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PAPER

Polycyclic aromatic hydrocarbons and ecotoxicological characterization of sediments from the Huaihe River, China†

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The distribution, source, ecological risk and ecotoxicity of polycyclic aromatic hydrocarbons (PAHs) of sediments from 7 sampling sites, named as Xinyang (XY), Huainan (HN), Bengbu (BB), Xuyi (XuY), Fuyang (FY), Mengcheng (MC) and Zhengzhou (ZZ), in the Huaihe River basin, China, have been investigated. The total concentrations of 16 USEPA priority PAHs ranged from 62.9 to 2232.4 ng g^{-1} dry weight (d.w.) with a mean concentration of 1056.8 ng g^{-1} d.w. Through the assessment of ecological risk, we found that the levels of PAHs in the Huaihe River should not exert adverse biological effects. The total benzo[*a*]pyrene toxicity equivalent (TEQ) values calculated for samples varied from 0.01 to 194.1 ng g^{-1} d.w., with an average of 65.9 ng g^{-1}. The toxicity data were accordant with the chemical analysis results in this study. HN, BB and ZZ showed the greatest pollution extent both in the chemical analysis and the study of ecotoxicological effects.

Introduction

The Huaihe River, one of the seven largest rivers in China, flows through Henan, Anhui, Shandong and Jiangsu provinces and into the Hongze Lake in Jiangsu Province.[1] Excessive economic growth, industrialization and urbanization have resulted in the deterioration of the water quality.[2] The river has become more and more vulnerable and probably one of the most polluted rivers in China.[3] Contamination of the water has seriously affected the living standard of the people along the river. In 1995, the China State Council (CSC) had promulgated a regulation to deal with the increasingly serious environmental problems of the Huaihe River.[4] During the past decade, great efforts have been made by the Chinese government to treat the Huaihe River. After the implementation of two five year plans,[5,6] significant progress has been made in the prevention and control of water pollution of the Huaihe River basin. However, affected by lots of factors, the targets of the two plans were not realized.

Sediments serve both as a sink and a source for a number of environmental pollutants and they exchange chemicals, such as polycyclic aromatic hydrocarbons (PAHs), with the water phase.[7] PAHs are chemical compounds that consist of fused aromatic rings and do not contain heteroatom or carry substituents.[8] As pollutants, they are of concern because some compounds have been demonstrated to be carcinogenic, mutagenic, and teratogenic.[9] Moreover, many PAHs have slow degradation rates and can persist in the environment for long periods and tend to bioaccumulate and biomagnify in the food

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Environmental impact

PAHs are an important class of persistent organic pollutants (POPs) in the environment and sediment is one of the most important reservoirs of such pollutants. Analysis of these sedimentary contaminants can be used for assessment and interpretation of their impact on aquatic environments. However, much work has focused on only chemical analysis. This paper attempted to combine chemical analysis and toxicity bioassay to investigate the distribution, source, ecological risk and ecotoxicity of PAHs in sediments of the Huaihe River, which was one of the most polluted large rivers in China. This work could provide some information about the PAH pollution in this region and is an exploration in connecting chemical analysis with ecotoxicological characterization.

chains.[10] Due to floods or human activities, residues associated with sediments can be resuspended and become bioavailable.

Some research on PAHs pollution in sediments of the Huaihe River have been reported.[11–15] However, their studies are often limited to the partial Huaihe River and focused on only chemical analysis. In this paper, we attempt to combine chemical analysis and toxicity bioassay to investigate the distribution, source, ecological risk and ecotoxicity of PAHs in sediments of the whole Huaihe River. The main object of this work is to disclose the pollution status of PAHs in the sediments of Huaihe River. We hope the preliminary results might be helpful for the investigation of water pollution in the Huaihe River basin.

Materials and methods

Sampling

The sampling took place during the summer of 2008. There were 7 sampling sites, named as Xinyang (XY), Huainan (HN), Bengbu (BB), Xuyi (XuY), Fuyang (FY), Mengcheng (MC) and Zhengzhou (ZZ). The distribution of 7 sampling sits is shown in Fig. 1. The descriptions of sampling sites are summarized in Table S1, ESI.† Triplicate sediment samples (0–10 cm depth) were collected from each site by stainless steel grab. The sediment samples from each site were mixed and sub-sampled to ensure the representation. Approximately 1 kg of the middle part of each sample was put in clean glassware (washed with washing-powder) and immediately stored in an icebox. Care was taken to avoid any contamination during their transport to the laboratory.

