

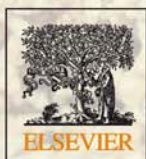
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Determination of 4-tert-octylphenol in surface water samples of Jinan in China by solid phase extraction coupled with GC-MS

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Abstract

Octylphenols, considered as xenoestrogens, mainly exist as 4-tert-octylphenol (OP) in aquatic environments. The high stability and accumulation of OP in aquatic systems have caused endocrine disruption. The OP in surface water in Jinan, China was analyzed by gas chromatography-mass spectrometry (GC-MS) coupled with solid phase extraction (SPE). Water samples were extracted by SPE on a cartridge system containing C-18 as sorbent. To increase sensitivity and selectivity, OP was derivatized to 4-tert-octyl-phenoxy silane. With the use of phenanthrene-d10 as internal standard, the detection limit based on signal-to-noise ratio ($S/N = 3$) was 0.06 ng/mL. The average recovery was from 84.67% to 109.7%. The precision of the method given as the relative standard deviations (RSD) was within the range 6.24%–12.96%. In the target water samples, the concentrations of OP were as follows: 15.88–71.24 ng/L for Jinxiuchuan Reservoir, 3.850–26.68 ng/L for the city moat, 6.930–41.56 ng/L for Daming Lake, 66.03–474.2 ng/L for Xiaoqing River, 14.66–17.72 ng/L for the Yellow River, and 10.60–26.43 ng/L for Queshan Reservoir. The Xiaoqing River was seriously polluted due to the discharge of wastewater from Jinan. Jinxiuchuan Reservoir had a higher concentration of OP compared with the Yellow River and Queshan Reservoir, which is ascribed to the surrounding human activities. These data are reported for the first time, providing strong support for the control of OP pollution in Jinan.

Key words: GC-MS; octylphenol; Jinan; solid phase extraction; derivatization; internal standard

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Introduction

4-tert-Octylphenol (OP), a kind of endocrine disrupting chemical, has been receiving increasing attention due to its widespread occurrence in the aquatic environment as a result of the discharge of its parent compounds, alkyl phenol polyethoxylates (APEO) (Tamagawa et al., 2006; Petrovic et al., 2002). OP has been shown to be the most estrogenic of the breakdown products of APEOs, and is up to 5 times more estrogenic than nonylphenol (Zhou, 2006; Jobling and Sumpter, 1993). Furthermore, OP can be concentrated by bioaccumulation along to the food chain and finally into animals and humans, causing greater harm. Because of its relatively high toxicity in aquatic organisms and long-term persistence in the environment, determination of the environmental concentration and distribution of OP is very important.

Recently, environmental OP contaminations have been

reported in water and sediments (Loyo-Rosales et al., 2003), marine water (Famiglini et al., 2005), surface and drinking water (Kuch and Ballschmiter, 2001), an urban estuary (Sacks and Lohmann, 2011), healthcare facility wastewaters (Nagarnaik et al., 2010), infant food (Raecker et al., 2011) and human tissue (Lopez-Espinosa et al., 2009) all over the world. In particular, domestic OP contamination has been reported in many places in China. Yang et al. (2007) reported 130 ng/L OP in Qingdao Shilaoren seawater in Shandong Province. OP concentrations in the Haihe River and Bohai Bay were 18–56 ng/L and n.d.–14 ng/L, respectively (Shen et al., 2005). In surface sediment samples from the Pearl River and its adjacent branches in Guangzhou City, the OP concentration varied from 0.36 to 498.54 ng/g (dry weight) (Xu et al., 2007). Among the methods used in previous studies, the technique of gas chromatography-mass spectrometry (GC-MS) coupled with solid phase extraction (SPE) has been demonstrated to be reliable to determine OP concentrations in various samples (Ferguson et al., 2000; Jeannot et al.,

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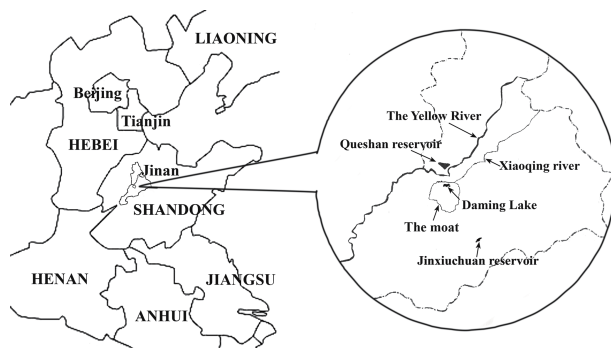


Fig. 1 Maps of water system of Jinan (Shandong Province, China).

