

Distribution and Characteristics of PAHs in Bivalves from Gwangyang Bay, Korea

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ABSTRACT - Polycyclic aromatic hydrocarbons (PAHs) were quantitatively determined in bivalves from Gwangyang Bay. Twenty-four kinds of target PAHs were detected in the entire samples. Total PAHs concentrations ranged 86.1 ~ 1,210 ng g⁻¹ dry weight with a average concentration of 309 ng g⁻¹, which is much lower than those of other industrialized bays in Korea. Strong tidal currents and artificial interferences like reclamation and dredging activity were suggested as major reason for disparity between highly industrialization and low contamination status. There was no correlation between total PAHs and lipid contents, implying that lipid content is not the one major parameter for the accumulation of PAHs by bivalves. Input sources were inferred as petrogenic and pyrogenic PAHs. With help of PCA analysis, signals of seasonal variations, not only concentrations but also compositions were found.

Key words : PAHs, Gwangyang Bay, bivalves, seasonality

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of widely distributed organic compounds consisting of hydrogen and carbon arranged in two or more fused benzene rings. PAH compounds originate from a variety of anthropogenic sources, including municipal and industrial effluents, creosote, oil spills, urban and agricultural runoff, fossil fuel combustion, asphalt production, and waste incineration (Latimer and Zheng 2003). About 43,000 metric tons of PAHs are discharged into the atmosphere each year, and another 230,000 tons enter aquatic environments (Eisler 1987).

Various aquatic organisms including macro-algae, mussel, fish, bird and seal have been widely used to monitor the pollution of anthropogenic contaminants since the concept of biomonitoring was introduced to

environmental pollution in the 1960s (Goldberg 1975; Aguilar *et al.* 2002). The use of biological tissues gives a chance to overcome the limitation for determinations of trace contaminants in water samples. PAHs readily partition into tissues from water column due to their lipophilicity, which cause several orders of magnitude higher PAHs concentrations in organisms than in water (Meador *et al.* 1995). Even if the contaminants are present at such low concentrations in water that is hard to be detected with normal analytical procedure, enhanced PAHs levels in aquatic organisms can be more easily detected. Among the marine organisms, bivalves such as mussel and oyster have been addressed as target species for monitoring trace toxic substances in coastal waters (Goldberg 1975).

A petrochemical complex, steel mill, thermoelectric power plant, and other manufacturing complexes are located around Gwangyang Bay. Reclaiming and dredging operation has been in progress all over Gwangyang Bay since 1970s. For demands of resource supplies,

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harbor facilities have been also co-developed within Gwangyang Bay. In spite of these vigorous industrial activities, there is only limited studies related with PAHs contamination (MOMAF 2002).

In this study, we aimed to find out the status of PAHs contamination and their possible sources around Gwangyang Bay by analyzing PAHs in indigenous intertidal bivalves.

METHODS

1. Sample collection

The sampling sites are shown in Figure 1. Twenty monitoring sites were selected which cover Gwangyang Bay and its vicinity area. Bivalve samples like oyster (*Crassostrea gigas*) and mussel (*Mytilus edulis*) were collected during three different sampling period depen-

ding on the accessibility and availability (Table 1). More than thirty individuals were collected at each site and immediately frozen using dry ice and transferred to the laboratory. Average shell length of the oysters and mussels was 62 mm and 48 mm respectively. Whole tissue samples were combined and macerated with tissue-mixer.

2. Analytical procedures

The analytical procedures of PAHs in biota followed the method of Sloan *et al.* (1993) after some modifications. Briefly, bivalve samples (approximately 15 g) were mixed with 50 g of sodium sulfate to dry the samples. The dried samples were spiked with 300 ng of deuterated surrogates (naphthalene- d_8 , acenaphthene- d_{10} , phenanthrene- d_{10} , chrysene- d_{12} , and perylene- d_{12}) and extracted by Soxhlet for 6~8 hrs with 200 mL of methy-

