Supplementary Information

Exploring the Cumulative Selectivity of Polycyclic Aromatic Hydrocarbons in Phytoplankton, Water, and Sediment in Typical Urban Water Bodies

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S1.1: Sample collection and pretreatment

Fifteen sampling sites were selected on October 15, 2021, along the Qinhuai River and Xuanwu Lake in Nanjing City (Figure 1) for analysis of a variety of physicochemical parameters (Table 1). Water samples were collected at each site and stored in brown glass bottles to be used for determining PAHs concentrations and physicochemical indices. An additional water sample (1 L) was collected for identification and counting of algae after addition of 1 % methanol. For the bioaccumulation analysis of PAHs in phytoplankton, a 25-L water sample was collected and passed through a #25 phytoplankton net (0.064-mm mesh). Phytoplankton were collected on the net and then eluted into a 500-mL glass bottle for later determination of PAHs concentrations. Several reagents were used in the analysis. Petroleum ether, acetone, hexane, methanol, and copper powder were pure analytical grade; anhydrous sodium sulfate was guaranteed reagent grade (Nanjing Chemical Reagent Limited Company, Nanjing, China). Methylene chloride was pesticide residue level (China Chemical Reagent Limited Company, Shanghai, China). Neutral alumina was chromatographically pure (Shanghai Ludu Chemical Reagent Plant, Shanghai, China); C18-bonded silica gel and non-bonded silica gels were chromatographically pure (Dalian Institute of Chemical and Physics, Dalian, China). After the addition of anhydrous sodium sulfate, neutral alumina and silica gel were baked for 3 h at 400 °C; 3% distilled water was then added for deactivation. Next, the neutral alumina and silica gel were placed in a desiccator to cool to room temperature, after which they were preserved in hermetically sealed containers.

S1.2: Analyses of environmental parameters

Dissolved oxygen (DO) were measured *in situ* using SX751-series portable electrochemical meters (San-Xin Instrumentation Inc., Shanghai, China). Total nitrogen (TN) and ammonia nitrogen (NH_4^+-N) were measured in the laboratory within 24 hours after fixing water samples with concentrated sulfuric acid; TN was measured by potassium persulfate oxidation–ultraviolet spectrophotometry and NH_4^+-N was measured by Nessler's reagent spectrophotometry [1]. Chemical oxygen demand (permanganate index, COD_{Mn}) was measured by titration analysis. Total phosphorus (TP) was measured by alkaline potassium persulfate digestion and Mo-Sb antiluminosity (Fusheng 2002). Chlorophyll a (*Chl-a*) was determined by spectrophotometry (UV-1201, Beifen-Ruili analytical instruments Co., Ltd., Beijing).

S1.3: Extraction of PAHs from water samples

After filtration of a 1-L sample through a 0.45-μm glass fiber filter (Whatman GF/C, 47 mm diameter, USA), PAHs were enriched using a C18 solid-phase extraction column (SPE: upper layer consisted of 0.5 g copper powder, lower layer consisted of 1.0 g C18) (Borui Co., Ltd., Tianjin, P.R. China), which was pre-activated using a 15-mL mixture containing petroleum ether, acetone, and methanol (1:1:1) and 15 mL of high-performance liquid chromatography (HPLC) water. The flow rate was controlled at 5 mL/min. After the water sample was enriched, the C18 SPE column was rinsed with 5 mL HPLC water, pumped for 30 min, and eluted with a 22-mL mixture of petroleum ether and methylene chloride (3:1). The eluent was collected in a 50-mL pear-shaped bottle and concentrated to approximately 1 mL with a vacuum rotary evaporator (RE-3000, Yarong Biochemical Instrument Plant, Shanghai, P.R. China) and N_2. After the concentrated solution was adjusted to 1 mL with *n*-hexane, the PAHs were determined by HPLC.

