

## **Variation in polycyclic aromatic hydrocarbons in sediments of shrimp farms**

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### **ABSTRACT**

Studies were conducted to find the variations of polycyclic aromatic hydrocarbons (PAH) in sediments of shrimp farms. Evaluation of their concentrations in different cropping seasons were studied in scientifically cultured ponds in comparison with that in a natural pond. The total PAH concentration was found to be higher in the scientific system in all cropping seasons. The levels of individual low molecular weight PAHs, i.e. from naphthalene to phenanthrene showed greater values during the juvenile stages that gradually got reduced at the harvest stage. Benzo[a]anthracene was the prominent component among the high molecular weight PAHs which also showed a maximum value during the juvenile stages of cropping. A slight increase in its value was observed in the harvest stage than late juvenile stages in scientific pond, which may probably be due to the addition of excess nutrients. Benzo[b]fluranthene was nondetectable in natural system, while it showed its presence in scientific system. Correlation data revealed that there is a strong positive correlation between organic matter and the levels of total PAHs, whereas negative correlation with clay which implies the minimum capacity of adsorption of PAH by clays.

### **Introduction**

Aquaculture industry is a major export earner of which shrimp aquaculture contributes a major part. In recent years pollution in aquaculture fields is of great concern. One of the major organic pollutants, which have been ubiquitous, is polycyclic aromatic hydrocarbon (PAH). Polycyclic aromatic compounds are fused compounds built on benzene rings. Fusion imparts chemical properties between those of benzene and those of olefinic hydrocarbons. The lowest among PAH is naphthalene, whereas the ultimate member is graphite, an allotropic form of car-

bon. The majority of environmental PAH range between naphthalene and coronene ( $C_{24}H_{12}$ ). Physical and chemical properties of PAH vary approximately in a regular trend with molecular weight. Susceptibility to redox reactions increases with increasing molecular weight, whereas aqueous solubility and vapour pressure decreases almost logarithmically with increasing molecular weight (Neff, 1979). Thus PAH differ in their environmental behaviour and interactions with biological systems. Some of the PAHs are well known carcinogens and mutagens.

The sources of polycyclic aromatic hydrocarbons in marine environments are assumed to be anthropogenic combustion (pyrolysis) and production, transport and use of oil and its refined products (petrogenic) (Woodhead *et al.*, 1999). They reach the marine environment *via* effluent discharges, urban runoff, atmospheric transport and the spillage or disposal of oil and petroleum products. The Cochin backwater area has many industries including oil refineries and also jetties for commercial and oil tanker ships. There is heavy mixing of water between the Cochin estuary and the Arabian Sea during tides (0.5 to 2m high). Cochin estuary provides water to the numerous aquaculture systems located in Vypeen Island. Large number of studies on PAH was conducted under marine environments and the data on aquaculture environment is scarce. The study was aimed for a preliminary evaluation of the concentration of PAH in sediments of shrimp farming system and its variation in different stages of culture.

### Materials and methods

Three aquaculture farms were selected from Varapuzha village of Vypeen Island of Kerala, since the island has maximum traditional prawn farming fields called Pokkali fields. Among these, two ponds (S1 and S2) followed scientific methods of culture and the third followed purely natural cropping (N1). In natural system of cropping, sluice gate valves were opened during high tide and the prawns entering the pond were allowed to grow. Here water exchange takes place with tidal variation. In scientific culture, seeds (*Penaeus monodon*) were purchased from outside and confined to grow in the fields with supplementary feeding and other required inputs. Water exchange was carried out every day between 0 and 4.00 hrs. The total cropping

season was 90-100 days (January-April) and after harvest the fields were used for traditional rice culture called pokkali.

