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Bioaccumulation of polycyclic aromatic hydrocarbons in Manila clam (*Ruditapes philippinarum*) exposed to crude oil-contaminated sediments

Chang-Hoon Lee[1], Ji-Hye Lee[1], Chan-Gyoung Sung[1,2], Seong-Dae Moon[1,3], Sin-Kil Kang[1], Jong-Hyeon Lee[1], Un Hyuk Yim[4], Won Joon Shim[4] and Sung Yong Ha[4]

[1]*Institute of Environmental Protection and Safety, NeoEnBiz Co., Bucheon 420-806, Republic of Korea*
[2]*Department of Energy and Environmental System Engineering, University of Seoul, Seoul 130-743, Republic of Korea*
[3]*Department of Biological Science, Chungnam National University, Daejon 305-764, Republic of Korea*
[4]*Oil & POPs Research Group, South Sea Research Institute, KIOST, Geoje 656-834, Republic of Korea*

ABSTRACT

The bioaccumulation of 16 United States Environmental Protection Agency (USEPA) priority polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs in the Manila clam, *Ruditapes philippinarum* exposed to sediments artificially contaminated by Iranian Heavy Crude Oil was measured and the biota-sediment accumulation factor (BSAF) was estimated through laboratory experiments. The proportion of 16 PAHs accumulated in the tissue of *R. philippinarum* was only from 3 to 7% of total PAHs. Among 16 PAHs, the concentration of naphthalene was highest in the tissue. Alkylated PAHs were highly accumulated more than 93% of total PAHs. The C3 dibenzothiophene was most highly accumulated. The relative composition of alkylated naphthalenes in the tissue of *R. philippinarum* was lower than in the sediments. In contrast, those of alkylated compounds of fluorenes, phenanthrenes, dibenzothiophenes were higher in the tissue than the sediments. The BSAF for sum of 16 PAHs was 0.11 to 0.13 g carbon/g lipid and that for alkylated PAHs was 0.05 to 0.06 g carbon/g lipid. Naphthalene showed the highest BSAF value. Alkylated PAHs with the same parent compound, BSAF tended to increase with the number of alkylated branch increased, except for alkylated chrysenes. BSAF of total PAHs lies between that of field-based values, and are also similar to those of other persistent organic pollutants (PCBs, DDTs, HCHs). This study provides the BSAF values of individual alkylated PAHs accumulated in *R. philippinarum* for the first time and will be used as a basis for further understanding the bioaccumulation of organic contaminants in the marine benthic organisms.

Key words: *Ruditapes philippinarum*, bioaccumulation, *Hebei Spirit* oil spill, polycyclic aromatic hydrocarbons (PAHs), biota-sediment accumulation factor (BSAF)

Introduction

Bivalve is one of the most dominant groups in marine benthic ecosystems. Since the embryo of bivalves is highly sensitive to environmental contaminants such as metals, pesticides and organic pollutants, standard toxicity test methods for bivalve embryo are well established (ASTM, 1994; USEPA, 1995). Many kinds of biomarkers in bivalves are also analyzed for evaluating the exposure levels and potential biological effects of toxic contaminants (Maioli *et al.*, 2010; Wang *et al.*, 2011; Moschino *et al.*, 2012). In contrast to embryo stage, adult stage of bivalves is not widely used for routine toxicity tests. When they are exposed to unfavorable environmental conditions, they can close their valves and withstand the conditions while protecting their body from outer environments. For this behavioral feature, the

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Corresponding author : Chang-Hoon Lee
Tel: +82 (32) 718-9430 e-mail: chlee@neoenbiz.com
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sensitivity of adult bivalve to contaminants is quite lower than similar stages of other taxonomic groups. In addition, when they close valves, it is not easy if they are live merely closing valves for a while, or they are really dead closing valves permanently. This gives a practical problem in determining mortality during toxicity tests. For these reasons, adult stage of bivalves is not widely used in toxicity tests, especially observing the acute responses. Instead, owing to high tolerance to toxic compounds, they are used for evaluating the bioaccumulation of various chemicals, such as metals (Boisson *et al*., 1998; Cardoso *et al*., 2009; Fukunaga and Anderson, 2011), organic contaminants (Maioli *et al*., 2010; León *et al*., 2013) and chemical mixtures (Wu *et al*., 2010). Because bivalves have quite lower enzyme activity to metabolizing persistent organic pollutants (POPs) than fish or crustaceans, tissue concentration in bivalves can be a good indicator of exposure level of environmental contamination of POPs (Otchere, 2005).

