



## Phthalate acid esters in *Potamogeton crispus* L. from Haihe River, China

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### ABSTRACT

This study was conducted in Haihe River, China, in order to assess the ability of *Potamogeton crispus* L. (*P. crispus*), a submerged plant, to accumulate phthalic acid esters (PAEs). Dibutyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) were measured in samples of water, sediment and *P. crispus* plant from March to May 2008. The results showed that the highest levels of DBP and DEHP in above-ground tissues appeared during the blooming period of *P. crispus*, which was consistent with the results obtained with the water samples. Regression analysis reveals that concentrations of PAEs in above-ground tissues were mainly influenced by PAE concentrations in the water of Haihe River. Enrichment of DBP and DEHP in above-ground tissues was observed, with bioconcentration factors (BCFs) of 4.82–83.65 L kg<sup>-1</sup> for DBP and 6.71–93.70 L kg<sup>-1</sup> for DEHP. The distribution pattern of DBP in roots, surface and near root sediments was different from that of DEHP. Concentrations of DBP in near root sediments were lower than those in roots and comparable to those in surface sediments, while concentrations of DEHP in roots were lower than those in near root sediments but higher than those in surface sediments.

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### 1. Introduction

Phthalic acid esters (PAEs) are one class of the most widely used man-made compounds. They have been used as plasticisers for a long time to enhance the flexibility and technical properties of material. The worldwide production of PAEs has reached 2.7 million metric tons per year (Bauer and Herrmann, 1997). PAEs have been found in all types of environmental and many biological samples (Sha et al., 2007; Huang et al., 2008; Wang et al., 2008a,b) and have been listed as 'priority pollutants' by the US EPA (Keith and Telliard, 1979). Recently, PAEs have been suspected to cause estrogenic effects in mammals including humans, and thus damage the reproductive systems (Parmar et al., 1995; Lee and Koo, 2007; Matsumoto et al., 2008). Aquatic plants have a great potential to function as in-site and on-site biosinks and biofilters of aquatic pollutants because of their abundance and limited mobility. These plants possess a large surface area that is covered by a lipid-rich cuticle and thus have the potential to take up hydrophobic organic chemicals (HOCs) (Sabljic et al., 1990; Gao et al., 2000; Miglioranza et al., 2004; Tront and Saunders, 2006). Therefore, the investigation of PAEs in aquatic plants can provide a valuable record of contamination in aquatic environment.

The Haihe River is the largest water system in north China. With the rapid economic growth, industrialization and urbanization, water pollution is becoming the major environmental issue (Jin et al., 2004; Yang et al., 2005; Liu et al., 2006; Jiang et al., 2007).

*Potamogeton crispus* L. (*P. crispus*) is a submerged herbaceous perennial plant, which can be found in freshwater lakes, ponds, paddy fields and rivers throughout China. A distinguishing characteristic of *P. crispus* is its life history, as its turions germinate in autumn and display early rapid growth during April–May, when most other submerged macrophytes are yet dormant (Jian et al., 2003). The purpose of this work is to elucidate the accumulation of PAEs by *P. crispus* from Haihe River during different growing periods as well as the bioavailability of these contaminants.

### 2. Materials and methods

#### 2.1. Site description and sample collection

Haihe River in the urban area of Tianjin is 20 km long with an average width of 100 m and depth of 4 m. Four sampling sites (S1–S4) were selected along the river in the urban area, as shown in Fig. 1. S1, from which Haihe River originates, is an important crossing point of Beiyun, Nanyun and Ziya rivers. S4 is about 16 km away from S1. Samples, including water, surface sediments, near root sediments and *P. crispus* plant, were collected from the four sampling sites. The field investigation was carried out three times from March to May 2008, which covered the seasons of early growing, blooming and dying off of *P. crispus*. Water samples were collected from the top layer (0–50 cm) using a pre-cleaned glass bottle. Sediment cores were obtained by a piston-sampler. The top 2-cm of the sediment core was sliced off, and referred to as surface sediment. The plants were removed from the sediment and the sediment that did not fall off the roots was defined as near root

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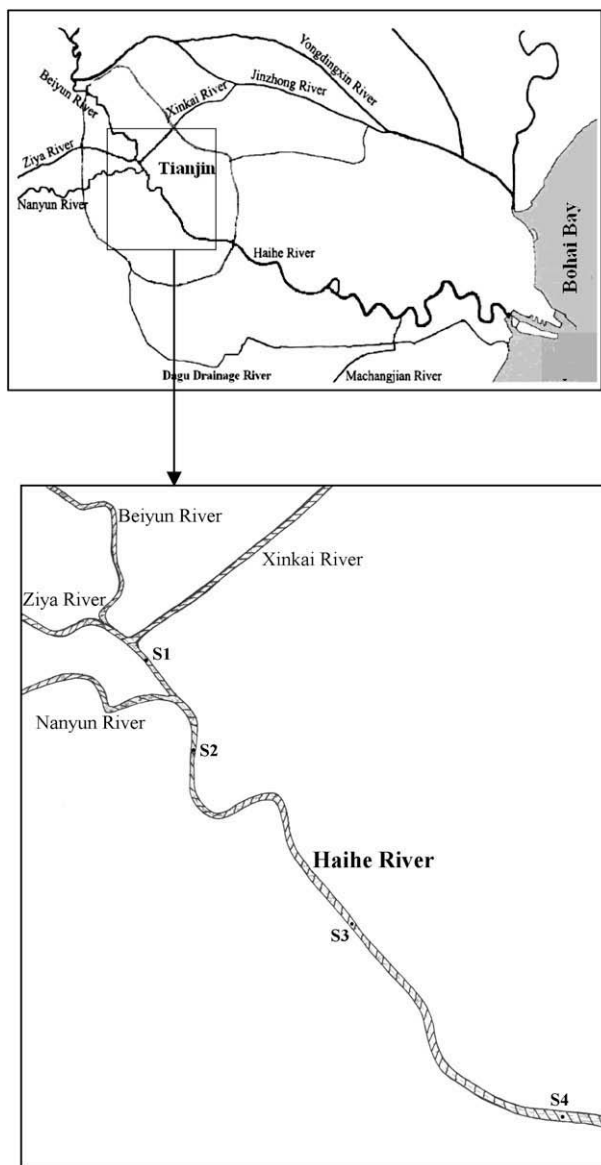


