

Supporting Information

MANUSCRIPT TITLE: Identifying organic toxicants in sediment using effect-directed analysis: a combination of bioaccessibility-based extraction and high-throughput midge toxicity testing

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FIGURES: 4

TABLES: 4

NO. OF PAGES: 16

Chemicals and Reagents. Analytical grade acetone was purchased from Tianjin Chemical Reagent Factory (Tianjin, China) and redistilled before use. Hexane and dichloromethane (HPLC grade) were purchased from Oceanpak Alexative Chemical (Gothenburg, Sweden). Acetonitrile and dimethyl sulfoxide (DMSO) (HPLC grade) were bought from Merck Millipore (Darmstadt, Germany) and Acros Organics (Morris Plains, NJ, USA), respectively. Ultrapure water was prepared using a Milli-Q ultrapure water system (Millipore Corporation, Bedford, MA, USA). Analytical grade Na₂SO₄ and NaCl were bought from Guangzhou Chemical Reagent Factory (Guangzhou, China) and baked at 450 °C for 4 h prior to use. Copper powder was purchased from Tianjin Kemiou Chemical Reagent Factory (Tianjin, China) and purified with 10% HCl (v/v), distilled water and acetone, sequentially. Humic acid was acquired from Sigma-Aldrich (St. Louis, MO, USA). Sulfur was purchased from Guangzhou Chemical Reagent Factory.

As listed in Table S1, 17 compounds with various polarities and structures were used to develop and validate the extraction and fractionation methods, including polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs) and pesticides. Standard compounds were all supplied as neat chemicals with purity above 96%. The PCBs and PBDEs were gained from AccuStandard, except for CB-209 which was acquired from ChemService (West Chester, PA, USA). Phenanthrene was purchased from Acros Organics and benzo(a)pyrene was from Alfa Aesar. Carbaryl and *p,p'*-DDE were purchased from AccuStandard, phoxim and fipronil were acquired from Dr. Ehrenstorfer (Augsburg, Germany) and the remaining pesticides were bought from ChemService. The surrogates (CB-67, ¹³C-CB-141 and CB-191) and internal standards (CB-24, CB-82 and CB-189) were purchased from AccuStandard. Versalide, tonalide and galaxolide were acquired from AccuStandard (New Haven, CT, USA) and their properties are shown in Tables S2 and S3.

Sediment Sampling. As shown in Figure S2, four sediment samples were collected from urban waterways in Guangzhou, China and detailed descriptions on the sampling sites can be found in Yi et al.¹ Previous TIE tests showed that sediment samples in the study area were highly toxic to benthic invertebrates and organic contaminants were one of the main classes of toxicants present.¹⁻³ Top 5-cm of sediments were collected, passed through a 2-mm sieve on site to remove large debris, and immediately transported to the laboratory. After being thoroughly mixed and sieved through a 500- μ m sieve, sediments were kept in the darkness at 4 and -20 °C for toxicity testing and chemical analysis, respectively.

Whole-Sediment Toxicity Testing. Acute toxicity of the original and XAD resin-amended sediments were assessed using 3rd instar midges with survival as the endpoint. The tests were conducted in five replicates in 400-mL beakers containing 60 g wet sediment, 300 mL of overlying water and 10 midge larvae. In the course of testing, temperature was maintained at 23 \pm 1 °C, with a photoperiod of 16:8 h. No water change or feeding occurred throughout the bioassays. Temperature, pH, conductivity, dissolved oxygen and ammonia in overlying water were monitored at 0, 24 and 48 h. At the end of the testing, surviving organisms were sieved from the sediment and counted. Control sediment was tested in the same manner as the site sediments.

GPC cleanup. Complexity of the extracts was considerably decreased after gel permeation chromatography (GPC) cleanup, which was not surprising as this method was used to remove macromolecules (such as humic acid) and small interfering molecules (such as sulfur). Humic acid and sulfur if not removed could have substantially confounded EDA diagnosis as humic substances accounted for about 80% of sediment organic matters, and sulfur caused severe overlapping of chromatographic peaks.

