Journal of Science, Technology, and Humanities : 2010, Vol. 8, No. 1, pp. 13-23

ACCUMULATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN GREEN MUSSELS (*Perna viridis*) COLLECTED FROM THE EAST COAST OF CHON BURI PROVINCE, THAILAND.

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ABSTRACT

The accumulation of polycyclic aromatic hydrocarbons (PAHs) in marine sediments and green mussels (Perna viridis) collected from the east coast of Chon Buri province, Thailand between March and July 2007 was investigated. The concentration levels of PAHs in sediment samples ranged from 0.07 to 0.28 μg/g dry weights in the dry season and 0.03 to 0.18 μg/g dry weights in the wet season. The highest concentration of PAHs in sediment was found in both seasons at Laem Thoa Thewa station, while those in suspended particulate matter in both seasons were low at all stations in both seasons. The bioaccumulation of PAHs in mussels was from 2.33 to 3.06 μg/g dry weights in the dry season and 2.49 to 3.15 μg/g dry weights in the wet season without significant differences between stations and seasons. The results from this study suggest that PAHs contamination in the marine environment during the two seasons can contribute to humans risk from the consumption of mussels contaminated with low PAHs concentration.

Keywords: Green mussel, bioaccumulation, polycyclic aromatic hydrocarbons, PAHs.

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) can be formed from incomplete burning of organic materials including coal, oil, gasoline, and garbage. These chemicals are introduced into the environment as persistent compounds and one potent carcinogen in animals, especially Benzo (a) pyrene, (BaP) (Noji et al., 1999). Recent investigation has reported the status of petroleum hydrocarbons in the Gulf of Thailand such as in the sediment and in marine food chains of economic areas along the east coast with significant indications of industrial pollution (Pollution Center Department, 1999; Suwanagosoom, 2001). PAHs and BaP were detected in seawater, sediments, and marine organisms. Jacob and his colleagues (Jacob et al., 1986) suggested that the strong lipophilic properties and low solubility of PAHs compounds enhance their adsorption onto the surface of particulate substrates such as silica, calcareous materials, and clay, as well as organic particles, which play roles in transport, and removal of these chemicals in the environment. PAHs adsorbed on organic and inorganic particulates are deposited onto bottom sediments and some are readily accumulated by aquatic organisms in food chains reaching toxic levels in that environment. The toxic effects of concern depend on metabolic pathways in the organisms regarding their roles in accumulation, elimination, and partitioning of the compounds. PAHs contamination, both on land and in aquatic environment, has the potential to affect the immune complex related conditions in aquatic organisms.

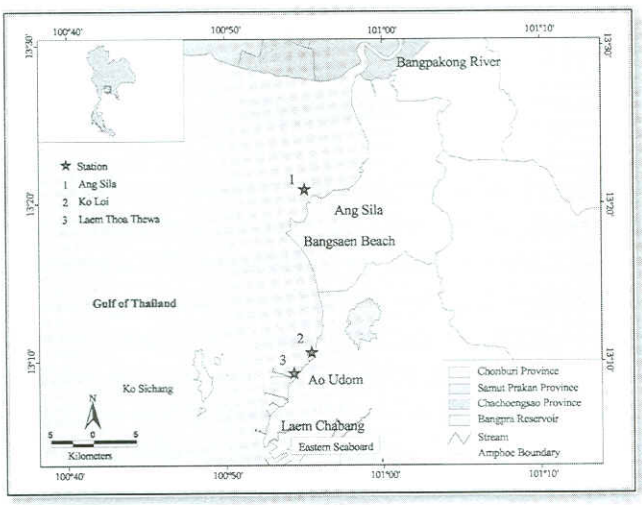

Figure 1. A map shows location of sampling stations from the east coast of Chon Buri province, Thailand. Sampling sites include Ang Sila (1), Ko Loi Sri Racha (2) and Laem Thoa Thewa (3).

