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PAH-biodegradation potential of indigenous microorganisms: evidence from the respiratory activity of surface sediments in the Quanzhou Bay in China

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Abstract
Seven stations were established in the Quanzhou Bay (24.73°–24.96°N, 118.50°–118.70°E) in China on three cruises to determine the concentrations of polycyclic aromatic hydrocarbons (PAHs) and the numbers of PAH-degrading bacteria in surface sediments. Assessing the biodegradation potential of indigenous microorganisms by measuring the respiratory intensity with the addition of PAHs in sediment samples was also one of the aims of this study. The results show that the total PAH concentrations of the sediments were 99.23–345.53 ng/g dry weight (d.w.), and the PAHs composition pattern in the sediments was dominated by phenanthrene, fluoranthene and pyrene. The numbers of phenanthrene, fluoranthene and pyrene-degrading bacteria during three cruises were 1.42×10^3–8.93×10^4 CFU/g d.w., 8.29×10^3–9.43×10^4 CFU/g d.w. and 7.05×10^3–$9.43\times^4$ CFU/g d.w., respectively. The addition of three model PAH compounds (phenanthrene, fluoranthene and pyrene) showed a great influence on the increasing of the microbial activity in the sediments. And there was a significant correlation among the change of respiratory activity, PAH concentration and the number of PAH-degrading bacteria. The change in respiratory activity under PAHs selective pressure could, to a certain extent, indicate the potential degradative activity of the PAH-degrading microbial community.

Key words: biodegradative potential, polycyclic aromatic hydrocarbons (PAHs), Quanzhou Bay in China, respiratory activity, sediment

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous, persistent environmental contaminants with high-mutagenicity and carcinogenicity, which are generated from natural combustion processes and human activities (Luan et al., 2006; Yu et al., 2005). Anthropogenic inputs of PAHs from oil spills, ship traffic, urban runoff, wastewater and industrial discharge, as well as atmospheric fallout of vehicle exhaust and industrial stack emissions, have caused significant accumulation of PAHs in the marine environment (Simpson et al., 1996). Possible fates for PAHs released into the environment include volatilization, photo-oxidation, chemical oxidation, bioaccumulation, adsorption on soil particles, leaching, and microbial degradation (Cerniglia, 1992). Environmental bacteria are generally considered to be the most important organisms in the natural biodegradation of polycyclic aromatic hydrocarbons (Bastiaens et al., 2000; Hughes et al., 1997; Simoneit and Mazurek, 1981).

PAHs are hydrophobic and readily adsorbed onto particulate matter, thus, coastal and marine sediments become the ultimate sinks for PAHs (Hughes et al., 1997; Kim et al., 2004). PAHs present in sediments

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may exhibit a toxic activity towards different plants, microorganisms and invertebrates. Microorganisms, being in intimate contact with the sediment environment, are considered to be the best indicators of sediment pollution. In general, they are very sensitive to low concentrations of contaminants and rapidly respond to sediment perturbation (Zheng et al., 2005). An alteration of their activity and diversity may reflect in a reduced sediment quality (Schloter et al., 2003; Maskaoui et al., 2005). Soil or sediment respiration mostly refers to the course of CO_2 release during microbial metabolism, and changes in its intensity represent one of the important indexes reflecting microbial activity (Zhu et al., 1999). The change in the degree of respiratory intensity could reflect the potential degrading activity of the environmental samples towards pollutants and so provide scientific evidence for in situ remediation of the polluted environment.

The Quanzhou Bay (24.73°–24.96°N, 118.50°–118.70°E), a semi-enclosed bay, is located in the southeast of Fujian Province, China, being the entrance to the sea of the Jinjiang River and Luoyang River westwards and abutting the Taiwan Strait eastwards. The Quanzhou Bay has an area of 136.42 km^2 and its depth ranges from 7 to 25 m. The line of Xiutu to Shihu is the dividing line of the inside and outside of the Quanzhou Bay. The Quanzhou Bay, the starting point of maritime Silk Road in China, was exploited long before. The rapid development of industry, agriculture and aquaculture has resulted in great environmental stress to the Quanzhou Bay (Yuan and Xie, 2003). Pollution in the Quanzhou Bay has been mostly rooted in industrial, agricultural, domestic and aquicultural sewage, terrestrial pollutants carried by the Jinjiang River, the Luoyang River and oil sewage emitted by shipping inside the bay. Now the Quanzhou Bay has become rather more severely polluted sea area in the shore of Fujian Province and poisonous persistent organic pollutants (POPs) greatly influenced the fishery and aquiculture of the Quanzhou Bay (Wang et al., 2006). However, in recent years, there were few researches on POPs in the Quanzhou Bay (Gong et al., 2007; Wang et al.,2006), and no data have been available on PAHs in this area.