Physicochemical properties analysis

The total organic carbon (TOC) was measured using a Multi N/C Total Organic Carbon Analyzer (Carl Zeiss Yena, Germany) by combustion oxidation method. Samples (5 mg) were combusted at 1300 °C. pH value was determined by ISO 10390 method,[16] after the suspension of the substrate in a 1 M aqueous solution of potassium chloride. Samples were also characterized by particle size distribution using an Easysizer20 Lzer Particle Sizer (OMCE Technology Co., Ltd., Zhuhai, China).

Sample extraction and purification

Sample extraction and cleanup for PAHs analysis was performed according to the literature.[17] 20 g of freeze-dried and homogenized sample, adding 1 g copper power to remove the interference of sulfur, was extracted by 160 mL *n*-hexane/acetone (1 : 1, v/v) in a water bath at 70 °C for 24 h using a Soxhlet Extractor. The extract was concentrated to about 2 mL by a rotary evaporator under the vacuum and further concentrated to 1 mL in highly pure nitrogen. The concentrated extract was passed through a silica gel column (6 cm, 200–300 mesh, wet packing method) with a layer of 0.5 g sodium sulfate on the top. The elution was repeated by 10 mL dichloromethane and 10 mL *n*-hexane, and the collected solution was concentrated by means of a rotary evaporator to nearly 2 mL under the vacuum and further concentrated to 1 mL in the high pure nitrogen.

Sample extraction and cleanup for bioassay was performed according to the literature.[2] 10 g freeze-dried and homogenized sample was mixed with anhydrous sodium sulfate and extracted with 60 mL dichloromethane for 24 h in a Soxhlet Extractor. The extract was then purified with a column consisting of silica gel (60–80 mess, deactivated, 4 cm long) and anhydrous sodium sulfate (4 cm long), treated with copper powder to remove sulfur and evaporated to dryness by blowing with a stream of high purity nitrogen.

Gas chromatography/mass spectrometry (GC/MS) analysis

16 USEPA priority PAHs were analyzed by GC/MS according to the method of USEPA 8270C.[18] GC/MS analysis was carried out with gas chromatography (GC, Hewlett-Packard 6890 Series II, Agilent Technology). A HP-1 25 m × 0.20 mm I.D., 0.11 μm film (Agilent Technology) fused silica capillary column was used. The GC oven temperature was programmed as follows: 45 °C hold for 1 min, raised at 6.0 °C min^{-1} to 200 °C, then at 8.0 °C min^{-1} to 300 °C (held for 1 min). Helium was the carrier gas, at a flow of