MATERIALS AND METHODS

Samples

Sediment, suspended particulate matter, and mussels were collected from three stations in the dry season from March-April 2007, and during the wet season from July-August 2007 along the coast of Chon Buri province. Results are summarized, as shown in Figure 1. Sediment samples were collected by using a Petersen Grab sampler at the same stations and seawater samples were also collected. The sediment samples were taken at 5-7 cm depth from the surface by using a stainless steel spoon. Suspended particulate matter (SM) was sampled in situ by pumping the water at a depth of about two meters or mid depth by following the method of Fernandes et al. (1997). SM was isolated by filtration of around 50 liters through pre-combusted GF/C Whatman glass fiber filters (0.7 μm pore size). Each of the filters was wrapped in aluminum foil and stored in a refrigerator maintained approximately at-20°C. Mussels of 4-7 cm shell length were grown on ropes at a depth of 3-8 meter were also collected from the study area.

Analytical procedures

After collection, sediment samples and SM were dried in a freeze dryer and kept in an amber glass bottle with Teflon line cap and stored in an ice box before being transferred to the laboratory. All sediment samples were analyzed for grain size composition and organic matter content (OM) following the method of the Institute of Marine Science (2001). The samples were analyzed by using the Soxhlet Extraction method modified by Gfrerer et al. (2002), Hwang and Curtright (2004), and Suwanagosoom (2001). The samples were homogenized and ground in a mortar and sieved to particle size smaller than 63 μm. Each 3 g of samples with 250 μl (or around 2.5 ppm) of surrogate standard were transferred into a pre-extracted extraction thimble. The samples were then extracted with 250 ml dichloromethane solvent for 24 hours. In addition, the empty thimbles were extracted in parallel to provide blank values. The extracts were concentrated in a rotary evaporator to remove sulfur by adding copper chips into the solutions. The concentrated solutions were cleaned up by using a silica gel column. The resulting volumes were then reduced to less than 5 ml by using a rotary evaporator. The extracts were finally adjusted to 5 ml with n-hexane for liquid chromatography (Merck, purity ≥ 96 %). The mussel tissue samples from each station were prepared in three pooled replicates comprising of 20 organisms. The tissue samples were homogenized in a high-speed homogenizer and dried in a freezer dryer. The samples of 2 g each were extracted and saponified with 300 ml dichloromethane and 1 N KOH in methanol (90:210, v/v) for 24 hours in Soxhlet Extraction sets. The extracted solutions were washed once with 50 ml hexane-washed water and extracted three times with 50 ml redistilled hexane. The combined extracts were then evaporated to 5 ml, and were cleaned up by using silica gel and a florisil column. Combined solutions were reduced following the procedure for sediment analysis by GC/MSD. Statistical significance was determined via two factor ANOVAs.

RESULTS

PAHs levels in marine sediment

The concentrations of total PAHs in surface sediment from three sampling stations were analyzed in the sum of sixteen PAHs (∑16 PAHs), namely: Naphthalene (NAP), Acenaphthylene (ACY), Acenaphthene (ACE), Fluorene (FLU), Phenanthrene (PHE), Anthracene (ANT), Fluoranthene (FTH), 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), Dibenz (a,h) anthracene (DahA), and Benzo (g,h,i) perylene (BghiP). In the dry season, the concentration of seven PAHs with carcinogenic potential was found in the range of 0.01-0.09 μg/g. The averaged concentrations at Ang Sila, Ko Loi, and Laem Thoa Thewa stations were 0.03±0.01, 0.03±0.02, and 0.07±0.01 μg/g, respectively. The concentrations of combined sixteen PAHs ranged from 0.07-0.28 μg/g. The averaged concentrations at Ang Sila, Ko Loi, and Laem Thoa Thewa stations were 0.10±0.02, 0.18±0.03, and 0.22±0.03 μg/g, respectively. In the wet season, the concentrations of seven PAHs ranged from 0.01-0.05 μg/g, with the averaged concentrations at Ang Sila, Ko Loi, and Laem Thoa Thewa stations of 0.02±0.00, 0.01±0.00, and 0.032±0.01 μg/g, respectively. Sixteen PAHs ranged from 0.030-0.183 μg/g resulting in averaged concentrations at Ang Sila, Ko Loi, and Laem Thoa Thewa stations of 0.10±0.01, 0.04±0.01, and 0.13±0.04 μg/g, respectively. There were significant differences of the concentrations ($p<0.05$) between different stations and different seasons, whereas no significant differences were observed with the combination of station and season. The PAHS concentration found at Laem Thoa Thewa station was higher than those found at the other stations, while lower concentrations were found at Ko Loi and Ang Sila stations as presented in Figure 2 and Table 1.

