The High Performance Liquid Chromatography (HPLC) Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) in Sediment, Mussels and Oysters from the Coastal Zone of Kwangyang Bay, Korea

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광양만 연안의 퇴적물과 이매패류 (진주담치, 굴)에 농축된 다환방향족 탄화수소의 HPLC에 의한 분석

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Abstract

다환방향족 탄화수소(이하 PAHs)는 오염된 연안역의 해양생물에 흡수, 농축되어 발암 및 돌연변이를 유발시키는 오염물질로 알려져 있으며 이를 섭취하는 인간에게도 영향을 미칠 것으로 판단된다.

PAHs가 해양환경으로 유입되는 대표적인 몇 가지 경로는 가정하수와 산업폐수, 해난사고에 의한 기름유출, 화석연료의 불완전 연소, 자연적인 경로 등이 있다.

여천석유화학단지, POSCO, 하동화력발전소 등 산업시설이 산재한 광양만 연안에서 채집된 퇴적물과 두 종류의 이매패류 (진주담치, 굴)에 농축된 PAHs를 HPLC를 이용하여 분석하였다.

퇴적물과 이매패류에서 분석된 16 가지의 PAH중 우세한 농도를 보인 PAH는 퇴적물에서는 acenaphthylene (1074.86 ± 150.21), pyrene (235.08 ± 81.74), naphthalene (251.25 ± 46.49), 진주담치에서는 acenaphthylene (688.83 ± 72.44), naphthalene (296.50 ± 83.01), pyrene (60.42 ± 9.76), 굴에서는 acenaphthylene (513.93 ± 63.73), naphthalene (235.57 ± 100.87), pyrene (100.83 ± 12.40) 으로 나타났다.

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Total PAHs의 농도범위는 퇴적물에서 1,430~3,601 ppb (평균 1,995 ppb dry wt), 진주담치에서 901~1,956 ppb (평균 1,296 ppb wet wt), 굴에서 681~2,269 ppb (평균 1,076 ppb wet wt)로 나타났다. 분석된 PAH 농도는 대체로 생물시료에서 낮은 경향을 보였다.

벤젠고리가 3개 이하인 PAHs와 4개 이상인 PAHs의 농도비는 각각 진주담치에서 5.3, 굴에서 3.0, 퇴적물에서 3.9로 나타났다. 또한 N (naphthalene) 과 P (phenanthrene)의 농도비는 진주담치에서 31.05±11.67, 굴에서 21.60±7.93, 퇴적물에서 18.29±2.67로 나타났다. 모든 시료에서 벤젠고리가 3개 이하인 PAHs가 우세한 농도를 보였으며, N (naphthalene) 과 P (phenanthrene)의 농도비가 1.0보다 훨씬 큰 수치를 보였으므로 본 연구지역에서 분석된 PAHs는 연소되지 않은 fresh petroleum에서 기원된 PAH가 대부분이라고 추정된다.

Ⅰ. INTRODUCTION

Oil pollution has recently received the greatest public attention because of the direct damage to fisheries, and the harmful effects on marine lives such as seabirds, marine mammals, seaweeds and so on. Spilled oil slick will destroy beaches, recreational areas as well as marine ecosystem, and decrease the size of the populations of flora and fauna, and further, modify their habitats, delay or prevent recolonization (Marshall and Coull, 1996; Al-lihaibi and Al-omran, 1996; Readman et al., 1996). The widespread use of petroleum and its products has inevitably resulted in the discharge of oil to the environment.

World War Ⅰ caused a large number of oil spills that had a noticeably adverse effect on marine birds. The subsequent conversion of the economy of the world from coal to oil, followed by World War Ⅱ, greatly increased petroleum threat to marine life. Efforts to deal with a growing number of oil spills and intentional oil discharges at sea continued during the 1950s and 1960s (Bourne, 1968). The wreck of the *Torry Canyon* off the coast of England in 1967 produced worldwide concern about the consequences of massive oil spills in marine environment. Research on the environmental fate and biological effects of spilled petroleum increased dramatically during the

1970s. Despite the considerable progress in developing methods to clean up oil spills, and adoption of numerous controls on shipping practices, petroleum nowadays continues to be a widespread environmental hazard.

Crude oil consists primarily of hydrocarbons which contain only carbon and hydrogen. Some crude oils contain as much as 98% hydrocarbons (Nelson Smith, 1972). In addition to hydrocarbons, the organic substances in crude oil include compounds containing sulfur, nitrogen, and/or oxygen, with sulfur being more abundant than nitrogen and nitrogen more abundant than oxygen. In addition, there are small concentrations of metals such as nickel, vanadium, and iron (NAS, 1975). The three principal class of hydrocarbons found in crude oil are alkanes (paraffins), cycloalkanes (naphthenes) and aromatics (Ryan, 1977).

