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Polycyclic Aromatic Hydrocarbons (PAHs) in the Sediments and Fish of the Mill River, New Haven, Connecticut, USA

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The headwaters of Mill River watershed originate in a sparsely populated region of central Connecticut but the river flows southward through the densely populated south-central regions of the state into New Haven Harbor. The southern reaches of the river serve as a resource for fishing and recreation. Of particular interest is the anecdotal evidence of considerable fish consumption from this portion of the river. A previous study focused on the distribution of polychlorinated biphenyls (PCBs) and, to a lesser extent, total polycyclic aromatic hydrocarbon (PAHs) concentrations in the Mill River below the tidegates (Wheeler et al., 1994). No studies have focused on the section of the river from Lake Whitney to the tidegates, the area of most intense fishing and recreation.

PAHs are ubiquitous contaminants in soils and sediments and are classified as Persistent Organic Pollutants (POPs)(Wania and Mackay, 1996). As with other POPs such as DDT and dioxin, certain PAHs have been shown to be mutagenic and carcinogenic (Alexander and Alexander, 1999). Concerns over POP toxicity and longevity in the natural environment are excerbated by their hydrophobicity, which leads to bioaccumulation in the fatty tissues of living organisms and biomagnification through food chains (Kawano et al., 1988; Muir et al. 1998).

The purpose of this study was to characterize the distribution of individual PAH constituents in the sediments and fish of the lower Mill River. In addition, eight commonly used chlorinated pesticides were monitored.

MATERIALS AND METHODS

The study site was the section of the Mill River between the Lake Whitney Dam (N41°20'12", W72°54'38") in Hamden, CT and a set of tide gates 2.7km downstream in New Haven, CT. Twenty-one surficial sediment samples were collected with an Eckman dredge in October 2000. The sites were chosen based proximity to both known fishing sites and storm-water runoff outflows. A small number of samples were collected from outside the study area, including locations below the tide gates and areas in the upper reaches of the watershed. Most samples were collected near the banks of the river, with the exception of samples 3,6, and 10, which were taken at the center of the channel. The dredge recovered

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the top 8-10 cm of sediment. Three separate samples were taken within a 3-5 m area at each site and were composited in a 1-L amber glass jar with a Teflon-lined cap. The organic carbon content of duplicate samples was determined on a CHN analyzer at 950°C (Letco CHN 600, St. Joseph, MI)(Table 1). Seventeen fish were collected from within the study area, five white perch (*Morone americana*) and one sunfish (*Lepomis gibbosus*) were collected at the site 10 and eleven white perch were collected at the downstream side of the tide gates (site 3). The sediment samples and fish were stored in a freezer prior extraction.

Sediment samples were wet-sieved and the size fraction between 0.1mm and 2.0mm was collected and mixed for homogeneity. This fraction was selected to allow for comparison between samples, but because fines were not included, PAH values reported are lower limits. For each site, duplicate 5-g portions of the sediment were added to 40-mL amber glass vials with Teflon-lined caps. The vials were amended with 25 mL of methanol, shaken vigorously, and were placed in a 70°C oven for 24 h. The vials were centrifuged at 1500 rpm for 15 min, and 10 mL of the supernatant was added to a 250-mL separatory funnel. The funnels were amended with 100 mL of distilled deionized water and 10 mL of hexanes. The funnels were capped, inverted 5 times, and allowed to phase separate for 15 min. The funnels were shaken 2 additional times and a saturated Na_2SO_4 solution was added as to dissolve the emulsion. The water/methanol solution was drained off and the hexanes were collected in 20-mL amber glass vials with Teflon-lined caps.