In this present study, we investigated the distribution characteristics of PAHs in the sediments of the Quanzhou Bay and applied the idea of respiratory activity to marine sediments firstly. The respiratory intensity changes in response to the addition of PAHs to the surface sediments were determined, combining the detection of PAH concentrations and the biodiversity of PAH-degrading bacteria, to evaluate the biodegradation potential of the indigenous microorganisms in the surface sediments, so as to provide scientific evidence for the control of pollution by PAHs in offshore sea areas.

2 Materials and methods

2.1 *Study area and sampling*

Sediment samples were collected from seven stations in the Quanzhou Bay on 13 September 2003 (level period), 28 February 2004 (dry period) and 9 July 2004 (wet period) (Fig. 1). Station S_1 was near the Jinjiang River entrance dock, located at the entrance to the bay of the Jinjiang River, the longest river into the bay. Station S_2 was near the Luoyang River entrance dock, located at the entrance to the bay of the Luoyang River. Station S_3 was near the Xiutu Harbor. Station S_4 was located outside the Quanzhou Bay. Station S_5 was located in Shihu coastwise. Station S_6 was located on the outer Xiangzhi Dock. And Station S_7 was located in Shuitou Town coastwise.

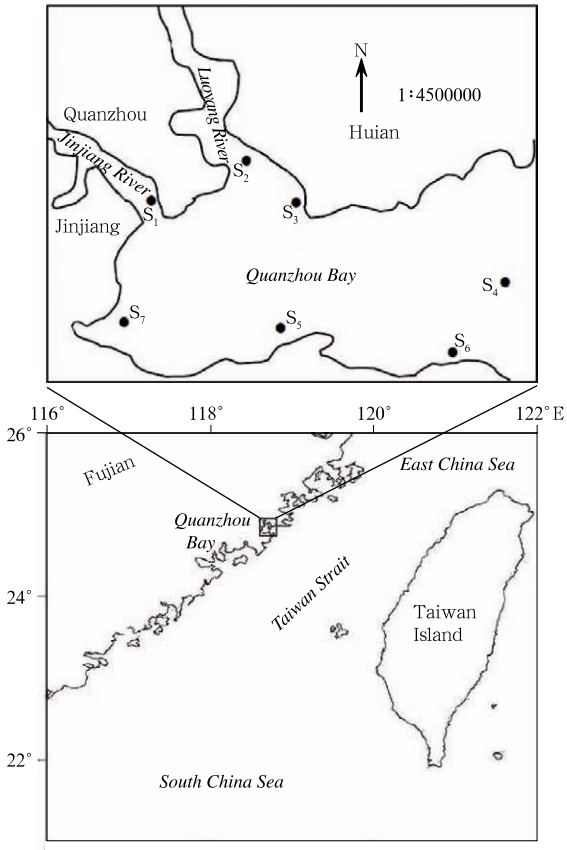

Fig.1. Location of sampling stations in the Quanzhou Bay in Fujian Province, China.

Surface sediments (0–5 cm) were gathered with a stainless steel bottom sampler (QNC6, China), and then transferred to plastic zipper bags. The determination of the respiratory activity, total bacteria and PAH-degrading bacteria in the sediments were carried out within 24 h of collection. Other samples were kept frozen for further analysis.