Petroleum is a major source of PAHs (polycyclic aromatic hydrocarbons) in marine environment. PAHs are found throughout the world, as their presence has been detected in living and nonliving components of marine ecosystems.

Polycyclic aromatic hydrocarbons (PAHs) consist of hydrogen and carbon arranged in the form of two or more fused benzene rings in linear, angular, or cluster arrangements with unsubstituted groups possibly attached to one or more rings (Eisler, 1987).

Most PAHs are formed by a process of thermal decomposition of organic molecules and subsequent recombination of the organic particles (pyrolysis). Incomplete combustion of organic matter produces PAHs in high-temperature (500-800℃) environment. All forms of combustion, except flammable gases well mixed with air, produce some PAHs. Subjection of organic material to low temperature (100-300℃) for long periods of time also results in PAH production (Neff, 1985; Grimmer, 1983). Although the PAH compounds formed by high- and low-temperature processes are similar, the proportional representation of individual compounds and isomers are different (Grimmer, 1983).

Natural sources of PAHs include forest and grass fires, oil seeps, volcanoes, and chlorophyllous and nonchlorophyllous (bacteria, fungi) plants (Eisler, 1987; McElroy et al., 1989). Anthropogenic sources of PAHs include petroleum spills

and discharges, electric power generation, refuse incineration, home heating, coke, carbon black, coal tar, asphalt production, and internal combustion engines (Eisler, 1987; Neff, 1985). The primary mechanism for atmospheric contamination by PAHs is incomplete combustion of organic matter (Baek et al., 1991). Sources of PAHs on land include petroleum spills, volcanoes, natural fires, industrial activities, waste disposal and incineration, home heating, and automobile exhaust. Polycyclic aromatic hydrocarbons (PAHs) are a class of chemical carcinogens and mutagenic pollutants which are suspected of toxicity to aquatic organisms (NAS, USA, 1972; Hoffman *et al.*, 1974; Mei-Tein Lo & Sandi, 1978; Baumann *et al.*, 1982; Malins *et al.*, 1984) and are known to be absorbed and accumulated by marine organisms from waters polluted by industrial and municipal wastes (Suess, 1976; Pancirov & Brown, 1977; Uthe, 1979).

PAHs enter the marine environment via several processes such as precipitation, land runoff, atmospheric fallouts, industrial and municipal waste discharges, and accidental oil spills (Whipple and Hunter, 1979; *e. g.* Hoffman *et al.*, 1984; Prahl *et al.*, 1984).

Once introduced into the aquatic system, PAHs rapidly adsorb onto particulate matter due to their characteristically low water solubilities (Whitehouse, 1984). They are removed from water column either by direct mixing to the sediment surface, by adsorption onto particle sand subsequent sinking, or by uptake into aquatic organisms. Because of general resistance to chemical and biological degradation as well as high lipophilicity, they are also preferentially accumulated in the lipids of organisms (Kahng, 1995).

The concentrations of PAHs in sediments, water column, and biota have been determined to assess the PAH levels in coastal areas using either Gas Chromatography (GC) or High Performance Liquid Chromatography (HPLC); in marine animals or plants (Coccheri and Arnese, 1990; Hellou et al., 1990; Ranio et al., 1986; Butler and Sibbald, 1986; Jones et al., 1986; Pancirov and Brown, 1977), sediments (Kiceniuk and Williams, 1987; Badawy et al., 1993; Smith et al., 1985; Pendoley, 1992; Bjorseth, 1979; Mattsson and Lehtinen, 1985; Kayal and Connell, 1989), and seawater (Theobald, 1989; Kayal and Connell, 1989;

Law and Whinnet, 1992; Green et al., 1992; Cripps, 1992).

Bivalves (mussel and oyster), have been thought to be valuable sentinel organisms reflecting the level of contamination of some pollutants in marine ecosystem (Farrington et al., 1980, 1982, 1983). Bivalves are generally preferred for assessing pollution level because of their wide geographic distribution, sedentary form of life, ability to bioconcentrate organic and inorganic contaminants, comparatively low enzyme activity metabolizing xenobiotics, ability to survive under extreme pollution conditions and commercial value (Goldberg et al., 1978; Farrington et al., 1983).

The toxic effects of oil fall into two general categories. The first category includes effects associated with coating or smothering of an organism with oil. Such effects are associated primarily with the higher-molecular-weight, water-insoluble hydrocarbons, the various tarry substances that coat the feathers on birds and cover intertidal organisms such as clams, oysters, and barnacles. Although some organisms such as tubeworms and barnacles are suprisingly little affected by such coatings (Nelson-Smith, 1972), the effect on organisms such as aquatic birds may be devastating.