Table 1. Summary of sampling locations for mussels and oyster from Gwangyang Bay

Station No.	Biota	Location	Sampling date
B1	<i>Crassostrea gigas</i>	Northward of Myodo	Feb. 2000
B2-1	<i>Crassostrea gigas</i>	Westward of Myodo	Feb. 2000
B2-2	<i>Mytilus edulis</i>		Sep. 2001
B2-3	<i>Mytilus edulis</i>		Feb. 2000
B3-1	<i>Crassostrea gigas</i>	Southward of Myodo	Sep. 2001
B3-2	<i>Mytilus edulis</i>		Jul. 2002
B4-1	<i>Crassostrea gigas</i>	Nakpo LG Harbor	Jul. 2002
B4-2	<i>Mytilus edulis</i>		Jul. 2002
B5-1	<i>Crassostrea gigas</i>	Wolnae LG Harbor	Jul. 2002
B5-2	<i>Mytilus edulis</i>		Jul. 2002
B6	<i>Crassostrea gigas</i>	Creek near Samil Harbor	Jul. 2002
B7	<i>Mytilus edulis</i>	Jungheung Harbor	Jul. 2002
B8-1	<i>Crassostrea gigas</i>	Beach near Yosu Airport	Feb. 2000
B8-2	<i>Crassostrea gigas</i>		Sep. 2001
B9-1	<i>Crassostrea gigas</i>	Yulchon Industrial Complex	Feb. 2000
B9-2	<i>Crassostrea gigas</i>		Sep. 2001
B10-1	<i>Crassostrea gigas</i>	Chonam Industrial Complex	Feb. 2000
B10-2	<i>Crassostrea gigas</i>		Sep. 2001
B11	<i>Crassostrea gigas</i>	Gwangyang-Shi Wawoo 1st Bridge	Feb. 2000
B12	<i>Crassostrea gigas</i>	Sueochon (River)	Jul. 2002
B13-1	<i>Crassostrea gigas</i>	Gwangyang-Shi Taein Bridge	Feb. 2000
B13-2	<i>Crassostrea gigas</i>		Sep. 2001
B14	<i>Mytilus edulis</i>	Gwangyang Steel Mill Outlet of Effluent	Sep. 2001
B15	<i>Crassostrea gigas</i>	Hadong Thermoelectric Powerplant	Feb. 2000
B16	<i>Crassostrea gigas</i>	Hadong Keumnam-Myon	Sep. 2001
B17	<i>Crassostrea gigas</i>	Namhae-Gun Gohyun-Myon	Nov. 2001
B18	<i>Crassostrea gigas</i>	Namhae-Gun Seo-Myon	Nov. 2001
B19	<i>Crassostrea gigas</i>	Namhae-Gun Nam-Myon	Nov. 2001
B20	<i>Crassostrea gigas</i>	Yosu-Shi Dolsando	Sep. 2001

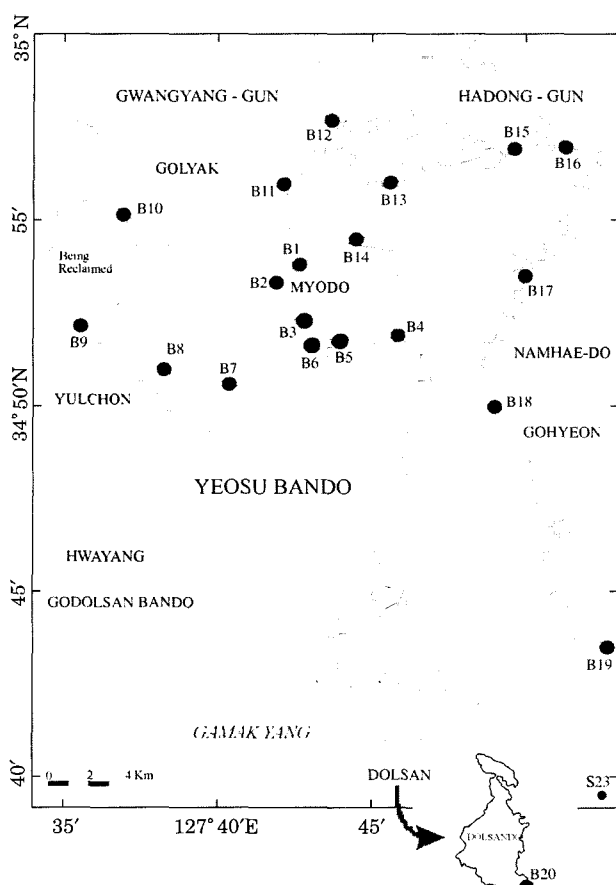


Fig. 1. Location map of sampling sites for intertidal bivalves in Gwangyang Bay.