2.2 *PAHs analysis*

Freeze-dried sediment samples were filtered through an 80–mesh screen-sieve, and then 20.0 g samples were transferred to 250 ml pre-washed conical flasks, adding 1 g activated copper powder and 20 μl internal standard prior to extraction (Tam et al., 2001). The mixtures were then shaken with 100 ml dichloromethane and methanol (volume ratio Being 2:1), and sonicated in an ultrasonic bath (50 °C) for 20 min. A second extraction was conducted with 100 ml dichloromethane and methanol (volume ratio Being 1:2). The two extracts were mixed, water added (volume ratio Being 1:0.45) and then they were extracted with separatory funnels. The lower liquid was collected and concentrated by rotary evaporation and under a gentle stream of high-pure nitrogen to 1 ml. The solvent extracts were fractionated using a silica gel/Alumina column (1 cm in diameter and 20 cm long), PAHs were eluted with 10 ml dichloromethane and hexane (volume ratio Being 2:1), and the eluted liquid was concentrated using high-purity nitrogen at 30 °C, to about 100 μl.

A suite of 16 USEPA (United States Environmental Protection Agency) priority PAH compounds were identified and quantified according to the method by Tian et al. (2008).

2.3 *Microbiological analysis*

A 10 g sediment sample was shaken (150 r/min) with 90 ml sterile seawater for 3 h in a 250 ml conical flask and then settled for 30 min. The supernatant was used in the following experiments.

The DAPI direct count method was used to estimate the number of total bacteria (Velji and Albright, 1993; Su et al., 2007) and the spray-plate technique with modification was used to count culturable PAH-degrading bacteria. The composition of the mineral salt medium (MSM) was as follows: $(NH_4)_2SO_4$ (1000 mg/L), Na_2HPO_4 (800 mg/L), KH_2PO_4 (200 mg/L), $MgSO_4$ (200 mg/L), $FeCl_3{\cdot}6H_2O$ (5 mg/L), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (1 mg/L), $CaCl_2{\cdot}H_2O$ (100 mg/L) and 15 g agar. The medium pH was 7.0–7.2. Each solid medium plate was incubated at 25 °C over night prior to inoculation. Stock PAH solutions were prepared at a concentration of 0.5 mg/ml acetone, and acetone solutions (0.5 ml) of the three model PAH compounds (phenanthrene, fluoranthene and pyrene) were added to the agar plates. 0.1 ml of the sediment sample supernatant or its dilution was added to the PAH layer after acetone volatilization. After incubation at 25 °C for three weeks in the dark, the numbers of PAH-degrading bacteria were determined by colony forming units (KäStationer et al., 1994; Tian et al., 2002).

2.4 *Measurement of respiratory activity*

Production of CO_2 was used to measure microbial activity in sediments. Sediment respiration was determined using the method described by Vokou and Liotiri (1999) and Zhou et al. (1996). Sediment samples, treated with a single model PAH compound (phenanthrene, pyrene and fluoranthene, at a final concentration of 50×10^{-6}), mixture of the three PAHs, glucose (5 mg/g sediment) and an untreated sediment sample as control, were placed in 100 ml conical flasks. A 10 ml vial with 5 ml 0.1 mol/dm^3 NaOH, to absorb the CO_2 released, was hung above each sample in an airproof culture system, and the experiment was conducted at 25 °C. The NaOH was titrated with 0.1 mol/dm^3 HCl after 3, 5, 7, 9, 12, 15 and 19 d, and respiratory intension was denoted by the release velocity [V_r,mg/(Kg·d)] of CO_2 according to

$$V_r = (V_1 - V_2)C \times 44 \times 1000/2mBT, \quad (1)$$

where V_1 is the volume of hydrochloric acid used to titrate the residual NaOH of the blank control (L); V_2 is the volume of hydrochloric acid used to titrate the residual NaOH of the sample (L); C is the concentration (mol/dm^3) of hydrochloric acid; m is the mass (kg) of the sample; B is the ratio of dry to wet mass of the sample; and T is the incubation time (d).

3 Results and discussion

3.1 *PAH concentrations in surface sediments of Quanzhou Bay*

The total PAH concentrations of the sediments (d.w.) from the Quanzhou Bay were 99.23–345.53, 117.93–281.60, 151.28–324.02 ng/g respectively for the three cruises (Table 1). The dominant PAH compounds were phenanthrene (three-ring PAH), fluoranthene and pyrene (four-ring PAH), accounted for 25.47%–60.89% of total PAHs.