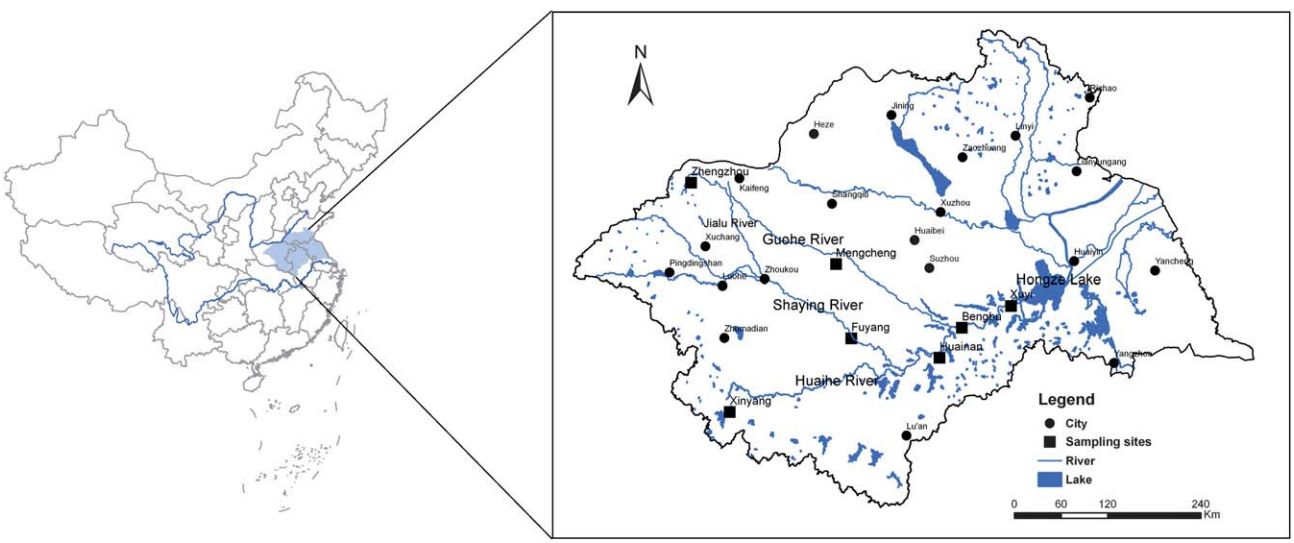

Fig. 1 Map of the Huaihe River showing the sampling sites.

0.615 mL min^{-1}. The injection was set on a splitless mode at 250 °C. The injection volume was 1.0 μL. The solvent delay was 3.00 min and total run time was 39.33 min. Detection was conducted by a mass selective detector with electron impact ionization in selected ion monitoring mode. MS transfer line temperature was at 280 °C. The MS was operated in full scan in electron ionization mode with an electron multiplier voltage of 2200 V. The mass scanning ranged from *m/z* 50 to 550. Mass spectra were compared to reference compounds in the National Institute of Standards and Technology (NIST) library. Identification of individual PAHs was based on the comparison of retention time data between samples and the standard solution containing 16 PAHs (Aladdin Reagent Inc., Shanghai, China). Quantification was performed by integration of the selected ion chromatograms extracted from the total ion current. The internal calibration method (hexamethylbenzene) for quantification was applied based on a five-point calibration curve for each individual component. The retention time of the internal standard was used to correct the variation of the retention time. This operation was performed automatically by the Hewlett-Packard MS chemstation.

Quality control and quality assurance

All analytical data were subject to strict quality control. The instruments were calibrated daily with the calibration standards and the relative percent difference between the five point calibration and the daily calibrations were less than 20% for all target analyses. Method blanks (solvent), spiked blanks (standards spiked into solvent), matrix spiked duplicates and sample duplicates were routinely analyzed together with the sediment samples. The PAH content of the method blanks was less than 2% of the content in the sediment samples. The average recovery of the PAHs was 83%, except for naphthalene which was 52%, with the relative standard derivation less than 18%.

Ecotoxicological bioassay of sediment extracts

The potential ecotoxicological effect of the sediment extracts were assessed by the ethoxy resorufin-*o*-deethylase (EROD) method.[17] Briefly, 2.5×10^3 H4-IIE rat hepatoma cells were seeded into individual wells of a 96-well microtiter tissue culture plate. The cells were grown for 24 h to about 60–70% confluence and exposed with samples for another 24 or 72 h. An 8-well lane was used for replication. The plate was incubated at 37 °C, in a 10% carbon dioxide incubator. The sediment extracts dissolved in dimethylsulfoxide (DMSO) were added directly to the medium. The final concentration of DMSO was less than 0.5%. The same amount of DMSO was added to control cultures. EROD enzyme activity was determined directly in intact rat hepatoma cells cultured on 96-well plates. Monolayers were washed twice with phosphate-buffered saline. Assays were started by addition of 200 μL per well of culture medium containing 8 μM 7-ethoxyresorufin and 20 μM dicumarol. After 30 min incubation at 37 °C, a 100 μL aliquot of cell medium was withdraw from each well and transferred to another 96-well plate. Then, 200 μL of ethanol was added to each well. Fluorescence of the product resorufin, as the relative EROD induction value, was recorded directly on a Microplate Reader (Labsystems Fluoroskan II, Finland) at 550 nm excitation and 585 emission wavelengths.

Toxicity test of sediments

In addition, we have carried on a toxicity test of sediments using *Mercenaria mercenaria* (*M. mercenaria*).[19] The juvenile clams (1.2–1.4 mm) were obtained from the Dafeng Demonstrational Park of Intertidal Land Fisheries Science and Technology (Yancheng, China). They were held at 20 °C in seawater, fed *Tetraselmis sp.* in the laboratory for 24 h before use. 100 mL glass beakers with 20 g of different sediments and 60 mL seawater were used as exposure chambers. Ten clams were placed into each triplicate exposure chamber. Clams with sediment from the demonstrational park were used as a control. An additional 40 clams were used to determine the initial weight for the growth staring point. After 7 days, clams were sieved from the sediments. Mortality was recorded and live clams were rinsed, dried, and weighed to determine growth.