Hebei Spirit oil spill, occurred near the Taean County, west coast of Korea on December 7, 2007, released about 10,900 tonnes of crude oil and affected about 375 kilometers of shoreline along the west coast of Korean Peninsula. Cleanup campaign was activated right after the spill by more than million volunteers, and scientific monitoring program and researches were organized. Thereafter, studies related to *Hebei Spirit* oil spill were performed on the chemical and biological changes in seawater and fishes (Kim *et al*., 2010; Jung *et al*., 2011; Jung *et al*., 2012; Kim *et al*., 2013), ecotoxicity, genotoxicity, endocrine-effects, and aryl hydrocarbon receptor-mediated activity in heavily contaminated sediments (Ji *et al*., 2011; Hong *et al*., 2012; Lee *et al*., 2013a; Lee *et al*., 2013b). However, there was no cleanup activity in the muddy tidal flats where there were active aquaculture grounds for oysters and clams. After 7 years of the accident, residual oils trapped within sediment layers are found and these sediments still exhibited significant toxicity to amphipods (Lee *et al*., 2013a).

The Manila clam, *Ruditapes philippinarum* (Class Bivalvia: Family Veneridae) is widely distributed along the coasts of Korea, China, Japan, and Spain. In Korea, it mainly inhabits in the intertidal and shallow subtidal zones of the south and west coasts of Korea (Yoo 1976; Kwon *et al*. 1993; Chung *et al*. 1994). This species is one of the most important marine resources for human consumption. Many aquafarms of this species were destructed by the *Hebei Spirit* oil spill. Moschino *et al*. (2012) noted that *R. philippinarum* is a good sentinel species for the study of environmental pollution, especially in bioaccumulation and biomarker studies. There are several studies on the bioaccumulation in *R. philippinarum* for metals (Baudrimont *et al*., 2005; Figueira *et al*., 2012; Giani *et al*., 2012), polychlorinated biphenyls (PCBs) and pesticides (Choi *et al*., 2014), benzo[a]pyrene (Wang *et al*., 2011; Liu *et al*. 2014), etc. However, bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) from petrogenic sources (originated mainly from oil spill), especially for alkylated PAHs in *R. philippinarum* is not well-understood yet. To this ends, we set the purpose of this study to know the bioaccumulation pattern of PAHs originated from crude oil in the *R. philippinarum*. We exposed *R. philippinarum* to artificially contaminated sediments by Iranian Heavy Crude Oil, which is one of major components released by the *Hebei Spirit* oil spill, and analyzed the concentration of each 16 United States Environmental protection Agency (USEPA) priority PAHs and alkylated PAHs in the sediments and the tissue of *R. philippinarum*.

Materials and Methods

1. Test organisms

Ruditapes philippinarum were collected at a tidal flat in Seokmun, Dangjin, west coast of Korea (37° 02′ 31″ N, 126° 35′ 40″ E). After collection, clams were transferred to an acclimation tank with an uplifting filter and 5 cm of clean sand on the bottom. Natural seawater was supplied and renewed daily by 20%. They were acclimated under constant temperature of 20 ± 2℃ for 1 month with mixed diets of microalgae (*Isochrysis galbana*, *Chaetoceros gracilis*, and *Skeletonema costatum*).

2. Experimental design

Clean sediments were collected at a muddy tidal flat in Songak, Dangjin. After collection, sediments was passed through a 300-㎛ standard sieve to remove larger particles and other macrofauna. Iranian Heavy Crude Oil was spiked into the sediments with predetermined oil-loading ratios. Five oil-loading ratios were set as 0.39, 0.78, 1.56, 3.13, and 6.25 g/kg (wet sediments), the range of which covers from light to heavy pollution level observed at tidal flats near oil-polluted area after the *Hebei Spirit* oil spill (MOF, 2013). Clean sediments without spiked oil were used as controls. Approximately 300 mL of oil-loaded sediments were transferred into a 1-L beaker into which 1-㎛ filtered natural seawater (FSW) was added up to 1-L level and stabilized for 24 hrs. Six replicated beakers were prepared for each oil-loading ratio. After 24 hrs, overlying water was exchanged by new FSW and experimental beakers were transferred into larger exposure tanks (40-L), in which beakers were completely submerged beneath the water level of exposure tank. Six individuals of *R. philippinarum* with shell height of 20.9 ± 1.35 mm (shell length of 29.2 ± 2.0 mm) were transferred into each beaker. The temperature of exposure tanks was controlled either by heater or cooler to 20 ± 2℃. Salinity of seawater during the experiment was 30 ± 2 psu. Photoperiod was 12 light:12 dark and seawater was renewed daily by 50% during the experiment. During the exposure, survival was checked every day. When dead individuals were observed, they were removed immediately. After 14 days, surviving individuals were transferred into a new beaker with FSW and depurated for 4 hrs. After depuration, the tissue of *R. philippinarum* was separated from the shells, frozen by liquid nitrogen, and then stored at - 80℃ before chemical analysis.