Table 1. Concentrations (ng/g) of model PAHs, Phenanthrene (Phe), Fluoranthene (Flu), Pyrene (Pyr) and total PAHs in surface sediments of the Quanzhou Bay and PAH's toxicity guidelines in sediments(Cd.w.)

Station	Sampling time	Phenanthrene	Fluoranthene	Pyrene	Total PAHs
S1	Sep.	16.97	10.20	10.08	99.23
	Feb.	10.02	4.19	34.46	182.28
	Jul.	25.53	19.79	18.43	169.45
S2	Sep.	72.92	50.38	51.08	345.53
	Feb.	22.86	19.84	18.32	117.93
	Jul.	25.61	25.92	22.40	283.24
S3	Sep.	31.25	27.88	26.79	331.11
	Feb.	32.40	32.77	28.57	197.42
	Jul.	23.84	16.61	15.09	208.63
S4	Sep.	23.59	24.33	20.30	189.81
	Feb.	44.89	20.78	27.22	157.80
	Jul.	21.68	12.61	9.86	151.28
S5	Sep.	43.49	19.35	24.53	150.83
	Feb.	46.22	41.57	36.94	238.44
	Jul.	22.17	24.39	14.17	163.90
S6	Sep.	22.80	20.41	17.06	207.56
	Feb.	34.31	49.15	37.38	281.60
	Jul.	79.92	54.28	63.09	324.02
S7	Sep.	26.44	17.57	17.01	185.66
	Feb.	27.02	21.14	18.60	262.13
	July.	21.90	16.57	15.35	189.38
ER-L①		240	600	660	4022
ER-M①		1500	5100	2600	44792

Notes: ① Cited from Long et al. (1995), ER-L, effects range-low; ER-M, effects range-median.

PAH concentrations in the sediments from the Quanzhou Bay were near those values of the sediments in the Shenzhen Bay (Connell et al., 1998), the Zhujiang Estuary (Luo et al., 2006; Chen et al., 2006) and the South China Sea (Yang, 2000), and lower than those in surface sediments of other harbours and gulfs around the world such as the Xiamen West Harbour (Tian et al., 2002), the Zhelin Bay (Dong et al., 2007), the Kaohsiung Harbor (Lee et al., 2005), the Richmond Harbour (Pereira et al., 1996) and the Santander Bay (Woodhead et al., 1999). These concentrations in the Quanzhou Bay were unlikely to cause any adverse biological effects as they were below the effects range-low values (Long et al., 1995). However, some high molecular mass PAH compounds such as benzo(b)fluoranthene, dibenz(a, h)anthracene, indeno(1, 2, 3-cd)-pyrene and benzo(ghi)-perylene were detected (data not shown) but their toxicity guidelines were not available, so this might need a more detailed study.

The sources of PAHs, whether from fuel-combustion (pyrolytic) or from crude oil (petrogenic) contamination, may be identified by ratios of individual PAH compounds such as phenanthrene/anthracene (Phe/Ant) and fluoranthene/pyrene (Flu/Pyr) (Sicre et al., 1987; Budzinski et al., 1997; Soclo et al., 2000; Tam et al., 2001; Yunker et al., 2002; Fang et al., 2007). Soclo et al. (2000) suggested that sediments with Phe/Ant greater than 10 were mainly contaminated by petrogenic inputs and Phe/Ant less than 10 was typical of pyrolytic sources. Sicre et al. (1987) suggested that a Flu/Pyr ratio of less than 1 was attributed to petrogenic sources and values greater than 1 were obviously related to a pyrolytic origin. In this study, the Phe/Ant and Flu/Pyr ratios belonged to different zones (Fig. 2), which indicated that surface

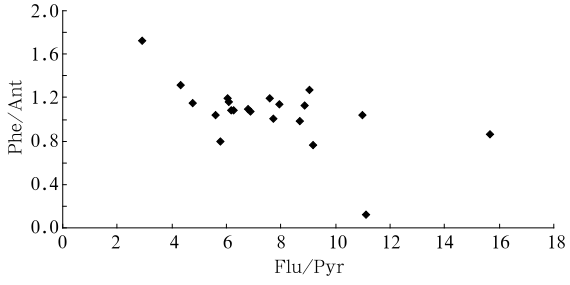

Fig.2. Ratios of phenanthrene/anthracene (Phe/Ant) and fluoranthene/pyrene(Flu/Pyr) in surface sediments from the Quanzhou Bay.

sediments of the Quanzhou Bay were characteristic of a mixture pattern of pyrolytic and petrogenic contamination.