*Penaeus monodon* seeds were introduced in the selected fields (S1 and S2) on 17 th January 1999 and the sediment samples were collected from the above aquaculture system in three growing stages, *viz.*, juvenile (01-02-1999), late juvenile (01-03-1999) and harvest stages (29-03-1999). Early harvest was carried out due to labour unrest. Sediments were collected from 7-10 different points of the farm, pooled, thoroughly mixed and divided into four portions out of which one portion was selected for the study. The samples were stored in polythene bags, transported immediately to the laboratory and kept under cold storage till the analysis was carried out. Water was drained by vacuum filtration (Upto 10-20% moisture) and finally dried under shade. The sediment (25gm) and 50ml dichloromethane were taken in a conical flask and shaken for 1hr. The filtrate was collected and the same procedure repeated for 3 to 4 times (Lizia Guzella and Paolis, 1994). The extracts were washed with water and the organic layer passed over sodium sulphate, evaporated to dryness and redissolved in 3ml n-hexane. The extract was purified in chromatographic columns packed with activated alumina and silicagel (neutral) and eluted with 25ml hexane initially and then with 1:1 hexane dichloromethane mixture. The extract was evaporated to dryness by flash evaporator and redissolved in HPLC grade acetonitrile. The quantification of PAH was carried out using Hewlett Packard 1090 HPLC fitted with EMerck PAH column, eluted with 100% acetonitrile. The maximum elution time was 30 minutes. The samples were analysed for sixteen PAH constituents *viz.* naphthalene, acenaphthylene, acenaphthene, fluorene, phen-

anthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo(ghi)perylene and indeno (1,2,3-cd)pyrene. All the standards were purchased from Supelco, USA and recovery studies on sediments were conducted which was 95%.

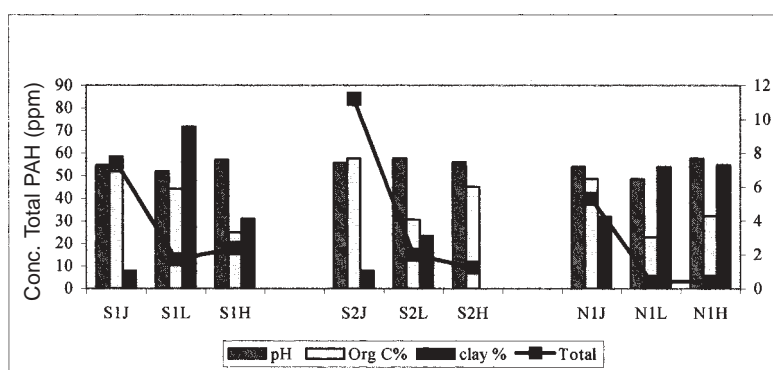
Organic carbon content was determined as per Wilkley and Black method and pH by Jackson (1973). Mechanical characteristics of sediments were analysed by pipette method (Day, 1965). Statistical and correlation analysis was done using excel available with MS Office software.

**Results and discussion**

Total PAH is the sum of the concentration of PAH constituents that are natural and anthropogenic origin, although the latter predominates. (Law and Biscaya, 1994). The total PAH in sediment was higher in S1 and S2 (55.90 and 84.01 µg/g) compared to N1 (39.72 µg/g) in the juvenile stage (Fig. 1). Overall in the scientific system the total PAH was reduced considerably in the late juvenile and harvest stages. The total PAH decreased significantly in N1 during late juvenile and at harvest (3.15µg/g each).

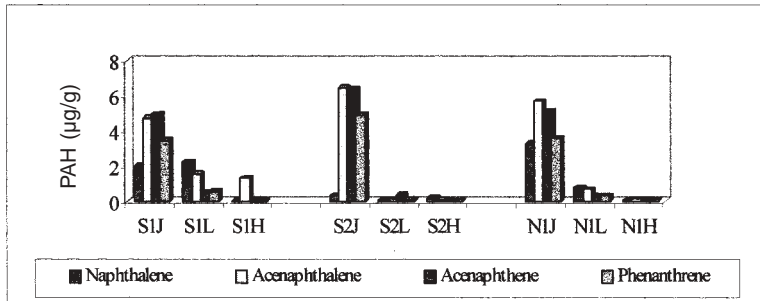
Pond S2 was having red tides during the initial days and recovered after few days. PAH concentration in sediments as low as 41.2 and high as 181 µg/gm dry weight have been associated with carcinogenic effects in brown bull head catfish from lake Manuscong and Cuyahoga River respectively (Baumann *et al.*, 1991). Literature on total PAH in sediments highlights its influence in aquatic organisms like growth reduction of larval surf smelt (Misitano *et al.*, 1994) and liver disease in english sole (*Pleuronectus vetulus*) (Malins *et al.*, 1984).