3. Chemical analysis

Wet sediment samples (approximately 20 g) or tissue samples of *R. philippinarum* were mixed with 50 g of sodium sulfate to absorb water from the samples. The dried samples were spiked with deuterated surrogates (naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12) and extracted by Soxhlet for 8 hrs with 200 mL of methylene chloride. The sample extracts were then fractionated and cleaned up by a Si/Al column chromatography. Terphenyl-d14 was spiked as GC internal standard and the final volume of eluate was adjusted to 500 ㎕. A 30 m × 0.25 mm I.D. DB-5MS capillary column was used in an Agilent Model 5890 GC with Agilent Model 5972 MSD. The mass spectrometer was operated under the selected ion monitoring mode using the molecular ions of the studied PAHs. Quantification was performed using the molecular ion (mass-to-charge ratio). Peaks were confirmed based on retention time and secondary ions. The oven temperature was programmed to start initially at 60℃ (1.5 min), increased to 300℃ at 4℃/min, and held for 10 min. Injector temperature was set at 300℃ and transfer line at 280℃. Sixteen USEPA priority PAHs and alkylated PAHs were analyzed. The studied PAHs ranged from the diaromatics (naphthalene) to the hexaaromatics (benzo[ghi]perylene). Alkylated PAHs include C1 to C4 naphthalene, C1 to C3 fluorene, C1 to C4 phenanthrene, C1 to C3 dibenzothiophene, and C1 to C3 chrysene. In this study, the concentration of total polycyclic aromatic hydrocarbons (TPAHs) is defined as the sum of concentrations of upper mentioned 16 PAHs and alkylated PAHs. Matrix spikes, laboratory sample duplicates, and laboratory blanks were processed with each batch of samples as part of the laboratory internal quality control. All the quality control procedures satisfied their acceptable ranges. Standard Reference Materials (SRMs), provided by the International Atomic Energy Agency (IAEA) and National Institute of Standards and Technology (NIST) were analyzed to monitor the performance of the analytical methods. The recoveries of PAHs in a certified material (EC-4, NIST 1941b) were within the acceptable ranges (certified values ± 20%).

4. Estimation of biota-sediment accumulation factors

The biota-sediment accumulation factor (BSAF) was estimated to know and compare the accumulation patterns of each PAH compounds between sediments

and *R. philippinarum*. BSAF (g carbon/g lipid) is defined as following equation (Ankley *et al*., 1992);

$$BSAF = (C_o/f_l) / (C_s/f_{soc})$$

where C_o is the PAH concentration (mg/kg) in the tissue of *R. philippinarum*, f_l is the lipid content (g/g) in the tissue of *R. philippinarum*, C_s is the PAH concentration (mg/kg) in sediments, and f_{soc} is the fraction (g/g) of the total organic carbon in sediments.

Results and Discussion

1. Concentration of PAHs in sediments

The concentration of total PAHs (TPAHs) including 16 priority PAHs and alkylated PAHs was 0.04 mg/kg DW in control sediments. TPAHs concentrations in experimental sediments increased as crude oil loading increased from 3.79 to 58.30 mg/kg DW (Table. 1). There was a good linear relationship between crude oil loading and TPAHs concentration (r^2 = 0.990). In the oil spiked sediments, the proportion of 16 PAHs was as low as 1.5-2.6% of total PAHs. The majority of TPAHs originated from crude oil was composed of alkylated PAHs. The most dominant groups of alkylated PAHs were C2 to C4 naphthalenes. Alkylated naphthalenes contributed to more than 50% of total PAHs. The lowest concentration (3.79 mg/kg DW) of crude oil spiked sediments is slightly higher than the LC_{10} (2.8 mg/kg DW) of the amphipod, *Monocorophium uenoi* (Lee *et al*., 2013a). The second highest concentration (30.48 mg/kg DW) is similar to the LC_{50} (36.0 mg/kg DW) of *M. uenoi* (Lee *et al*., 2013a).