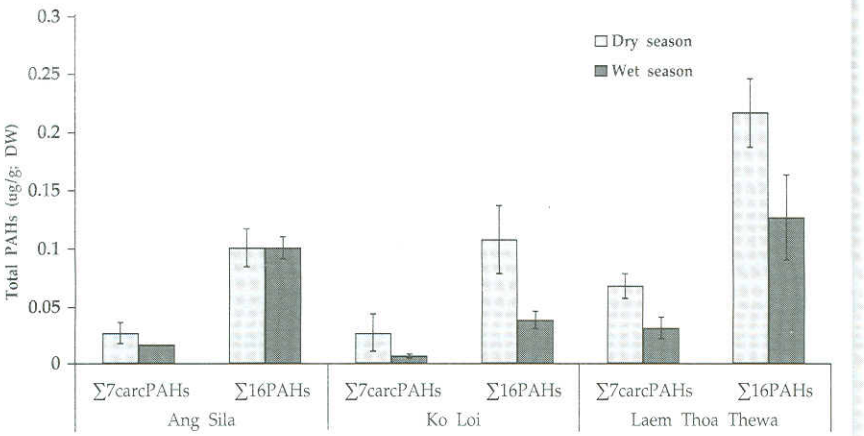

Figure 2. Accumulation of PAHs in marine sediments at different stations in dry season (□) and in wet seasons (■). ∑7 carcinogenic PAHs: chrysene, benzo[a]anthracene,benzo[k] fluoranthen, benzo[b]fluoranthene, benzo[a]pyrene, indeno(123-cd]pyrene, and dibenzo [ah] anthracene.

Table 1. The concentration of total PAHs in sediment at different stations in dry and wet seasons.

Stations	PAHs (μg/g, DW)		
	Dry season	Wet season	Mean
Ang Sila	0.101±0.017	0.101±0.010	0.101±0.000
Ko Loi	0.108±0.030	0.039±0.008	0.073±0.028
Laem Thoa Thewa	0.217±0.030	0.127±0.037	0.172±0.037 *
Mean	0.142±0.026 *	0.089±0.018 *	0.115±0.021

Note: * Statistical significantly difference at $p<0.05$.

The comparison of data at Ang Sila and Ko Loi stations showed no differences, whereas data at Laem Thoa Thewa station were significantly different ($p<0.05$) as compared to the other stations.

In addition, the sediment samples from three sampling stations were analyzed for the grain size as shown in Figure 3, and organic matter. The data were expressed as mean of the grain size and the organic matter showed significant differences ($p<0.01$) with stations and seasons. Table 2 is a summary of four groups of the organic matter in the sediment samples collected from the three stations.

Table 2. The percentage in concentrations of the organic matter (OM) in sediments at three stations.

Stations	OM (%)		
	Dry season	Wet season	Mean
Ang Sila	6.68±0.000	5.72±0.220	3.194±2.062 **
Ko Loi	2.68±0.650	1.52±0.080	2.100±0.474 **
Laem Thoa Thewa	2.48±0.200	2.41±0.080	2.445±0.029 **
Mean	3.947±0.283 **	3.217±0.127 **	2.580±0.855

Note: * Statistical significantly difference at $p<0.05$, ** Statistical significantly difference at $p<0.001$.

The level of organic matter were significantly different ($p<0.01$) with stations and seasons. The highest organic matter content was found at Ang Sila station in the dry season, while the lowest content was found at Ko Loi station in the wet season. There was no difference in organic matter content at Ko Loi and Laem Thoa Thewa stations in the dry season.

