

DISTRIBUTION AND SOURCES APPORTIONMENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE EDIBLE BIVALVES AND SIPUNCULIDA FROM COASTAL AREAS OF BEIBU GULF, CHINA

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(Received 16th Nov 2016; accepted 7th Mar 2017)

Abstract. Concentrations, composition and sources apportionment of 16 polycyclic aromatic hydrocarbons (PAHs) were investigated in the edible bivalves (oysters, mussels and clams) and sipunculida samples collected from coastal areas of Beibu Gulf, China. The results showed that the total PAH concentrations ranged from 36.4 to 403.3 ng/g dry weight. Compared with other bays and coastal areas, current contamination levels of PAHs were in a low range. Relative abundances and isomer ratios indicated a probable pyrogenic origin for the PAHs contamination. Further quantitative analysis showed that the percentages contributions of biomass and coal combustion, vehicular emission, coke oven emissions and crude oil pollution were 67.67%, 17.39% and 14.94%, respectively.

Keywords: *benthos; seafood; PAH; source contributions; PCA-MLR*

Introduction

Polycyclic aromatic hydrocarbons are compounds containing two or more fused aromatic rings in linear, angular or clustered arrangements (Perugini et al., 2007). PAHs are hydrophobic compounds with very low water solubilities, they become more hydrophobic as molecular weight increases (Juhasz and Naidu, 2000). Owing to their toxicity, carcinogenic and mutagenic properties, 16 PAHs have been identified as priority pollutants by the United States Environmental Protection Agency (USEPA) in the early 1980s (Ramdine et al., 2012). PAHs are common in the marine environment and originated from different emission sources, some of them natural, but mostly related to human activities (Cortazar et al., 2008; Fasano et al., 2016), which can be differentiated into petrogenic, pyrogenic or diagenetic (Fang et al., 2007; Pongpiachan et al., 2015). Benthic organisms are believed to have relatively sessile character and relatively low capability to metabolize PAHs (Baumard et al., 1998; Oros and Ross, 2005). The accumulation of PAHs in benthic organisms is of global concerns, owing to the fact that consumption of contaminated benthic organisms poses a threat to human health (Wang et al., 2010; Khairy et al., 2014).

As a semi-enclosed bay, Beibu Gulf is surrounded by the land territories of Vietnam and China's Hainan, Guangxi and Guangdong provinces and has a weak self-purification capability (Zhang et al., 2014; Gu et al., 2015). With the establishment of the Beibu Gulf Economic Rim and ASEAN-China Free Trade Area (ACFTA), the

Beibu Gulf has now become one of the most important economic development areas in China for its bountiful resources and strategic location to both China and Southeast Asian Countries (Yu and Mu, 2006; Li et al., 2015). As a result of an accelerated industrialization process and rapid population growth in the coastal region in the past two decades, it consequently suffers increasing anthropogenic pollution (Xia et al., 2011), such as heavy metal (Xia et al., 2011; Gan et al., 2013; Gu et al., 2015) and organic pollutants (Zhang et al., 2014; Li et al., 2014, 2015; Kaiser et al., 2016). The Beibu Gulf is also one of the most important parts of Chinese mariculture zone, with mariculture production of 1.09 million tons in 2014. Bivalve mollusks and sipunculida are the traditional and principal seafood of coastal populations in this region. However, information on PAH pollution in local bivalve mollusks and sipunculida is limited in this region.

The principal objective of the present study was to investigate the distribution, composition and pollution levels of PAHs in bivalve mollusks and sipunculida sampled from coastal areas of Beibu Gulf. Possible sources were identified by diagnostic ratios and quantitative sources apportionment was carried out using principle component analysis-multiple linear regression (PCA-MLR).

Materials and methods

Area description and sampling

The Beibu Gulf is a marginal shallow shelf region in the northwest of the South China Sea, covering an area of approximately $12.8 \times 10^4 \text{ km}^2$. It has an average depth of approximately 38 m and maximum depth of less than 100 m. The climate around this gulf is subtropical and monsoonal. Four sampling areas were selected along Beibu Gulf coast as shown in Fig. 1. The species selected are the most common seafood within the study area, including oysters (*Crassostrea* sp, with $6.2 \pm 1.2 \text{ cm}$ in shell length), mussels (*Mytilus* sp. and *Perna viridis*, with $4.2 \pm 0.5 \text{ cm}$ and $7.1 \pm 1.1 \text{ cm}$ in shell length), clams (*Ruditapes philippinarum*, ranging from 4.2 to 5.3 cm in shell length) and sipunculida (*Phascolosoma esculenta* and *Sipunculus nudus*, ranging from 12 to 19 cm in body length). At least 15 specimens were collected and pooled as a composite sample at each sampling location in October 8. The whole soft tissues were dissected from the shells with a stainless steel knife, packed with aluminum foil, transported to the laboratory on ice, and stored at -20°C until analysis.