2002; Kuklenyik et al., 2003; Ye et al., 2005).

Jinan, called the Spring City, is the capital of Shandong Province, China. The complex terrain, which is low in the north while high in the south, can be roughly divided into three zones (Fig. 1): the belt close to the Yellow River in the north, the central piedmont zone and the southern mountainous area. The limestone in the southern mountains is highly permeable. However, the north igneous rock is impermeable. Because of abundant rainfall in southern mountainous areas, water can infiltrate along the limestone fissures underground, and then flows along the topography towards the north where the water pressure increases due to the impermeable igneous rock in the north. Once a crack breaks out, a spring forms. This is the reason why springs are formed in Jinan.

Accordingly, the surface water system in Jinan mainly includes three sections: the Yellow River (Queshan Reservoir) and Xiaqing River in the north, Daming Lake and the city moat system (including springs, for instance, Baotu Spring and Heihu Spring) in the center (downtown), and surface water in the southern mountainous area (Jinxiuchuan Reservoir) (Figs. 1 and S1). There are two water paths for the surface water system. The first one is described as follows: water from the southern mountainous area feeds the springs in the city, forming the moat and flowing into Daming Lake. Then, water merges into the Xiaqing River and flows away. The second path is the Yellow River, an important source of drinking water in Jinan City. However, there is no close relation between the river and other surface water in Jinan except that Queshan Reservoir uses water from the Yellow River.

So far in China, there are no relevant laws or regulations to restrict the use and discharge of APEOs in the environment. Importantly, little is known about the concentration of OP in surface water in Jinan City. It is of great significance to survey the distribution of OP in Jinan so as to assess the ecological risks of such chemicals. In this study, we determined for the first time the concentrations of OP in surface water samples in Jinan by GC-MS coupled with SPE. Before GC-MS analysis, OP was derivatized to 4-tert-octyl-phenoxy silane and phenanthrene-d10 was added as an internal standard.

1 Experimental

1.1 Reagents and standard solutions

OP standard, derivatization reagent (BSTFA+TMCS, 99:1) and internal standard phenanthrene-d10 were obtained from Sigma-Aldrich. Anhydrous sodium sulfate was made by baking sodium sulfate in a muffle furnace at 400°C for 4 hr.

OP standard stock solution (1.0 mg/mL) was prepared by dissolving 10.0 mg OP with 10.0 mL acetone. Working solutions of 10–1000 ng/mL OP were then prepared and used to generate calibration curves.

To prepare an internal standard stock solution (0.2 mg/mL), 1 mg of phenanthrene-d10 was dissolved with 5.0 mL of acetone. To obtain the working internal standard solution, 10.0 μ L of the stock solution was added to the final sample vials to obtain the ultimate internal standard concentration of 4.0 μ g/mL. Both the OP and internal standard stock solutions were stored in glass vials at 4°C in lightproof containers. Working solutions were freshly prepared each time from the stock solutions.

1.2 Sample collection and storage

Surface water samples were collected from 3–10 sites in Daming Lake, the city moat, the Xiaqing River, Jinxiuchuan Reservoir, the Yellow River and Queshan Reservoir (Fig. S1), respectively. According to national guidance on sampling techniques from lakes and reservoirs (GB/T 14581-93, China), a 1.5-L water sample at each site was divided into three aliquots of 500.0 mL in plastic bottles which had been washed with ultra-purity water. Afterward, samples were placed in a refrigerator at 4°C to reduce the impact of microbial action.

1.3 Solid-phase extraction

All water samples were filtered through a filter paper, and then the pre-concentration step was performed. SPE with Agela Cleanert C-18 (500.0 mg, 3.0 mL) sorbent was used with a buffer device and an external AP-02B vacuum pump. The SPE cartridges were conditioned by 10.0 mL of methanol to wash away the impurities in the sorbent, and subsequently with 10.0 mL of ultra-purity water to activate the SPE column. A 500.0-mL of filtered samples were percolated through the SPE-cartridges at a flow rate of 5.0 mL/min. After pre-concentration, the C-18 cartridges were dried, and the elution was carried out using 10.0 mL acetone. Anhydrous sodium sulfate of 1.0 g was added to the eluent to remove the moisture. The extracts from SPE were evaporated to dryness in a gentle stream of N₂ at 55°C.