The fatty tissue and muscle of the fish were removed, composited based on sampling location, and added to 1-quart blender jars. The tissue was blended on high speed for 30 sec for homogeneity. Twenty five-gram portions of the tissue were then weighed into separate 1-quart blender vessel containing with 25-mL of 2-propanol (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA) and 20 μg of fluorene (internal standard). The sample was blended at high speed for 30 sec and amended with 50-mL volume of petroleum ether (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA). The sample was blended at high speed for 5 min. The extract was decanted through a funnel containing glass wool and collected in a 500-mL glass separatory funnel with a Teflon stopcock. The solids were drained for 10 min and 100 mL of distilled water and 10 mL of saturated sodium sulfate solution were added to each funnel. The funnels were capped, shaken gently, and allowed to phase separate for 15 min. The water was drawn off and the ether was rinsed three additional times with 100 mL distilled water. Saturated sodium sulfate solution was added as needed to break any emulsion. The final ether extracts were added to pre-weighed 35-mL amber glass vials. The ether was volatilized under nitrogen and the mass of extracted lipids was determined. The samples were dissolved in 5 mL of petroleum ether for saponification. Three mL of 10%KOH in methanol was added to each vial and the samples were placed in a 90°C hot water bath for 35 min. and reduced to near dryness. Each vial was amended with 5 mL of hexanes and 2 mL of 1:1 methanol:water. The vials were then shaken vigorously for 1 min and the phases were allowed to separate for 6 h.

The hexanes was removed by pipette, an additional 5 mL of hexanes was added to each vial, the procedure was repeated. Triplicate extractions were performed on composited fish tissue from each site.

The sediment and fish extracts were analyzed for PAHs by gas chromatography (GC)(Hewlett Packard 5890) with flame ionization detection. The column (30m x 0.25mm, 0.25μm thickness) contained a PTE-5 film (Supelco, Inc., Bellefonte, PA, USA). The GC program was as follows: initial temperature of 100°C for 1 min, ramped at 5°C/min to 250°C, and held at 250°C for 10 min. A 2 μL splitless injection was used, and the injection port was maintained at 290°C. The carrier gas was He flowing at 10mL/min.

A stock solution containing the following PAHs was used for quantitation: naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, 1,2-benzanthracene, and chrysene. The stock was diluted to create a series of calibration standards of PAHs at 0.1, 0.25, 0.5, 0.75, 1.0, 2.0, 5.0, and 10 μg/mL. The detection limit was approximately 0.02 μg/mL for each PAH.

The sediment and fish extracts were also analyzed for eight chlorinated pesticides commonly found in Connecticut soils and occasionally produce. These compounds include Botran, Chlorothanil, Vinclozolin, Captan, Endosulfan I, Endosulfan II, *p,p'*-DDE, and Iprodione. Here the samples were analyzed by GC (Hewlett Packard 5890) with a ^{63}Ni electron capture detector (ECD). The column (30m x 0.53mm, 0.5μm thickness) contained a SPB-1 film (Supelco, Inc., Bellefonte, PA, USA). The GC program was as follows: initial temperature of 175°C, ramped at 1°C/min to 210°C, then ramped at 2°C/min to 250°C, and held at 250°C for 15 min. A 2-μL splitless injection was used, and the injection port was maintained at 250°C. The carrier gas was He and the make-up gas was 5% CH_4 in Ar at 20mL/min.

To validate the extraction and analytical procedures, a Standard Reference Material (#1944) was purchased from The National Institute of Standards and Technology. The sample is a marine sediment from the New York/New Jersey Waterway and is certified to contain known amounts of 24 PAHs. Duplicate extractions were performed on SRM 1944. A PAH-free sediment (Pleasant Point, Brandford, CT;supplied by Dongye Zhao, CAES) was also extracted in duplicate.

RESULTS AND DISCUSSION

Percent recovery values from the extractions of the Standard Reference Sediment varied considerably with individual PAHs (Table 1). The recoveries of naphthalene and phenanthrene were 101 and 87% respectively, but the recovery of 1,2-benzanthracene was only 13%. Based on the extraction efficiencies determined from SRM 1944, a correction factor was applied for each of PAH constituents from the Mill River sediments (Table 1).

None of the eight chlorinated pesticides were detected in any of the sediment samples. Two random samples were analyzed by mass spectrometry (MS)(Hewlett Packard 5970 Mass Selective Detector), but the only non-PAH peak was sulfur. PCBs were not detected in the samples analyzed by MS.