Extraction and cleanup

All the samples were freeze dried for 24h with a Topcon vacuum freeze drier (Topcon Co., USA), grounded into a fine powder (about 100 mesh). Approximately 10 g of sample were extracted with a *n*-hexane: acetone (1:1) mixture, in a MSP-1000 microwave extraction system (CEM Co., Matthews, NC, USA), a method developed and verified by the authors (Zhang et al., 2004; Lu et al., 2005). Briefly temperature was raised from room temperature to 110°C at a rate of $15^\circ\text{C min}^{-1}$, and pressure was raised from 14.5 to 25 psi, and then held for 10 min. The extract was transferred to a 250 ml conical flask and 1 g of activated copper powder was added to remove sulfides. The extract was then filtered through a funnel filled with anhydrous sodium sulfate (dried at 440°C for 4 h) and concentrated to 2 ml by rotary-evaporation and under a gentle stream of nitrogen. The concentrated extracts were fractionated by a column (40 cm $\times$ 1.3 cm

i.d.) packed in sequence with 9 g neutral aluminum oxide, 18 g silica gel and 2 g anhydrous sodium sulfate. The column was then eluted with a *n*-hexane : pentane (1:1) mixture. The eluent was concentrated to 2 ml by rotary-evaporation and under a gentle stream of nitrogen and sealed in an amber vial for GC-MS analysis.

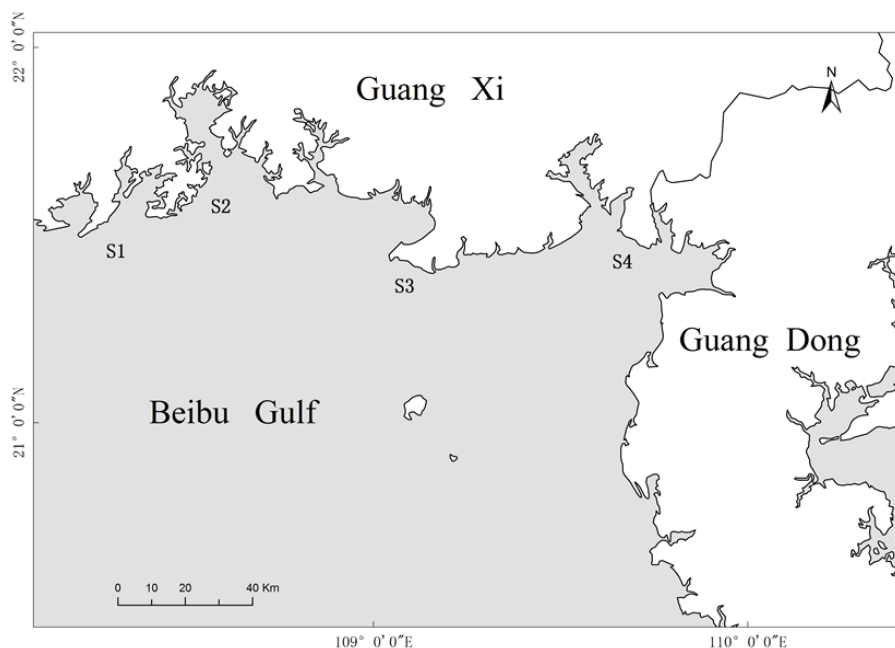


Figure 1. Bivalves and sipunculida sampling locations in Beibu Gulf

Instrument analysis

The purified extracts were analyzed for PAHs with a Hewlett-Packard 6890N/5973 gas chromatography/mass spectrometer (GC/MS) operated in selected ion monitoring mode and a 30m×0.25mm (i.d.) HP-5MS column (Agilent Co., USA). The temperatures of transfer line, injector and detector were set at 300, 280 and 320°C, respectively. The column temperature was raised from 60 to 150°C with 15°C min⁻¹, from 150 to 220°C at 5°C /min, from 220 to 300°C at 10°C min⁻¹, and held at 300°C for 10 min. The PAHs studied were naphthalene (Nap), acenaphthylene (Acp), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno[1,2,3,cd]pyrene (IcdP), dibenzo(ah)anthracene (DahA), and benzo(ghi)perylene (BghiP).