The GPC cleanup was conducted using a BioBeads S-X3 preparative column (20 × 300 mm, 40–80 μm). Isocratic elution program was performed with dichloromethane as the mobile phase at a flow rate of 5 mL/min. Test compounds were all dissolved in dichloromethane. Firstly, 0.5 mL of the 17 standard compounds (SI Table S1) (3 μg/mL) was injected into the GPC column via a 2-mL injection loop and eluted with 5 mL/min of dichloromethane. The time window for the standard compounds was 10.5–18.0 min. Secondly, 0.5 mL of humic acid (10 μg/mL) was injected and the retention time was 9.6 (8.6–10.6) min. Lastly, 0.5 mL of sulfur (10 μg/mL) was injected and the retention time was 19.5 (18.0–21.0) min. So, time window for GPC cleanup was 10.5–18.0 min. Three quarters of GPC eluent were solvent exchanged to hexane for further LC fractionation and the remaining quarter was exchanged to DMSO for high-throughput midge toxicity testing.

Sediment Extraction and Cleanup. Contaminants in sediment samples were extracted using accelerated solvent extraction (ASE, Dionex ASE-350, Thermo Scientific, USA). In brief, 10 g of freeze-dried sediment, 2 g of activated copper powder and 25 ng of surrogates (4,4-dibromooctafluorobiphenyl and decachlorobiphenyl) were mixed with diatomaceous earth and then added to 22-mL cells. The sediments were extracted using a mixture of hexane and acetone (1:1, v/v). Static time, extraction cycles, system pressure and temperature for ASE were set at 5 min, 3 cycles, 1500 psi and 100 °C, respectively. The extracts were concentrated and solvent exchanged to 1 mL of hexane, and then divided to two equal portions. One portion was cleaned using a solid phase extraction (SPE) cartridge containing 600 mg of primary secondary amine, 300 mg of graphite carbon black (supelco) and 50 mg of anhydrous Na₂SO₄, and used to analyze pyrethroids and organophosphate pesticides. The other portion was cleaned using a glass column (i.d. 1 cm) packed with 6 cm of alumina, 12 cm of neutral silica gel and 2 cm of

anhydrous Na₂SO₄, and analyzed for musks. After the extracts were loaded onto the columns, 7 mL and 70 mL of a mixture of hexane and dichloromethane (7:3, v/v) was added to elute pesticides and musks, respectively. The effluent was concentrated and solvent exchange to 1 mL of hexane, and 20 ng of internal standards were added before instrumental analysis.

Instrumental Analysis. Suspected toxicants in sediment, GPC-cleaned XAD extracts and laboratory spiked samples were quantified using a Shimadzu TQ8040 GC-MS/MS in electron impact ionization mode. A SH-RXi-5Sil-MS column (30 m × 0.25 mm × 0.25 μm) was used to separate the analytes. One μL of sample was injected at 250 °C in splitless mode. Oven temperature was set at 60 °C for 2 min, ramped to 300 °C at 20 °C/min, and then held at 300 °C for 5 min. Helium was used as carrier gas at 1 mL/min. Temperature of transfer line and ion source was 300 and 200 °C, respectively.

Total organic carbon (TOC) contents in the sediment samples were analyzed using an elemental analyzer (ElementarVavio EL III) after removing inorganic carbon with 1 mol/L HCl.

Data Analysis. The LC50 of the test sediment (LC50_{test sediment}) was calculated to describe sediment toxicity based on EDA bioassays. Relative enrichment factor (REF) is used as a dose metric in constructing dose response relationship to obtain LC50_{test sediment}, which is dimensionless. The REF is calculated using Eq. S1 and detailed steps for the calculation are presented below.

$$\text{REF} = \frac{\text{Enrichment factor}}{\text{Dilution factor}} \quad (\text{S1})$$

(1) Enrichment factor (EF) of bulk sediment sample: 500 g of dry sediment was extracted and the extract was concentrated to 4 mL. So EF = 500 g / 4 mL = 125.

(2) Dilution factor (DF): A series of volume of the XAD extract (2, 5, 10, 20 and 50 μL) was firstly diluted to 100 μL, and then 10 μL of the diluted extract was added to 3.99 mL of

reconstituted water to perform bioassay using the high-throughput in vivo toxicity testing method.

