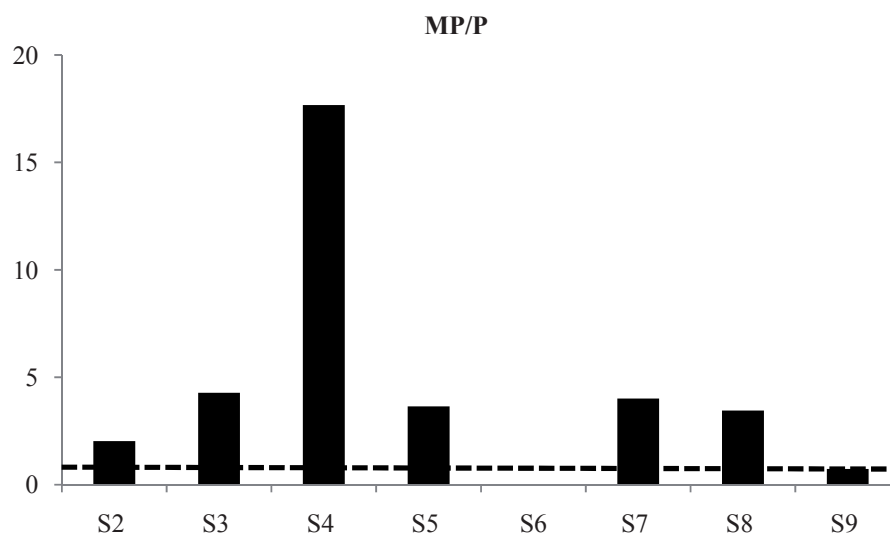


Figure 5. PAHs ratio of MP/P in rural soil

3.3. Ecotoxicological risk assessments of PAHs

Effects on the organisms are typically considered to be an early warning indicator of potential human health impacts. In Iran, no environmental standards have been established for PAHs in soil. Therefore, ecotoxicological effects of these pollutants in this study area were approximately evaluated by applying published guidelines from National Oceanography and Atmospheric Administration (NOAA) (Buchman, 1999) and from Canada (CCME, Canadian Council of Ministers of the Environment, 2002).

Table 2 shows the recommended guideline concentration of these pollutants in soils from environmental agencies. Maximum concentrations of individual PAHs were comparable with guideline values. For the case of fluoranthene there was not mentioned any concentration and the concentration of fluoranthene for the present study showed high amount (1392.9 ng g^{-1}) and possibly it may have some harmful and toxic effects on the organisms living in soil. However, more evaluations and biological studies are needed to decide if this concentration has toxic effects on the organisms or no. Fortunately, almost all

Table 2. Guidelines for individual PAHs in soil ($\mu\text{g kg}^{-1} \text{ dw}$): comparison with this study

PAHs	Soil Guidelines*		
	NOAA	CCME	Present study (Max. concentration)
naphthalene	5000	600-22 \times 10 ³	76.2
acenaphthylene	0	0	-
acenaphthene	0	0	-
fluorene	0	0	-
phenanthrene	5000	5 \times 10 ³ -50 \times 10 ³	1655.5
anthracene	0	0	127.03
fluoranthene	0	0	1392.9
pyrene	10 \times 10 ³	10 \times 10 ³ -100 \times 10 ³	912.9
benzo (a) anthracene	1000	0	-
chrysene	0	1000-10 \times 10 ³	73.14
benzo (b) fluoranthene	1000	1000-10 \times 10 ³	-
benzo (k) fluoranthene	1000	1000-10 \times 10 ³	173.91
benzo (a) pyrene	1000	700	67.4
benzo (ghi) perylene	0	0	-
dibenzo (a,h) anthracene	1000	1000-10 \times 10 ³	685.7
Indene (e1,2,3-cd) pyrene	1000	1000-10 \times 10 ³	-

*guidelines for residential, park, community and industrial soil, NOAA-National Oceanic and Atmospheric Administration, CCME-Canadian Council of Ministers of the Environment

Table 3. LD₅₀ values of some representative PAHs

Material	Number of carbon rings	LD ₅₀ value (mg/Kg)	Test Subject	Exposure route
Naphthalene	2	533-710	Male/Female mice respectively	Oral
Phenanthrene	3	750	Mice	Oral
Anthracene	3	>430	Mice	Intrapretoneal
Fluoranthene	4	100	Mice	Intravenous
Pyrene	4	514	Mice	Intrapretoneal
Benzo(a)pyrene	5	232	Mice	Intrapretoneal

Normally toxicity increases with an increase in number of benzene rings, but data should be examined using careful concern of the exposure route, etc. (Data taken from the risk assessment Information System: RAIS) <http://risk.Isd.ornel.gov>.

molecular weight PAHs were lower than the guideline values and assumed no adverse effects to the biota.

The relative toxicity of PAHs can be measured using LD₅₀ values (the lethal dose in 50% of cases). These are expressed as milligrams of toxic material per kilogram of the subject's body weight that will cause death in 50% of cases. It is important to specify the route by which the toxic material was administered to the test animal (such as oral or intraperitoneal), and the animal upon which the toxic material was tested (ie rat, mouse) (Bamforth and Singleton, 2005). Table 3 shows the LD₅₀ values of some representative PAHs.

According to the tables 2 and 3 and comparing the result of the study it was revealed that there is not any risk of high toxicity for the organisms living in soil except for Fluoranthene which was high and station S₉ which had high concentration of PAHs. Although it should be considered that microorganisms and invertebrates of the soil are different from their tolerance to these contaminants and careful and more precise examinations are needed to evaluate how the situation is for the smaller organisms. Since none of these samples showed values higher than mentioned LD₅₀, they do not suppose risk for human health.

4. Conclusion

Total concentration of the PAHs in Stations S₁, S₄, S₅ and S₁₀ were significantly ($p < 0.05$) lower than the other stations. S₁ is situated in a protected region of Bushehr Province so it is obviously evident that the concentration will be low. For the cases S₄ and S₅, they are located in Hormozgan Province. They are far away from the oil fields so as a result the concentration of total PAHs is lower than the other stations. On the whole, the other stations showed higher amount of PAHs components, however, it does not have significant harmful effects comparing to global scales and LD₅₀ of some of the PAHs components.

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