The second category of toxic effects involves disruption of an organism's metabolism due to the ingestion of oil and the incorporation of hydrocarbons into lipid or other tissues in sufficient concentrations to upset the normal functioning of the organism. With respect to this second category of effects, it is generally agreed that aromatic hydrocarbons are the most toxic, followed by cycloalkanes, then olefins, and lastly alkanes. There is also a definite tendency for toxicity to be correlated positively with the molecular size of the hydrocarbons. Most toxic effects caused by ingestion of oil in water, however, are believed to be due to low-molecular-weight (C_{12} - C_{21}) alkanes and low-molecular weight aromatics, since these compounds are the most soluble in water (NRC, 1985). Based on studies of the concentrations of hydrocarbons in water-soluble fractions of various oils, the NRC (1985) concluded that the contribution of compounds of higher molecular weight than alkylnaphthalenes was very small and probably insignificant in terms of acutely toxic effects.

Thus the compounds of greatest concern from the standpoint of ingestion are the low-molecular-weight aromatics such as benzene and toluene.

In general, aromatic hydrocarbons have more undesirable effects on biota than do the aliphatic hydrocarbons. Compared to the aliphatics, most aromatics degrade more slowly, persist in tissues for longer, and are usually more toxic. One group of aromatics, the PAHs, including some compounds that are toxic or carcinogenic, is widely distributed in the environment, and is one of the U. S. EPA's Priority Pollutants. PAHs can interfere with metabolic process and tend to be concentrated and retained in tissues more effectively than other hydrocarbons (Boylan and Tripp, 1971; Neff, Anderson, Tatem, and Hightower, 1974; Neff, Cox, Dixit, and Anderson, 1976). The relation of skin cancer to the occupation of chimney sweeping was made in England during the late 18th century. By the early 20th century, soot, coal tar, and pitch were all known to be carcinogenic to humans. In 1918, benzo(a)pyrene was identified as a major carcinogenic agent; several other PAHs have since been similarly identified. Numerous toxic and carcinogenic effects of PAHs on the wildlife have been documented (Eisler, 1987).

In Korea, one can hardly find any research on the PAH determination in sediment or marine organisms except a few (Lee, 1997; Sul, 1997).

In the present study, I determined the concentrations of PAHs in sediment and bivalves (mussels and oysters) from the coastal zone of Kwangyang Bay. The data on the PAH levels in mussels, oysters and sediment will be utilized for dignosing PAH contamination in Kwangyang Bay and will contribute to the data accumulation for the PAH levels in sediment and bivalves in Korea.

Ⅱ. MATERIALS AND METHODS

1. Study area

Sampling was performed in Kwangyang Bay, where many kinds of industries, such as Yeochen industrial complex, POSCO, Hadong fire-power

plant, and so on are located in the vicinity.

Kwangyang Bay is 17 km long along its east ~ west axis, and 9km wide at maximum. The Sumjin River flows into the bay at a rate of 120m^3/s and 5.8 ~ 8.7×10^{7}ton/year. Small islands including Myo Is., Kumho Is., and Taein Is. are scattered in the center of the bay. Water depths are relatively shallower; ranging from 5m to 20m (Lee, 1994).

Total of eight sampling sites in this study area were taken as follows: 2 sampling sites (site 1, 2) near the Namhae Great Bridge which is thought to be relatively an unpolluted area, 2 sampling sites (site 7, 8) near the Yeochen industrial complex which are considered to be severely polluted, and the intermediatly polluted sites (site 3, 4, 5, 6) between the two areas (Figure 1).

2. Sampling of mussel, oyster, and sediment

Oyster (*Crassostrea gigas*), mussel (*Mytilus edulis*), and sediment samples were collected at 8 sites (Fig. 1) in the intertidal zone of the Kwangyang Bay in March, July, and September 1997.

Oysters and mussels were collected by chisel and sediment samples were taken with shovel at low tide.

PAHs are hydrophobic and adsorb onto the inner surface of sampling devices, which must be rinsed with solvent in order to avoid contamination. Therefore sampling devices shouldn't be made of plastics, and such as glass, PTFE and stainless steel are recommended. When marine sediment and organisms are sampled from ship for PAHs analysis, care must also be taken to avoid contamination from discharges from engine room, lubricated winches, wires, and shackles, etc. If analysis cannot be done immediately after sampling, samples should be stored with care. Storage under the wrong conditions can radically alter the concentration of analyses, yielding incorrect data and wasting the analytical effort devoted to these samples. Storage poses particular problems when the aim is the identification of the chemical form (speciation) in which an element is present, as the original equilibria may be

altered during storage (Law et al., 1994). Extracts may be stored in freezer satisfactorily for a period of months prior to analysis.