3.2 *PAH-degrading bacteria in surface sediments*

The numbers of PAH-degrading bacteria in sediments from the Quanzhou Bay in three cruises are shown in Table 2. In September, the numbers of phenanthrene-degrading bacteria, fluoranthene-degrading bacteria and pyrene-degrading bacteria were 1.42×10^3–8.93×10^4, 8.29×10^3–5.47×10^4 and 7.55×10^3–7.55×10^4 CFU/g, respectively. In February, the numbers of phenanthrene, fluoranthene and pyrene-degrading bacteria were 8.24×10^3–4.83×10^4, 1.73×10^4–9.43×10^4 and 1.87×10^4–3.46×10^4 CFU/g. In July, their numbers were 1.92×10^3–8.29×10^4, 3.41×10^4–8.93×10^4 and 7.05×10^3–9.43×10^4 CFU/g. The mean number of phenanthrene-degrading bacteria did not change with seasons. While those of fluoranthene and pyrene-degrading bacteria during the July cruise were higher than the other two cruises.

Table 2. Numbers of culturable PAH-degrading bacteria and total bacteria in surface sediments of the Quanzhou Bay

Station	Sampling time	Phenanthrene-degrading bacteria/(CFU·g^{-1})	Fluoranthene-degrading bacteria /(CFU·g^{-1})	Pyrene-degrading bacteria/(CFU·g^{-1})	Total bacterial number/(cell·g^{-1})
S1	Sep.	4.11×10^4	2.12×10^4	2.12×10^4	3.63×10^{10}
	Feb.	8.24×10^3	3.53×10^4	1.87×10^4	2.17×10^{10}
7	Jul.	1.92×10^3	3.41×10^4	3.73×10^4	1.19×10^{11}
S2	Sep.	8.93×10^4	5.47×10^4	7.55×10^4	6.53×10^{10}
	Feb.	3.65×10^4	1.73×10^4	3.09×10^4	6.20×10^{10}
	Jul.	6.09×10^4	3.61×10^4	5.47×10^4	6.69×10^{11}
S3	Sep.	4.64×10^4	1.47×10^4	3.36×10^4	1.13×10^{11}
	Feb.	3.91×10^4	2.33×10^4	2.41×10^4	4.54×10^{10}
	Jul.	1.03×10^4	4.01×10^4	2.55×10^4	3.54×10^{11}
S4	Sep.	2.02×10^3	2.31×10^4	7.55×10^3	5.43×10^{11}
	Feb.	4.33×10^4	2.01×10^4	2.81×10^4	1.63×10^{11}
	Jul.	2.64×10^4	8.24×10^4	7.05×10^3	8.90×10^{11}
S5	Sep.	1.22×10^3	8.29×10^3	2.02×10^4	1.01×10^{11}
	Feb.	4.83×10^4	2.53×10^4	3.20×10^4	1.89×10^{11}
	Jul.	5.02×10^3	3.83×10^4	2.36×10^4	5.44×10^{11}
S6	Sep.	5.39×10^4	4.01×10^4	2.94×104	$1.0^4\times10^{11}$
	Feb.	3.63×10^4	9.43×10^4	3.46×10^4	1.78×10^{11}
	Jul.	8.29×10^4	8.93×10^4	9.43×10^4	4.29×10^{11}
S7	Sep.	1.42×10^3	1.13×10^4	1.20×10^4	1.20×10^{11}
	Feb.	2.82×10^4	2.94×10^4	2.43×10^4	1.67×10^{11}
	Jul.	3.11×10^4	4.23×10^4	8.09×10^3	4.55×10^{11}

The number of PAH-degrading bacteria also showed a definite positive relationship with the corresponding component of PAHs in the sediments. The correlation coefficients for phenanthrene, pyrene and fluoranthene were 0.557, 0.470 and 0.480 (Table 3). This suggests that the abundance of PAH-degrading bacteria might reflect the degree of PAHs pollution.