lene chloride. The sample extracts were extensively cleaned up by a Si/Al column chromatography and HPLC with size exclusion column (Krahn *et al.* 1988). Terphenyl- d_{14} was used as GC internal standard.

A 30 m \times 0.25 mm I.D. DB-5MS capillary column was used in a Hewlett-Packard Model 5890 GC with Hewlett-Packard Model 5972 MSD. Injector temperature was set at 300°C and transfer line at 280°C. Temperature program of GC oven was as follows: initial temperature 1.5 min at 60°C; first rate 4°C min⁻¹ to 300°C; isothermal pause 10 min at 300°C. Two or more target and confirmation ions were selected for identification and quantitation of the compounds.

3. Quality assurance/Quality control

Quality control samples were processed in a manner identical to actual samples. A minimum of one method blank was made for every batch of samples (14 sampl-

es). Blank levels were no more than 3 times the method detection limit. Method procedural blank results showed that none of individuals exceed the 3 times the method detection limit. Surrogate standards were used for examining the recovery of each sample and quantifying the analytes. The acceptable range of surrogate standards recovery was between 40 and 120%. If the recovery of surrogate standards deviated from the accepted range, the sample was reanalyzed. Average recovery of naphthalene- d_8 , acenaphthene- d_{10} , phenanthrene- d_{10} , Chrysene- d_{12} , and perylene- d_{12} was 68.6, 74.0, 85.4, 92.7, and 91.5%, respectively. Although recovery of naphthalene was low compared to other surrogate standard due to several steps of concentration, this value was within an acceptable range. Matrix spike sample was processed with a sample set. The acceptable matrix spike recovery criteria for sediment and tissue analysis was that the average recoveries for all 24 compounds must fall between 40 and 120%. The recovery of each analyte was 80~111%.

4. Statistical analysis

One-way analysis of variation (ANOVA) tests were made by MINITAB® for windows version 10.1 software. A confidence level of 95% has been used. Principal component analysis were made using SIMCA version 6.0 software (Umetri AB & Erisoft AB). To compensate for strongly skewed distributions and to prevent high concentrations from dominating the mathematical modeling, all data in the SIMCA analysis in this study were total PAH normalized.

RESULTS AND DISCUSSIONS

1. PAHs residues in bivalves and horizontal distribution

The entire target 24 PAHs were detected in the bivalve samples analyzed in this study. Total PAH concentrations in bivalves were in the range of 86.1~1,210 ng g⁻¹ dry weight (Fig. 2). Oyster samples collected at Taein Bridge (St. 13) showed the highest concentration, while mussel from B5 the lowest. The average concentration was 309 ng g⁻¹ dw with standard deviation of 269 ng g⁻¹ dw. The level of PAHs concentrations in

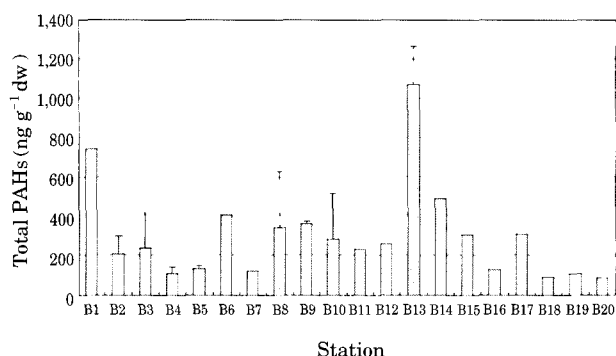


Fig. 2. Distribution of total PAHs accumulated in bivalves from Gwangyang Bay. Error bar indicate 1 standard deviation.