The lowest member among PAHs, naphthalene (Fig. 2) was 2.03, 0.30 and 3.25 µg/g in S1, S2 and N1 respectively at the juvenile stage. In the late juvenile stage there was a significant reduction of naphthalene in S2(0.035µg/g) and N1(0.78µg/g) compared to the juvenile stages, whereas in S1 there was a slight increase. At harvest, the concentrations were 0, 0.17 and 0.40 µg/g respectively in S1, S2 and N1. Similarly 4.73, 6.44 and 5.68 µg/g of acenaphthalene was recorded respectively in S1, S2 and N1 in the juvenile stages and it was reduced to 1.31, 0.11 and 0.14 µg/g during harvest stages. Acenaphthene concentration was 4.95, 6.38 and 5.08 µg/g in S1, S2 and N1 respectively during juvenile stages which



J - JUVENILE, L - LATE JUVENILE, H - HARVEST  
 Fig. 1. Sediment characteristics with total PAH

reduced gradually and was nondetectable at the harvest stage in all the ponds. Phenanthrene also showed a similar trend. The low molecular weight PAHs are non-persistent, relatively less toxic to the aquatic organism and easily volatilised due to the in-

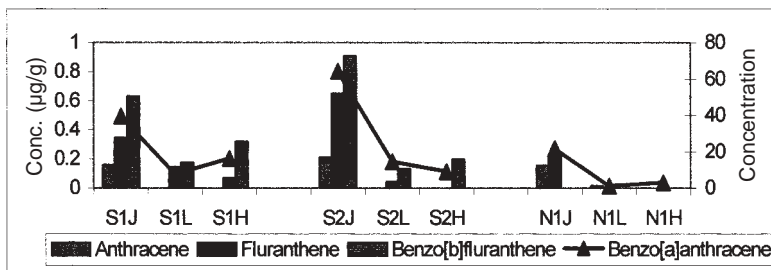


J - JUVENILE, L - LATE JUVENILE, H - HARVEST

Fig. 2. Low molecular weight PAHs in µg/g

creased activity of microorganisms and plankton in the bottom fauna.

Anthracene was detected in all the samples at the juvenile stages, which recorded 0.16, 0.21 and 0.16 µg/g in S1, S2 and N1 respectively, but was not detected in the later stages (Fig. 3). In the scientific system, fluranthene level was 0.35 (S1) and 0.65 µg/g (S2) initially and reduced to non-detectable levels at harvest stage. Fluranthene was 0.22 µg/g in the natural system initially and was not detected in other stages. The results reveal that increased farming activity, water exchange and addition of nutrients have profound influence in the faster degradation of anthracene in scientific pond, whereas undisturbed environment helped quicker degradation of fluranthene in natural pond.



J - JUVENILE, L - LATE JUVENILE, H - HARVEST

Fig. 3. High molecular PAHs in µg/g (B[a]A is in secondary axis)

Among the high molecular weight PAHs Benzo[a]anthracene was present in highest concentrations which varied from 21.74- 64.19 µg/g during juvenile stage (Fig. 3). There was a significant reduction in Benzo[a]anthracene of about 77 to 80%

in the scientifically cultured pond and 95% in natural pond during late juvenile stage. At the harvest stage there was a significant enhancement of benzo[a]anthracene in S1(112%) and N1(189%), except in S2 where a 62% decrease was noted compared to late juvenile stage. According to the US sediment quality criteria, 1600/55000 ng/g of benzo[a]anthracene is toxic. Klammer *et al.* (1990) reported the Benzo[a]anthracene concentration in sediments of oyster grounds of North Sea, Netherland. This high molecular weight PAH constituents are not acutely toxic but have been proven carcinogenic (Woodhead *et al.*, 1999) and also influences the disruption of endocrine ability (Tran *et al.*, 1996). Benzo[a]anthracene is a major PAH constituent seen in prawn aquaculture systems in all the stages of cropping and

probably it is mainly derived from fossil fuel combustion and hydrocarbon pollution. This is not surprising in the case of Varapuzha as large number of heavy industries are situated on the banks of Periyar

River. Benzo[b]fluranthene, another member of the PAH in the scientific ponds ranged from 0.63-0.91  $\mu\text{g/g}$  initially and reduced to 0.13 to 0.18  $\mu\text{g/g}$  in the late juvenile stages. However, a slight increase was seen at the harvest stage (0.20- 0.32  $\mu\text{g/g}$ ). It is to be highlighted that benzo[b]fluranthene was absent in N1 in all the stages. The sources of the PAH determined in this study may be assumed to represent both pyrolytic and petrogenic. The degradation of these products is enhanced due to the intensive biological activity prevailing in the ponds by way of microbial and chemical influence.