3.3 *Effect of adding PAHs on microbial activity in surface sediments*

Microbial activity in soil has been measured traditionally using the rates of soil metabolic processes, since microorganisms make up most of the soil biomass (Huang et al., 1999). Some of the metabolic processes used to measure microbial activity in soil are the production of CO_2 and the consumption of O_2 (Regno et al., 1998). In this study, the production of CO_2 was used to measure microbial activity in surface sediments treated with PAHs and glucose of high concentrations, in order to determine the response and PAH-biodegradation potential of microorganisms in surface sediments of the Quanzhou Bay.

The trends of the respiration intensity after the addition of PAHs during the three cruises and among different stations were generally consistent. The result of September cruise was discussed for example. In Fig. 3, the ratio of respiratory intensity between the sediment samples with additives (glucose, phenanthrene, pyrene, fluoranthene and mixed PAHs) and the untreated ones (Y-axis) was used to denote the influence

Table 3. Correlation between the change of respiratory intensity with the addition of PAHs, the number of PAH-degrading bacteria and PAH concentrations in surface sediments from the Quanzhou Bay(n=21)

Item	Phenanthrene		Pyrene		Fluoranthene	
	r	P	r	P	r	P
Relationship between the change of respiratory intensity and the number of PAH-degrading bacteria	0.466	*	0.372	**	0.269	**
Relationship between the change of respiratory intensity and PAH concentration	0.778	*	0.818	*	0.724	*
Relationship between PAH concentration and the number of PAH-degrading bacteria	0.557	*	0.470	*	0.480	*

Notes: r is Spearman's rank correlation coefficient; *P <0.01; **P <0.05.

of additives on the respiration of the sediment samples. The effect of additives on respiration intensity in sediments of the three cruises all generally represented the order of glucose (easily used carbon source), mixed PAHs and single PAH from high to low, which might result from complex structures, low biological availability of PAHs and their cometabolism by the microorganisms. After the addition of PAHs, the respiration of the sediment samples (except those of Sta. S_2) was restrained to a different extent during the first three days (Fig. 3). Then, the stimulation on the sediment respiration by PAHs appeared and reached their peaks during 7th–12th day. The higher respiratory stimulation by PAH was found mostly at Sta. S_2 and Sta. S_6, which also had a higher proportion of PAH-degrading bacteria (Table 2). The respiratory intensity of Sta. S_6 samples with mixed PAHs exceeded 90% of that of the control samples while in Sta. S_2 samples it was doubled on their peaks. Subsequently, the stimulation of PAHs on the sediments gradually weakened. Those results suggest that the microorganisms in sediments have high bioactivity, the capability of adapting to an unfavourable environment, and a high potential to degrade PAHs.

3.4 *Correlation analysis of PAH concentrations, PAH-degrading bacteria and respiratory activity in the sediments*

There were significantly correlation between PAH's stimulation on respiration, the concentration of PAH and the number of PAH-degrading bacteria (Table 3). The higher the PAH concentration in the sediments, the more PAH-degrading bacteria were present, and the stronger the stimulation of respiration in sediments by PAH. Bacteria are the principal microorganisms in the oceanic environment, and they dominate the microbial abundance and respiratory activity of the oceanic sediments. Especially in a severely polluted environment, bacteria play a significant role in the degradation of pollutants (Huang et al., 1999; Tamar and Yosef, 2006).

The addition of PAHs provided abundant carbon sources for PAH-degrading bacteria in the sediments, which gradually became the dominant bacteria with inducement and enrichment by PAHs. The PAH-degrading bacteria in the sediments could more rapidly adapt to external pollution (PAHs) by alteration of the microbial metabolic pathway, resulting in a change of the microbial community structure, and could effectively utilize and degrade PAHs, showing their strong potential to degrade PAHs. These could be primely proved by the significant correlation between the variation of respiratory intensity with the addition of PAHs to the sediment and the number of PAH-degrading bacteria.

4 Conclusions

Total PAHs concentrations in surface sediments in the Quanzhou Bay were less than those in other harbours and gulfs. However, the microorganisms in the sediments could respond to the addition of PAHs and degrade them rapidly, and there was significant correlation between PAH's stimulation on respiration, the concentration of PAH and the number of PAH-degrading bacteria. Our results show that microbial community of the sediments in the Quanzhou Bay had a powerful capability of adapting to an unfavourable environment and the potential to degrade PAHs, which could provide scientific evidences for future studies on pollutant bioremediation in the Quanzhou Bay.

