POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENT AND AMERICAN OYSTER FROM MARSH AND ESTUARINE ECOSYSTEM IN SAVANNAH, GA, USA

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Abstract

Sediment and oyster tissue collected from nine different marshes/estuarine ecosystem in Savannah GA USA, were analyzed for 16 predominant polycyclic aromatic hydrocarbons (PAHs). Total concentrations of PAHs were 7.5-48 (sediment) and 4.0-264 (oyster) on ng/g dw. There was no significant ($P<0.5$) contamination variation in target analytes in between the nine estuarine sites. Observed PAHs were lower than ERL-ERM guidelines. Overall, this baseline data can be used for regular ecological monitoring, considering the domestic and industrial growth around this important marsh/estuarine ecosystem.

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of common environmental contaminants. They originate from anthropogenic sources such as waste incineration, coal gasification, accidental oil spills, as well as natural processes like fossil fuel and wood combustion[1,2]. Due to their hydrophobicity, low water solubility, and vapor pressures, PAHs tend to accumulate in sediment and various organic components [1,3]. Sediment concentrations of total PAHs vary depending on the location, and range from a few parts-per-billion to several parts-per-million. Occurrence of PAHs in the environment is of great concern due to their carcinogenic properties, and ability to exert toxic effects through the aryl hydrocarbon receptor (AhR) mediated mechanism, similar to those of dioxins[4].

PAHs can be present in high concentrations (parts-per-million) in the coastal marine environment[5]. They can affect the productivity of marine organisms, and can ultimately be hazardous to human health. The Savannah River estuary is typical of many estuaries within the South Atlantic Bight. The port of Savannah located in this estuary handles kaolin, coal, ferrous minerals, fuel oil, and raw and processed chemicals. Many industries have developed around the port including a paper factory, and fertilizer and chemical manufacturing companies[6]. Additionally, the Savannah River estuary receives domestic waste water and other contaminants from several industries, and mosquito control operations along its upstream waters. Considering the significant quantities of wastes, both of industrial and domestic origin being released into the Savannah River estuary every year, it is of particular interest to evaluate the presence of PAHs in archived sediment and oyster tissue (*Crassostrea virginica*), collected during late 1990's, 2000 and 2001.

Materials and Methods

In consultation with Fort Pulaski's staff and administration regarding industrial activity, nine sampling sites were chosen along the Cockspur Island and Mc Queen's Island within the Fort Pulaski National Monument salt marsh ecosystem. Details of sampling location and sampling sites are described elsewhere[7,8]. At each site, water, sediment, and oyster's (*Crassostrea virginica*) were collected during a five-day period in November, 2000 and 2001. For water, approximately 2 liters of water was collected from 1 m below the surface. In the field, the following water quality parameters were measured: Secchi disk depth, temperature, salinity, total dissolved solids, conductivity, dissolved oxygen, and dissolved oxygen percent saturation (Table 1). Water samples were transported back to the lab for additional analysis which included: pH, turbidity, settleable solids,

nitrate and phosphate (Table 1). In case of sediment; at each sampling site, five sub-samples of sediment from depths of 1-5 m were collected using a bottom grab sampler (clam shell type). The oysters were collected by hand or rake at the same sites, from the mid and lower intertidal zone. Oysters were immediately opened, and the oyster edible tissue was directly transferred into acetone-washed I-Chem bottles, sealed, labeled, transported to lab with ice, and placed in deep-freezer until chemical analysis. Edible tissue of 25-36 oysters, were collected at each site to form a single composite oyster sample from each site.

Table 1. Water quality parameters.