In the presented study sediment samples were stored in cleaned glass jars and bivalve samples were wrapped whole in aluminum foil before storage in a freezer (Law and Biscaya, 1994). All samples were stored at -20℃ in a freezer prior to analysis.

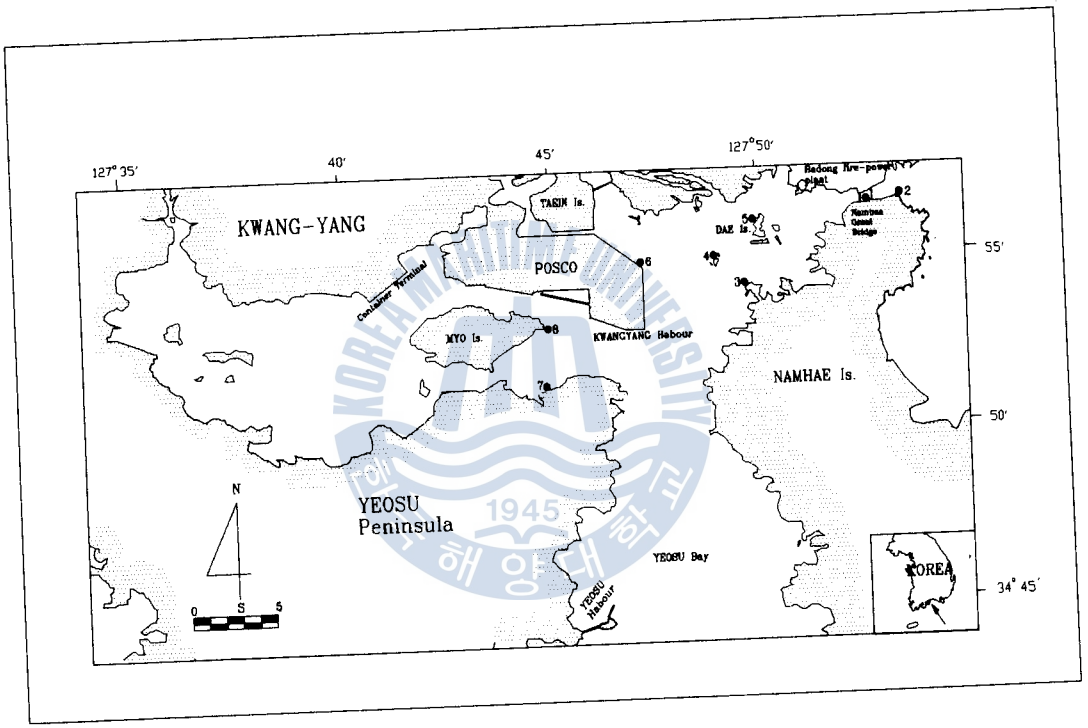

Fig. 1. Map showing the sampling sites in the study area.

3. The extraction and isolation of PAHs

16 PAHs were extracted and analyzed in this study.

For analysis, bivalves (oysters and mussels) were partially thawed, the shells were removed and a composite sample of at least 10 individuals was made. The mean size of mussels and oysters is given in Table 1. And then, the samples were homogenized with a macerator. 20g (wet wt) of bivalves homogenate was

dried with anhydrous sodium sulphate and hydrocarbons together with the fats were Soxhlet-extracted with a mixture of hexane, acetone, diethylether, and petroleum ether (2.5:7.5:1:9 v/v) at 40~60 ℃ for 6 hours. The solvents were evaporated to near dryness and the fatty residue was saponified by refluxing in aqueous methanolic (1:9 v/v) 2N KOH solution for 3 hours.

Following the digestion, the methanol-KOH supernatant was extracted with cyclohexane according to the method of Grimmer and Böhnke (1975). To separate PAHs from the aliphatic hydrocarbons, the liquid-liquid extraction procedure developed by Natusch and Tomkins (1978) was applied. The cyclohexane extract was partitioned three times with equal volumes of dimethylsulfoxide (DMSO). The DMSO layers, which contained the PAHs, were then combined.

For clean-up of PAHs, two volumes of water were added to the combined DMSO extracts and the resulting solution was partitioned three times with equal volumes of cyclohexane. The cyclohexane layers were washed once with same volumes of water (Rainio et al., 1986), and were dried nearly to a volume of 2.0㎖ using a rotary evaporator, reduced to a final volume of approximately 1 ㎖ under the flow of nitrogen gas. The sample was filtered through a 0.45 ㎛ PVDF filter unit (Whatmman) prior to analysis by HPLC (Kiceniuk and Williams, 1987).