Quality assurance and quality control

The standard mixture of 16 PAHs, deuterated PAH surrogate (acenaphthene-*d*₁₀, phenanthrene-*d*₁₀, chrysene-*d*₁₂ and perylene-*d*₁₂) and internal standard (pyrene-*d*₁₀) were obtained from Supelco, Inc. (Supelco, USA). Method blanks, spiked blanks and sample duplicates were routinely analyzed with field samples in this study. Surrogate standards were spiked to all the samples to correct procedural performance and matrix effects. The average recoveries of surrogate standards ranged from 96% to 115%. The variation of PAH concentrations in duplicates was less than 12 %. The method detection

limits ranged from 0.05 to 0.18 ng/g. All reported concentrations were given on a dry weight (dw) basis and corrected using surrogate recoveries.

Statistical analysis

Statistical analyses, including Pearson correlation analysis and principal components analysis followed by multivariate linear regression, were performed using SPSS 22.0 software for windows (SPSS Inc., Chicago, IL, USA).

Results and discussion

PAHs concentrations and composition

The 16 individual parent PAHs and total concentrations determined in bivalves and sipunculida collected from four sampling areas of Beibu Gulf were shown in *Table 1*. The PAHs concentrations was fairly constant in four sampling areas, presenting a range of concentrations from 117.3 to 403.3 ng/g in oyster, from 50.9 to 80.1 ng/g in mussels and from 36.4 to 81.0 ng/g in sipunculida. The PAHs concentration found in oysters were obviously higher than that in mussels, clams (70.4 ng/g) and sipunculida. The total PAHs detected in oysters from the San Francisco Bay and the Pacific Coast of Japan was also higher than that in mussels and clams (Oros and Ross, 2005; Onozato et al., 2016). Palma-Fleming and Gutierrez (2004) found that oysters can't metabolize and excrete PAHs well, probably due to inefficient mixed-function oxidase (MFO) enzymes that is induced by and plays a critical role in the oxidation of numerous xenobiotics, including PAHs. The activities of MFO enzymes in oysters were also found lower than that in mussels and clams (Solé et al., 1994). Moreover, oysters may have a different capability to regulate filtration, and therefore to bioaccumulate chemical pollutants more efficiently (León et al., 2013).

The composition patterns of PAHs in bivalves and sipunculida from Beibu Gulf were shown in *Fig.2*. The percentage composition of PAHs did not vary much from one sample to another, indicating similar PAH sources over the whole area. In most of the samples, the 4-ring PAHs were the most abundant, accounting for an average of 47.7% of the total PAHs. The 3-ring and 5-ring PAHs followed, with an average of 30.8% and 28.0%, respectively. Generally, the tri- and tetra-aromatics are concentrated at larger levels in bivalves or other marine organisms than the heavier molecular weight compounds. Such composition pattern was consistent with that in bivalves reported in some previous studies (Porte and Albaiges, 1994; Baumard et al., 1998; Hong et al., 2016). Bivalves can accumulate PAHs directly by the uptake of lower molecular weight PAHs through their gills, and indirectly by ingestion of the fine grain size fraction of sediment and suspended particles, then assimilation of higher molecular weight PAHs through the digestive tract (Oros and Ross, 2005).

A comparison of PAH concentrations in oysters, mussels and clams collected from other bays and coastal areas around the world was given in *Table 1*. The total PAHs was nearly similar to the levels present in coastal area of Dalian and Guangdong (China), Persian Gulf (Iran), Tongyeong (Korea) and Tanoura Bay (Japan), and it was lower than in most other bays and coastal locations shown in *Table 2*, such as Bohai Bay in China, San Francisco bay in USA, Corral Bay in Chile, Guanabara Bay in Brazil, Shoreham-by-Sea in UK and Pays Basque in France. Overall, the comparison indicated that the PAHs pollution level in bivalves and sipunculida from Beibu Gulf were in the low range.