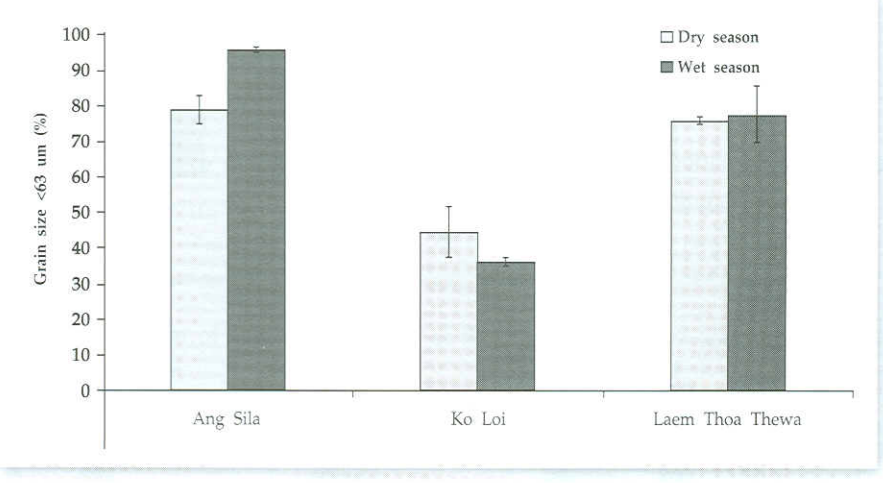

Figure 3. The grain size (< 63μm) of sediments at three stations in dry (□) and wet seasons (■).

The data showed that organic matter content in sediment from Laem Thoa Thewa station in both seasons were the same as those from Ko Loi station in the dry season. However, there were concentration differences in sediment from Ang Sila and Ko Loi stations in the wet season (see Figure 4). The sediments from Ang Sila, Ko Loi, and Laem Thoa Thewa stations with the grain size less than 63 μm. were found having an averaged percentage of organic matter of 95.71±0.74, 36.31±1.23, and 77.70±7.99, respectively. The concentration of PAHs in sediment samples in this study displayed no correlation with organic matter content. The concentrations of PAHs in suspended particulate matter

from the three sampling stations are presented in Figure 5. In the dry season, the concentration of seven carcinogenic PAHs were undetectable to 0.270 μg/g showing the averaged concentrations at Ang Sila, Ko Loi, and Laem Thoa Thewa stations from undetectable limit to 0.023±0.023 and 0.090±0.090 μg/g, respectively. The averaged concentrations of sixteen PAHs at Ang Sila, Ko Loi, and Laem Thoa Thewa stations were 0.236±0.236, 0.154±0.103, and 0.090±0.090 μg/g, respectively. In the wet season, the concentrations of seven carcinogenic PAHs were undetectable (non-detectable, nd) to 0.655μg/g, with the averaged concentrations at Ang Sila, Ko Loi, and Laem Thoa Thewa stations of 0.218±0.218, 0.019±0.019, and nd μg/g, respectively. The total sixteen PAHs concentration was from nd to 0.655 μg/g with averaged concentrations at Ang Sila, Ko Loi, and Laem Thoa Thewa stations of 0.218±0.218, 0.216±0.138, and 0.146±0.034 μg/g, respectively. Total PAHs suggested no significant differences ($p<0.05$) in concentrations observed between different stations and seasons.

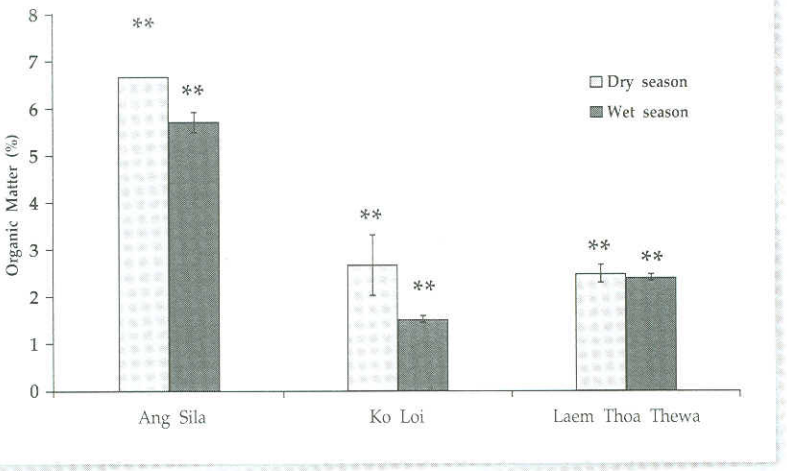

Figure 4. Organic matter content in sediments at three stations in dry (□) and wet seasons (■), ** statistical significantly difference at p<0.01.