S No.	Water Temperature (^{o}C)	Secchi (cm)	Turbidity (NTU)	Settleable Solids ml/l	Salinity pg/mL	TDS mg/l	Conductivity mS	Dissolved Oxygen mg/l	Oxygen Percent Saturation	pH	Nitrate mg/l	Phosphate mg/l
1	19.4	97	17	trace	31.2	30,100	48.3	6.13	93	7.66	0.57	0.18
2	19.3	98	14	trace	31.6	30,400	48.8	5.98	91	7.64	0.48	0.04
3	19.1	66	20	trace	30.9	29,700	47.8	6.57	99	7.51	0.44	0.12
4	18.4	80	12	trace	29.5	28,400	45.8	6.21	92	7.49	0.53	0.11
5	18.9	56	27	0.1	27.6	26,500	43.1	7.72	110	7.81	1.06	0.10
6	17.6	57	22	0.1	28.2	27,100	44.0	7.66	110	7.56	0.70	0.07
7	19.8	51	51	0.3	25.2	24,400	39.9	7.73	109	7.79	0.53	0.14
8	19.8	78	24	trace	25.6	24,600	40.2	7.29	104	7.70	0.18	0.07
9	18.6	65	24	0.1	31.2	30,000	48.1	7.65	115	7.80	0.48	0.03

PAHs were analyzed following methods described elsewhere[9]. Sediment and oyster tissue samples were Soxhlet extracted for 20-h using dichloromethane. Extracts of sediment were then treated with acid-activated copper granules to remove sulfur. Aliquots of extracts were concentrated to approximately 5-mL by rotary evaporation (39^{o}C), and then to 1-mL under a gentle stream of nitrogen. Extracts were passed through 10-g of activated Florisil, packed in a glass column for clean up. PAHs were eluted using 100-mL 20% dichloromethane (DCM) in hexane. Recoveries through all the analytical steps were between 79% and 101%. PAHs were quantified using a Hewlett Packard 5890 series II gas chromatograph equipped, with a 5972 series mass spectrometer detector (GC-MSD). The PAH standard (AccuStandard, New Haven, CT, USA) consisted of 16 priority pollutant PAHs identified by the U.S. Environmental Protection Agency (U.S. EPA; Method 8310). The mass spectrometer was operated under a selected ion monitoring (SIM) mode, using the molecular ions selective for individual PAHs[10]. Calibration standards were prepared at 0.25, 0.5, 1, 2, and 5 µg/mL. Concentrations based on individually resolved peaks, were summed up to obtain the total PAH concentrations. The detection limits of total PAHs for sediment and oyster samples were 0.1 ng/g wet wt. Blank analysis were performed with each batch containing 5 samples, however none of the samples detected PAHs at the detection limit of 0.1 ng/g dry wt.

Results and Discussion

Water quality parameters (Table 1) were normal and typical of Georgia salt marsh ecosystems during autumn. Mean depth of sediment collection varied from 1.4-3.8. Average sediment moisture content were 47-73%, whereas, the pH ranged from 5.0 to 8.1. The lowest pH (3.5) was noticed at site-6 whereas, the highest (8.3) at site-4. Most sediment samples had pH values of 7-8. Site 6 was different from the other sites in having relatively low pH values in four of five sediment sub samples collected at the site. Most of the sediment samples were classified as pelite (silt-clay) (<0.063 mm in diameter of grains), sand (0.063-2 mm) and gravel (>2 mm). The fat percent in oyster tissue was 1.2 - 1.5%, while moisture content in oyster tissue was between 20 - 21 %.

Concentrations of total PAHs in sediments ranged from 1.2-160 ng/g dw (Table 2). Overall contamination pattern of total PAHs at the different sites, were in the following decreasing order: site-8>site-9>site-5>site-6=site-4>site-1>site-2>site-7>site-3. Fluoranthene was prevalent at site- 1, 2, 3, 5, 6 and 7; pyrene was slightly

higher atn site-8; benzo[k]fluoranthene was higher in site-4, and benzo[a]pyrene was also high at site-9. Slight variation in PAH concentrations within these sites, suggests different sources of contamination. Changes in the PAH pattern may reflect changes in the sources of PAHs such as coal, oil, gas, and petroleum combustion, along with the effect of combustion conditions. However, PAHs originate mainly from petrogenic and pyrolytic sources. Four- and five-ringed PAHs were the most abundant compounds in sediment, whereas two- and three-ringed PAHs were less abundant. In terms of the composition pattern of PAHs in sediment; it is dominated by the 5-ring PAHs. Pereira et al. (1996)[11] showed that 4-ringed PAHs dominated PAH distributions in sediments from San Francisco Bay. Pyrolysis/combustion of fossil materials yields such PAH assemblages, which are subsequently introduced into the marine environment by coastal and river runoff, and by direct dry or wet deposition from the atmosphere. Industrial and/ or domestic wastes are often another important local source. Greater benzofluoranthenes concentration suggests a major source from high temperature pyrolytic processes. In this study, sediment from site-4 contained slightly higher benzofluoranthene, and therefore thermal industries in Bull River would have been influenced. On average, fluoranthene, pyrene, benzo[a]pyrene, and benzo[k]fluoranthene together accounted for 75% of the total PAHs in sediment.