Take 2 μL as an example: $\text{DL} (2 \mu\text{L}) = (100/2) * (4000/10) = 20000$.

(3) Relative enrichment factor (REF): $\text{REF} (2 \mu\text{L}) = \text{EF} / \text{DF} = 125 / 20000 = 0.006$. A series of the REF values were 0.006, 0.016, 0.031, 0.063 and 0.156.

(4) LC_{50} of the test sediment was obtained from the dose (presented as the REF)—response (mortality of the midges at each REF level) curve (SI Figure S3). $\text{LC}_{50_{\text{test sediment}}}$ of sediments S1, S2, S3 and S4 were 0.025, 0.041, 0.019 and 0.025, respectively.

Table S1. Information of the test compounds selected to develop and validate extraction and fractionation methods

Compound	Class	Log K_{ow}	Retention time (min)	
			NPLC	RPLC
Carbaryl	pesticide	2.36 ⁴	32.9	3.3
Triazophos	pesticide	2.92 ⁵	28.3	4.9
Fipronil sulfone	pesticide	3.70 ⁶	33.6	6.1
Fipronil	pesticide	4.00 ⁶	20.5	5.2
Phoxim	pesticide	4.39 ⁵	14.6	6.9
CB4	PCB	4.65 ⁷	7.0	8.0
Phenanthrene	PAH	4.57 ⁸	7.8	8.8
EPN	pesticide	5.02 ⁴	15.6	7.6
Bifenthrin	pesticide	6.00 ⁴	10.4	18.4
<i>p,p'</i> -DDE	pesticide	6.09 ⁴	6.3	15.9
Benzo(a)pyrene	PAH	6.13 ⁸	9.6	17.7
Permethrin	Pesticide	6.60 ⁴	11.6, 13.4	15.5, 16.8
BDE47	PBDE	6.81 ⁹	7.1	17.5
CB156	PCB	7.18 ⁷	5.9	22.3
BDE99	PBDE	7.32 ⁹	7.2	20.6
BDE154	PBDE	7.82 ⁹	7.0	23.3
CB209	PCB	8.18 ⁷	4.7	32.2

Table S2. Organic carbon-water partitioning coefficient ($\log K_{oc}$) and medium lethal concentration (LC50) of three musks and three pyrethroids used for calculating toxic unit.

Class	Chemical	$\log K_{oc}^a$	LC50 _{water} (ng/mL)	LC50 _{sediment} used for calculating toxic unit (ng/g OC)
musk	Galaxolide	4.10	224 ^b	51220 ^e
	Tonalide	4.10	119 ^b	27336 ^e
	Versalide	4.10	6.27 ^b	1440 ^e
pyrethroid	Bifenthrin	5.32	NA ^c	6200 ^f
	Cypermethrin	5.14	0.53 ^d	1340 ^g
	Permethrin	5.07	NA ^c	24500 ^f

^a The $\log K_{oc}$ values were obtained from US EPA EPI Suite™

(<https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface>)

^b The LC50 values of the three musks for 72-h water only toxicity were determined using the high-throughput in vivo toxicity testing method in the present study

^c Not available

^d The LC50 value of cypermethrin for 96-h water only toxicity was obtained from Mehler et al.¹⁰

^e The LC50 values of three musks for calculating toxic unit (LC50_{sediment}) were calculated by the

following equation: $LC50_{sediment(i)} = \frac{LC50_{water(i)}}{LC50_{water(cypermethrin)}} \times \frac{K_{oc(i)}}{K_{oc(cypermethrin)}}$, where i is one of the three

musks.