Table 2. Comparison of total PAHs concentration in this study with other major industrialized bays in Korea

	Min.	Max.	Mean	Std.	References
Youngil Bay	354	7,491	1,452	1,777	Yim (2003)
Ulsan Bay	501	11,390	1,912	3,979	
Busan Bay	260	2,515	1,085	816	
Masan Bay	151	1,110	562	340	
Kyunggi Bay	369	1,279	702	399	
Gwangyang Bay	86	1,210	309	269	This study

Gwangyang Bay were compared with other industrialized bays in Korea (Table 2). As much as 11 ppm was reported from Ulsan Bay. And most of contaminated regions showed more than ppm levels. However in this study only one station reached 1 ppm level and the average concentration was much lower than those of bivalves from Youngil, Ulsan, and Busan Bay.

Stations. B1, B13, and B14 showed high level of PAHs, which indicate the presence of nearby local input sources. All these three stations are located around Gwangyang Steel Mill Complex and reflect emissions of PAHs related with steel production. Stations B4~B7 were selected for monitoring of petrogenic PAHs from petroleum spill and waste effluent from oil refineries. The concentration of total PAHs were relatively low (100 ng g^{-1}) which could be comparable with control sites (Sts. B18~19). Only st. B6 showed elevated level (402 ng g^{-1}), indicating the influence of waste discharge from oil refinery. West side of Gwangyang Bay were monitored with sts. B8~B10. Reclamation and construction of industrial complex is under way and this

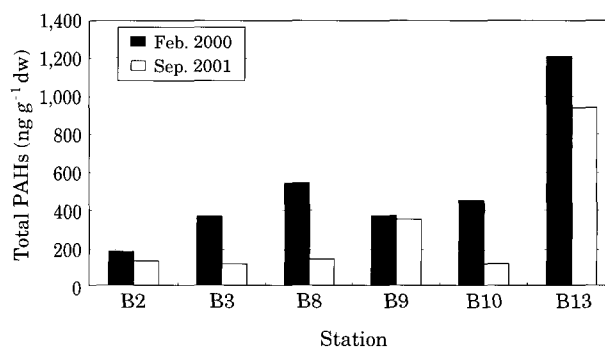


Fig. 3. Temporal variations of total PAHs in selected stations.

area showed rapid geomorphological changes. When compared with control sites sts B8~B10 showed almost four times higher level. Sueochon (River) is the second largest river which flows into Gwangyang Bay. Stations B11 and B12 were selected for the monitoring of riverine input from Sueochon, and their contribution was low (average: 250 ng g^{-1}). Right side of the bay (Sts. B15~B17) showed higher concentration (average: 248 ng g^{-1}) than control sites.

There was difference between 1st sampling campaign (February 2000) and 2nd sampling campaign (September 2001) (Fig. 3). Six sites (Sts. B2, B3, B8, B9, B10, and B13) were revisited and checked for changes of PAHs accumulation. Samples collected at winter showed higher levels than those of autumn. This will be discussed later.

There exists disparity between highly industrialization and relatively low contamination levels in Gwangyang Bay. Atmospheric pollution related with Yeochon Petrochemical Complex and Gwangyang Steel Mill Complex became so serious that this region has been managed by 'Special Atmospheric Management Region' plan since 1995. Another serious pollution source came from waste effluents that had been reported to be responsible more than 90% of contaminants influx into the Gwangyang Bay. Oil spill accident has been also main issue in this region. In the year 1995, two large oil spill accident (supertanker *Sea Prince & Sapphire*) released about 6,400 tons of crude and fuel oil into this region. All these are typical and main sources of PAHs into marine environment, that is atmospheric deposition, riverine input of wastewater, and oil spill accident. Youngil Bay where Pohang Steel Mill Complex is locat-

ed is highly contaminated with pyrogenic PAHs and Ulsan Bay has been also reported to be influenced by oil refineries and shipping activities (Khim *et al.* 2001; Yim 2003). However, bivalves in Gwangyang Bay accumulated relatively small amount of PAHs compared with similar source region. Moreover, PAHs, OCs (organochlorines), TBT, and trace metals in sediments also showed low concentration than other industrialized regions (Cho *et al.* 2000; MOMAF 2002).