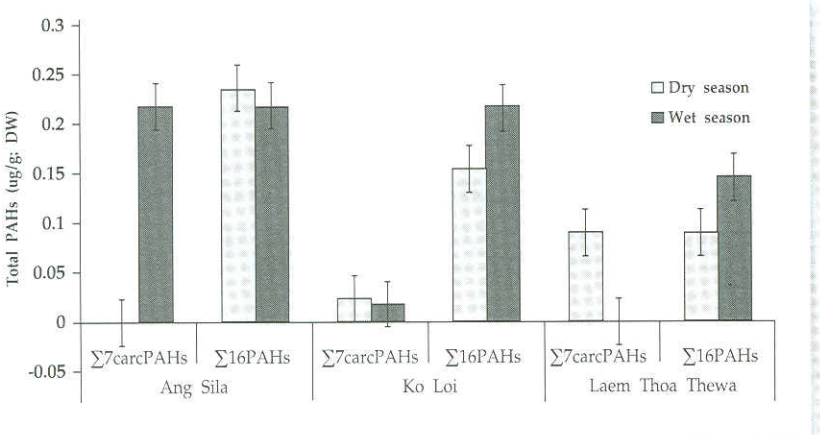

Figure 5. Concentration of PAHs in marine suspended particulate matters (SM) at three stations in dry (□) and wet seasons (■).

PAHs levels in marine organisms

The range of seven carcinogenic PAHs content in mussels was 1.285-1.336 μg/g, with the averaged concentrations at Ang Sila, Ko Loi, and Laem Thoa Thewa stations found to be 1.312±0.013, 1.317±0.016, and 1.320±0.014 μg/g, respectively. The total sixteen PAHs concentration was found in the range of 2.332-3.055 μg/g with averaged concentrations at Ang Sila, Ko Loi, and Laem Thoa Thewa stations of 2.947±0.016, 3.016±0.020, and 2.795±0.232 μg/g, respectively. However, the concentrations of the seven carcinogenic PAHs and total PAHs in the wet season were in the range of 1.229-1.393 and 2.490-3.145 μg/g, respectively. The averaged concentrations of the seven carcinogenic PAHs and total PAHs at Ang Sila, Ko Loi, and Laem Thoa Thewa station are shown in Table 3. PAHs showed no significant differences ($p<0.05$) in concentration among different stations and seasons (see Figure 6 and Table 3).

In addition, the results on the analysis of the organic matter and lipid contents in the mussel samples from the three stations are presented in Figures 7 and 8. The mean values of organic matter content in mussels were significantly different ($p<0.05$) between the seasons. The concentration of PAHs in mussel samples in this study was not correlated with respective organic matter content. However, the lipid content in mussel samples was found to be significantly different ($p<0.05$) among stations and seasons as presented in Table 5. In addition, the organic matter content in mussel samples studied was not correlated with the lipid content.