Table 1. The concentrations of 16 PAHs in bivalves and sipunculida collected from Beibu Gulf

Sampling area	Species	PAH (ng/g dw)																Total
		Nap	Acp	Ace	Flu	Phe	Ant	Flt	Pyr	Chr	BaA	BbF	BkF	BaP	IcdP	DahA	BghiP	
S1	<i>Perna viridis</i>	8.07	1.19	0.13	-	12.67	1.83	8.37	9.03	0.88	6.13	3.65	-	1.13	-	-	0.66	53.8
	<i>Crassostrea</i> sp.	14.02	2.53	0.42	-	32.00	8.91	28.72	20.85	7.46	32.41	93.45	29.23	1.56	0.96	2.10	1.72	276.3
	<i>Ruditapes philippinarum</i>	11.44	1.43	0.14	2.26	13.07	0.77	11.91	10.23	1.80	9.81	4.86	2.66	-	-	-	-	70.4
	<i>Phasolosma esculenta</i>	13.22	0.68	0.14	2.76	18.68	2.17	15.11	9.73	1.39	6.13	8.00	-	3.04	-	-	-	81.0
S2	<i>Crassostrea</i> sp.	9.63	2.06	0.36	3.70	34.68	5.96	15.61	11.62	1.58	12.98	12.53	6.59	-	-	-	-	117.3
	<i>Sipunculus nudus</i>	12.02	0.45	-	1.81	5.75	-	7.65	6.39	0.24	2.13	-	-	-	-	-	-	36.4
	<i>Mytilus</i> sp.	0.99	0.65	-	-	14.47	1.55	8.57	7.62	0.94	7.78	5.92	-	0.88	-	-	1.50	50.9
S3	<i>Crassostrea</i> sp.	16.09	3.55	0.84	4.23	83.51	11.49	75.01	45.03	8.61	47.50	86.23	21.22	-	-	-	-	403.3
	<i>Phascolosoma esculenta</i>	20.31	-	-	3.28	12.41	-	34.40	7.85	1.36	-	-	-	-	-	-	-	79.6
	<i>Sipunculus nudus</i>	15.36	1.18	-	-	10.08	2.05	11.40	7.80	0.23	1.76	-	-	-	-	-	-	49.9
	<i>Mytilus</i> sp.	11.71	2.29	0.28	-	21.64	2.04	14.35	10.25	0.91	8.04	3.74	3.65	-	-	-	1.19	80.1
S4	<i>Sipunculus nudus</i>	13.28	0.94	-	2.08	6.74	-	6.81	6.19	0.31	1.49	-	-	-	-	-	-	37.8
	<i>Crassostrea</i> sp.	11.15	2.16	0.53	5.11	44.95	5.14	26.95	16.73	5.66	18.86	46.70	21.22	4.02	1.63	1.79	1.62	214.2

Table 2. Total concentrations of PAHs in oysters, mussels and clams from coastal locations in the world

Sampling site	Species	Σ PAH (ng/g)	References
China (Beibu Gulf)	Oyster (<i>Crassostrea</i> sp.)	117.3-403.3 ^a	This study
	Mussel (<i>Mytilidae</i> sp.)	50.9-80.1 ^a	
	Clam (<i>Ruditapes philippinarum</i>)	70.4 ^a	
China (coastal area of Dalian)	Oyster	60-216 ^b	(Hong et al., 2016)
China (Bohai Bay)	Clam (<i>R. philippinarum</i>)	171.4±31.9 ^e	(Wan et al., 2007)
China (Eastern Guangdong coast)	Oyster (<i>C. rivularis</i>)	231-1178 ^a	(Yu et al., 2016)
Iran (Persian Gulf)	Oyster (<i>Saccostrea cucullata</i>)	126 -226.1 ^a	(Mirza et al., 2012)
Korea (Tongyeong)	Oyster (<i>C. gigas</i>)	194.5-375.9 ^a	(Cho, 2010)
Japan (Tanoura Bay)	Oyster	57-350 ^f	(Nakata et al., 2014)
Guadeloupe	Oyster (<i>C. rhizophorae</i>)	66.9-961.2 ^b	(Ramdine et al., 2012)
Tanzania (Dar es Salaam)	Oyster (<i>S. cucullata</i>)	83.0-241.7 ^a	(Gaspere et al., 2009)
France (Pays Basque)	Oyster (<i>Crassostrea</i> sp.)	301-1406 ^c	(Cortazar et al., 2008)
Mexico (Terminos Lagoon)	Oyster (<i>C. virginica</i>)	387.97-2245.67 ^a	(Noreña-Barroso et al., 1999)
USA(San Francisco bay)	Oyster (<i>C. gigas</i>)	184-6899 ^a	(Oros and Ross, 2005)
	Mussel (<i>Mytilus californianus</i>)	21-1093 ^a	
	Clam (<i>Corbicula fluminea</i>)	78–720 ^a	
Chile (Corral Bay)	Mussel (<i>M. chilensis</i>)	138-877 ^a	(Palma-Fleming and Gutierrez, 2004)
UK (Shoreham-by-Sea)	Mussel (<i>Mytilidae</i> sp.)	33.2-2280.6 ^d	(Law et al., 2002)
Brazil(Guanabara Bay)	Mussel (<i>Perna perna</i>)	9-273 ^a	(Francioni et al., 2005)