This paradox could be partly explained by environmental factors and artificial activities related with construction of industrial complex. Firstly, environmental factor like physical movement of seawater has strong effect on the fate of PAHs in the water column. Tidal current responsible most of water movement in Gwangyang Bay, its velocity reaches about 100 cm sec^{-1} in the south of Myodo. The east side and bay proper regions are also strongly affected by tidal energy, while west side is relatively away from this effect. And about 10^9 m^3 of seawater is exchanged through Yeosu and Noryang Waterway during ebb and flood tide (Yun 1976). Therefore there is not sufficient time for mixing, partitioning, deposition of contaminants into the bay proper and east side where most of industrial complexes are located.

Another crucial factor is human interruption like dredging and reclamation. Since 1970s reclamation of shallow intertidal area has been continued that 25% of Gwangyang Bay now becomes land (Choi *et al.* 2002). These reclamation activities remove relatively stable and calm environments where particle bound contaminants are preferentially deposited. Moreover dredging activities for the safe operation of ocean going vessels and construction of harbor disturb and get rid of accumulated contaminants. Because resuspension of PAHs from sediment and bioaccumulation of sediment related PAHs are another major input source of PAHs for nearby biota (Boehm and Farrington 1984; Baumard *et al.* 1999), reclamation and dredging activity can distort status of contamination monitored by bivalves.

2. Correlation with lipid content

Filter-feeding organisms can absorb xenobiotics from two pathways: the direct one is the absorption of com-

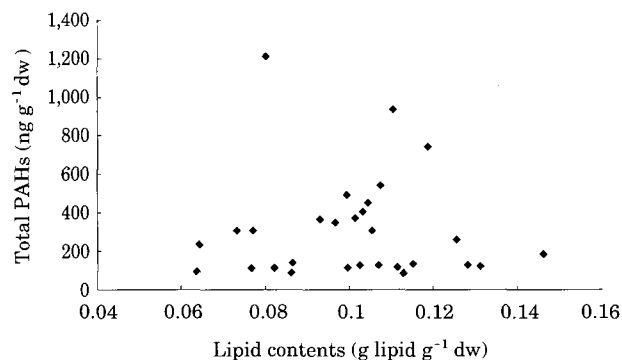


Fig. 4. Correlation between lipid contents and total PAHs concentration in bivalves from Gwangyang Bay.

pounds present in the water phase through the gills, and the indirect one is the absorption of xenobiotics adsorbed on the small particles through the digestive system. For the more water-soluble PAHs ($\log K_{ow} < 5.5$), the main route of uptake is believed to be through ventilated water, whereas the hydrophobic compounds ($\log K_{ow} > 5.5$) are taken in mainly through ingestion of food or sediment (Meador 1995). And lipid content is thought to be one important factor that can determine the amount of hydrophobic compound that is accumulated (McElroy *et al.* 1989).

Lipid content of bivalves analyzed in this study ranged from 6.4% to 14.6%. There was no significant correlation between lipid contents and total PAHs accumulated in bivalves (Fig. 4). Firstly this could be due to the surrounding environmental that PAHs in water column are not distributed uniformly in this bay (MOMAF, 2002). As Gwangyang Bay is affected locally by strong tidal currents and rapid water exchange, the concentration of PAHs in water varies regionally. Therefore, bivalves in this study didn't have similar chance to absorb or digest PAHs in water column. Other appealing suggestion is that total lipid content is not the only factor governing PAH accumulation, storage, and retention in organisms. Mix *et al.* (1982) found that gametogenesis and spawning did not significantly alter whole-body PAH concentrations.

3. Source identification

PAHs can be generated by three main processes: (1) pyrogenic (combustion of organic matter); (2) petrogenic

(related with petroleum); (3) diagenetic processes (degradation of the organic matter) (Neff 1979). The molecular PAH patterns generated by each source are like fingerprints and it is possible to determine the processes that generate PAHs. Pyrolytic PAHs are characterized by the dominance of high molecular weight PAHs, while petrogenic PAHs are dominated by lower molecular weight PAHs and alkylated PAHs.