S1.4: Extraction of PAHs from algal samples

After zooplankton in the algal samples were removed with a 150-μm screen mesh, the filtrate was passed through a 0.2-μm glass fiber filter (Borui Co., Ltd., Tianjin, P.R. China) under <50 kPa

pressure. The filter membrane was then folded in half with the face containing the phytoplankton on the inside. Water was blotted with ordinary filter paper (Borui Co., Ltd., Tianjin, P.R. China) and the filter membrane was cut into pieces and placed in a 50-mL glass centrifuge tube. A 30-mL extraction mixture of petroleum ether and acetone (3:1) was added and the sample was extracted ultrasonically for 0.5 h before being left to stand overnight. The extract liquor was transferred to a 50-mL pear-shaped bottle. An additional 10 mL of petroleum ether was added to the original sample centrifuge tube and the tube was then extracted for a second time (0.5 h). The extracts were combined in the 50-mL bottle and concentrated to 1 mL with a vacuum rotary evaporator. The concentrated solution was then purified using a solid-phase purified column (upper layer, 1.5 g anhydrous sodium sulfate; middle layer, 1 cm nonbonded silica gel; bottom layer, 1 cm neutral alumina), which was pre-activated with 5 mL *n*-hexane at a flow rate that was controlled dropwise. The column was washed twice with 10 mL *n*-hexane, and the eluent was concentrated to approximately 1 mL with a vacuum rotary evaporator (RE-3000, Yarong Biochemical Instrument Plant, Shanghai, P.R. China) and N_2 for HPLC analysis.

S1.5: Algae identification and counting

The 1-L water samples were left to stand for 48 h to allow algae to settle out and the volume was concentrated to 30 mL using a small siphon tube (the underwater end of the siphon was sealed with a #25 bolting cloth). After the concentrate was shaken, 0.1 mL of liquid from the center of the sample was injected into a 0.1-mL (20 mm × 20 mm) plankton-counting chamber (Purity instrument Co., Ltd., Beijing, P.R. China) and carefully covered with a glass microscope slide (22 mm × 22 mm) to prevent bubble formation in the counting chamber. Specimens were observed under a microscope (CX31, Olympus, Japan); phytoplankton were classified and identified, and the numbers and biomass of algal cells were calculated according to the relevant literature [2].

S1.6: Determination of PAHs [3].

The PAHs were analyzed by Agilent1100 HPLC with fluorescence and UV – adsorption detector. A 250 mm × 4.6 mm × 5 μm reversed phase C18 column (Agilent ZORBAX Eclipse XDB-C18) served as the stationary phase. Mixed solution of acetonitrile and ultrapure water were delivered as the mobile phase in a gradient programme at 0.75 ml/min. The initial volume ratio of acetonitrile and water is 60 : 40, then the ratio of organic phase is increased to 100 : 0 within 60 minutes. PAHs were quantified by using external standard solutions obtained from Ehrenstorfer (Augsburg, Germany).

Detect wavelength: time programming with FLD signals, 0 min - 9min: Exλ=260 nm, Emλ=380 nm; 9 min - 16min: Exλ=260 nm, Emλ=340 nm; 16 min - 18 min: Exλ=260 nm, Emλ=380 nm; 18 min - 21 min: Exλ=260nm, Emλ=380 nm; 21 min - 23 min: Exλ=289 nm, Emλ=462 nm; 23 min - 30 min: Exλ=320 nm, Emλ=380 nm; 30 min - 36 min: Exλ=266 nm, Emλ=403 nm; 36 min - 52 min: Exλ=294 nm, Emλ=430 nm; 52 min: Exλ=290 nm, Emλ=500nm. The investigated PAHs in this study included naphthalene (Nap), phenanthrene (Phe), anthracene (Ant), fluorene (Flu), fluoranthene (Fla), pyrene (Pyr), acenaphthylene (Ace), chrysene (Chr), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DahA), and benzo[g,h,i]perylene (BghiP).

S1.7: Quality Assurance/Quality Control (QA/QC) [4-6].