- a. Σ 16PAH: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)Anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, dibenz(a,h)anthracene, benzo(ghi)Perylene.
b. Σ 20PAH: Σ 16PAH plus triphenylene, benzo(e)pyrene, dibenzo(ac)anthracene and perylene.
c. Σ 13PAH: Σ 16PAH minus naphthalene, acenaphthylene and acenaphthene.
d. Σ 14PAH: Σ 16PAH plus benzo(j)fluoranthene, minus acenaphthylene, acenaphthene and fluorene.
e. Σ 18PAH: Σ 16PAH plus benzo(e)pyrene and perylene.
f. Σ 20PAH: Σ 16PAH plus 2-methylnaphthalene,1-methylnaphthalene,1,2-dimethylnaphthalene, benzo(e)pyrene

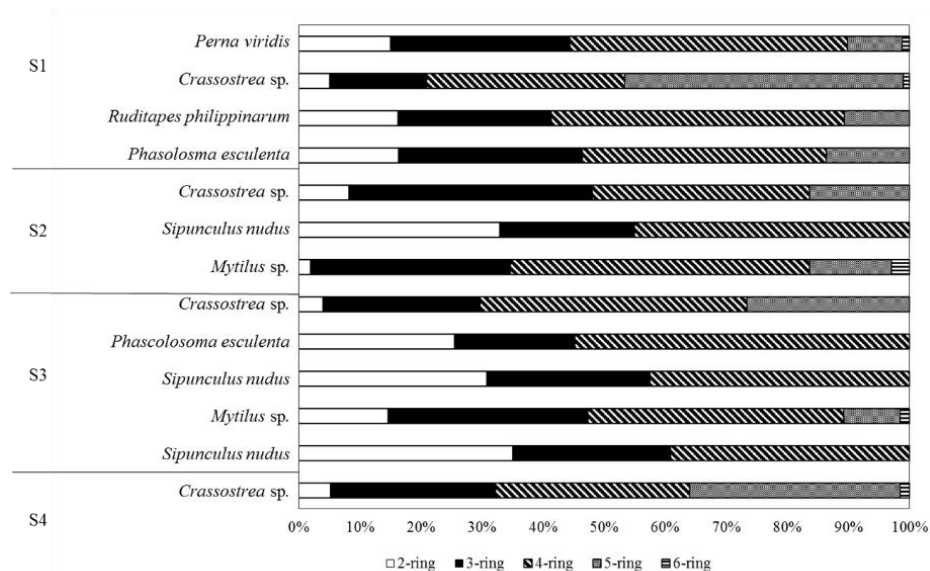


Figure 2. The composition pattern of PAHs by ring size in bivalves and sipunculida from Beibu Gulf

Sources identification by ratio analyses

PAHs usually do not occur as individual compounds but in complex mixture of hundreds of compounds (Neff et al., 2005). Each source generates a characteristic PAH pattern, through analysis of the composition of these mixtures we can assess the origins of the PAHs.

The relative abundances of low molecular weight (LMW) and high molecular weight (HMW) PAHs could be used as an index for the origins of PAHs. Generally, LMW PAHs come mainly from petroleum and its products, especially the refined oil, while HMW PAHs are linked mainly to the combustion of the fossil fuel and the coking reaction in the industrial activity (Soclo et al., 2000). If the value of LMW/HMW above 1, thus the PAHs mainly come from oil contamination. On the contrary, when the ratios below 1, the PAHs most likely originate from the high temperature combustion of the fossil fuel. In this paper, the ratios in most of samples were less than 1, indicating the probable pyrogenic origins for the PAHs.