For analysis, sediment samples were partially thawed, and the supertant water was removed after centrifugation (2000 rpm, 15min). The excess moisture of sediment sample was removed in the dry-oven at 40 ℃ for 24 hours. 20g (dry wt) of sediment sample was taken to extract.

4. HPLC system

The analysis of PAHs was carried out by reverse phased HPLC (Linear Instruments co.) using a gradient solvent program at room temperature. The HPLC system consists of binary solvent delivery system (Linear Instruments Model S-1100), an automatic gradient controller (Linear Instruments Model

S-2000), an injector with 20 ㎕ sample loop fitted with a Spherisorb S5 ODS 2 column (4.6 ㎜×25㎝, 5 ㎛ particle size).

The pressure fluctuated over a 8 ~ 15 Mpa range. The flow rate of solvents, binary solvent delivery time-steps, gradient of solvent values and start and stop of HPLC system are controlled by the automatic gradient controller system.

Solvent A of acetonitrile and solvent B of distilled water/acetonitrile (1:1, v/v) were utilized as mobile phases, and a binary gradient solvent system for the elution of PAHs employed in this study is as follows; solvent delivery was programmed at 0% solvent A for the initial condition, and then 30% solvent A at 5 min, 80% solvent A at 15 min, 90% solvent A at 20 min, 95% solvent A at 25 min, and 100% solvent A at 30 min. The flow rate was held constant 0.8 ㎖/min.

Sample was injected by 20 ㎕ syringe. Analytical blank test were carried out between the each sample run.

5. Identification and quantification

The peaks of PAHs were identified, and then quantified using a fluorescence detector (Model LC 304 fluorescence detector) plus uv/vis detector (Model 200 uv/vis detector). The fluorescence detector was set up excitation wavelength at 270 nm and emission wavelength at 400 nm, and the absorption of uv/vis detector was set up at 254 nm. The fluorescence detector was only made use of authenticating peaks of PAHs.

PAH concentrations were determined comparing the peak area obtained from the samples with those obtained from the reference standard (PAH mixture 610-M Supelco Inc., Bellefonte, PA, USA).

The management of chromatograms, integration and calibration of data were carried out using Peaksimple Serial Data Program system (SRI Model 202).

Ⅲ. Result

The chromatograms of PAHs in sediment, mussels, and oysters from

Kwangyang Bay are given in Fig. 2, 3, and 4.

The mean concentrations of individual PAHs in sediment were as follows: NPTHL (251 ± 46), ANCPL (1075 ± 150), ACNPN (32 ± 8), FLURN (15 ± 9), PHEN (24 ± 13), ANTHR (21 ± 10), FLUOR (102 ± 42), PYR (235 ± 82), CHRY (29 ± 15), BbF (17 ± 7), BkF (60 ± 38), BaP (17 ± 4), DahA (64 ± 19), BghiP (53 ± 16), and $I_{123cd}P$ (23 ± 6). The predominant group of PAHs in sediment consisted of ANCPL (1075 ± 150), PYR (235 ± 82), NPTHL (251 ± 46), and FLUOR (102 ± 42). The second dominant group of PAHs in sediment consisted of DahA (64 ± 19), BkF (60 ± 38), and BghiP (53 ± 16). And the least dominant group of PAHs in sediment consisted of ACNPN (32 ± 8), CHRY (29 ± 15), PHEN (24 ± 13), $I_{123cd}P$ (23 ± 6), ANTHR (21 ± 10), BaP (17 ± 4), BbF (17 ± 7), FLURN (15 ± 9) and BaA (not detected).

The mean concentrations of individual PAHs in mussels were as follows: NPTHL (297 ± 83), ANCPL (689 ± 72), ACNPN (19 ± 6), FLURN (7 ± 2), PHEN (11 ± 1), ANTHR (5 ± 1), FLUOR (49 ± 19), PYR (60 ± 10), CHRY (8 ± 3), BbF (7 ± 3), BkF (8 ± 5), BaP (9 ± 2), DahA (27 ± 5), BghiP (55 ± 24), and $I_{123cd}P$ (16 ± 6). The predominant group of PAHs in mussels consisted of ANCPL (689 ± 72), NPTHL (297 ± 83), and PYR (60 ± 10). The second dominant group of PAHs in mussels consisted of BghiP (54.60 ± 24.30), FLUOR (49 ± 19), DahA (27 ± 5), ACNPN (19 ± 6), and $I_{123cd}P$ (16 ± 6). And the least dominant group of PAHs in mussels consisted of PHEN (11 ± 1), BaP (9 ± 2), CHRY (8 ± 3), BkF (8 ± 5), FLURN (7 ± 2), BbF (7 ± 3), ANTHR (5 ± 1), and BaA (not detected).

