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Title: Assessment of Environmental Pollution of Taihu Lake by Combining Active Biomonitoring and Integrated Biomarker Response

Summary of the number of pages: 7

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Chemical analyses. Sediment samples were dried-frozen. Dry sediment sample of 5 g was spiked with surrogate standards and extracted for 48 h using 200 mL of *n*-hexane/acetone (1:1, v/v) mixture in a soxhlet apparatus. The extract was concentrated to 2 mL in a rotary evaporator. Cleanup was performed using a multilayer column composed of 10 g of silica gel (deactivated with water, 5% w/w), 10 g of Florisil, 1 g of anhydrous sodium sulphate and approximately 0.5 cm of activated powdered copper at the top. Target analytes were eluted from the column with 70 mL of hexane/dichloromethane (3:2, v/v) (for polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs)) or 50 mL of hexane (for polychlorinated biphenyls (PCBs)). The volume of eluant was reduced to approximately 200 μL using a gentle stream of nitrogen gas.

The concentrations of 16 PAHs, identified as priority pollutants by United States Environmental Protection Agency (EPA), were determined using a gas chromatograph (Thermo Fisher) equipped with a splitless injector and coupled to a Flame Ionization Detector (FID) according to method described in EPA 8100. Ten PCB congeners (PCB28, 52, PCB101, 112, 118 and PCB138, 153, 155, 180, 198) and OCPs (hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethanes (DDTs)) were determined using a gas chromatograph (Thermo Fisher) equipped with a splitless injector and coupled to a ^{63}Ni Electrical Capture Detector (ECD), as described in EPA 8082 and EPA 8081A, respectively.

Quantification of organic pollutants was performed from the GC profiles using the external standard method. A method blank with each sample batch was checked. The recovery of isotope labeled PAH was 87.19±9.33% for naphthalene-D8, 86.35±6.37% for chrysene-D12 and 88.14±8.65% for perylene-D12. The surrogate standards of OCPs were PCB30 and PCB204 and their recoveries were 88.18±5.57% and 90.67±8.38%, respectively. The recoveries of PCB surrogate standards were 85.24±8.40% for 2,4,5,6-tetrachloro-m-xylene and 88.26±9.13% for PCB209. The limits of detection were 0.9 to 12.8 μg/kg for PAHs and 0.01 to 0.2 μg/kg for PCBs and OCPs. No target compounds were detected in the method blanks.

A mass of 1 g of dried-frozen sediment was weighed into 100 mL conical flask and 3 mL of nitric acid and 7 mL of hydrochloric acid were added and allowed to dryness on a hot plate. Then 10 mL of

nitric acid was added and the mixture was heated to dryness. The samples were then cooled to room temperature. Each sample was dissolved with 1 mL of nitric acid, and transferred quantitatively to a 50 mL volumetric flask by adding ultrapure water. The solution was used to measure the concentrations of Zn, Cu, Ni and Pb elements using an ICP-MS (Leeman Labs).

Biomarker assays EROD GSH GST TBARS CAT MT. EROD activity was quantified at 572 nm using a microplate reader. The reaction mixture consisted of 140 µL buffer (0.1 mol/L Tris, 0.15 mol/L KCl, pH 8.0), 10 µL of 2 µmol/L 7-ethoxyresorufin and 10 µL extracts. The reaction was initiated at 25 °C by the addition of 40 µL of 2.1 mg/mL nicotinamide adenine dinucleotide phosphate (NADPH). GSH content (nmol mg^{-1} protein) was determined at 405 nm, using 50 µL of GSH or GSSG standards, samples, or blanks and 100 µL of reaction mixture (0.225 mM 5,5'-dithio-bis(2-nitrobenzoic acid), 0.3 mM NADPH, and 2.8 U/mL GSSG reductase). GST activity was determined at 340 nm by adapting to a microplate reader, using 30 µL of homogenate and 150 µL of the reaction solution (100 µL of 0.1 mM potassium phosphate, 10 µL of 1.0 mM 1-chloro-2,4-dinitrobenzene, 10 µL of 1.0 mM GSH, and 880 µL H_2O). The thiobarbituric acid reactive substances, termed as TBARS (nmol mg^{-1} protein), was determined at 532 nm, using 10 µL of tissue homogenate and 190 µL of the reaction solution (40 µL of 8.1% sodium dodecylsulfate, 300 µL of 20% acetic acid buffer (pH 3.5), 300 µL of 1% 2-thiobarbituric acid, and 200 µL of distilled water). Catalase (CAT) activity was determined by the method of ammonium molybdate. 200 µL of the homogenate was incubated with 1 mL of substrate (65 mM hydrogen peroxide in 60 mM potassium phosphate buffer, pH 7.4) in 37 °C for 60 s. The enzymatic reaction was terminated by adding 1 mL of 32.4 mM ammonium molybdate and yellow complex of molybdate and hydrogen peroxide was measured at 405 nm. The concentration of MT was determined by Cd- hemoglobin saturation method. 500 µL of 200 mg/L $CdCl_2$ solution is added into 200 µL of tissue homogenate and allowed to incubate at room temperature for 15 minutes. 200 µL of 2% bovine hemoglobin solution (w/v) is added into the sample, then the sample is mixed, heated and centrifuged, and the supernatant is collected. The Cd concentration in the supernatant is measured using a flame

atomic absorption equipment (TAS-986, Purkinje General Instrument Co. Ltd., Beijing, China) and MT concentration is calculated from the Cd concentration measured in the supernatant.