Results and discussion

Physicochemical properties

The physicochemical properties of sediments collected from the 7 different locations of the Huaihe River are presented in Table 1. TOC values in these sediments ranged from 5.4% to 15.4% dry weight (d.w.). High TOC value were observed in XY, possibly an influence of the residual weed roots in the sediments. pH values ranged from 7.9 to 8.9 and showed alkalescency. This is mainly because the Huaihe River has received large quantities of alkalescent industrial effluents. The percentage of particles with a size below 65 μm in the HN, BB, FY, MC and ZZ samples was higher than 85% suggesting that these sediments mainly consisted of the fine particles. However, the percentage in XY and XuY was only approximately 70% because the River at XY and XuY is narrow and the high flow velocity makes it hard for the fine particles to deposit.

Concentration and distribution of PAHs

Table 1 lists the concentrations of the 16 PAHs in the 7 samples. As shown in Table 1, all 16 PAHs were detected in these sample extracts. The total concentration of the 16 PAHs in the sediments ranged from 62.9 to 2232.4 ng g^{-1} d.w. with a mean concentration of 1056.8 mg kg^{-1} d.w. The spatial distribution of the PAHs in the sediments was site-specific. HN and BB from the midstream of the Huaihe River had the highest concentrations, indicating that the midstream of the Huaihe River was the most seriously polluted. Both the two sites were affected by industrial effluent from a nearby chemical plant. Moreover, the placer dredges and power station could significantly affect HN. The PAH concentrations at XY from the upstream of the Huaihe River were much smaller than that at HN and BB. This suggested that the pollution of the upstream of the Huaihe River was less. Around the XY site, there was a village and the industrial pollution was weak. XuY, which is located in the downstream of the Huaihe River, is an ecological agriculture area and is not heavily polluted by domestic or industrial water discharge, but the total PAHs concentration of XuY was higher than that of

Table 1 Physicochemical properties and PAHs concentrations of sediments from 7 sampling sites

	XY	HN	BB	XuY	FY	MC	ZZ
TOC (% d.w.)	1.54	0.88	0.75	0.94	1.02	0.54	1.48
pH	7.9	8.9	8.7	8.1	8.4	7.8	8.2
Particles sizes < 65 μm (%)	68.75	87.23	86.65	70.57	97.84	94.58	86.51
PAH concentration (ng g^{-1} d.w.)							
Naphthalene (Nap)	1.6	81.1	124.7	ND[a]	56.1	ND[a]	178.0
Acenaphthylene (Acy)	1.0	42.1	127.8	ND[a]	ND[a]	ND[a]	71.4
Acenaphthene (Ace)	3.6	152.1	38.9	175.9	20.1	5.1	73.2
Fluorene (Fl)	11.6	114.3	12.4	172.2	68.7	21.1	283.1
Phenanthrene (Phe)	81.5	567.9	217.7	2.5	91.6	14.6	60.6
Anthracene (Ant)	8.1	301.8	181.6	9.3	115.4	1.4	181.3
Fluoranthene (Fla)	72.0	465.9	414.8	8.0	20.3	12.0	285.0
Pyrene (Pyr)	55.6	204.8	222.2	2.5	10.5	7.7	156.2
Benz[*a*]anthracene (BaA)	75.1	29.6	31.8	ND[a]	94.6	ND[a]	132.6
Chrysene (Chr)	11.3	39.8	49.4	4.6	ND[a]	ND[a]	142.1
Benzo[*b*]fluoranthene (BbF)	12.0	39.9	14.9	ND[a]	69.5	ND[a]	107.9
Benzo[*k*]fluoranthene (BkF)	5.0	15.8	20.5	25.8	29.6	1.0	121.4
Benzo[*a*]pyrene (BaP)	6.7	2.1	4.4	16.3	92.5	ND[a]	98.1
Indeno[1,2,3-*c*,*d*]pyrene (InP)	ND[a]	0.9	ND[a]	8.0	ND[a]	ND[a]	147.7
Dibenzo[*a*,*h*]anthracene (DahA)	1.1	1.9	0.6	23.6	79.6	ND[a]	55.8
Benzo[*g*,*h*,*i*]perylene (BghiP)	1.2	1.5	0.9	11.3	21.0	ND[a]	138.0
ΣPAH[b]	347.4	2061.5	1462.6	460.0	770.5	62.9	2232.4
ΣCPAH[c]	99.9	90.2	72.2	73.7	365.8	1.0	663.5
TEQ	16.6	11.2	9.9	41.0	188.8	0.01	194.1

