Baseline

Concentration of polycyclic aromatic hydrocarbons in zooplanktons of Bushehr coastal waters (north of the Persian Gulf)

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ABSTRACT

In this study, polycyclic aromatic hydrocarbon contents in zooplanktons of coastal waters of Bushehr Province at the north of the Persian Gulf were evaluated. Zooplankton samples were collected from 18 stations in both summer and winter (2017), and their polycyclic aromatic hydrocarbon contents were measured by gas chromatography–mass spectrometry after ultrasound-assisted extraction. Results revealed that among 16 compounds that were analyzed, only 7 compounds were recognizable at both seasons. Pyrene was the most common individual compound of polycyclic aromatic hydrocarbons, whereas acenaphthene was the least individual compound found at both seasons. Additionally, the highest content of the measured compounds in both summer and winter was 16.4–75.9 ng/g and 17.3–93.7 ng/g, respectively. On the basis of the obtained results, the total concentration of polycyclic aromatic hydrocarbons was significantly higher in winter than in summer ($P < 0.05$).

Zooplanktons are considered as the most important herbivorous organisms, and their feeding habit is of ecological importance. They play a role as energy transmitters from primary producers to pelagic fish (secondary producers). Zooplanktons are mostly filter feeders, which mainly feed on phytoplankton. They are bio-indicators in aqueous ecosystems, and their distribution and growth rate depend on abiotic (temperature, salinity, nutrient, and others) and biotic (food sources and competition) factors (Ferdous and Muktadir, 2009; Alcaraz et al., 2010).

Recently, zooplanktons have been commonly applied as pollution indicators for water monitoring, as they respond more quickly than fish to environmental changes and are more identifiable than phytoplankton. Several species of zooplanktons are highly susceptible to water quality changes and respond differently in terms of reproduction and distribution (Sladecek, 1983; Duggan et al., 2001; Ferdous and Muktadir, 2009).

Zooplanktons have a high nutritional value in terms of energy transfer to aquatic stocks in a food pyramid (Gowen et al., 2003). Through diurnal vertical migrations, they act as a biological pump and transfer carbon dioxide from surface waters to water depth. Owing to their short lifetime, they are regarded as an important indicator for monitoring environmental pollution and climatic changes. Hence, any

biodegradable effects of contamination on zooplankton communities would have direct effects on fish stocks (Richardson, 2008).

There are many derivatives of petroleum hydrocarbons, among which polycyclic aromatic compounds (PAHs) are one of the contaminants in the Earth and water ecosystem. Moreover, these compounds are dangerous or hazardous to the aquatic system in nature (Baker, 1976 and Vo-Dinh, 1989). A low percentage of PAHs originate from natural processes (i.e., biological activities); however, the level of PAHs has recently increased following human activities in coastal ecosystems.

The presence or increased level of PAHs is critically harmful for both marine organisms and human health because of their toxic, mutagenic, carcinogenic, and estrogenic effects. PAH compounds differ in physical and chemical properties according to their specific group, for example, these compounds have a high degree of melting and boiling points but low solubility and vapor pressure (Neff, 1979). Their solubility increases with increased temperature and decreased salinity and molecular weight. As a result, these compounds are lipophilic and tend to be absorbed by organic and inorganic matters of suspended material and sediments (Meyer et al., 2001). This is the reason why PAHs are found in abundance in the ecosystem with high loadings of organic matter. In addition, their accumulation is much higher in the body of