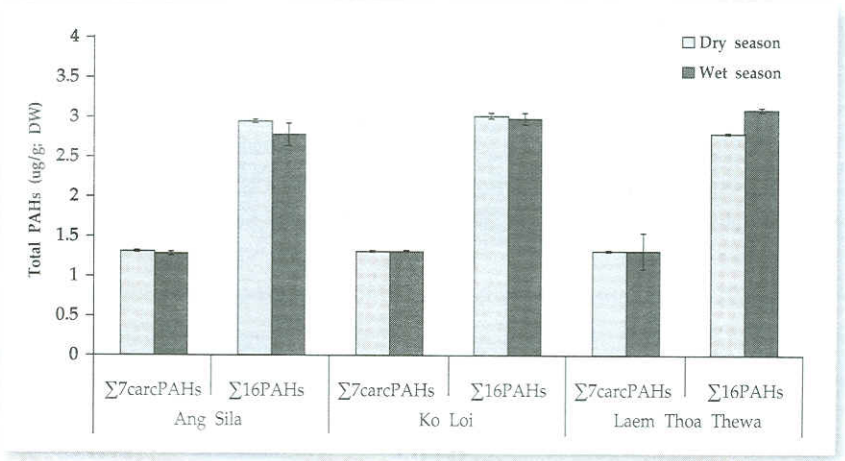

Figure 6. Concentration of PAHs in mussels at three stations in dry (□) and wet seasons (■).

Table 3. The concentration of PAHs in mussels at three stations in dry and wet seasons.

Stations	PAHs (μg/g, DW)		
	Dry season	Wet season	Mean
Ang Sila	2.947±0.016	2.783±0.147	2.865±0.067
Ko Loi	3.016±0.020	2.980±0.079	2.998±0.015
Laem Thoa Thewa	2.795±0.232	3.089±0.030	2.942±0.120
Mean	2.919±0.089	2.951±0.085	2.935±0.067

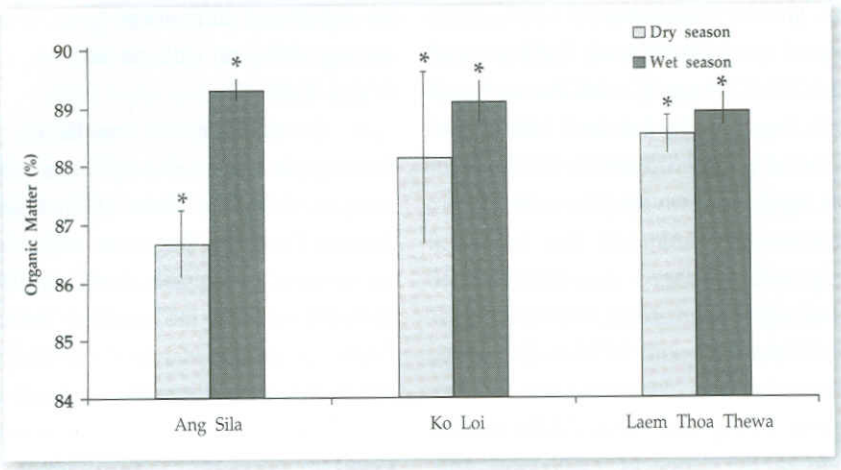

Figure 7. Organic matter content in mussels at three stations in dry (□) and wet seasons (■) was significantly different (* = $p<0.05$) only between seasons.

Table 4. Concentration of organic matter in mussels at three stations in dry and wet seasons.

Stations	OM (%)		
	Dry season	Wet season	Mean
Ang Sila	86.68±0.55	89.32±0.19	88.00±1.08
Ko Loi	88.16±1.48	89.10±0.36	88.63±0.38
Laem Thoa Thewa	88.54±0.33	88.93±0.24	88.74±0.16
Mean	87.79±0.79*	89.12±0.26*	88.46±0.54

Note: * Statistical significantly difference at $p<0.05$.