The mean concentrations of individual PAHs in oysters were as follows: NPTHL (236 ± 101), ANCPL (514 ± 64), ACNPN (13 ± 4), FLURN (7 ± 1), PHEN (9 ± 2), ANTHR (3 ± 1), FLUOR (63 ± 17), PYR (101 ± 12), CHRY (7 ± 1), BbF (7 ± 2), BkF (14 ± 4), BaP (5 ± 1), DahA (27 ± 6), BghiP (26 ± 11), and $I_{123cd}P$ (13 ± 5). The predominant group of PAHs in oysters consisted of ANCPL (514 ± 64), NPTHL (236 ± 101), PYR (101 ± 12), and FLUOR (63 ± 17). The second dominant group of PAHs in oysters consisted of DahA (27 ± 6), BghiP (26 ± 11), BkF (14 ± 4), ACNPN (13 ± 4), and $I_{123cd}P$ (13 ± 5). The least dominant group of PAHs in oysters consisted of PHEN (9 ± 2), BbF (7 ± 2), FLURN (7 ± 1), CHRY (7 ± 1), BaP (5 ± 1), ANTHR (3 ± 1), and BaA (not detected).

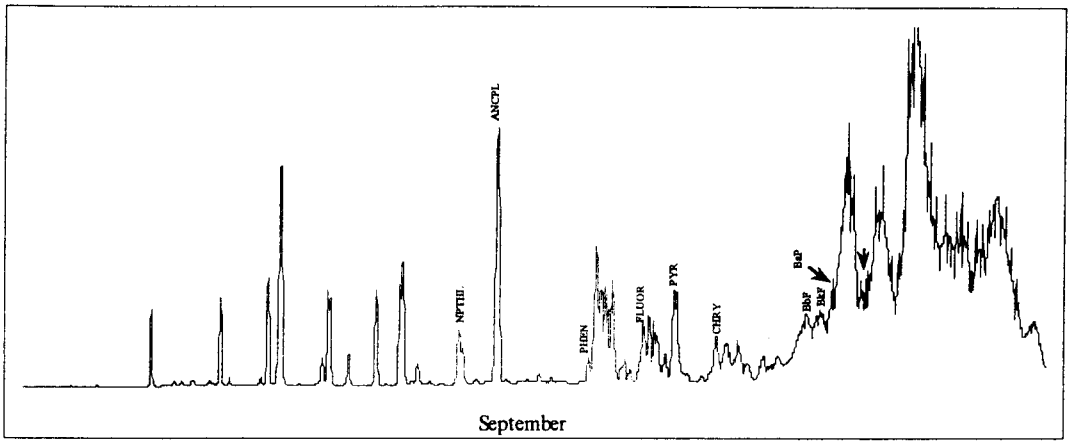

Fig. 2. Chromatogram of PAHs in sediment detected by uv/vis detector at site 4.

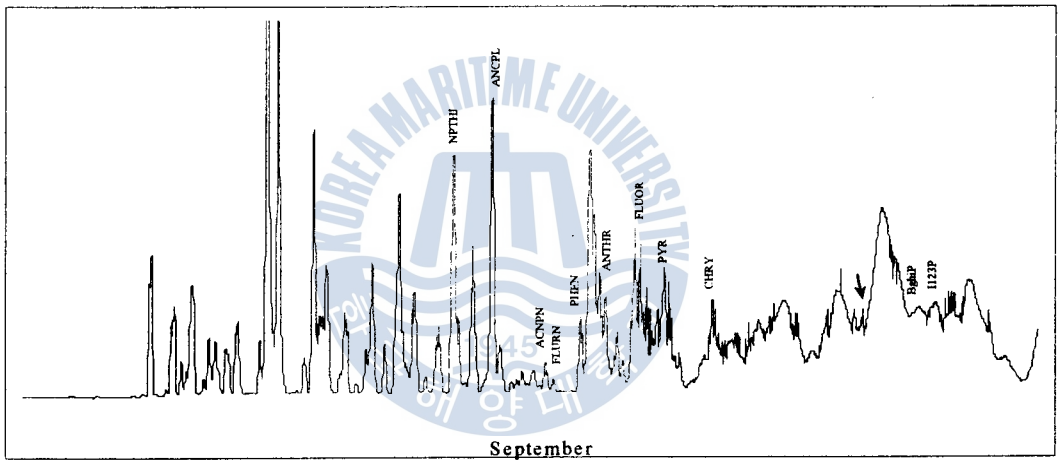

Fig. 3. chromatogram of PAHs in mussels detected by uv/vis detector at site 2.