Table 2. Mean (n=5 samples from each sites) concentrations of PAHs in sediment.

PAHs	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6	Site-7	Site-8	Site-9
Naphthalene	0.16	0.21	0.07	0.46	0.87	0.21	0.55	0.07	0.2
Acenaphthalene	0.25	0.23	0.13	0.57	0.42	0.36	0.19	0.24	0.69
Acenaphthene	0.06	0.18	0.07	0.11	0.4	0.21	0.1	0.15	0.15
Fluorene	0.19	0.51	0.12	0.23	0.57	0.43	0.17	0.38	0.3
Phenanthrene	0.79	1.5	0.24	1.1	2.3	1.1	0.4	1.3	1.6
Anthracene	0.24	<0.1	<0.1	0.06	0.13	0.16	0.11	1	<0.1
Fluoranthene	2.4	2	1.3	3.5	8.2	6.4	1.5	5.5	5.7
Pyrene	2	0.2	0.51	2.1	4.1	3.3	1.6	9.9	2.4
Benzo[a]anthracene	0.57	0.06	<0.1	0.46	0.77	1.3	<0.1	8.6	<0.1
Chrysene	1.5	0.57	0.35	1.2	1.9	1.7	1.7	2.7	6.4
Benzo[b]fluoanthene	2	1.25	<0.1	4.2	2.9	1.7	<0.1	6.3	<0.1
Benzo[k]fluoranthene	0.32	<0.1	0.21	<0.1	<0.1	<0.1	1.3	4.2	7.3
Benzo[a]pyrene	0.92	<0.1	<0.1	2.9	<0.1	0.16	1.2	1.1	6.5
Indeno(1,2,3-cd)pyrene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Dibenz(a,h)anthracene	<0.1	<0.1	<0.1	<0.1	<0.1	0.32	<0.1	<0.1	<0.1
Benzo(g,h,i)perylene	0.29	<0.1	<0.1	<0.1	<0.1	0.04	<0.1	<0.1	<0.1
Total PAHs (ng/g wet wt)	12	6.7	2.9	17	22	17	6.1	29	25
Total PAHs (ng/g dry wt)	43	17	7.5	34	46	46	10	44	48

The possible sources of PAHs in sediments may be assessed by the ratios of individual PAH compounds[12]. A ratio of phenanthrene/anthracene <10 and fluoranthene/pyrene >1 tends to indicate that the PAH contamination is from combustion processes. In contrast fluoranthene/pyrene ratios >1 are attributed to pyrolytic origin, whilst values <1 are related to petrogenic sources. Except site-4 and 5, the ratio of phenanthrene/anthracene was <10, therefore these results indicated the combustion source of PAHs in majority of sites. On the other hand, fluoranthene/pyrene ratio was <1 at sites 7 and 8. Petrogenic sources at site 7 and 8 may have possible influence in contamination while other sites are considered to be a pyrolytic source. Overall, it is apparent that multiple source would have accounted for the PAH contamination in Savannah Estuary. Temporal variation of total PAHs were not pronounced between the 2000 (32 ng/g dw) and 2001 (34 ng/g dw) sediment samples. The measured concentrations of PAHs in sediment were relatively low compared to sediments from the Detroit River, Michigan River, Casco Bay, Chesapeake Bay, Penobscot Bay, and San Diego Bay in USA; also several

aquatic bodies in Japan, Poland, United Kingdom, White Sea, Barents Sea, Hong Kong, Puerto Rico, The Caribbean Islands, Adriatic Sea, Korea, China, and Canada.