Calculation of the IBR. The procedure of IBR calculation for each biomarker is: (1) Calculation of mean and standard deviation (SD) for each station; (2) Standardization of data for each station: $F_i'=(F_i-\text{mean}F)/S$, where F_i' is the standardized value of the biomarker, F_i is the mean value of a biomarker from each station, mean$F$ is the mean of the biomarker calculated for all the stations, and S is the standard deviation calculated for the station-specific values of each biomarker; (3) Using standardized data, Z was computed as $+F_i'$ in the case of activation and $-F_i'$ in the case of an inhibition, and then the minimum value for all station for each biomarker was obtained and added to Z. Finally the score B was computed as $B=Z+|\min|$, where $B\geq 0$ and $|\min|$ is the absolute value. The corresponding IBR value is: $\{[(B_1\times B_2)/2]+[(B_2\times B_3)/2]+\cdots[(B_{n-1}\times B_n)/2]+[(B_n\times B_1)/2]\}$.

TABLE S1. Mean concentrations of PAHs in the surface sediments at seven biomonitoring sites

PAH concentration (μg kg^{-1})	S1	S2	S3	S4	S5	S6	S7
Naphthalene	2.0	2.0	0.9	3.2	2.2	1.5	1.2
Acenaphtylene	3.0	2.0	1.0	3.7	4.6	1.7	1.5
Acenaphtene	6.9	8.9	10.0	13.0	17.4	12.5	9.3
Fluorine	57.1	30.8	37.2	98.0	72.6	42.6	12.8
Phenanthrene	68.7	61.0	92.6	138.9	112.5	72.5	39.8
Anthracene	60.5	40.8	39.0	98.3	71.6	44.0	27.9
Fluoranthene	27.9	28.8	27.7	82.7	57.4	32.5	39.4
Pyrene	27.1	28.9	27.6	72.4	42.4	20.3	19.3
Benzo(a)anthracene	29.9	43.9	34.0	57.5	62.3	27.6	35.0
Chrysene	20.5	41.0	54.1	108.2	56.7	28.0	34.1
Benzo(b)fluoranthene	59.9	40.9	40.2	120.5	112.2	57.3	40.1
Benzo(k)fluoranthene	35.4	33.8	43.6	72.7	51.4	54.3	27.8
Benzo(a)pyrene	18.7	25.3	20.2	38.9	27.4	13.1	20.7
Indeno(1,2,3-c,d)pyrene	18.9	4.6	11.0	18.5	17.8	8.0	11.8
Dibenzo(a,h)anthracene	13.1	7.5	13.0	31.5	19.3	9.1	8.5
Benzo(g,h,i)perylene	54.8	62.2	40.2	90.2	72.5	37.4	49.7
∑PAHs	503.8	462.0	491.8	1047.8	800.0	462.1	378.6

TABLE S2. Mean concentrations of PCBs in the surface sediments at seven biomonitoring sites

PCB concentration ($\mu g\ kg^{-1}$)	S1	S2	S3	S4	S5	S6	S7
PCB-28	1.38	1.62	0.89	2.08	1.95	1.86	0.23
PCB-52	0.23	0.05	0.06	0.02	0.17	0.05	0.01
PCB-155	0.02	0.01	0.01	0.02	0.02	0.05	0.01
PCB-101	0.20	0.24	0.15	0.20	0.17	0.18	0.15
PCB-112	0.10	0.10	0.10	0.10	0.10	0.10	0.10
PCB-118	0.95	0.37	0.15	0.62	0.24	0.33	0.16
PCB-153	0.27	ND[a]	ND	0.06	0.01	0.26	ND
PCB-138	0.11	0.09	0.10	0.10	0.10	0.11	0.08
PCB-180	0.01	0.02	0.01	0.02	0.01	0.01	0.01
PCB-198	0.01	0.01	0.01	0.02	0.01	0.01	ND
$\sum$PCB	3.27	2.52	1.49	3.25	2.79	2.97	0.76

[a] Not detected

TABLE S3. Mean concentrations of OCPs in the surface sediments at seven biomonitoring sites

OCP concentration ($\mu g\ kg^{-1}$)	S1	S2	S3	S4	S5	S6	S7
α -666	0.06	0.15	0.03	0.04	0.03	0.24	0.01
β-666	0.18	0.10	0.12	0.11	0.12	0.04	0.03
γ-666	0.16	0.07	0.31	0.32	0.33	0.34	0.12
δ-666	0.67	0.29	0.64	0.55	0.68	0.37	0.31
p,p-DDT	0.02	0.10	0.06	ND[a]	0.05	0.02	ND
p,p-DDD	0.17	0.39	0.89	0.20	1.07	0.61	0.37
p,p-DDE	ND	0.06	0.49	0.23	0.77	0.33	0.29
o,p-DDE	0.12	0.10	0.09	0.09	0.03	0.10	0.01
∑OCPs	1.36	1.25	2.60	1.53	3.08	2.03	1.12

[a] Not detected

TABLE S4. Metal contents in the surface sediments at seven biomonitoring sites

Metal content ($mg\ kg^{-1}$)	S1	S2	S3	S4	S5	S6	S7
Zn	45.56	40.74	85.04	48.32	31.82	65.66	39.31
Cu	16.43	13.96	91.02	16.42	13.04	64.86	17.51
Ni	6.51	7.45	28.66	7.31	4.96	21.18	7.82
Pb	9.33	10.56	22.44	11.84	20.16	16.12	10.49
Total	77.83	72.71	227.16	83.89	69.98	167.82	75.13