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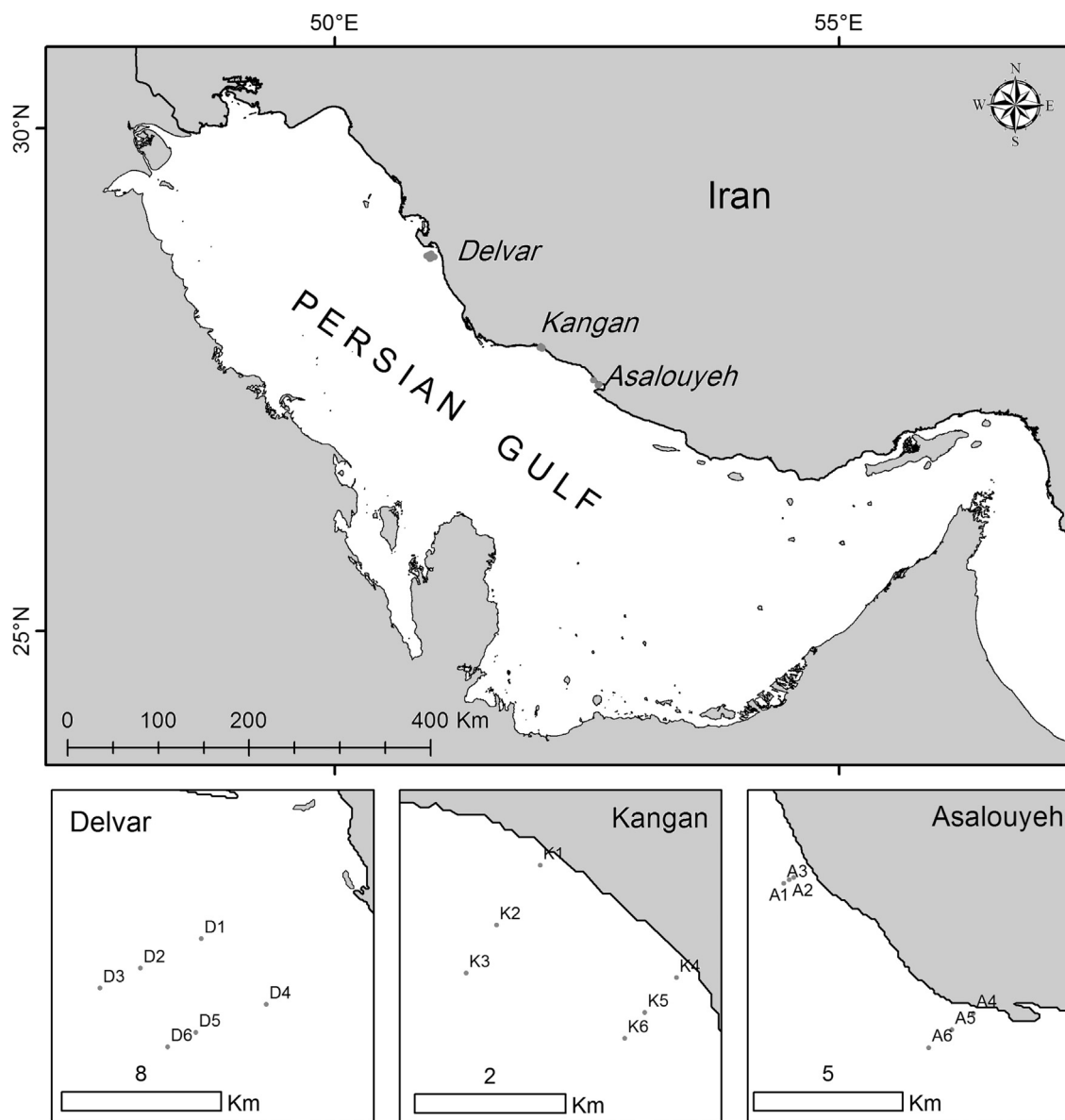 E-mail address: p_nejatkhah@yahoo.com (P.N. Manavi).

Fig. 1. The locations of the sampling sites at the Persian Gulf.

small organisms than in that of large organisms.

The Persian Gulf is a warm sea, and primary production and biodiversity level in this gulf are considerable. Unfortunately, during the last two decades, aquatic species diversity of this unique and vulnerable sea ecosystem has been damaged through human activities such as oil transit, war, fishing, vessel traffic, and development on the beaches. Phytoplankton and zooplankton are the primary and secondary producers in the sea and ocean, respectively. Bioaccumulation from water to phytoplankton and biomagnification from phytoplankton to zooplankton are the first two steps for chemical pollutants to enter the food web (Berglund et al., 2000).

Little information is known about PAH compounds in zooplanktons. Therefore, in this investigation, we aimed to evaluate these compounds in zooplankton samples in the coastal waters of the Bushehr Province in the northern part of the Persian Gulf.