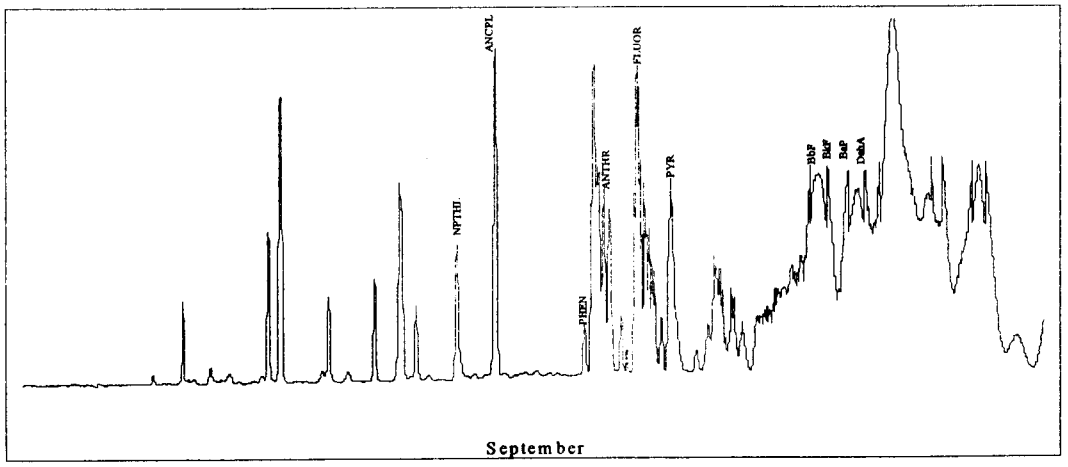

Fig. 4. Chromatogram of PAHs in oysters detected by uv/vis detector at site 4.

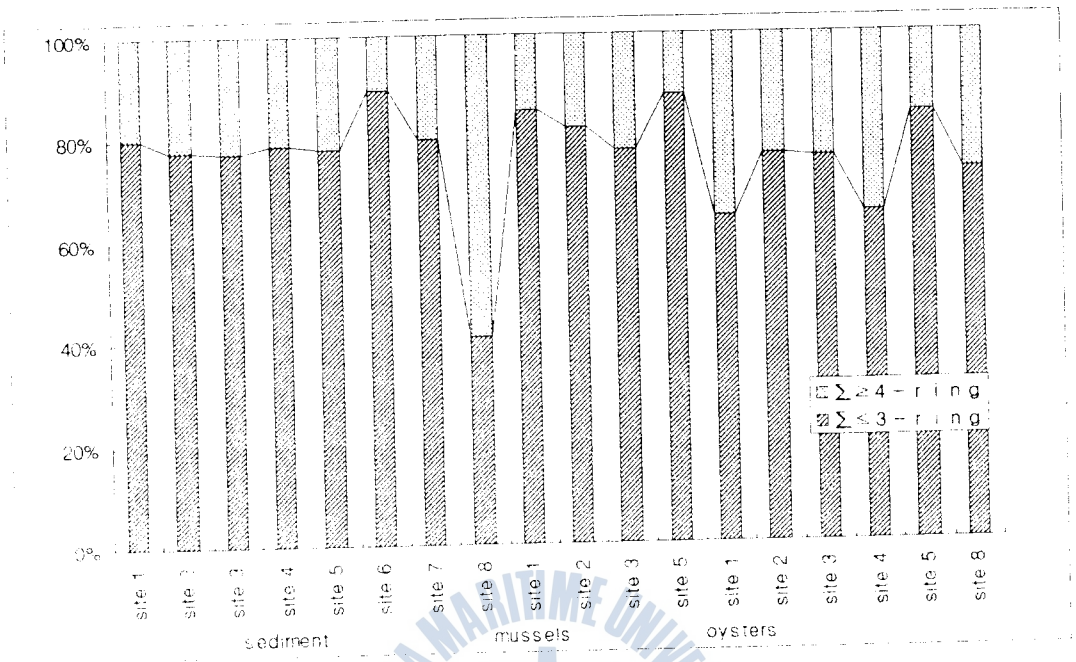

Fig. 5. The mean ratio of $\Sigma \leq 3$ rings to $\Sigma \geq 4$ rings in sediment, mussels, and oysters from Kwangyang Bay.