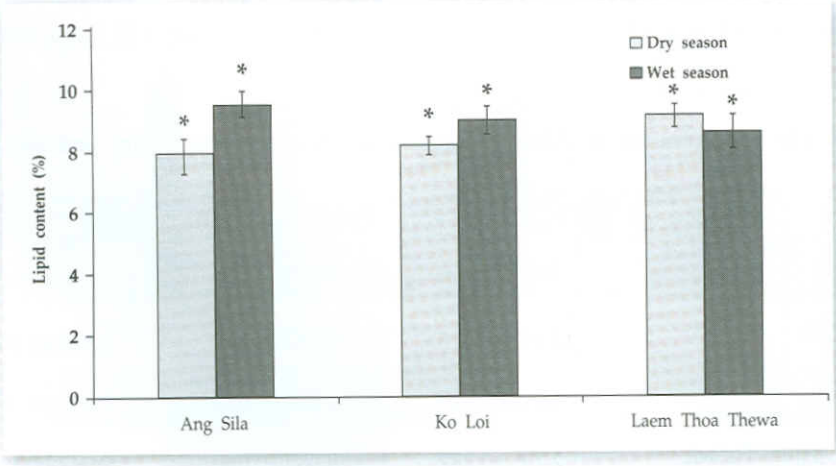

Figure 8. Lipid content in mussels at the three stations in dry (□) and wet seasons (■) was statistical significantly difference at $p<0.05$.

Table 5. Variability of lipid content in mussels at the three stations in dry and wet seasons.

Stations	Lipid (%)		
	Dry season	Wet season	Mean
Ang Sila	7.96±0.48	9.54±0.45	8.750±0.65
Ko Loi	8.20±0.26	8.98±0.48	8.590±0.32
Laem Thoa Thewa	9.17±0.34	8.60±0.54	8.885±0.23
Mean	8.443±0.36*	9.040±0.49*	8.742±0.40

Note: * Statistical significantly difference at $p<0.05$.

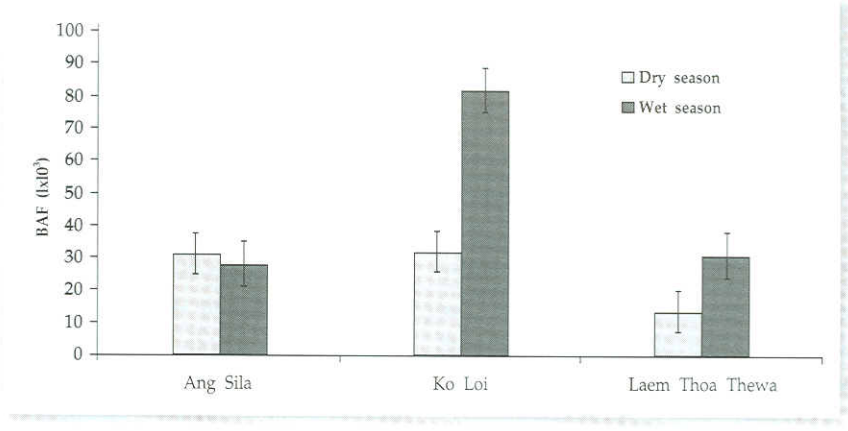

Figure 9. Bioaccumulation Factor (BAF) in the mussels at the three stations.

Bioaccumulation factor (BAF)

The Bioaccumulation Factor (BAF) was calculated as the ratio between the average total PAHs concentration in whole tissue of mussels and the average concentration of PAHs in sediments in the two seasons, as shown in Figure 9. The range of BAF in the dry season was 13.55-31.82, whereas in the wet season was 27.83-81.67. The highest BAF value was found in the wet season at the Ko Loi. The BAF values between the PAHs in mussels and suspended particulate were not calculated because of very low PAHs concentrations.

DISCUSSION

The highest concentration of seven PAHs in sediment from three stations, both in dry and wet seasons, was found in the sediments at the Laem Thoa Thewa station which is situated near oil refineries adjacent to the Ao Udom market and Laem Chabang port. These sites have been used in heavy water transportation, fishing piers, and loading or unloading of oil products. Wastes associated with such anthropogenic activities may have increased the inputs of contaminants, including PAHs. The accumulation of PAHs obviously decreased from the Ko Loi station and Ang Sila stations. Because both sampling stations were located on the north side of Ko Loi which has been used as a pier, this could also contribute to PAHs contamination in the wet season in contrast to the dry season, whereas the water circulation in the Gulf of Thailand is clockwise in the dry season (January-June) and counterclockwise in the wet season (July-October) according to the south west monsoon (Suwanagosoom, 2001). Therefore,