[a] ND = not detected. [b] ΣPAH = total concentration of the 16 PAHs. [c] ΣCPAH = sum concentration of BaA, BaP, BbF, BkF, DahA and InP.

XY. This is possibly related to the exposure to the effluent of Anhui province in the past decades. The river Shaying and the river Guohe are the main tributaries of the Huaihe River. However, their pollution statuses are different. The total PAH concentration of FY was much higher than at MC and MC had the lowest PAH concentration. In addition, we analyzed the PAH concentration at ZZ, located on the Jialu River, which is upstream of the Shaying River and high PAH concentration was observed. This is possibly related to the effect of garbage dumps and a food factory. Domestic rubbish pollution is a great problem in the Jialu River and large quantities of garbage dumps along the river are needed.

Some previous studies involved the PAH concentrations of the sediments from some sites on the Huaihe River. Wang *et al.*[11] analyzed the normalized concentrations of 13 PAHs (without Acy, Inp and BghiP) in the sediment samples from Xinyang city and Huainan city with semipermeable membrane devices (SPMD) and disclosed that the total concentrations of 13 PAHs were 7.7 and 9.73 ng mg^{-1} of organic carbon (o.c.), respectively. This is far below the pollution level of XY and HN (22.56 and 234.26 ng mg^{-1} o.c.) observed in our study and the use of SPMD might affect the analysis results. Huang *et al.*[12] also investigated the PAHs in surface sediments in Jiangsu section and found the total concentrations of 12 PAHs (without Nap, Acy, BaA and BbF) were 690–6630 ng g^{-1} d.w., which are higher than that at XuY in our study.

A comparison of PAH concentrations in surface sediments collected from different rivers is given in Table 2. The PAH concentrations in surface sediments from the Huaihe River in this study are similar to those detected in the river Gomti, India[20] and the Yellow River, China,[21] and higher than those found at the Yalujiang River, China,[22] the Sava River, Serbia,[23] the Luan River, China[24] and the Daliao River, China.[25] However, the levels were lower than those reported in the Zhujiang River, China,[26] the Passaic River, USA,[27] the Morava River, Czeck Republic[28] and the Scheldt River, France.[29]

In addition, the TOC contents of the sediments did not appear to correlate with the total PAH concentrations ($r = 0.234$, $p > 0.05$), suggesting that sediment contamination in the study area may be dictated more predominantly by anthropogenic inputs than by natural processes.[26] There was also no correlation between the total PAH concentrations and particle sizes ($r = 0.202$, $p > 0.05$), which is different in some marine sediments.[30]

Composition and source of PAHs

The composition pattern of PAHs by ring size in sediments is shown in Fig. 2. As shown in Fig. 2, 3-ring PAHs were the most abundant in HN, XuY and MC samples, whereas 4-ring PAHs were the most abundant in XY and BB samples. However, 3- and 5-ring PAHs were the most common in the FY sample. 3- and 4-ring PAHs were comparable in the ZZ sample.

Based on characteristics in PAH composition and distribution pattern, the sources of anthropogenic PAHs can be distinguished by ratios of the individual PAH compounds.[31] In general, a Phe/Ant ratio < 10 is seen as characteristic of combustion processes, whereas a ratio > 10 is characteristic of a petroleum input or diagenesis.[32] Fla/Pyr < 1 is attributed to a petrogenic source, while a ratio > 1 is obviously related to pyrolytic origins.[33] Fla/(Fla + Pyr) ratio < 0.4 is attributed to a petrogenic source, a ratio > 0.5 suggests grass, wood and coal combustion, while between 0.4 and 0.5 is characteristic of petroleum combustion.[34] As shown in Table S2,† except at XY and MC, a Fla/Pyr ratio > 1 and Phe/Ant ratio < 10 indicated a pyrolytic origin. A Fla/(Fla + Pyr) ratio > 0.5 confirmed this kind of source and further indicated this source was a biomass and coal combustion process. However, the Phe/Ant ratios at XY and MC were close to 10, suggesting that the PAH pollution in the two sites was also

Table 2 A comparison of PAHs concentrations in surface sediments collected from different rivers (ng g^{-1} d.w.)