^f The LC50 values of bifenthrin and permethrin for 10-d sediment toxicity were obtained from Maul et al.¹¹

^g The LC50 value of cypermethrin for 10-d sediment toxicity was obtained from Maund et al.¹²

Table S3. Information of the three musks (versalide, galaxolide and tonalide)

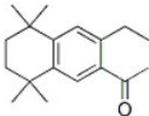
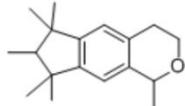
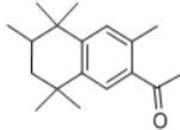
Compound	CAS No.	Molecular formula	Molecular weight	Structure	log K_{ow} ¹³	Solubility (mg/L) ¹⁴	GC-MS/MS quantification			
							Target m/z	Reference m/z	Voltage	Retention time (min)
Versalide	88-29-9	C ₁₈ H ₂₆ O	258.4		5.7	1.27	258>243	243>187 243>57	15	10.24
Galaxolide	1222-05-5	C ₁₈ H ₂₆ O	258.4		5.7	1.75	243>213	258>243 243>143	15	10.72
Tonalide	1506-02-1	C ₁₈ H ₂₆ O	258.4		5.7	1.25	258>243	243>187 243>57	15	10.76

Table S4. The total organic carbon (TOC) contents (%) of the four sediment samples and concentrations (ng/g dry weight) of three pyrethroids and three musks in sediments.

	S1	S2	S3	S4
TOC content (%)	7.10%	3.00%	1.40%	9.00%
Galaoxlide	468	422	559	4060
Tonalide	25.3	47.0	289	170
Versalide	118	31.4	89.6	39.2
Bifenthrin	6.55	1.22	4.58	27.5
Cypermethrin	167	43.7	361	486
Permethrin	73.0	21.7	69.6	314