2. Mortality of *R. philippinarum*

The cumulative mortality of *R. philippinarum* exposed to oil contaminated sediments at the end of experiment was 0% at TPAHs concentrations ranging from 0.04 to 30.48 mg/kg DW (Table 2). Mortality was found at only the highest concentration of TPAHs (58.3 mg/kg DW). At this concentration, the cumulative mortality of *R. philippinarum* at day 7 was 14% and that at day 14 was 57%. Compared to the sensitivity of amphipod, *M. uenoi*, of which the LC_{50} is 36.0 mg/kg DW (Lee *et al*., 2013a), *R. philippinarum* is more tolerant to TPAHs than the amphipod.

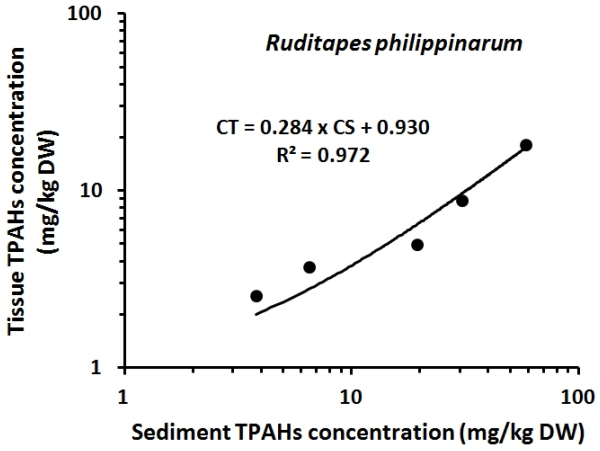

Fig. 1. Relationship between the concentrations of total polycyclic aromatic hydrocarbons (TPAHs) in the crude oil contaminated sediments (CS) and the tissue of *Ruditapes philippinarum* (CT) after 14 days' exposure.

3. Bioaccumulation of PAHs in *R. philippinarum*

The concentration of TPAHs in the tissue of *R. philippinarum* exposed for 14 days to oil contaminated sediments increased as sediment TPAHs concentration increased. There was a good linear relationship between the TPAHs concentration in sediments and tissue of *R. philippinarum* (r^2 = 0.972, Fig. 1). Tissue TPAHs concentration of *R. philippinarum* exposed to the highest sediment TPAHs concentration was 18.22 mg/kg DW (Table 3), which is about 30 times higher than that exposed to control sediments (0.61 mg/kg DW). The proportion of 16 PAHs was from 3 to 7% of TPAHs. It was lower in the tissue of *R. philippinarum* exposed to higher level of crude oil loading. The concentration of naphthalene was highest in the tissue among 16 PAHs. In contrast, alkylated PAHs composed more than 93% of the TPAHs in the tissue. The C3 dibenzothiophene was most highly accumulated in the tissue of *R. philippinarum* among the alkylated PAH compounds in all treatments. It contributed to 15-20% of TPAHs. The next highly accumulated compound was C2 dibenzothiophene followed by C4 naphthalene. PAHs with more alkylated groups tended to accumulate more in the

Table 1. Concentration of 16 priority polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs in the control and each treatment for the bioaccumulation test with *Ruditapes philippinarum* (TR1: 0.39, TR2: 0.89, TR3: 1.56, TR4: 3.13, TR5: 6.25 g Iranian Heavy Crude Oil/kg wet sediments)