the average total PAHs concentrations at Ang Sila and Ko Loi stations were not significantly different, whereas the values at Laem Thoa Thewa were significantly different during the two seasons. It was possible that the sediments in the area were stirred and re-suspended due to the winds and currents during the wet season. Consequently, the PAHs concentrations found during the dry season were high and similar to those reported by Sunwanich (1991) of higher concentration of PAHs in March than in August from the mouth of the Tha Chin River. From sediment analysis, even though the amount of sediment less than 63 μm was high at Ang Sila station, this did not reflect high adsorption of PAHs on sedimentary organic matters. The PAHs contamination on surface sediments in the coastal area of Thailand has been attributed to oil spills and oil refineries (Boonyatumanond et al. 2006) studies in the coastal environment of the upper Gulf of Thailand have found that total PAHs concentration in the sediments was in the range of 0.08-2.0 μg/g dry weight. For example, Duangkaew (2003) studied the accumulation of PAHs in sediments from the east coast of Thailand and found that the concentration range was from 0.24-1.15 ng/g dry weights depending on activities at each location such as a fishing pier. A study by Terdteppitak (1999) suggested that the accumulation of petroleum hydrocarbons in surface sediments from the eastern coastal waters were high, especially at Sriracha, as compared to those at Ang Sila, Bangsaen, Laem Chabang, and Pattaya. The mentioned station located on the west part of Ko Loi station is near Laem Thoa Thewa station. Chumchuchan et al. (1998) reported higher petroleum hydrocarbons contamination in coastal environment of the Upper Gulf of Thailand to be in the range of 0.08 to 2.0 μg/g dry weight from stations near Sattahip, which might have resulted from different methods of analysis used. The highest values of PAHs concentration detected at Laem Thoa Thewa in both seasons were probably due to contamination from related domestic and industrial activities in the area, especially in the adjacent Laem Chabang port. The increase in concentration of PAHs causes high accumulation in the environment particularly in the marine food chain. As there is a lack of data on PAHs accumulation in these marine environments, the comparison on the impacts of these chemicals is still limited.

PAHs accumulation in suspended particulate matter from the three stations both in dry and wet seasons were found to be low in carcinogens and total PAHs, except total PAHs in a dry season at Ang Sila station. However, this study showed lower concentrations of PAHs at the three stations as compared to those at other polluted areas. The composition of particulate matter can vary with the amount and types of planktonic sediments and the organic content which can reflect the concentration of PAHs sources. The accumulation of total petroleum hydrocarbons in planktonic particulate was found high in samples from Phe Bay, Rayong province that is near the Mab Taput industrial area. The high concentrations of total particulate PAHs found in the dry season at Ang Sila station might be brought down from the Bangpakong River, which has many fishing activities. As there have been no extensive available data on the particulate PAHs in the coastal areas, so that it is limited information to present an overview of the environmental contamination due to the accumulation of PAHs in the study area.

PAHs accumulations in mussels from this study were similar to those studied in green mussels (*P. viridis*) from the east coast of Thailand by Duangkaew (2003) and Suwanagosoom (2001) at Phe Bay, Rayong province. Sunwanich (1991) also found the same patterns of PAHs accumulations in mussels from the mouth of the Tha Chin River. PAHs enter the coastal marine environment from three general categories, namely, pyrogenic, petrogenic (or petroleum) and natural diagenesis (Zakaria and Azril, 2006). The sediments might not be the only source of PAHs accumulated in mussels, as suspended PAHs distributed in the water column could also be absorbed through filter feeding by the organisms. Nevertheless, the PAHs content in mussels showed no significant difference ($p<0.05$) among stations and seasons, even though Ang Sila station is far from industrial area as compared to Ko Loi and Laem Thoa Thewa stations. This may

suggest various possible sources of PAHs contamination in mussels. Domestic and household wastewater could increase the inputs of contaminants, including PAHs. However, there are limited information on PAHs contamination in coastal marine environment in Thailand.

This study suggested possible risk due to high BAF in the wet season at Ko Loi station similar to the Suwannagosoom (2001) reporting the risk of PAHs contamination at the Phe Bay, Rayong province. Therefore, consumers should avoid the consumption of contaminated mussels from area at the highest risk.

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