PAH isomer pairs have similar physical-chemical properties, thus they have similar dilution and distribution with particulate matter and other environmental phases (Oros and Ross, 2005; Dickhut et al., 2000). Flt/(Flt+Pyr) was plotted against BaA/(BaA+Chr) to assess contamination sources (Fig.3). For Flt/(Flt+Pyr), ratios below 0.4 indicate pyrogenic origins, ratios above 0.5 represent wood, grass or coal combustion origins, while ratios between 0.4 and 0.5 stand for petroleum combustion origins. In the case of BaA/(BaA+Chr), ratios below 0.2 suggest petrogenic origins, while ratios above 0.35 indicate pyrogenic origins (Yunker et al., 2002). As shown in Fig. 3, the ratios for most of samples indicated a major wood, grass or coal combustion origins. Due to no detectable BaA in some samples, the ratios of BaA/(BaA+Chr) was zero, but its Flt/(Flt+Pyr) ratios also represented wood, grass or coal combustion origins.

Overall, the results of relative abundances (LMW/HMW) and isomer ratios indicated a probable pyrogenic origins for the PAHs contamination, especially the wood, grass or coal combustion origins.

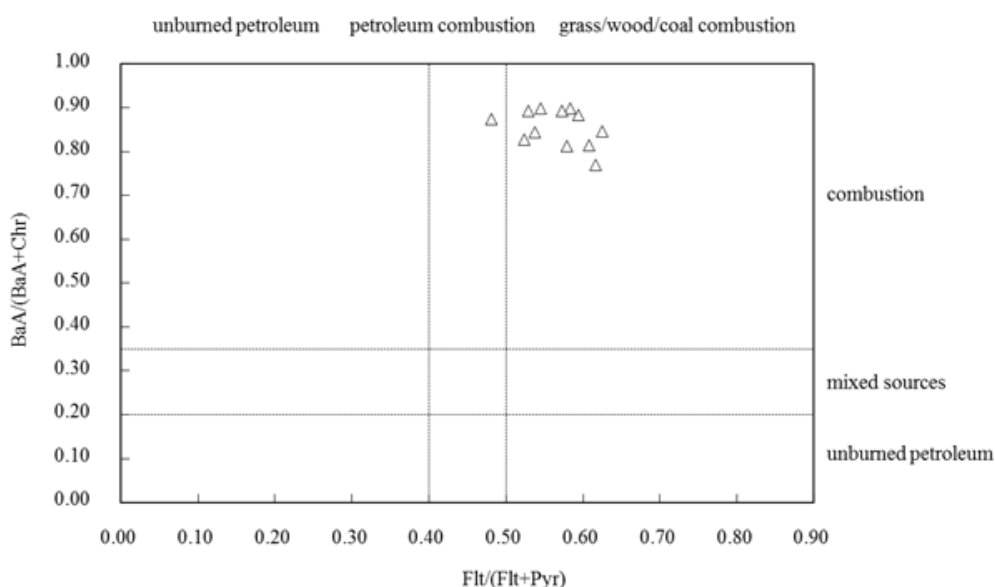


Figure 3. PAH cross plots for the ratios of $Flt/(Flt+Pyr)$ vs. $BaA/(BaA+Chr)$

Source apportionments by principal component analyses

In general, a quantitative proportion of sources of PAHs cannot be given by molecular diagnostic ratios. In most cases, more detailed information about the source apportionment is necessary for the control of PAH contamination. Principal component analysis (PCA) is a multivariate analytical tool used for receptor modeling in environmental source apportionment studies. Concentrations of variables can be regarded as linear combinations of a number of underlying factors (or sources) (Sofowote et al., 2008; Cao et al., 2011). The PCA analysis is to extract feature, concentrate information and reduce dimension from the raw information, then deduce one or more comprehensive characteristic indexes to identify environmental samples, by comparing the loadings values to conjecture the information of pollution source (Liu et al., 2009). The concentrations were Kaiser-normalized and varimax rotation was used as the preferred transformation so that loadings values could be clustered around 1 and 0, making them more physically interpretable. As shown in Table 3, three principal components (PC1, PC2 and PC3) with eigenvalue higher than 1 were extracted and the percent variances of three principal components were 52.79%, 23.23% and 12.37% respectively, which accounted for 88.4% of the total variability.

The first rotated component was responsible for 52.79% of the total variance. In this component, Acp, Ace, Phe, Ant, Flt, Pyr, Chr, BaA, BbF and BkF showed higher loadings. Acp is a predominate PAH compound emitted by grass or wood combustion (Lee et al., 2005). Phe, Ant, Flt, Pyr and BaA are related to the combustion of coal (Simcik et al., 1999). BbF, BkF and Chr are predominate PAH components emitted by domestic coal combustion in China (Cao et al., 2011; Chen et al., 2005). Thus, this component was highly related to the combustion of biomass and coal. This conclusion is consistent with the energy structure of Beibu Gulf.