Zooplankton sampling was performed at 3 regions (with 6 stations at each region) during both summer and winter, 2017 (Fig. 1). A 300 μ-mesh plankton net was applied for sampling. After lifting up the net, samples were collected at the end of the net by washing the outer layer of the net. Then, samples collected from each station were rinsed with

distilled water and filtered through a Whatman paper No. 42. Finally, samples were transferred to the laboratory and kept at −20 °C until further analysis.

For analysis of PAHs, samples were freeze-dried at −50 °C for 24 h. Thereafter, each sample was carefully rubbed in a mortar, and 2 g of each sample was weighed and placed in an extracting container. For extraction of PAHs, an ultrasound-assisted extraction (UAE) method (EPA methods 3550B, 1996) was used, wherein dichloromethane (DCM) and acetone were applied as solvents in a 50:50 ratio. Briefly, each sample (15 ml) was extracted by ultrasonic waves for three times (each for 15 min). The mixture was centrifuged and transferred into a rotary evaporator to reduce the volume of the extract to the value of 1 ml. A silica column was used to clean up the extract, followed by complete evaporation of the elution solvent (n-hexane:DCM, 90:10) under a gentle stream of N_2. Then, the residue was redissolved in 1 ml of n-hexane and injected into GC–MS for analysis. The external calibration method was applied to calculate the concentration of each PAH compound. To evaluate the efficiency of the method for PAH extraction, three samples were randomly selected and analyzed after spiking different concentrations of all PAH compounds. Extraction recoveries were

Table 1
Polycyclic aromatic hydrocarbons in zooplankton samples (ng/g dry weight) in different seasons.