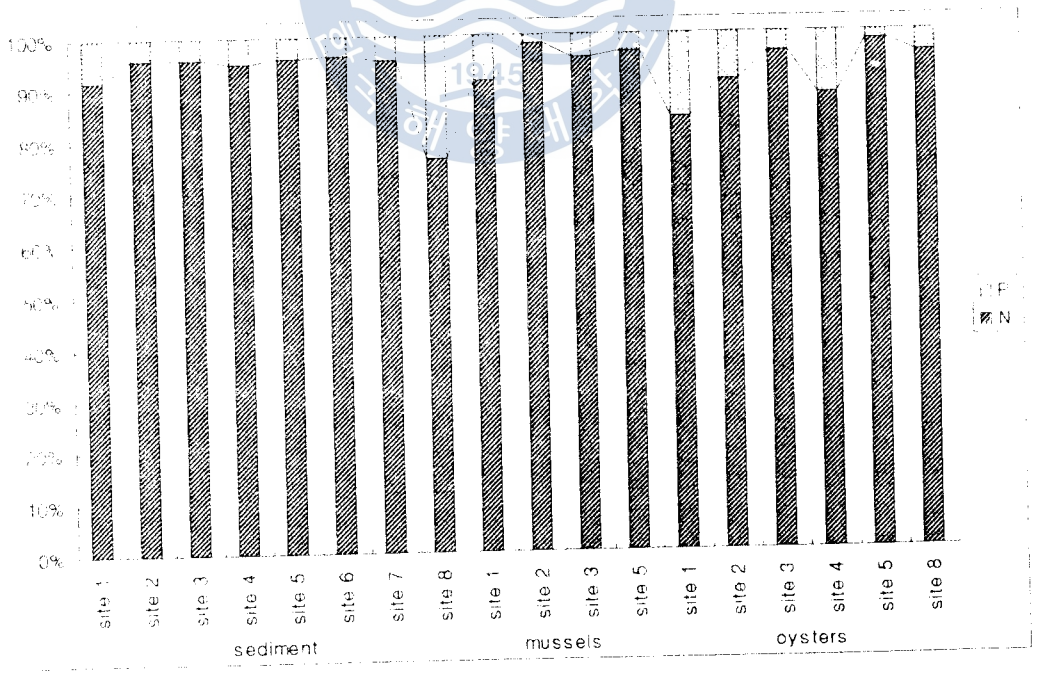

Fig. 6. The ratio of N (naphthalene) to P (phenanthrene) in sediment, mussels, and oysters from Kwangyang Bay.

Ⅳ. SUMMARY

PAHs are widely distributed in sediment and bivalves in Kwangyang Bay. The concentration of total PAHs in sediment was relatively higher in sediment, with a range of 1,430-3,601 ppb (dry weight) than those in mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas*), with a range of 901-1,956 ppb (wet weight) and 681-2,269 ppb (wet weight), respectively. The well known carcinogenic benzo(a)pyrene comprised 0.3-1.2% of the total PAHs in sediment, 0.6-1.0% in mussels, and 0.1-2.1% in oysters. At sites near Yeochen industrial complex, POSCO, and container terminal were highest in PAH level in sediment.

The concentrations of total PAHs (3,601 ppb) in sediment in Kwangyang Bay, though relatively high, did not exceed the ER-L value (4,000 ppb dry wt), whereas individual PAHs such as naphthalene, anthracene, pyrene, and dibenzo(a,h)anthracene exceeded ER-L value. However, they did not exceeded OAET (overall apparent effect thresholds) value.

The mean ratio of $\Sigma \leq 3$-rings to $\Sigma \geq 4$-rings of sediment, mussels, and oysters was 3.9 (range of 0.7-8.5), 5.3 (range of 3.5-7.3), and 3.0 (range of 1.8-5.4), respectively. The dominance of $\Sigma \leq 3$-rings in all samples from the study area suggests that the PAHs in samples were possibly non-pyrogenic or uncombusted petroleum derived. The PAHs of $\Sigma \leq 3$-rings seem to directly enter the bay as a result of discharged or spilled oil from ships (commercial sea-traffics, fishing boat activities), and industrial effluents. And those of $\Sigma \geq 4$-rings seem to be largely introduced into the bay by rainfall runoff from surface land and an atmospheric input route.

The ratio of N (naphthalene) to P (phenanthrene) in sediment was 18.29 ± 2.67, in mussels, 31.05 ± 11.67, and in oysters, 21.60 ± 7.93. The ratio of N to P is much greater than 1.0 for most sediment, mussel, and oyster samples from the study area, and this again suggests that the PAHs in sediment and bivalves in Kwangyang Bay were derived from uncombusted fresh petroleum, maily from spilled or discharged oil.

For further studies, seasonal variation of PAHs in sediment and biota needs to be analyzed.

And the experiments on the acute and chronic effects of the PAHs on marine organisms, e. g. environmental toxicological study, should be performed in subsequent studies in order to fully understand the deleterious effects of PAHs on marine organisms or risk assessments.

And further, it's very important to understand the behavior and fate of PAHs in order to set up a model about the diagenesis of PAHs once introduced into marine environment.