Location	Range	Mean	Reference
Gomti River, India	68–3153	1182	20
Yellow River, China	464–2621	1414	21
Yalujiang River, China	68–1500	290	22
Sava River, Serbia	416.2–593.3	501.6	23
Luan River, China	6.7–1585.7	342.9	24
Daliao River, China	61.9–840.5	287.3	25
Zhujiang River, China	1434–10811	4892	26
Passaic River, USA	220–8000000	145000	27
Morava River, Czeck Republic	636–13205	4997	28
Scheldt River, France	4900–96000		29
Huaihe River, China	62.9–2232.4	1056.8	This study

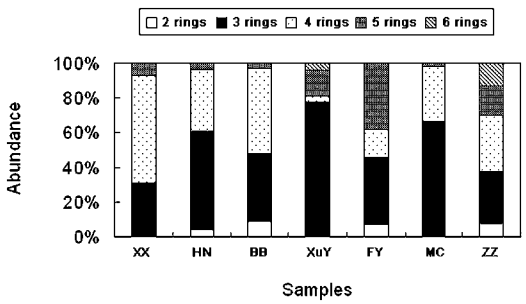

Fig. 2 Distribution of 2-, 3-, 4-, 5- and 6-ring PAHs in the sample extracts.

influenced by petrogenic sources. The results of individual PAH ratios were also confirmed by a principal component analysis (PCA). As shown in Fig. S1†, DahA, BaP, BghiP, InP, BaA, BkF, BbF and Fl constituted the first cluster. High loadings of these heavy-weighted PAHs indicated combustion of fossil fuel (ESI†).

Ecological risk of PAHs

Sediment quality. According to Long *et al.*'s research,[35] when concentration of the environmental PAHs is less than the effects range-low (ERL), the incidence of adverse effects is small. Whereas when the concentration is higher than the effects range-median (ERM), the adverse effects will be frequently produced. In order to evaluate the ecological risk of PAHs from the Huaihe River, we took risk assessment guideline values for PAHs (Table 3) as controls. Table 3 misses the values of BbF, BkF, DahA and BghiP, because these PAHs have no minimum safety values. As long as they exist in the environment, they will exhibit adverse effects on an organism. As shown in Table 3, the total PAH concentrations at all sites were below the ERL. And for all sites, individual PAH did not exceed ERM, but at some sites there was at least one PAH that might occasionally pose biological impairment (with a concentration higher than ERL). These findings indicated that in some samples there might be individual PAHs that occasionally pose biological impairment, but no samples had constituents that might frequently pose biological impairment (with concentration higher than the ERM).

Table 3 Risk assessment guideline values for PAHs in sediment matrix (ng g^{-1})

			This study	
Compound	ERL	ERM	Average	Maximum
Nap	160	2100	63.1	178.0
Acy	44	640	34.6	127.8
Ace	16	500	67.0	175.9
Fl	19	540	97.6	283.1
Phe	240	1500	148.0	567.9
Ant	853	1100	114.1	301.8
Fla	600	5100	182.6	465.9
Pyr	665	2600	94.2	222.2
BaA	261	1600	52.0	132.6
Chr	384	2800	35.3	142.1
BbF	NA[a]	NA[a]	34.9	107.9
BkF	NA[a]	NA[a]	31.3	121.4
BaP	430	1600	31.4	98.1
InP	NA[a]	NA[a]	22.4	147.7
DahA	63.4	260	76.42	173.67
BghiP	NA[a]	NA[a]	23.2	79.6
∑PAH	4000	44792	1056.8	2232.4

[a] NA, not available.