Contaminated sediments can directly affect bottom-dwelling organisms, and represent a continuing source for toxic substances in aquatic environments that may affect wildlife and humanss via the food chain. This was true in the present study, because of greater PAHs that were detected in oyster tissues (Table 3) compared to the sediments (Table 2). Concentrations of PAHs in oysters ranged from 4.0-264 ng/g dw. Overall contamination pattern of total PAHs at the various sites, were in the following decreasing order; site-2>site-1>site-5>site-6>site-3>site-8>site-4>site-7>site-9. With contrast to the sediment concentration, PAH bioaccumulation varied in oyster tissues. Fluoranthene was predominant in the oysters at site 7 and 8; pyrene was abundant in the oysters at site-2, 6 and 9, and indeno(1,2,3-cd)pyrene was abundant in the oysters at sites-1, 3, 4 and 5 (Table 3). This suggests greater bioaccumulation potential of higher molecular weight PAHs compounds. In particular, Indeno (1,2,3-cd)pyrene was prevalent in all the oyster samples. Consumption of contaminated oysters may lead to human exposure.

Table 3. Concentration of PAHs in oyster tissue.

PAHs	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6	Site-7	Site-8	Site-9
Naphthalene	0.30	<0.1	<0.1	0.40	<0.1	<0.1	<0.1	<0.1	0.73
Acenaphthalene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Acenaphthene	0.53	0.55	<0.1	0.32	0.38	<0.1	0.12	0.28	<0.1
Fluorene	<0.1	1.0	0.69	0.64	0.96	<0.1	<0.1	<0.1	0.20
Phenanthrene	<0.1	6.5	1.9	1.6	3.3	1.7	0.84	2.7	0.43
Anthracene	<0.1	<0.1	<0.1	<0.1	0.28	<0.1	<0.1	<0.1	<0.1
Fluoranthene	2.2	15	1.3	0.30	3.7	5.3	2.1	9.8	0.71
Pyrene	10	106	7.2	0.07	5.5	33	0.59	6.2	0.78
Benzo[a]anthracene	0.24	<0.1	<0.1	<0.1	0.28	0.30	0.60	2.4	<0.1
Chrysene	<0.1	<0.1	<0.1	<0.1	0.58	0.83	<0.1	<0.1	0.30
Benzo[b]fluoanthene	<0.1	<0.1	<0.1	1.4	4.4	<0.1	<0.1	<0.1	<0.1
Benzo[k]fluoranthene	11	<0.1	<0.1	<0.1	3.7	<0.1	<0.1	<0.1	<0.1
Benzo[a]pyrene	40	7.2	<0.1	<0.1	48	<0.1	<0.1	<0.1	<0.1
Indeno(1,2,3-cd)pyrene	109	73	43	14	64	20	<0.1	<0.1	<0.1
Dibenz(a,h)anthracene	6.4	<0.1	0.77	<0.1	3.8	<0.1	<0.1	<0.1	<0.1
Benzo(g,h,i)perylene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total PAHs (ng/g wet wt)	179	210	55	18	139	60	4.2	21	3.2
Total PAHs (ng/g dry wt)	223	264	70	23	174	76	5.3	27	4.0

Seven of the 16 priority PAHs tested induced significant dioxin-like responses. Benzo[a]anthracene and dibenzo[a,h]anthacene induced significant responses in the MVLN bioassay[4]. Numerous studies have shown PAHs to be capable of inducing dioxin-like responses in vitro in both fish[13,14], and mammalian cell lines. PAHs have also been shown to induce ethoxyresourufin-O-deethylase (EROD) activity in vivo[15]. Three PAHs-benzo[a]pyrene, chrysene, and benzo[a]anthracene have been reported to elicit estrogenic responses in vitro[16]. For sediments; chrysene was detected at all sites, benzo[a]anthracene was detected at 6 sites (sites-1,2,4,5,6,8); benzo[a]pyrene was detected at 5 sites (sites 1,4,6,7,8,9), and dibenzo(a,h)anthracene was detected at site-6. In oyster tissue benzo[a]anthracene was detected at 5 sites (sites-1, 5, 6, 7, 8), chrysene was detected at 3 sites

(sites-5,6,9), benzo[a]pyrene was detected at 3 sites (sites-1,2,5), and dibenzo(a,h)anthracene was detected at 3 sites (sites 1,3,5). Consequently presence of all toxic PAH compounds in sediments and oyster from site 1, 5, 6 are of major concern.

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