In order to ensure the accuracy and precision of experimental data, replicate samples, certified reference materials HS-5 (sediments, provided by NRCIMB of Canada), matrix spikes samples (sedimental samples of known PAH levels spiked with a mixture consisting of 1 μg each of PAHs),

and procedural blanks were used as quality control procedures. Analyses were run in batches of 10 samples plus four quality controls (QCs) including one reagent blank, one matrix blank, one matrix spikes sample, and one random sample in duplicate. The recovery efficiency of PAHs ranged from 89% to 111% for HS-5 and from 78% to 105% for the spiked samples with relative standard deviation (RSD) 3.2%–10.6%. The method detection limits were in the range of 0.5–2.0 ng/g dry weight. PAHs standard solution was diluted with acetonitrile, six concentrations of the mixed standard were configured to quantify peak area by establishing a working curve with external standard method, and the correlation of the standard curve was above 0.99. All of the measurements were performed in triplicate, the results were not corrected according to the percent recovery of HS-5 and spiked samples, and the means were used for calculations.

S2: Figure S1. The Pearson correlation analysis between PAHs concentration and algae biomass

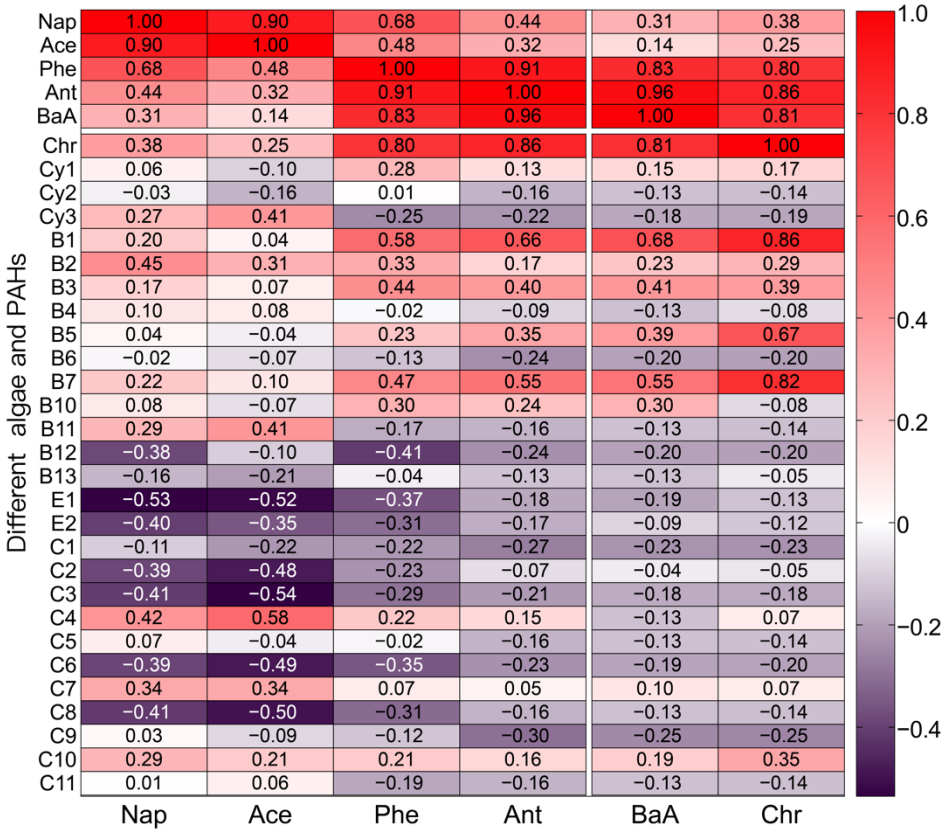


Figure S1. The Pearson correlation analysis between PAHs concentration and algae abundance. Note: 1) : naphthalene (Nap), acenaphthylene (Ace), phenanthrene (Phe), anthracene (Ant), benzo[a]anthracene (BaA), chrysene (Chr). 2) The codes of phytoplankton were listed in Table 1.

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