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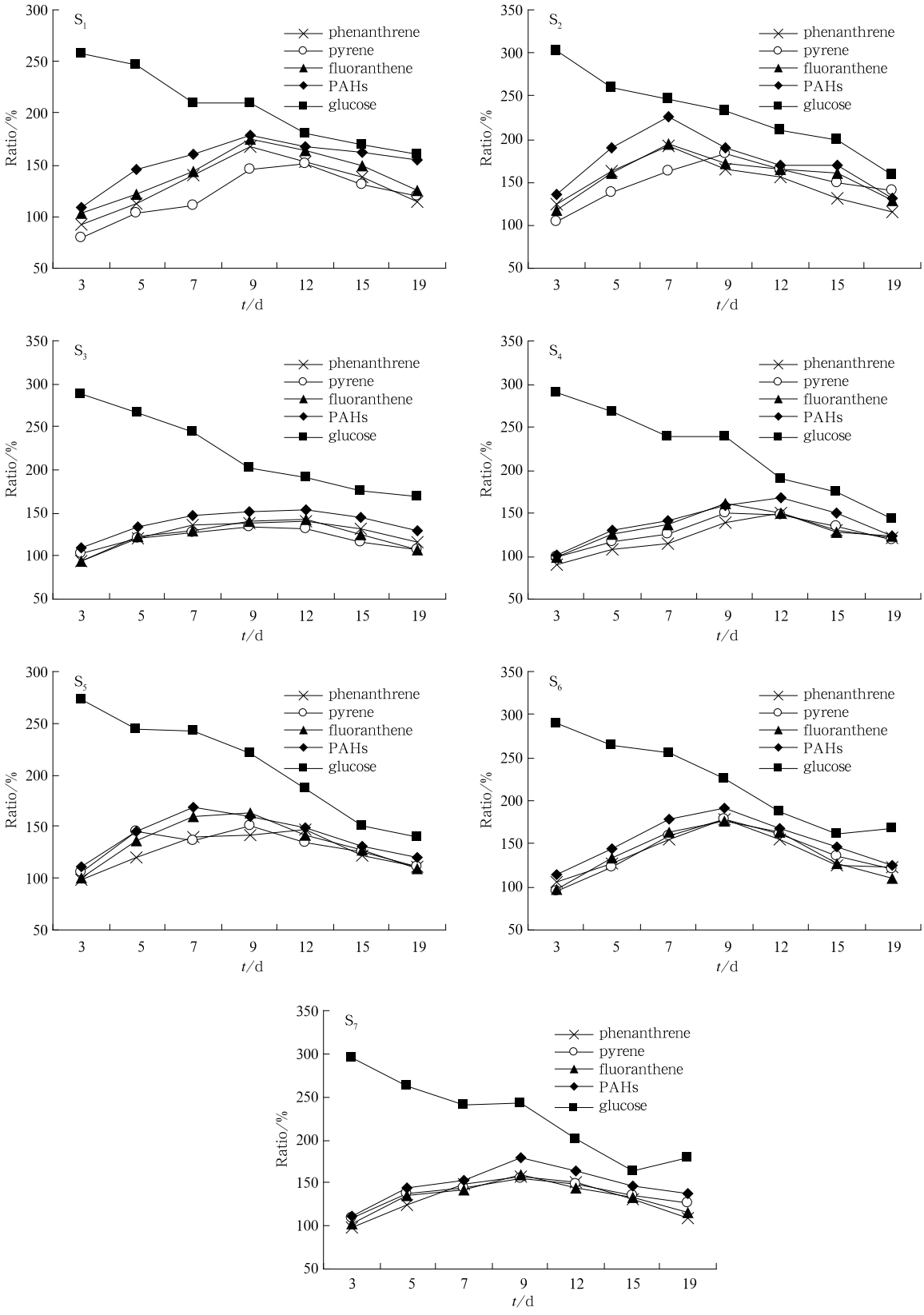

Fig.3. Effect of adding PAHs on the respiratory activity of surface sediments from the Quanzhou Bay (Sept. cruise).

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