| Sites	Na	Acl	Ace	F	Ph	An	Flt	Py	BaA	Chr	B(b)Fl	B(k)Fl	B(a)Py	IPy	DB(a,h)A	B(g,h,i)Pe
Summer																
1A	ND	2.97	1.67	1.96	5.86	2.52	4.59	11.05	ND	ND	ND	ND	ND	ND	ND	ND
2A	ND	ND	ND	1.74	5.68	ND	7.09	18.60	ND	ND	ND	ND	ND	ND	ND	ND
3A	ND	3.04	ND	2.13	5.19	ND	10.06	29.68	ND	ND	ND	ND	ND	ND	ND	ND
4A	ND	2.98	ND	1.69	3.31	ND	2.82	5.73	ND	ND	ND	ND	ND	ND	ND	ND
5A	ND	2.94	1.66	2.66	5.47	ND	6.42	17.40	ND	ND	ND	ND	ND	ND	ND	ND
6A	ND	2.39	0.67	2.04	5.10	0.50	6.20	16.49	ND	ND	ND	ND	ND	ND	ND	ND
1K	ND	3.44	ND	3.33	6.23	1.59	2.10	2.93	ND	ND	ND	ND	ND	ND	ND	ND
2K	ND	3.44	1.90	4.33	8.34	2.20	6.56	14.77	ND	ND	ND	ND	ND	ND	ND	ND
3K	ND	3.50	ND	ND	8.50	ND	9.68	29.31	ND	ND	ND	ND	ND	ND	ND	ND
4K	ND	4.18	ND	ND	7.40	ND	9.09	25.95	ND	ND	ND	ND	ND	ND	ND	ND
5K	ND	3.19	ND	ND	6.73	1.77	2.04	2.67	ND	ND	ND	ND	ND	ND	ND	ND
6K	ND	3.55	0.38	1.53	7.44	1.11	5.89	15.13	ND	ND	ND	ND	ND	ND	ND	ND
1D	ND	3.32	ND	2.69	7.07	ND	15.02	47.82	ND	ND	ND	ND	ND	ND	ND	ND
2D	ND	3.21	ND	3.81	7.57	ND	4.67	11.39	ND	ND	ND	ND	ND	ND	ND	ND
3D	ND	3.48	2.15	4.24	7.41	ND	6.75	18.43	ND	ND	ND	ND	ND	ND	ND	ND
4D	ND	3.07	0.00	1.87	10.39	ND	13.80	42.30	ND	ND	ND	ND	ND	ND	ND	ND
5D	ND	3.15	1.94	4.40	13.16	2.76	4.61	9.37	ND	ND	ND	ND	ND	ND	ND	ND
6D	ND	3.25	0.82	3.40	9.12	0.55	8.97	25.86	ND	ND	ND	ND	ND	ND	ND	ND
Winter																
1A	ND	3.60	ND	2.54	4.47	ND	3.74	7.98	ND	ND	ND	ND	ND	ND	ND	ND
2A	ND	3.67	ND	ND	4.38	5.74	16.23	25.93	ND	ND	ND	ND	ND	ND	ND	ND
3A	ND	4.81	ND	38.52	9.47	1.87	6.57	16.60	ND	ND	ND	ND	ND	ND	ND	ND
4A	ND	4.34	ND	3.36	8.24	1.59	8.91	25.55	ND	ND	ND	ND	ND	ND	ND	ND
5A	ND	4.09	ND	48.48	6.61	1.35	8.72	23.96	ND	ND	ND	ND	ND	ND	ND	ND
6A	ND	4.10	ND	18.58	6.63	2.11	8.83	20.00	ND	ND	ND	ND	ND	ND	ND	ND
1K	ND	4.39	ND	3.87	8.34	1.53	8.32	22.30	ND	ND	ND	ND	ND	ND	ND	ND
2K	ND	3.93	ND	ND	9.50	1.66	16.63	49.07	ND	ND	ND	ND	ND	ND	ND	ND
3K	ND	3.39	ND	2.74	6.40	ND	5.30	12.98	ND	ND	ND	ND	ND	ND	ND	ND
4K	ND	3.57	1.83	4.35	7.71	1.87	6.51	16.48	ND	ND	ND	ND	ND	ND	ND	ND
5K	ND	3.43	2.05	4.32	9.88	2.28	5.21	10.40	ND	ND	ND	ND	ND	ND	ND	ND
6K	ND	3.74	0.78	3.06	8.37	1.47	8.39	22.25	ND	ND	ND	ND	ND	ND	ND	ND
1D	ND	2.51	ND	2.06	5.10	1.61	2.44	3.62	ND	ND	ND	ND	ND	ND	ND	ND
2D	ND	3.98	ND	ND	8.11	1.50	9.75	25.55	ND	ND	ND	ND	ND	ND	ND	ND
3D	ND	4.16	ND	ND	10.12	1.80	19.03	55.17	ND	ND	ND	ND	ND	ND	ND	ND
4D	ND	3.27	ND	4.93	5.50	1.31	3.52	7.49	ND	ND	ND	ND	ND	ND	ND	ND
5D	ND	3.68	ND	5.34	8.87	1.47	19.14	55.23	ND	ND	ND	ND	ND	ND	ND	ND
6D	ND	3.52	ND	2.47	7.54	1.54	10.78	29.41	ND	ND	ND	ND	ND	ND	ND	ND

ND: Not Detected.
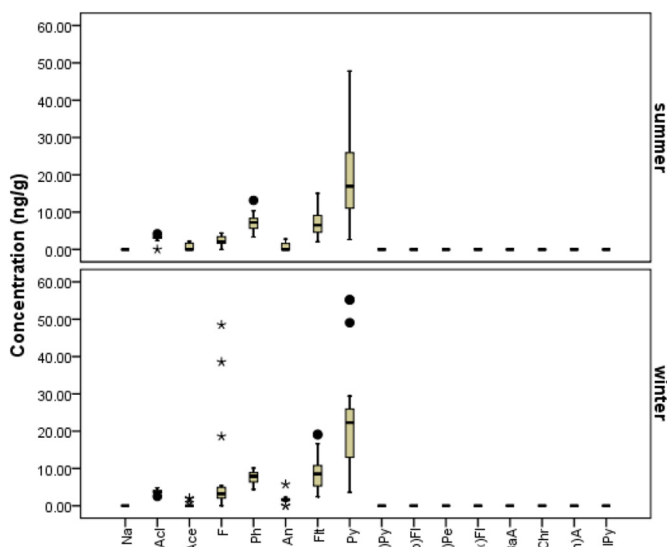
Fig. 2. Boxplots of individual PAH in zooplanktons (ng/g dry weight) of the northern part of the Persian Gulf (in both summer and winter).
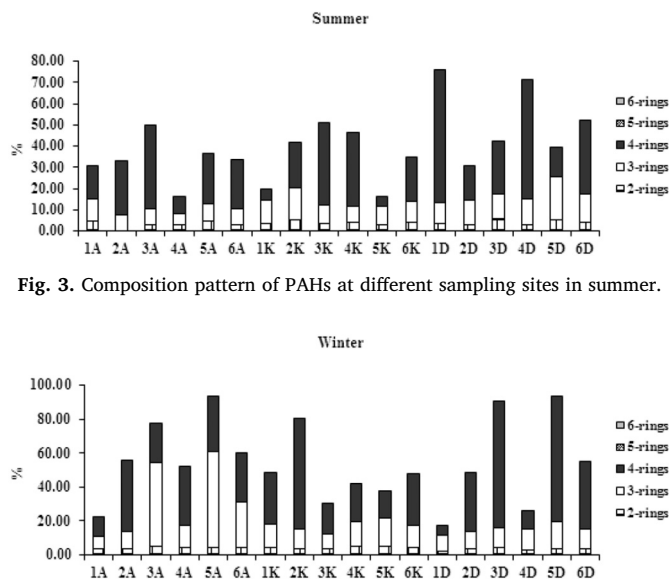