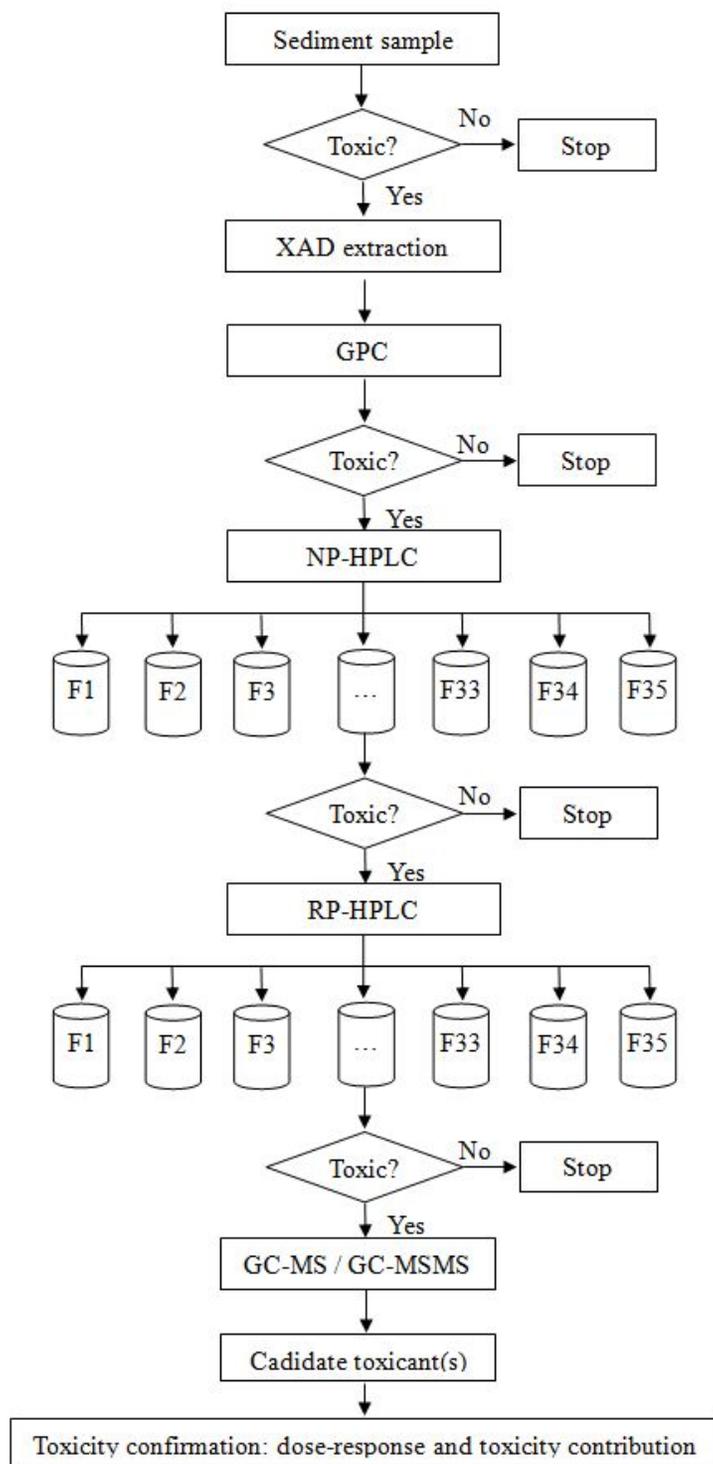


Figure S1. Flowchart of the stepwise procedure of the EDA

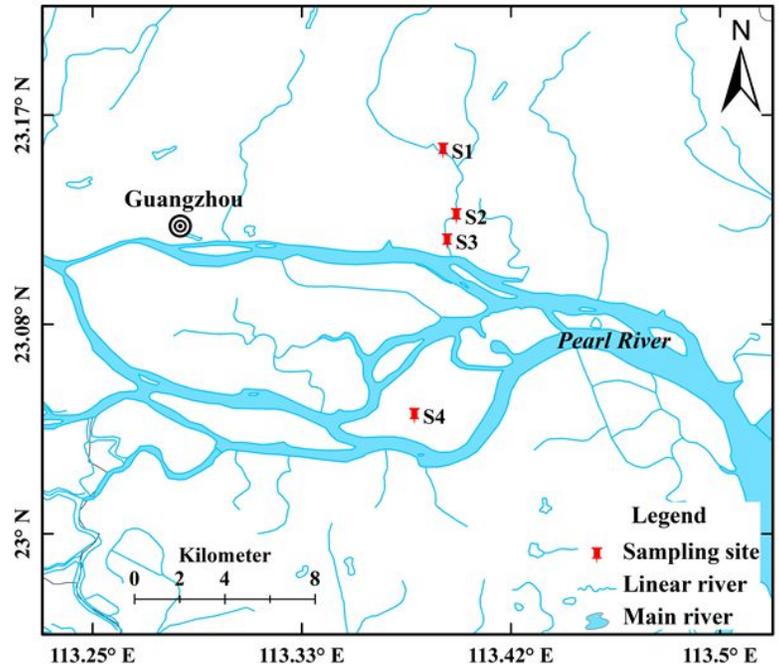


Figure S2. Map of sampling sites in urban waterways of Guangzhou, China.