Table 3. The rotated component matrix of 16 PAHs in the bivalves and sipunculida

	Component ^a		
	PC1	PC2	PC3
Nap	0.139	-0.086	0.752 ^b
Acp	0.908	0.114	-0.085
Ace	0.917	0.226	0.117
Flu	0.241	0.196	0.810
Phe	0.923	0.100	0.251
Ant	0.947	0.191	0.039
Flt	0.833	-0.044	0.445
Pyr	0.959	-0.015	0.214
Chr	0.884	0.399	0.159
BaA	0.976	0.162	0.038
BbF	0.880	0.391	0.036
BkF	0.798	0.561	0.039
BaP	-0.014	0.851	0.098
IcdP	0.235	0.947	0.057
DahA	0.321	0.893	-0.076
BghiP	0.202	0.697	-0.603
Percent of variance	52.79%	23.23%	12.37%

^a Rotation method: Varimax with Kaiser normalization

^b Loading values exceeding 0.7 are highlighted in bold face

The second rotated component had higher loadings of BaP, IcdP, DahA and BghiP, responsible for 23.23% for the total variance. IcdP and BghiP are related to the diesel and gasoline engines emission, respectively (Liao et al., 2011). BaP are also typically from the combustion of gasoline and coal (Khalili et al., 1995; Zuo et al., 2007). IcdP and DahA could be indicators for vehicular emission (Motelay et al., 2005). Therefore, this rotated component was attributed to vehicular emission.

The third rotated component was responsible for 12.37% for the total variance. This component was predominately composed of Nap and Flu. Nap constitutes a significant fraction of crude oils and petroleum products. And Flu is often indicated pyrogenic origins, especially the emission from coking. While Nap is the predominant PAH in the coke oven emissions, and Flu is the primary composition of the crude oil, too. Thus, we can tentatively infer this component was indicative of the complex origins of coke oven emissions and crude oil pollution.

Analyzing the correlation of individual PAHs would give more evidence of the PAHs origins and give an idea whether they all originate from the same source or not (Soclo et al., 2000). As shown in *Table 4*, the correlation analysis basically agreed with the principal component analysis. Acp, Ace, Phe, Ant, Flt, Pyr, Chr, BaA, BbF and BkF basically had highly significant paired correlation ($r = 0.625$ - 0.978). BaP, IcdP, DahA and BghiP had a good correlation between each other ($r = 0.504$ - 0.936). Nap and Flu had no significant correlation with the others, which would be related to their complex origins.

Table 4. Correlation factors between the PAH concentrations in bivalves and sipunculida from Beibu Gulf

Nap	Acp	Ace	Flu	Phe	Ant	Flt	Pyr	Chr	BaA	BbF	BkF	BaP	IcdP	DahA	BghiP	
1.000	0.069	0.155	0.340	0.172	0.135	0.485	0.265	0.242	0.127	0.206	0.181	-0.163	0.011	0.059	-0.459	Nap
	1.000	0.918**	0.206	0.844**	0.877**	0.625*	0.816**	0.782**	0.871**	0.778**	0.786**	0.062	0.354	0.395	0.290	Acp
		1.000	0.496	0.965**	0.915**	0.793**	0.902**	0.885**	0.919**	0.833**	0.832**	0.255	0.449	0.431	0.240	Ace
			1.000	0.545	0.294	0.511	0.395	0.379	0.300	0.232	0.287	0.286	0.299	0.100	-0.299	Flu
				1.000	0.905**	0.882**	0.949**	0.863**	0.915**	0.799**	0.751**	0.177	0.325	0.291	0.128	Phe
					1.000	0.764**	0.893**	0.904**	0.954**	0.919**	0.871**	0.178	0.390	0.482	0.257	Ant
						1.000	0.925**	0.816**	0.822**	0.743**	0.638*	-0.002	0.166	0.179	-0.048	Flt
							1.000	0.889**	0.953**	0.850**	0.742**	0.047	0.213	0.250	0.060	Pyr
								1.000	0.952**	0.978**	0.949**	0.324	0.584*	0.652*	0.361	Chr
									1.000	0.945**	0.873**	0.146	0.370	0.456	0.267	BaA
										1.000	0.960**	0.284	0.561*	0.686**	0.403	BbF
											1.000	0.377	0.735**	0.819**	0.511	BkF
												1.000	0.737**	0.624*	0.504	BaP
													1.000	0.936**	0.677*	IcdP
														1.000	0.631**	DahA
															1.000	BghiP

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

Contribution of PAH sources

PCA with multivariate linear regression (MLR) uses the linear transformation to choose PCs to represent the total variability of the original PAHs data in a minimum number of factors, and is considered as a more suitable tool to further identify the apportionment of the possible PAH sources. MLR was performed using PCA factor scores and normalized total PAH concentrations as independent and dependent variables, respectively (Larsen and Baker, 2003). The regression was run by a stepwise method. The standardized regression coefficients were used to represent the relative contributions from various sources (Larsen and Baker, 2003). Factor scores of PCAs for each sample are then used to calculate the contribution percent of the major sources using MLR.