Fig. 3. Composition pattern of PAHs at different sampling sites in summer.
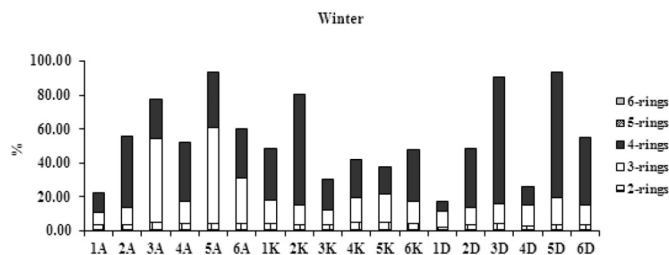
Fig. 4. Composition pattern of PAHs at different sampling sites in winter.

found to be at the range of 56.0% to 99.7%, with an average of 72%. Method detection limits (MDLs) were at the range of 0.5 to 3.0 ng/g for different PAH compounds.

The abbreviations used for the 16 PAHs are as follows: naphthalene, N; acenaphthylene, AC; acenaphthene, ACE; fluorene, F; phenanthrene, Ph; anthracene, A; fluoranthene, Fl; pyrene, Py; benzo (a)anthracene, B (a)A; chrysene, Ch; benzo(b)fluoranthene, B(b)Fl; benzo(k)
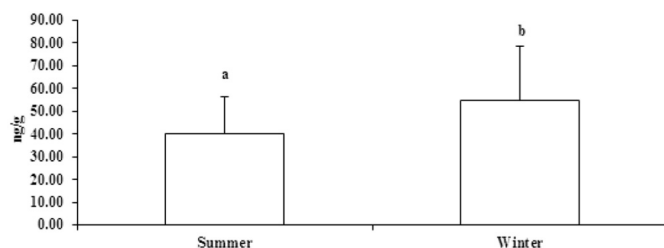
Fig. 5. Comparison of ΣPAH in zooplankton (ng/g dry weight) in different seasons.

Table 2
Isomer ratio plots of PAHs in different seasons.