Chemical name	Concentration (mg/kg DW)					
	Control	TR1	TR2	TR3	TR4	TR5
16 PAHs						
Naphthalene	0.003	0.014	0.017	0.022	0.034	0.050
Acenaphthylene	0.000	0.000	0.000	0.001	0.001	0.001
Acenaphthene	0.000	0.001	0.003	0.006	0.010	0.023
Fluorene	0.000	0.014	0.021	0.063	0.102	0.194
Phenanthrene	0.001	0.041	0.057	0.122	0.192	0.367
Anthracene	0.000	0.004	0.006	0.017	0.021	0.044
Fluoranthene	0.001	0.003	0.003	0.004	0.005	0.015
Pyrene	0.001	0.005	0.006	0.014	0.019	0.033
Benz[a]anthracene	0.000	0.003	0.004	0.007	0.010	0.019
Chrysene	0.001	0.011	0.013	0.031	0.040	0.075
Benzo[b]fluoranthene	0.001	0.003	0.004	0.006	0.008	0.012
Benzo[k]fluoranthene	0.000	0.001	0.000	0.001	0.001	0.001
Benzo[a]pyrene	0.000	0.001	0.002	0.003	0.004	0.006
Indeno[1,2,3-cd]pyrene	0.000	0.001	0.001	0.001	0.001	0.001
Dibenz[a,h]anthracene	0.000	0.000	0.000	0.001	0.001	0.002
Benzo[g,h,i]perylene	0.000	0.001	0.001	0.002	0.003	0.005
Alkylated PAHs						
C1-Naphthalene	0.003	0.073	0.168	0.304	0.677	1.596
C2-Naphthalene	0.009	0.569	1.240	2.703	6.306	12.611
C3-Naphthalene	0.005	0.688	1.156	3.372	4.941	9.603
C4-Naphthalene	0.003	0.583	0.898	3.157	4.459	7.363
C1-Fluorene	0.000	0.060	0.086	0.325	0.499	0.817
C2-Fluorene	0.001	0.126	0.177	0.705	1.088	1.739
C3-Fluorene	0.001	0.133	0.217	0.985	1.410	2.298
C1-Phenanthrene	0.001	0.177	0.241	0.585	0.857	1.609
C2-Phenanthrene	0.002	0.267	0.389	0.912	1.286	2.447
C3-Phenanthrene	0.001	0.238	0.347	0.816	1.113	2.155
C4-Phenanthrene	0.000	0.148	0.214	0.530	0.697	1.343
C1-Dibenzothiophene	0.000	0.089	0.180	0.790	1.211	2.464
C2-Dibenzothiophene	0.001	0.216	0.431	1.845	2.653	5.524
C3-Dibenzothiophene	0.001	0.229	0.466	1.769	2.454	5.196
C1-Chrysene	0.000	0.021	0.028	0.064	0.087	0.165
C2-Chrysene	0.000	0.037	0.053	0.106	0.154	0.270
C3-Chrysene	0.000	0.033	0.046	0.101	0.136	0.254
Sum of 16 PAHs	0.010	0.104	0.139	0.300	0.451	0.849
Sum of Alkylated PAHs	0.030	3.686	6.337	19.069	30.030	57.454
Total PAHs	0.039	3.790	6.476	19.369	30.481	58.303

tissue. There were positive linear relationships between tissue concentration and sediment concentration for most of PAHs except for fluoranthene, pyrene, and dibenz[a,h]anthracene.

The relative compositions of each PAH compound between sediments and tissues were different (Fig. 2).

Table 2. Cumulative mortality of *Ruditapes philippinarum* exposed to crude oil contaminated sediments with different concentration of total polycyclic aromatic hydrocarbons (TPAHs) for 7- and 14 days

Sediment TPAHs concentration (mg/kg DW)	Mortality of *Ruditapes philippinarum* (%)	
	Day 7	Day 14
0.04	0	0
3.79	0	0
6.48	0	0
19.37	0	0
30.48	0	0
58.30	14	57

While the relative composition of low molecular weight PAHs, such as alkylated naphthalenes in the sediments was more than 50% of TPAHs, that in the tissue of *R. philippinarum* decreased to 29.4%. Meanwhile, the relative compositions of high molecular weight PAHs, such as alkylated fluorenes, alkylated phenanthrenes, alkylated dibenzothiophenes were higher in the tissue than the sediments. This indicates that, the bioaccumulation of PAHs in the tissue of *R. philippinarum* is not proportional to the composition of sediments.

The tissue concentration is determined by the balance between uptake and elimination. Uptake is mainly controlled by externally the partitioning of contaminants, and elimination is controlled by both passive diffusion and enzymatic pathways that convert hydrophobic parent compounds to more polar metabolites that can be more readily excreted (Meador *et al*., 1995a).