Usually low molecular weight (LMW) PAHs are defined as PAHs whose molecular weight are less than 202 and high molecular weight (HMW) PAHs vice versa. In this study correlation between HMW PAHs and total PAHs clearly divided into two groups (Fig. 5). Considering that absorbing pathway of PAHs into bivalves is mainly passive and controlled by diffusion pressure and that water-soluble fraction is dominated by more soluble LMW PAHs, determination of sources using molecular weight is more or less arbitrary. However, predominance of HMW PAHs could be inferred from pyrogenic sources. Twelve stations were categorized as LMW rich and seventeen stations as HMW rich stations. Compound profile of each group is presented at Fig. 6, which clearly shows the difference between LMW and HMW rich sites. Phenanthrene, fluoranthene, and naphthalene were detected as most dominant compounds in LMW rich group. And alkylated naphthalens (2- and 1-methyl naphthalene, 2, 6-dimethylnaphthalene, and 1, 3, 5-trimethylnaphthalene) and alkylated phenanthrene also prevailed in that group, which clearly indicate petrogenic sources. However in HMW rich group pyrene, benzo[a]anthracene, chrysene, and especially highest molecular weight compounds (MW 276)

were detected predominantly. There were not site specific trends that can tell point sources. For example at st. B13, B13-1 which was sampled in February 2000 showed clearly petrogenic sources, while B13-2, sampled same location in September 2001, revealed totally different profile.

With help of PCA (principal component analysis) source discrimination were investigated more detail (Fig. 7). The SIMCA (Soft Independent Modeling of Class Analogy) pattern recognition method and applications in PAHs distribution in marine environment were described by Votgt *et al.* (1986). Two significant principal components were found and together they explain 61%

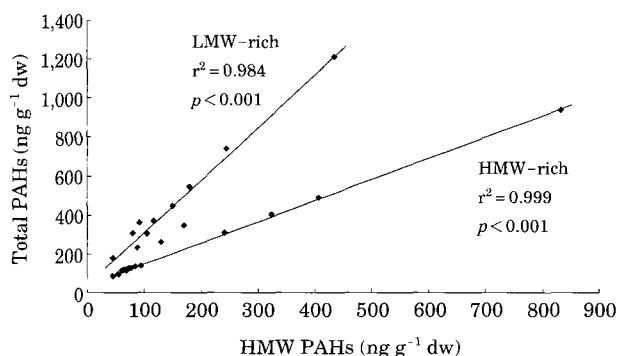


Fig. 5. Relationship between high molecular weight PAHs and total PAHs.