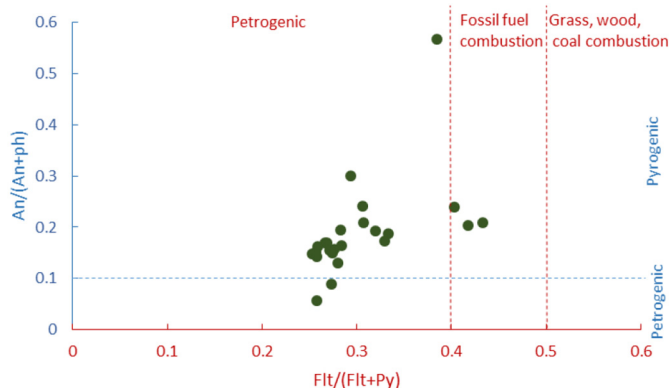| Sites	An/(An + Ph)	Flt/(Flt + Py)
1A	0.3	0.29
2A		0.27
3A		0.25
4A		0.33
5A		0.27
6A	0.09	0.27
1K	0.20	0.42
2K	0.21	0.31
3K		0.25
4K		0.26
5K	0.21	0.43
6K	0.13	0.28
1D		0.24
2D		0.29
3D		0.27
4D		0.24
5D	0.17	0.33
6D	0.06	0.26
1A		0.32
2A	0.57	0.38
3A	0.16	0.28
4A	0.16	0.26
5A	0.17	0.27
6A	0.24	0.31
1K	0.15	0.27
2K	0.15	0.25
3K		0.29
4K	0.19	0.28
5K	0.19	0.33
6K	0.15	0.27
1D	0.24	0.40
2D	0.16	0.28
3D	0.15	0.26
4D	0.19	0.32
5D	0.14	0.26
6D	0.17	0.27

Fig. 6. Diagnostic ratios applied for the identification of PAH pollution sources in zooplankton samples (in both summer and winter).

fluoranthene, B(k)Fl; benzo(a)pyrene, B(a)Py; indeno(1,2,3-c,d)pyrene, IPy; dibenz(a,h)anthracene, DB(a,h)A; and benzo(g,h,i)perylene, B(g,h,i)Pe.

One-way analysis of variance (ANOVA) was first applied to determine significant differences in PAH content among various seasons and regions. Then, mean comparison was conducted using Duncan's multiple range test when α = 0.05. Statistical analysis was carried out using software package of SPSS ver. 18.

On the basis of the obtained results, temperature, salinity, and dissolved oxygen in summer and winter were 33–34 °C, 41–42 ppt, and 6 mg/L and 24–26 °C, 37–40 ppt, and 6–8 mg/L, respectively. Overall, mean values of temperature and salinity were higher in summer than in winter. Moreover, the number of identified zooplankton species was more in winter than in summer, as 17 zooplankton species were isolated in winter, whereas 7 species were detected in summer.

Oil hydrocarbons are widely the most common environmental contaminants. Thus far, > 175 hydrocarbons have been isolated from the crude oil, among which 108 hydrocarbons are saturated aliphatic compounds and the remaining are classified as aromatic hydrocarbons (Harris et al., 2011). The presence of polycyclic aromatic hydrocarbons (PAHs), especially at high amounts, is dangerous to marine organisms and human health because of their harmful attributes such as toxicity, mutagenicity, carcinogenicity, and estrogenicity. For instance, both benzopyrene-a and benzopyrene-c react with amino acids in animal bodies, which cause various genetic diseases (Hendricks et al., 1985; Schirmer et al., 1998; Pacheco and Santos, 2001).

A comparison of measured PAHs in both winter and summer indicated that values of naphthalene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(K)fluoranthene, benzo(a)pyrene, indeno(1,2,3)pyrene, dibenz(a,h)anthracene, and benzo(g,h,i)perylene were lower than the detection limit in zooplanktons, and only 6 compounds were recognized (Table 1). Individually, pyrene had the highest concentration (19.16 ± 12.48 ng/g), whereas acenaphthene showed the lowest concentration (0.62 ± 0.83 ng/g) in summer. Boxplots illustrated in Fig. 2 show the distribution of PAH concentrations at all stations in both summer and winter. Four-ring and 3-ring compounds indicated the highest percentage of all the measured compounds in summer at stations 1D and 5D, respectively (Fig. 3). In this investigation, the total concentration of the measured PAHs ranged from 16.40 ng/g to 75.92 ng/g in summer.

In winter, the highest and the lowest individual concentration was 24 ± 15 ng/g for pyrene and acenaphthene, respectively (Fig. 2). Like the summer, the highest percentage of all compounds was measured for 4-ring and 3-ring compounds at stations 5D and 5A, respectively (Fig. 4). The total amount of the measured PAHs was between 17.34 ng/g and 93.73 ng/g in winter, with the highest content at station 5D and the lowest content at station 1D. A comparison of total PAHs between summer and winter showed a higher concentration of total compounds in winter than in summer (P < 0.05) (Fig. 5).