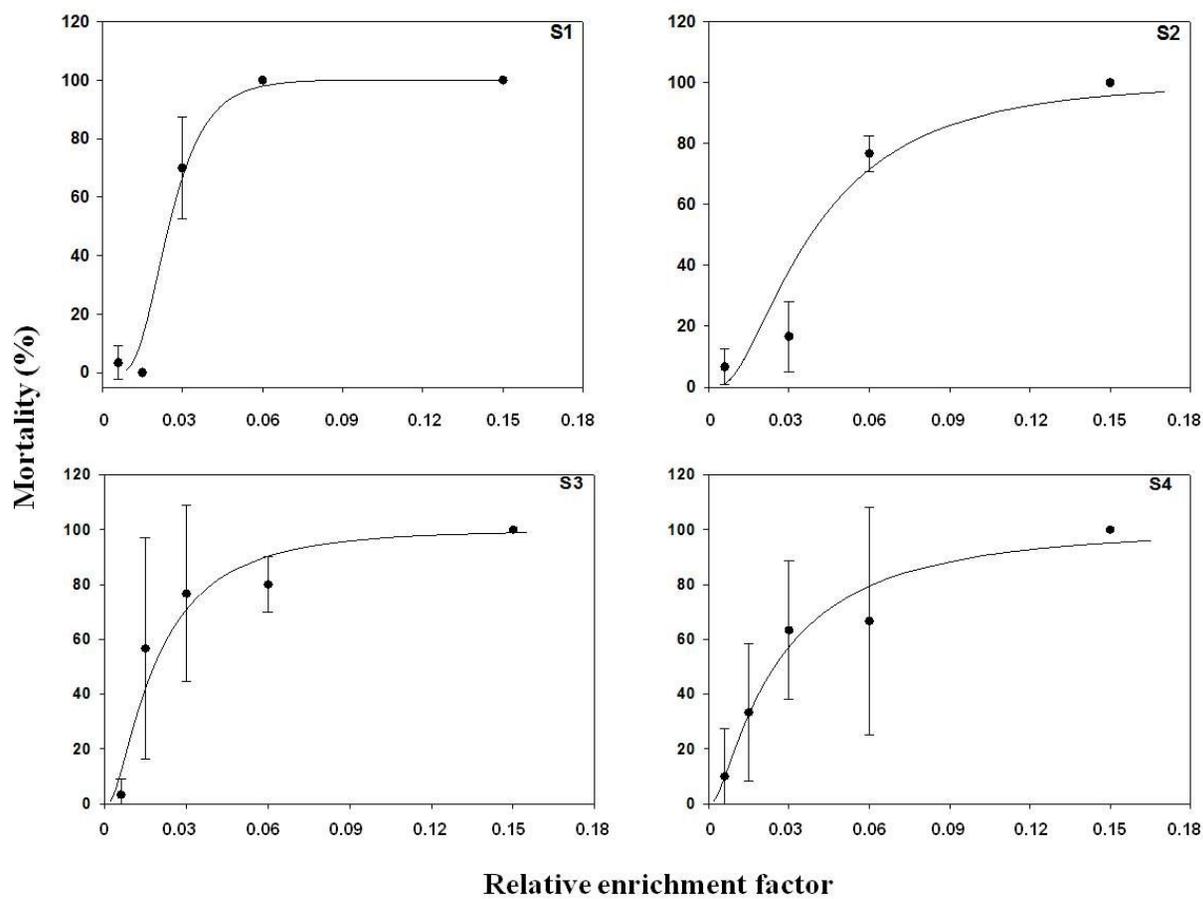


Figure S3. Dose-response curves of XAD extracts to *Chironomus dilutus* for the sediments from urban waterways in Guangzhou, China

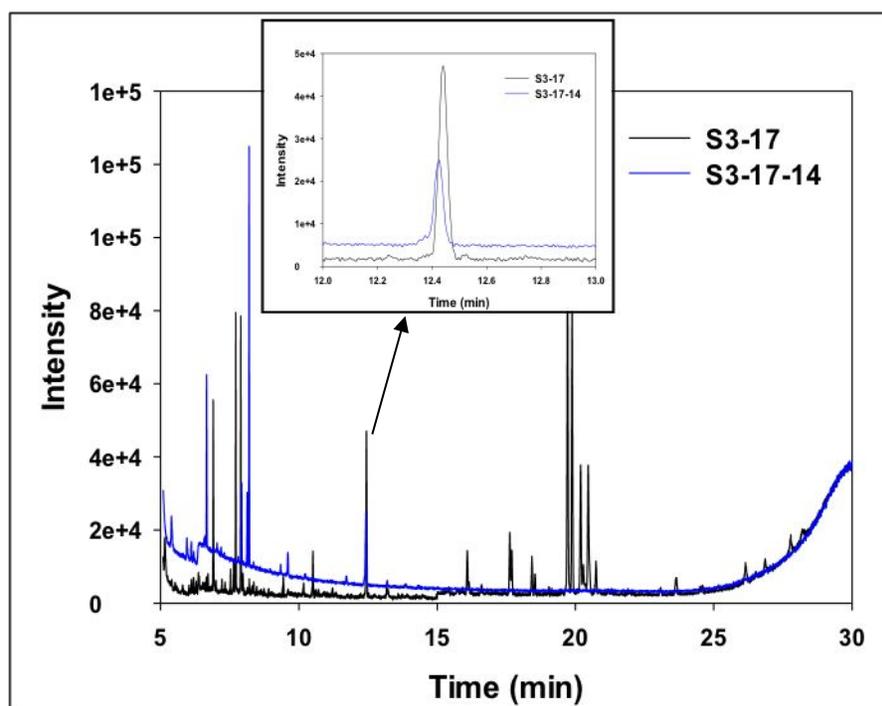


Figure S4. GC-MS spectrograms of NPLC (S3-17) and RPLC (S3-17-14) fractions of the XAD extracts

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