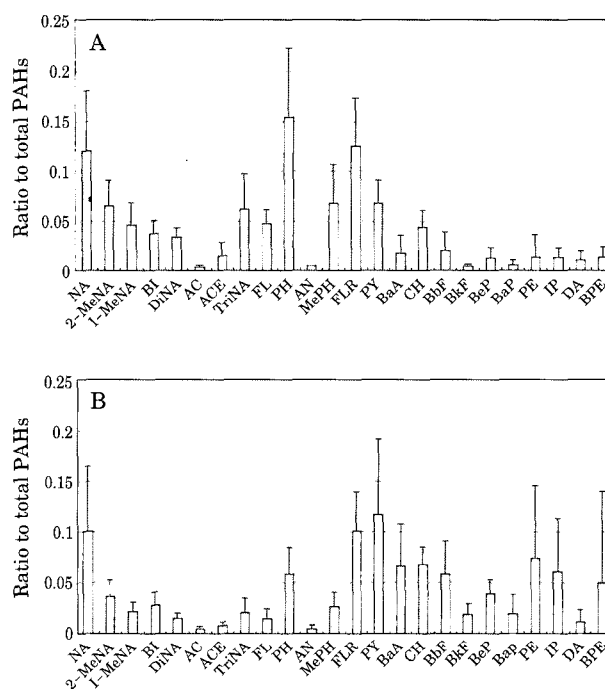


Fig. 6. PAHs composition profiles based on grouping (A) low molecular weight PAHs rich group and (B) high molecular weight PAHs rich group. Compound abbreviations indicate- NA: naphthalene, 2-MeNA: 2-methylnaphthalene, 1-MeNA: 1-methylnaphthalene, BI: biphenyl, DiNA: 2, 6-dimethylnaphthalene, AC: Acenaphthylene, ACE: Acenaphthene, TriNA: 1, 3, 5-trimethylnaphthalene, FL: fluorine, PH: phenanthrene, AN: anthracene, MePH: 1-methylphenanthrene, FLR: fluoranthene, PY: pyrene, BaA: benzo[a]anthracene, CH: chrysene, BbF: benzo[k]fluoranthene, BkF: benzo[k]fluoranthene, BeP: benzo[e]pyrene, BaP: benzo[a]pyrene, PE: perylene, IP: indeno[1, 2, 3-cd]pyrene, DA: dibenz[a, h]anthracene, BPE: benzo[ghi]perylene.

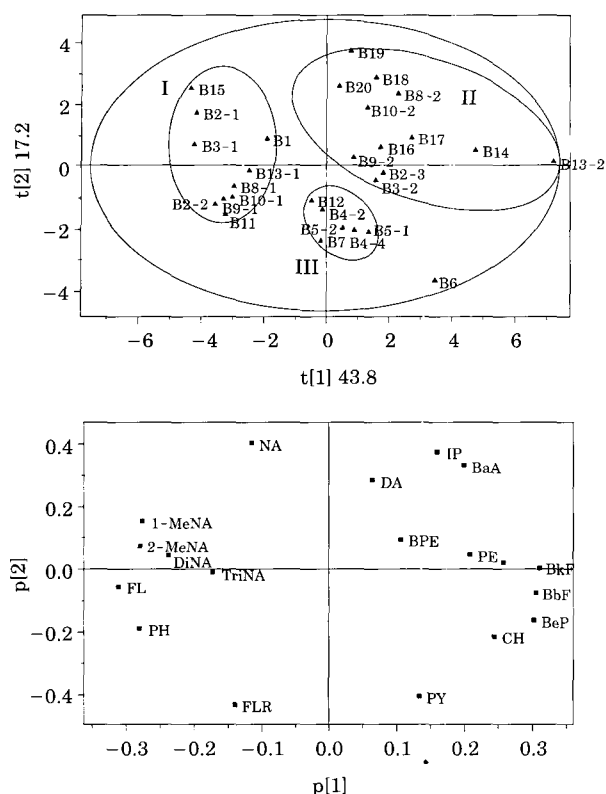


Fig. 7. Results of principal component analysis (A) score plot and (B) loading plot. For compound information, refer to caption of Fig. 6.

of the variance in the data set. Three groups could be divided according to their loadings. Accidentally these grouping almost correspond to the sampling time. Group I matches 1st sampling campaign, group II, 2nd sampling campaign, and group III, 3rd sampling campaign. Group I is strongly related with low molecular weight and alkylated PAHs and group II high molecular weight PAHs, while group III lies between LMW and HMW loadings. As previously mentioned each sampling campaign represent 1st for winter, 2nd for autumn and 3rd for summer season. These seasonal changes of PAHs compositions well correspond with quantitative differences between 1st and 2nd sampling shown at Fig. 3.

Baumard *et al.* (1999) reported seasonal variations of PAHs concentrations in mussels, and these variations were attributed to filtering rate changes of mussel, differences in waterborne PAHs, and reproductive cycle of the mussels. However their inference did not explain all the variables and they only considered the concentration differences. Yim (2002) reported seasonal changes

of PAHs accumulated in bivalves in terms of concentration and composition. The main focus was depuration of PAHs in bivalves that were affected by the *Sea Prince* oil spill in 1995. Not only concentration but also composition changes were observed. There are many factors that affect bioconcentration or bioaccumulation of PAHs in the marine environment. Most of studies focused on relatively short term changes like oil exposure experiment in the laboratory (Baussant *et al.* 2001) and there are not integrated studies which cover environmental factors and physiological changes of bivalves itself. Therefore when designing the monitoring studies using bivalves, time of sampling and frequencies should be carefully considered to avoid complex parameters affecting interpretation of the results.

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