Sediment toxicity. Some PAHs and especially their metabolic products are of great concern due to their documented carcinogenicity. These potentially carcinogenic PAHs (CPAHs) include BaA, BaP, BbF, BkF, DahA and InP.[36] The total concentrations of CPAHs in sediments from the Huaihe River varied from 1.0 to 663.5 ng g^{-1} d.w. with the average of 195.2 ng g^{-1}, and accounted for 1.6–47.5% of the total PAH concentrations (Table 1).

Among the CPAHs, BaP is the only PAH for which toxicological data are sufficient for derivation of a carcinogenic potency factor.[37] In order to assess the sediment toxicity, the concentrations of the above mentioned CPAHs and Chr were converted into the total BaP toxicity equivalent (TEQ) using the corresponding toxic equivalency factors (TEFs) (ESI†).[38] Total TEQ values calculated for samples from the Huaihe River varied from 0.01 to 194.1 ng g^{-1} d.w., with an average of 65.9 ng g^{-1}(Table 1). The maximum total TEQ value was found at FY and ZZ. Among different PAHs, contribution to the total TEQ decreased in the order: BaP (47.9%) > DahA (35.4%) > BaA (7.9%) > BbF (4.8%) > Inp (3.4%) > BkF (0.48%) > Chr (0.0054%) (Fig. S2†). In comparison with other studies (Table S3†), the total TEQ values in sediments of the Huaihe River were lower than that detected in Meiliang Bay, Taihu Lake, China,[39] the Gulf of Gemlik, the Marmara sea, Turkey[40] and the Guba Pechenga, the Barents Sea, Russia.[36] However, the TEQ levels were close to that reported in the Sundarban Mangrove, Wetland, India,[41] and higher than that reported in the coastal lagoons of central Vietnam.[42]

Ecotoxicological effect

EROD test. Extensive studies have suggested that EROD activity in the liver can serve as a biomarker of exposure to PAH and PCB types of pollutants[43] and for assessing the risks of environmental samples *in vitro*.[44] Fig. 3(a) is the EROD induction of the sample extracts as a function of the extract dosage. It could be seen that all 7 sample extracts could induce the activity

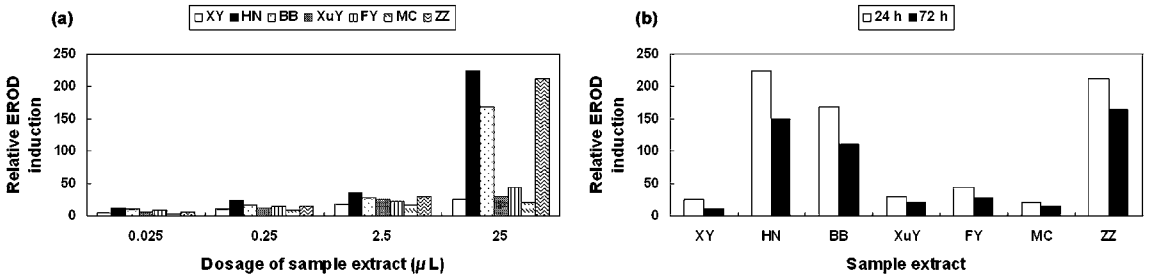

Fig. 3 EROD test: (a) EROD induction as a function of the dosage of the extracts; (b) EROD induction after 24 and 72 h exposure to extracts.

of EROD during a 24 h incubation with dose-response relationships. Higher dosage of sample extract showed greater induction of EROD. When the dosage of sample extract was 25 μL, extracts of HN, BB and ZZ showed potential induction of EROD, suggesting that the sediment extracts contained plenty of aryl hydrocarbon receptor-active residues which could induce the activity of EROD.[45] EROD inductions of the cells exposed to 25 μL extracts after 24 and 72 h incubation were illustrated in Fig. 3(b). Generally most organic chemicals could be metabolized after a 72 h incubation,[46] and then the level of induced EROD activity decreased. However, it is obvious that after 72 h of incubation, the high EROD activity still remained, indicating the presence of the persistent organic contaminants in all samples, such as PAHs. In addition, based on the preliminary ecotoxicological results, the pollution extent of HN, BB and ZZ was higher than at the other sites.