PAHs were detected in all 14 sediment samples from within the main study site and in the 3 samples taken below the tidegates (Table 1), as well as in 3 of the 4 samples from the rural upstream areas of the river. The mean concentration of total PAHs within study site was 10.6 mg/kg, but considerable site variability existed. For example, sites 5 and 8 had a total PAH concentrations of 0.59 mg/kg 39 mg/kg, respectively. Phenanthrene, fluoranthene, and pyrene were detected at most sites. Naphthalene was not detected at any of the sites and fluorene was detected at only site #13. None of the other PAH peaks in the SRM extract matched retention time with unidentified peaks from the Mill River.

Triplicate samples of fish tissue from the both the Orange Street Bridge and from the tidegates were extracted. Of the six samples, pyrene was detected in one sample from the bridge site (1.7 mg/kg) and 2 samples from the tidegates (1.7 mg/kg). These values are based on a total wet weight of 25 g of extracted tissue. The weight of extracted lipids was 1-2% of the total tissue, yielding 170 mg pyrene kg lipid. No chlorinated pesticides were detected in the tissues, but unidentified peaks on the ECD were investigated by MS. The presence of pyrene was confirmed, but no chlorinated pesticides or PCBs were present in the fish.

The data indicate that the sediments of the Mill River contain measurable levels of PAHs throughout the study site and most likely throughout the length of the water body. The average total PAH concentration of 10.6 mg/kg, as well as the maximum value of 39 mg/kg, are significantly less than the PAH burden in sediments of areas known to be heavily contaminated. Sediments from areas such as the Passaic River (NJ) and Hudson River Raritan Estuary contain 145 and 57 mg/kg total PAH, respectively (Huntley et al., 1995). Conversely, the National Status and Trends Program of the National Oceanic and Atmospheric Administration monitored estuarine sediments throughout the United States and the average total PAH concentration in the Mill River exceeds more than 90% of the areas in that study (NOAA, 1991). Also, as noted earlier, our results represent lower limits on the amount of PAHs in the Mill River sediments. Four of the five highest total PAH samples (sites 8,11,13,20) were from areas adjacent to combined sewer overflows (CSOs) or stormwater outflows. These findings imply a relationship between urban run-off and sediment PAH levels, although sites 4-7 were also near CSOs but contained low levels of contamination. The data indicate that the highest PAH levels are found in the sediments between East Rock Park and Orange street, an area of the river routinely used for fishing. Surprisingly, PAH levels below the tidegates were low, and one of the highest values (site 20) was found in one of the upstream more rural areas. PAH concentration can be normalized to organic carbon (Table 1) but doing so does not dramatically alter the relative distribution of PAH contamination within the study site. One

Table 1. Individual and total PAH concentrations in sediments of the Mill River

Site (% OC)[a]	Naphth.[b]	Fluor.[b]	Phenan.[b]	Anthr.[b]	Fluroan.[b]	Pyr.[b]	1,2-Benz.[b]	Chrys.[b]	Total PAH	Total PAH/g OC
1 (6.7)	*ND*[c]	*ND*	1.3[d]	1.0	2.9	2.9	*ND*	*ND*	8.1	59
2 (0.85)	*ND*	*ND*	0.37	*ND*	0.81	0.98	*ND*	*ND*	2.2	120
3 (0.45)	*ND*	*ND*	2.6	*ND*	2.0	2.1	*ND*	3.2	9.9	1100
4 (0.49)	*ND*	*ND*	*ND*	*ND*	1.0	0.98	*ND*	*ND*	2.0	170
5 (0.29)	*ND*	*ND*	*ND*	*ND*	0.27	0.32	*ND*	*ND*	0.59	82
6 (0.39)	*ND*	*ND*	*ND*	*ND*	0.34	*ND*	*ND*	0.90	1.2	120
7 (0.26)	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	2.3	2.3	330
8 (2.5)	*ND*	*ND*	2.8	1.8	9.8	9.3	9.2	5.8	39	570
9 (1.5)	*ND*	*ND*	0.65	0.51	2.3	2.2	*ND*	0.97	6.6	200
10 (0.64)	*ND*	*ND*	0.83	*ND*	2.3	2.7	*ND*	1.0	6.9	500
11 (2.0)	*ND*	*ND*	2.3	1.2	5.6	4.6	2.5	1.5	18	370
12 (1.9)	*ND*	*ND*	1.5	*ND*	2.4	3.9	1.2	2.5	11	270
13 (1.8)	*ND*	*ND*	4.3	3.0	9.8	9.5	8.5	3.2	38	860
14 (1.1)	*ND*	*ND*	*ND*	*ND*	1.3	0.39	*ND*	2.1	3.8	140
15 (0.86)	*ND*	*ND*	*ND*	*ND*	0.51	0.71	*ND*	*ND*	1.6	100
16 (0.30)	*ND*	*ND*	*ND*	*ND*	1.0	1.0	*ND*	2.0	4.3	620
17 (0.65)	*ND*	*ND*	2.9	1.5	4.9	4.9	6.2	3.7	24	1400
18 (1.5)	*ND*	0.65	*ND*	*ND*	1.5	0.71	1.7	6.1	11	230
19 (2.1)	*ND*	*ND*	0.70	0.24	0.90	1.0	*ND*	*ND*	2.9	73
20 (0.17)	*ND*	*ND*	2.3	1.4	6.1	4.6	6.2	8.7	29	6700
21 (9.4)	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	0	0
CF[e]	0.98	NA[f]	0.87	0.37	0.41	0.41	0.13	0.38		