Negative correlation exist between naphthalene and all other PAH constituents during juvenile stage (Table 1). The pH and organic carbon were positively correlated with all PAH constituents except acenaphthalene and negatively correlated with naphthalene. This further emphasizes the distribution of naphthalene in water, where naphthalene is the major PAH constituent. There is a significant negative correlation of clay with Benzo[a]anthracene and Benzo[b]fluranthene. In the late juvenile stage, pH was positively correlated with benzo[a]anthracene (Table 2). The organic carbon content was positively correlated with acenaphthene, fluranthene and bezo[b]fluranthine during late juvenile stage. Clay content negatively correlated with benzo[a]anthracene and benzo[b]fluranthene in the juvenile stages and in the late juvenile stages it positively correlated with acenaphthene, fluranthene and benzo[b]fluranthene. At harvest, clay was in perfect negative correlation with acenaphthalene, fluranthene, benzo[a]anthracene and benzo[b]fluranthene (Table 3). In all the three stages clay content positively correlated with naphthalene, which implies that low molecular weight PAH are easily adsorbed over

TABLE 1. Correlation between PAH and sediment characteristics at juvenile stage

	Naphthalene	Acenaphthalene	Acenaphthene	Phenanthrene	Anthracene	Fluranthene	Benzo(a)anthracene	Benzo(b)fluranthene	pH	Org C%	Clay %	Total
Naphthalene	1											
Acenaphthalene	-0.52806	1										
Acenaphthene	-0.87196	0.87620	1									
Phenanthrene	-0.86610	0.88184	0.99992	1								
Anthracene	-0.92902	0.80480	0.99122	0.98959	1							
Fluranthene	-0.99149	0.63410	0.92826	0.92380	0.96928	1						
Benzo(a)anthracene	-0.99998	0.52283	0.86893	0.86301	0.92672	0.99067	1					
Benzo(b)fluranthene	-0.95051	0.23809	0.67670	0.66794	0.76809	0.90198	0.95240	1				
pH	-0.99999	0.52721	0.87146	0.86560	0.92865	0.99136	0.99998	0.95082	1			
Org C%	-0.99917	0.56207	0.89109	0.88566	0.94326	0.99595	0.99890	0.93713	0.99913	1		
Clay %	0.81514	0.06146	-0.42718	-0.41645	-0.54294	-0.73280	-0.81869	-0.95477	-0.81572	-0.79097	1	
Total	-0.99834	0.57602	0.89867	0.89342	0.94876	0.99733	0.99797	0.93107	0.99828	0.99985	-0.78048	1

TABLE 2. Correlation between PAH and sediment characteristics at late juvenile stage

	Naphthalene	Acenaphthalene	Acenaphthalene	Phenanthrene	Anthracene	Fluranthene	Benzolanthracene	Benzofluranthene	pH	Org C%	Clay %	Total
Naphthalene	1											
Acenaphthalene	0.99369	1										
Acenaphthalene	0.82812	0.76006	1									
Phenanthrene	0.96949	0.99086	0.66545	1								
Anthracene	-0.18117	-0.06978	-0.70131	0.06541	1							
Fluranthene	0.80734	0.73610	0.99934	0.63807	-0.72659	1						
Benzolanthracene	-0.34150	-0.44471	0.24404	-0.56146	-0.86245	0.27890	1					
Benzofluranthene	0.41209	0.30735	0.85200	0.17618	-0.97072	0.87034	0.71563	1				
pH	-0.46081	-0.55740	0.11587	-0.66429	-0.78931	0.15166	0.99150	0.61873	1			
Org C%	0.75491	0.67664	0.99278	0.57113	-0.78174	0.99646	0.35858	0.90863	0.23415	1		
Clay %	0.94435	0.97527	0.59765	0.99617	0.15239	0.56831	-0.63166	0.08945	-0.72710	0.49718	1	
Total	0.00208	-0.11003	0.56227	-0.24309	-0.98382	0.59176	0.93916	0.91199	0.88653	0.65738	-0.32697	1