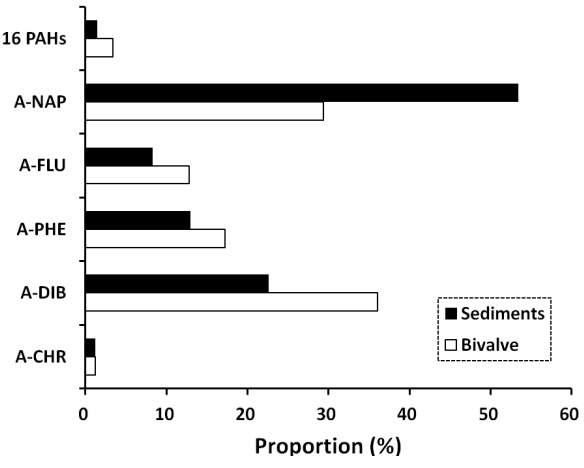

Fig. 2. Comparison of relative compositions of polycyclic aromatic hydrocarbon (PAH) groups in the crude oil contaminated sediments (solid bar) and the tissue of *Ruditapes philippinarum* (empty bar) after 14 days' exposure (A-NAP: alkylated naphthalenes, A-FLU: alkylated fluorenes, A-PHE: alkylated phenanthrenes, A-DIB: alkylated dibenzothiophenes, A-CHR: alkylated chrysenes).

The major route of exposure for PAHs is different according to the octanol-water partitioning coefficient (K_{ow}). Many studies revealed that the major route of exposure for low molecular weight PAHs (LPAHs) is pore water and that for high molecular weight PAHs (HPAHs) is sediment particles (Roesijadi *et al*., 1978a; Roesijadi *et al*., 1978b, Meador *et al*., 1995b). In this study, *R. philippinarum* is a filter feeding bivalve and food was not supplied during the experiment, we can assume that most of PAHs compounds were absorbed through the contact with pore water. From this viewpoint, the accumulated concentrations of each PAH compounds in the tissue of *R. philippinarum* should be proportional to the concentration in sediments and K_{ow}. The concentrations of lower molecular weight PAHs are predicted to be higher than higher molecular weight PAHs. But, our data did not accord to this prediction. The higher molecular weight PAHs, alkylated dibenzothiophenes were most dominant in the tissue of *R. philippinarum*. Therefore, differences in elimination rate of *R. philippinarum* for each compound seem to be more important.

Since most of PAHs exhibit a range of lipophilic affinity, elimination by passive diffusion should be slower for the more hydrophobic PAHs. In mussel (*Mytilus edulis*), the half-lives of more water soluble PAHs were shorter than those of more hydrophobic PAHs (Lake *et al*., 1985). Many of bivalve species are known to have very limited ability to metabolize PAHs and hence reflect environmental exposure more accurately than other taxonomic groups (Meador *et al*.,

Table 3. Concentration of 16 priority polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs in the tissue of *Ruditapes philippinarum* exposed to control and each treatment (TR1: 0.39, TR2: 0.89, TR3: 1.56, TR4: 3.13, TR5: 6.25 g Iranian Heavy Crude Oil/kg wet sediments)