Fig. 1. Map of the study area and sampling sites.

sediment. To evaluate the temporal variation of PAE inputs into the urban reach of Haihe River, sediment cores were collected at S1. Five sequential sediment slices were separated from the top 0–2 cm down to 20 cm depth. Sediment and plant samples were sealed in polytetrafluoroethylene bags. All samples were cooled in a refrigerator (4 °C) during transport to the laboratory.

## 2.2. Sample preparation and analysis

Forty milliliters of water sample was extracted three times with 3 mL of hexane. The hexane layers were combined, and were concentrated to 0.2 mL under a gentle nitrogen stream for GC-FID analysis. Analyses of the samples were conducted in triplicate.

Samples of surface sediments and near root sediments were freeze-dried, ground and passed through an 80-mesh sieve. Dried samples (1 g) were sonically extracted with 5 mL dichloromethane. Each sample was extracted three times and each for 15 min. After 5 min of centrifugation at 1600g, the extracts were combined and concentrated to a final volume of 1.0 mL for GC-FID analysis. Analyses of the samples were conducted in triplicate.

After transport to the laboratory, the plants were thoroughly washed with running tap water and divided into above-ground and root tissue subsamples, which were then rinsed with distilled water before extraction. One gram of fresh plant sample was extracted in a histoid grinding tube with 4 mL dichloromethane at 200 g for 10 min. The solution was centrifuged at 1600g for 5 min. After removal of the water phase, the dichloromethane layer was reduced to a final volume of 0.1 mL for GC-FID analysis. Analyses of the samples were conducted in triplicate.

Samples were analyzed by an Agilent 6890N gas chromatograph fitted with a splitless injector, a fused-silica capillary column (HP-5, 0.32 × 30 m) and a flame ionization detector. The temperature of injector and detector were both set at 250 °C. Nitrogen was used as a carrier gas at a flow rate of 50 mL min<sup>-1</sup>, while the flow rates of hydrogen and air were 37 and 550 mL min<sup>-1</sup>, respectively. Injection volume: 1 µL. DBP and DEHP were eluted with the following temperature program: 120 °C (2 min) → 15 °C/min (8.7 min) → 250 °C (3 min). The retention times of DBP and DEHP were 8.25 min and 12.17 min, respectively.

## 2.3. Quality assurance and quality control

All data were subject to strict quality control procedures. With each set of samples analyzed, a solvent blank, a procedural blank and a standard mixture of DBP and DEHP were run to check for the interference, contamination and quantification. None of the target compounds was detected in solvent blanks and procedural blanks for water, sediment and plant. Quantitative analysis by GC-FID was performed using external standard calibration. A series of injections of target compounds in the concentration range from 0.05 to 10 mg L<sup>-1</sup> were performed to determine the linear concentration range. Good linearity was observed with correlation coefficients above 0.999. The existence of target compounds was confirmed by the comparison of GC retention times and GC/MS spectra of target compounds with those of the calibration standards.

The limits of detection (LOD) of DBP and DEHP for the analytical methods were determined as the concentration of an analyte in a sample that gives rise to a peak with a signal-to-noise ratio of 3. The LODs of DBP and DEHP were both 0.1 µg L<sup>-1</sup> for water, 0.02 mg kg<sup>-1</sup> for sediment and 0.002 mg kg<sup>-1</sup> mg kg<sup>-1</sup> for plant. The recovery studies were performed by spiking water samples (40 mL), sediment samples (1 g), and plant samples (1 g) at a level of 10 µg L<sup>-1</sup>, 0.5 mg kg<sup>-1</sup>, and 0.05 mg kg<sup>-1</sup> of each target compound. Average recoveries of DBP and DEHP in samples of water, sediment, and plant were higher than 91.8% and 84.2%, 85.9% and 84.7%, 89.1% and 88.4%, respectively. Relative standard deviations (RSD) were 3–15% for water, 5–16% for sediment and 6–18% for plant.

## 2.4. Other analyses

Sub-samples were used to determine physicochemical properties of water and sediment (including temperature and pH of water, grain size, water content and total organic carbon of sediment; Miglioranza et al., 2004), and lipid content of plant (Barbour et al., 2005). The results are shown in Table 1.

## 3. Results and discussion

### 3.1. PAEs in water

The variations of DBP and DEHP in the water samples from Haihe River are shown in Fig. 2. DBP and DEHP were detected in all samples. Concentrations of DBP and DEHP ranged from 0.35 to

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