Yet, several studies have been focused on PAH compounds in zooplankton at Iran and worldwide. PAH compounds were 610–1310 ng/g in Valdez Port, 160–3650 ng/g in the Mediterranean Sea, and 560–2480 ng/g in the Black Sea and Marmara Sea. Ziyaadini et al. (2016) measured PAH component levels in zooplanktons from Chabahar Bay (190–1470 ng/g), which were more than those reported in the present research. Generally, PAH components in zooplanktons vary according to the study area, their environmental condition, the identified zooplankton species, and other factors.

PAHs constitute a wide range of chemical and environmental contaminants, and they can be formed by different processes in an environment. Their distribution differs according to production sources, chemical composition, and combustion degree of organic matter (Neff, 1979). Physicochemical properties of some PAHs such as chemo-reactivity (oxidation and photo-oxidation) can help recognize the main pattern of distribution for release sources (Butler and Crossley, 1981). In marine ecosystems, PAH compounds destruct by water surface

photo-oxidation and microbial activities in sediments. However, the presence of PAH compounds in sediments implies that density phenomena prevail in the destruction processes (Butler and Crossley, 1981). Molecular indices have been proposed to evaluate different origins of PAHs according to their physical and chemical behavior. The formation process of these compounds in a study area is determined by the simultaneous cooperation of several molecular indices (Neff, 1979). The application of specific PAH compounds with comparative thermodynamic stability and evolutionary trend in the environment is essential for distinction between anthropogenic and natural sources as well as for more identification of petrogenic, pyrogenic, and household waste inputs. Therefore, the ratio of these isomers is a suitable method to determine the probable origin of PAH compounds in the sediment (Hu et al., 2010; Leite et al., 2011).

Although distinction ratios are highly effective in origin determination, they should be carefully used after an accurate evaluation at particular occasions, as their stability varies with time by degradation or environmental variables. Environmental factors include a group of processes that occur after PAH release in the ecosystem. A set of factors including evaporation, volatility, biodegradability by microorganisms, and solubility influence these processes (Morrison and Murphy, 2006).

Table 2 and Fig. 6 present diagnostic ratios used to determine origins of PAHs in zooplankton samples and their content at different stations and seasons. Results of the present investigation revealed that the ratio of Flt/(Flt + Py) at all stations (except for the stations 1 K and 5 K in summer) was lower than 0.4, which indicates their petrogenic origin. However, the An/(An + Ph) ratio was higher than 0.1 at all stations (except for the stations 6A and 6D in summer), which implies their pyrogenic origin. Several published reports have shown that Flt/(Flt + Py) is more conservative than An/(An + Ph), which is particularly sensitive to photodegradation (Tobiszewski and Namieśnik, 2012). In fact, the An/(An + Ph) ratio is sensitive to environmental changes, and its values for the identification of particular processes lie within a narrow range, which makes it difficult to use. For example, the study of Brändli et al. (2008) confirmed the pyrogenic origin of PAHs in Swiss soils. However, the An/(An + Ph) ratio indicated the petrogenic origin of PAHs, which is an improbable result. In another study, Katsoyiannis et al. (2007) indicated the Flt/(Flt + Py) ratio as relatively stable even throughout the treatment processes. In the Persian Gulf, oil spills and fuel combustion are potential sources of PAHs in water, sediment, and biota. Therefore, on the basis of obtained results and published reports, mixed sources of petroleum and petroleum combustion could be considered as the sources of pollution of zooplankton samples collected from coastal waters of the northern part of the Persian Gulf, with the higher contribution to the petrogenic part.

In the present investigation, PAH compounds in zooplanktons were studied during both summer and winter in the north of the Persian Gulf. In both seasons, six compounds of PAH were identified in zooplankton tissues and other compounds were less than the detectable limit. Additionally, pyrene and acenaphthene showed the highest and the lowest individual level of PAHs in both seasons, respectively. Four- and 3-ring components were dominant in both seasons. Results also revealed that total PAHs in winter was more than that in summer. As zooplanktons are primary consumers in a food chain, they play a key role in transitions of contaminations. For this reason, pollution monitoring in these organisms is essential to obtain more detailed information about the transmission of contamination to upper trophic levels.

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