Chemical name	Concentration (mg/kg DW)					
	Control	TR1	TR2	TR3	TR4	TR5
16 PAHs						
Naphthalene	0.156	0.067	0.116	0.085	0.104	0.350
Acenaphthylene	0.001	0.001	0.002	0.001	0.002	0.003
Acenaphthene	0.001	0.001	0.002	0.002	0.002	0.004
Fluorene	0.002	0.006	0.011	0.010	0.012	0.026
Phenanthrene	0.006	0.012	0.022	0.026	0.028	0.071
Anthracene	0.001	0.002	0.001	0.003	0.005	0.012
Fluoranthene	0.011	0.017	0.016	0.006	0.006	0.010
Pyrene	0.019	0.052	0.071	0.023	0.032	0.051
Benz[a]anthracene	0.003	0.006	0.006	0.004	0.006	0.010
Chrysene	0.004	0.016	0.014	0.023	0.037	0.062
Benzo[b]fluoranthene	0.001	0.003	0.003	0.003	0.005	0.007
Benzo[k]fluoranthene	0.000	0.001	0.001	0.000	0.000	0.001
Benzo[a]pyrene	0.000	0.001	0.001	0.001	0.002	0.003
Indeno[1,2,3-cd]pyrene	0.000	0.000	0.000	0.000	0.000	0.001
Dibenz[a,h]anthracene	0.000	0.000	0.000	0.000	0.000	0.000
Benzo[g,h,i]perylene	0.000	0.000	0.001	0.001	0.001	0.002
Alkylated PAHs						
C1-Naphthalene	0.049	0.051	0.085	0.096	0.088	0.208
C2-Naphthalene	0.114	0.230	0.310	0.324	0.415	1.188
C3-Naphthalene	0.057	0.262	0.377	0.490	0.720	1.707
C4-Naphthalene	0.055	0.281	0.366	0.522	1.033	2.247
C1-Fluorene	0.011	0.025	0.048	0.054	0.080	0.200
C2-Fluorene	0.015	0.078	0.151	0.171	0.302	0.699
C3-Fluorene	0.030	0.132	0.263	0.315	0.681	1.440
C1-Phenanthrene	0.010	0.061	0.101	0.131	0.185	0.476
C2-Phenanthrene	0.001	0.154	0.218	0.310	0.513	1.108
C3-Phenanthrene	0.014	0.150	0.195	0.323	0.611	1.046
C4-Phenanthrene	0.000	0.071	0.102	0.180	0.350	0.504
C1-Dibenzothiophene	0.007	0.090	0.147	0.187	0.291	0.763
C2-Dibenzothiophene	0.022	0.356	0.508	0.738	1.377	2.859
C3-Dibenzothiophene	0.020	0.397	0.544	0.857	1.738	2.946
C1-Chrysene	0.001	0.008	0.010	0.026	0.052	0.077
C2-Chrysene	0.002	0.010	0.010	0.035	0.079	0.092
C3-Chrysene	0.000	0.007	0.005	0.018	0.042	0.045
Sum of 16 PAHs	0.208	0.186	0.266	0.189	0.241	0.612
Sum of Alkylated PAHs	0.407	2.363	3.441	4.777	8.558	17.605
Total PAHs	0.615	2.549	3.707	4.966	8.799	18.217

1995a). From these, we can explain the differences in the relative compositions of PAHs groups between sediments and tissue of *R. philippinarum* were due to the different balances between uptake and elimination among PAH groups. Among absorbed PAH groups, the rate of elimination by either passive diffusion or metabolism of alkylated naphthalenes seems to be higher than other PAH groups. Only its relative proportion in tissue was lower than sediments. For other PAHs groups, The relative proportions in tissue increased by a quite similar factor of those in sediments. This indicates that the uptake rates and elimination rates of alkylated fluorenes, alkylated phenanthrenes, alkylated dibenzothiophenes and alkylated chrysenes in *R. philippinarum* are controlled by similar pathways.

Table 4. Biota-sediment accumulation factors (BSAFs) for each 16 priority polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs accumulated in the tissue of *Ruditapes philippinarum* exposed to each treatment (TR3: 1.56, TR4: 3.13, TR5: 6.25 g Iranian Heavy Crude Oil/kg wet sediments)

Chemical name	BSAF (g carbon/g lipid)		
	TR3	TR4	TR5
16 PAHs			
Naphthalene	0.81	0.62	1.45
Acenaphthylene	0.34	0.60	0.47
Acenaphthene	0.06	0.04	0.04
Fluorene	0.03	0.02	0.03
Phenanthrene	0.04	0.03	0.04
Anthracene	0.04	0.05	0.06
Fluoranthene	0.28	0.21	0.14
Pyrene	0.34	0.35	0.31
Benz[a]anthracene	0.12	0.11	0.11
Chrysene	0.15	0.19	0.17
Benzo[b]fluoranthene	0.10	0.12	0.12
Benzo[k]fluoranthene	0.14	0.09	0.13
Benzo[a]pyrene	0.09	0.09	0.08
Indeno[1,2,3-cd]pyrene	0.08	0.07	0.09
Dibenz[a,h]anthracene	0.06	0.06	0.00
Benzo[g,h,i]perylene	0.07	0.09	0.08
Alkylated PAHs			
C1-Naphthalene	0.07	0.03	0.03
C2-Naphthalene	0.02	0.01	0.02
C3-Naphthalene	0.03	0.03	0.04
C4-Naphthalene	0.03	0.05	0.06
C1-Fluorene	0.03	0.03	0.05
C2-Fluorene	0.05	0.06	0.08
C3-Fluorene	0.07	0.10	0.13
C1-Phenanthrene	0.05	0.04	0.06
C2-Phenanthrene	0.07	0.08	0.09
C3-Phenanthrene	0.08	0.11	0.10
C4-Phenanthrene	0.07	0.10	0.08
C1-Dibenzothiophene	0.05	0.05	0.06
C2-Dibenzothiophene	0.08	0.11	0.11
C3-Dibenzothiophene	0.10	0.15	0.12
C1-Chrysene	0.08	0.12	0.10
C2-Chrysene	0.07	0.11	0.07
C3-Chrysene	0.04	0.06	0.04
Sum of 16 PAHs	0.13	0.11	0.15
Sum of Alkylated PAHs	0.05	0.06	0.06
Total PAHs	0.05	0.06	0.06