Multiple linear regression (MLR) analysis was performed on the PCA factor scores in order to obtain mass apportionment of the three sources to the total PAH burden in each samples. A MLR of elements in the factor scores matrix (t_i) against the normal standard deviate of the SumPAH values obtained the best correlation between observed and predicted SumPAH values for each sample (Fig.4). The three factor scores had coefficients (B_i) which were determined by the MLR analysis with a stipulated minimum 95% confidence limit. The multiple linear regression equation for the standard deviate of the SumPAH values was determined to be:

$$\hat{Z}_{\text{SumPAH}} = 0.942t_1 + 0.242t_2 + 0.208t_3 \quad (\text{Eq.1})$$

The percentage contributions to the mean for the three factors can be calculated as:

$$i(\%) = 100 \times (B_i / \sum B_i) \quad (\text{Eq.2})$$

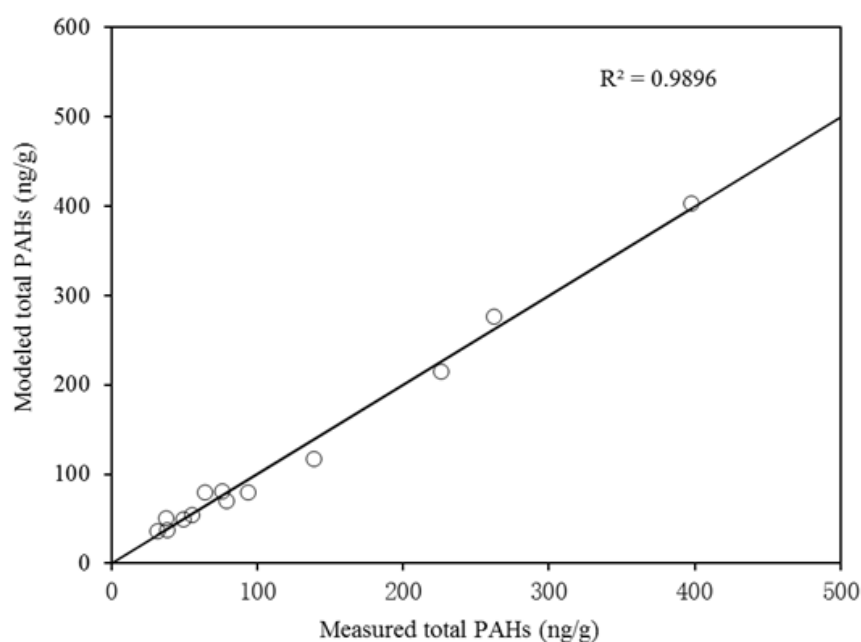


Figure 4. Correlation of the value of SumPAH between PCA-MLR prediction and measurement

The percentages contributions were 67.67% for t_1 (attributed to combustion of biomass and coal), 17.39% for t_2 (vehicular emission), and 14.94% for t_3 (coke oven emissions and crude oil pollution), respectively. According to the isomer ratios analysis above, combustion of biomass or coal was the primary source of the PAHs in most of samples. Thus, the two analyses of PAHs sources were in good agreement. Furthermore, high temperature combustion of biomass and coal was also regarded as dominant PAH source in surface sediments from Beibu Gulf (Li et al., 2015; Kaiser et al., 2016), which was in consistent with Chinese energy consumption patterns in rural areas and small industrial centers.

Conclusions

The present study has provided important data on the distribution characteristics of PAHs in the edible bivalves and sipunculida from Beibu Gulf. The current contamination levels of PAHs were relatively low compared to other coastal areas. 4 ring PAHs were found to be the most abundant PAHs in most samples. Most of the PAHs (67.67%) were mainly from biomass and coal combustion.

Acknowledgements. The research was supported by the Nature Science Foundation of Fujian (Project No. 2015J01169).

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