TABLE 3. Correlation between PAH and sediment characteristics at harvest stage

	Naphthalene	Acenaphthalene	Acenaphthalene	Phenanthrene	Anthracene	Fluranthene	Benzolanthracene	Benzofluranthene	pH	Org C%	Clay %	Total
Naphthalene	1											
Acenaphthalene	-0.70105	1										
Acenaphthalene	0	0	1									
Phenanthrene	0	0	0	1								
Anthracene	0	0	0	0	1							
Fluranthene	-0.68312	0.99969	0	0	0	1						
Benzolanthracene	-0.27751	0.87965	0	0	0	0	1					
Benzofluranthene	0.89119	1										
Benzofluranthene	-0.09555	0.77683	0	0	0	0.79223	0.98284	1				
pH	-0.81652	0.16075	0	0	0	0.13618	-0.32802	-0.49663	1			
Org C%	0.98971	-0.79583	0	0	0	-0.78054	-0.41206	-0.23694	-0.72556	1		
Clay %	1	-1	0	0	0	-1	-1	-1	1	1		
Total	-0.30714	0.89396	0	0	0	0.90482	0.99952	0.97665	-0.29860	-0.44009	-1	1

clays. pH has tremendous influence on the adsorption of high molecular weight PAHs and it is evident in late juvenile stage where the soil pH was between 6.48-7.69, only benzo[a]anthracene positively correlated. The results further emphasize that most of the PAH constituents are derived from organic fraction of the sediments and naphthalene is distributed over clays.

Total organic carbon and grain size has an important role in sediment PAH distribution (Evans *et al.* 1990). PAHs are relatively insoluble and adsorbed over suspended solids and dissolved organic matter (NRCC, 1983; Mackay *et al.*, 1992; Law *et al.* 1997). It is certain that a variable proportion of sediment associated PAH is available for uptake by both demersal fish (Hellou *et al.*, 1995) and benthic invertebrates (Forbes *et al.*, 1996). Organic matter concentration is the major factor for determining the proportion of PAH availability from sediments (De Witt *et al.*, 1992). The organic matter in turn controls the percentage which is strongly adsorbed and therefore less available for uptake *via* the interstitial waters. The strong adsorption of high molecular weight PAH (Mackay *et al.*, 1992) implies that these are more strongly bioaccumulated by selective deposit feeders than by organisms that live merely in the sediment, but feed on the

overlying detritus or plankton (Meador *et al.*, 1995).

Total PAH showed significant positive correlation with pH and organic carbon content and negative correlation with clay during juvenile stages (Fig. 1). There was a significant positive correlation of PAH and organic carbon content in the late juvenile stages and perfect negative correlation with clay was observed during harvest stage. There was a profound negative influence on the adsorption of PAH in clay content, while organic matter enhances the adsorption of PAH.

Correlation ratios of various PAH compounds of the same molecular mass (isomers) have been used to identify potential sources to many studies (Klamer and Fomsguard, 1993). The ratio between the phenanthrene vs anthracene varied from 21.61 to 23.27 in the juvenile stages, and in the natural system recorded 24.69 at the juvenile stage and at the harvest it was reduced to zero (Table 4). The ratio (phenanthrene + anthracene + fluranthene) / (benzo[a]anthracene + benzo[b]fluranthene) was 0.097 and 0.089 in S1 and S2 respectively and that of N1 was 0.182 at the juvenile stages. There was a decrease in the ratio in the late juvenile stage in S1 and S2 respectively as 0.08 and 0.007, whereas it was doubled in N1 (0.325). At harvest, the ratio almost reduced to zero in S2 and N1 and

TABLE 4. Correlation ratios of various PAH compounds

Ratios	Juvenile			Late juvenile			Harvest		
	S1	S2	N1	S1	S2	N1	S1	S2	N1
Phenanthrene/ Anthracene	21.610	23.044	23.273	0	24.692	0	0	0	0
(Phenanthrene +Anthracene+ Fluoranthene) /(Benzo[a] anth +Benzo[b]fluo ranthene)	0.0979	0.182	0.089	0.085	0.325	0.007	0.004	0	0

in S1 it was 0.004. The Cochin backwater area has many industries including oil refineries and also jetties for commercial and oil tanker ships. Moreover, the tidal amplitude of 1 to 2 meters from bar mouth to downstream of Cochin estuary may also influence the distribution of petrogenic and pyrolytic pollutants in the water channels of Vypeen islands.

The increased shrimp farming activity has influenced faster degradation of polycyclic aromatic hydrocarbons under aquaculture environments. Benzo[a]anthracene was the major PAH constituent seen in all the ponds at all stages of cropping. pH, clay and organic matter has prominent role in retention and distribution of PAH constituents in the environment. The correlation studies have revealed that clays have minimum capacity to adsorb high molecular weight PAHs but encourage the retention of naphthalene.

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