4. Biota-sediment accumulation factors (BSAFs)

BSAFs for each 16 PAHs and alkylated PAHs accumulated in the tissue of *R. philippinarum* exposed to each treatment are listed in Table 4. Since BSAF estimated from lower 2 treatments (0.39 and 0.79 g crude oil/kg WW) did not accord to the higher 3 treatments (1.56, 3.13, and 6.25 g crude oil/kg WW), we listed BSAFs estimated from 3 high sediment concentrations. The BSAF for sum of 16 PAHs was 0.11 to 0.13 g carbon/g lipid and that for sum of alkylated PAHs was 0.05 to 0.06 g carbon/g lipid. BSAF of alkylated PAHs was about half lower than that of 16 PAHs. Among individual PAHs, naphthalene showed the highest BSAF value (0.96 ± 0.43 g carbon/g lipid) and acenaphthylene the second (0.47 ± 0.13 g carbon/g lipid). Alkylated PAHs with the same parent

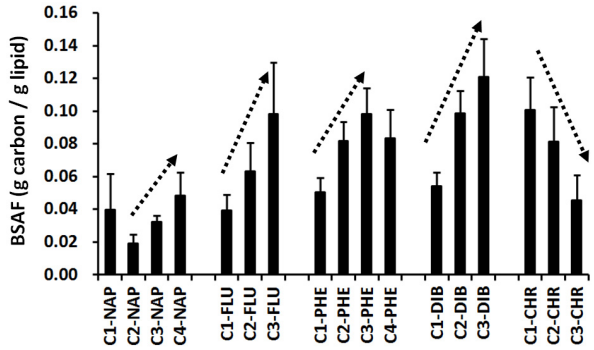

Fig. 3. Comparison of biota-sediment accumulation factors (BSAFs) of alkylated PAHs among different number of alkyl branches estimated from laboratory experiments with *Ruditapes philippinarum* exposed to crude oil contaminated sediments for 14 days (NAP: naphthalene, FLU: fluorene, PHE: phenanthrene, DIB: dibenzothiophene, CHR: chrysene). Vertical bar indicates the standard deviation of 3 different treatments (1.56, 3.13, and 6.25 g crude oil/kg WW).

compound, BSAF tended to increase with the number of alkylated branch increased, except for alkylated chrysenes (Fig. 3). BSAFs for C2 to C4 naphthalenes, C1 to C3 fluorenes, C1 to C3 phenanthrenes, and C1 to C3 dibenzothiophenes increased as the number of alkylation increased, whereas BSAFs for C1 to C3 chrysene decreased as the number of alkylation increased.

BSAF of TPAHs (0.05-0.13 g carbon / g lipid) from this study (laboratory-based BSAF) lies between the lower ranges of field-based BSAF values (0.07-3.72 g carbon / g lipid) estimated in *R. philippinarum* (Moschino *et al*., 2012). Our values are similar to those of other POPs, such as PCBs (0.01-0.91 g carbon/g lipid), DDTs (0.02-1.61 g carbon/g lipid), and HCHs (0.01-0.92 g carbon/g lipid) estimated by field data of the same species (Choi *et al*., 2014).

This study provides for the first time the BSAF values of individual alkylated PAHs accumulated in *R. philippinarum*. However, our values are merely rough estimation of BSAF for some reasons. In the real environments, clams contact with suspended particles in the water column, filter them, and intake them. But, in this study, clams were exposed to relatively calm conditions without re-suspension of sediment particles so that they had less chance to contact with the suspended particles. That is, the route of exposure to PAHs is limited to pore water, so that the BSAF values might be underestimated to an extent lower than that in the wild conditions. In addition, we did not check after 14 days' exposure if the tissue concentration of PAHs reached steady state. If our values were estimated before steady state, it might also lead to underestimation of them. Therefore, additional researches to understand the effects of suspended particles on the accumulation of PAHs in clams and to reveal the time required to reach steady state of tissue concentrations of PAHs are needed.

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