***M. mercenaria* test.** *M. mercenaria* is an ecological and economically important species. Ho *et al.*[47] used a series of experiments to demonstrate the toxicity of sediments to *M. mercenaria* was caused mainly by PAHs. Therefore, we also carried out the toxicity test to investigate the ecotoxicological effect of sediments from the Huaihe River. The results are plotted in Fig. 4. As shown in Fig. 4, clams exposed to Huaihe River sediments resulted in significantly lower growth than those in the control experiment. ZZ with the highest PAH concentration showed the most toxicity to clams and MC with the lowest PAH concentration showed the least toxicity. Reduced growth rates seemed to be linked to the pollution extent.

Correlation between PAHs contents and ecotoxicological effect

Chemical analysis of PAHs in sediment help in determining the magnitude of anthropogenic contamination by identifying the pollutants and also provide estimates of their concentration. However, they do not provide any information about contaminant effect on the biota. Toxicity bioassays, on the other hand, furnish us with reasonable information concerning the biological impacts of sediment contaminants, but provide no indication as to the causes of the observed toxic responses.[48] To fully evaluate the environmental impact of sediment contaminants, both chemical and biological analysis should be carried out.[49] However, it is evident that the measured toxicity of sediments vary with the biotest applied and the concentrations of pollutants in the sediment are not always related to their toxicity.[50]

However, in our experiment, a significant correlation between the total PAH concentrations and the relative EROD induction ($r = 0.977$, $p < 0.01$) was observed. For instance, HN and ZZ samples with the highest PAH concentrations were the most toxic and MC sediment with the lowest PAH concentration exhibited the least toxicity. This suggested that PAH content was possibly the main factor that contributed to the toxicity of these sediments. EROD activity can serve as a biomarker of exposure to PAH and PCB types of pollutants. Though we did not evaluate the effect of PCBs, our preliminary results demonstrated the PAHs contributed to the EROD activity. This is possibly because the concentrations of PAHs are usually much higher than that of PCBs. However, we could not say that the EROD assay could indicate the PAH content in the sediments and more observations are needed in a future study.

Likewise, there seemed to exist a good correlation between total PAH concentration and the deceleration of the clam's growth ($r = 0.848$, $p < 0.05$). Ho *et al.*[47] carried out a chemical analysis of *M. mercenaria* with DNA damage, which were exposed to contaminative sediments, and found high concentrations of PAHs in the clam digestive gland tissues but no detectable or low levels of PCBs and pesticides. This suggested that PAHs were bioavailable compounds for *M. mercenaria* and the toxicity for *M. mercenaria* was mainly caused by these PAHs. However, this is just one case of toxicity of sediments caused mainly by PAHs. The ecotoxicological effects of sediments are complex and affected by many factors. PAHs, the most abundant pollutants in the sediments, must play an important role during the ectoxicological process.

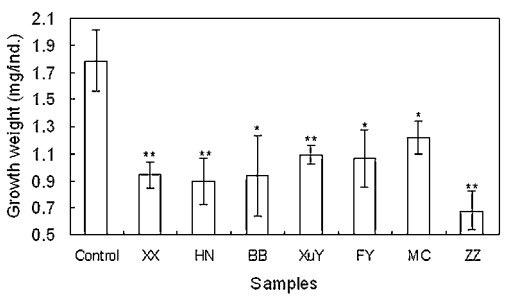

Fig. 4 *M. mercenaria* test. The values were obtained from the dry weight of clams minus their initial weight. Each value is the mean ± S.D. * $p < 0.05$, ** $p < 0.01$ *vs.* Control (Dunnett's test).

Conclusions

In this paper, we have combined chemical analysis and toxicity bioassay to investigate the distribution, source, ecological risk and ecotoxicity of PAHs in sediments of the whole Huaihe River. Based on the results, we found that the extent of PAH pollution

at HN, BB and ZZ was higher than at the other sites. By fingerprint analysis, PAHs in the sediments were found to be of mostly pyrogenic origins. However, the PAH pollution in XY and MC were also influenced by petrogenic sources. When assessing the ecological impairment, it was found in some samples that there may be individual PAH that occasionally pose biological impairment, but no samples had constituents that may frequently pose biological impairment. When the potentially carcinogenic PAHs were normalized by multiplying by respective TEFs, the maximum total TEQ was found at FY and ZZ. There are good functional correlations between PAH content and ecotoxicological effects, including the EROD and *M. mercenaria* tests. However, we could not say definitively that these biological assays could indicate the PAH content in the sediments and more observations are needed in a future study.

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