[a] % organic carbon of sediment sample

[b] Naphth=naphthalene, Fluor=fluorene, Phenan.=phenanthrene, Anthr.=anthracene, Fluoran=fluoranthene, Pyr=pyrene, 1,2-Benz=1,2-benzanthracene, Chrys=chyrsene. All values are mg/Kg.

[c] Total PAH=sum of individual PAHs detected

[d] ND=not detected

[e] Correction factors (CF) based on percent recovery of individual PAHs from Standard Reference Material 1944

[f] Fluorene was not present in SRM 1944

difference is that the relative level of PAHs in the sediments between East Rock Park and Orange street becomes consistent with the remainder of the study sites. On the basis of organic carbon, there is still no correlation between locations of urban run-off and PAH concentration. Perhaps the most significant finding of the study is PAH contamination along the entire length of the river, irrespective of the rural or urban character of the surrounding area. Such distribution is not uncommon with other POPs (Wania and Mackay 1996). It is noteworthy that although PAH contamination was present throughout the study site, pyrene was the most frequently detected contaminant, being absent from only 3 sites. The fish were also found to contain only pyrene, a logical finding given the relatively high concentrations of pyrene found in the associated sediments. The Orange street bridge sites (9-10) and the tidegates (sites 2-5) contained summed pyrene concentrations of 4.9 and 4.4 mg/Kg, respectively.

The risk posed from weathered fractions of POPs is unknown. Substantial evidence in the literature suggests that the bioavailability of organic compounds in natural solids declines with aging time (Alexander 2000). This process of time-dependent reductions in availability has been termed sequestration and has been demonstrated with a wide range of physical and biological assays (Alexander 2000). This list of compounds shown to undergo sequestration is extensive and includes such POPs as PAHs, PCBs, DDT, and dieldrin. It has been suggested that the practice of relying on total pollutant burden to estimate exposure is inappropriate due to the lack of consideration of bioavailabilty. Arguments have been offered that risk estimation and remedial endpoints have been overstated because of the presumed reduced hazard posed by sequestered fractions of contaminants (Alexander 2000). It is noteworthy that in the laboratory studies demonstrating the declines in contaminant availability, at no point does the contaminant assume complete unavailability. Chung and Alexander (1998) showed that in certain soils, phenanthrene availability declined during the first 4 months of aging but that pollutant availability remained constant thereafter. Nam et al. (1998) observed that phenanthrene sequestration was only evident if the organic carbon content of the soil was greater than 2.0%. Similarly, Reeves et al. (2001) fed rats pellets containing coal-tar amended soil and observed that aging time (0 and 270 d) had no effect on PAH concentrations in the liver and urine. Similar contradictory data regarding sequestration exists for other POPs. For example, certain vegetable crops such as zucchini, pumpkin, lettuce, spinach, and cucumbers have been shown to accumulate significant levels of weathered chlordane, *p,p'*-DDE, and dioxins in their roots and edible tissues (Hulster et al. 1994; Mattina et al. 2000; White 2001). Clearly, the evidence regarding the risk estimation of sequestered pollutants in the environment is contradictory and much investigation is required prior to any changes in regulatory guidelines.

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