1.4 Derivatization and internal standard

The derivatization reagent 50.0 μ L were added to the test vial containing extracts from SPE. Next, the vial was closed and placed at room temperature for 30 min. Then,

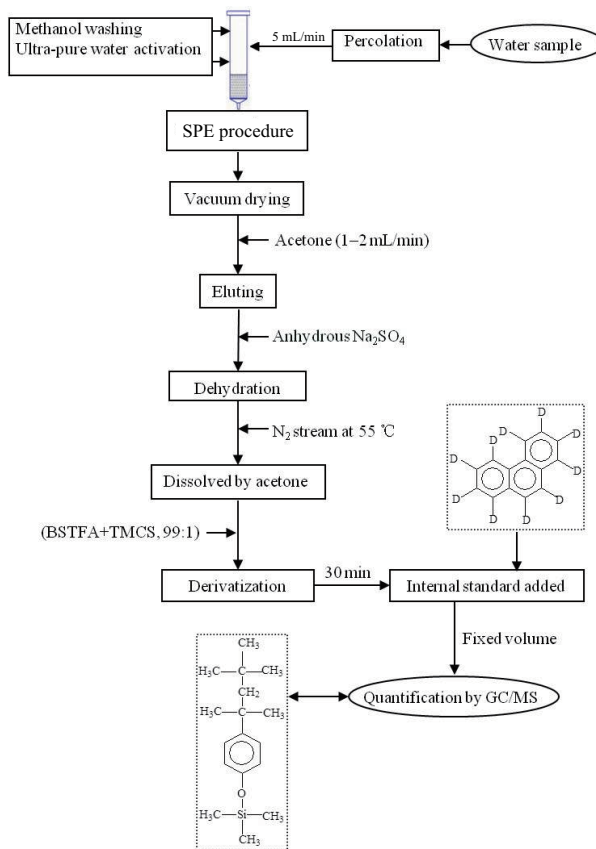
10.0 μL phenanthrene-d10 was added as internal standard. The sample was diluted with acetone to 0.5 mL for the subsequent analysis by GC-MS.

After SPE, OP was derivatized to 4-tert-octyl-phenoxy silane (**Scheme S1**) to increase the selectivity and sensitivity of the method, which was confirmed by the total ion chromatograms of OP and 4-tert-octyl-phenoxy silane (**Fig. S3**). The effects of derivatization time were examined as shown in **Fig. S4**, the responses of the target compound reached almost 100% at 30 min and then leveled off. Thus, a derivatization time of 30 min was selected.

1.5 Determination by GC-MS

The surface water samples were analyzed with GC-MS (Clarus 500 GC and EI-MS, PerkinElmer, USA). A PE-5MS capillary column (30 m \times 0.25 mm i.d. = 0.25 μm) was used for separation. The carrier gas was high-purity He at constant flow rate of 1.0 mL/min. The injector temperature was set at 280°C and the 1 μL injections were made at a split ratio of 10:1. For each run, the oven temperature was held at 50°C for 1 min and then increased to 290°C at a rate of 20°C/min. The GC transfer line temperature was set at 280°C. The parameters of the MS ion source were set as follows: temperature at 230°C, electron energy at 70 eV, multiplier at 480 V and solvent delay at 5 min. All the mass spectra were collected in both full scan for qualitative analysis and selective-ion monitoring (SIM) mode for quantitative analysis. PE TurboMass software was used for data acquisition and processing. Retention times of 4-tert-octyl-phenoxy silane and phenanthrene-d10 were 9.76 and 10.85 min, respectively. The ions (m/z = 41, 57, 207, 208 and 209) were used as characteristic ions for 4-tert-octyl-phenoxy silane, while those for phenanthrene-d10 were m/z = 80, 160, 184, 188 and 189. The structural formula and MS spectra of the OP derivative and phenanthrene-d10 are shown in **Fig. S2**.

The total flowchart for OP determination and analysis is shown in **Scheme 1**.



Scheme 1 Total flowchart for the OP determination and analysis.

2 Results and discussion

2.1 Quality control of the method

2.1.1 Calibration curve

Figure 2a shows a comparison of the total ion chromatograms of 4-tert-octyl-phenoxy silane and phenanthrene-d10. The ratios of the respective areas for peaks representing 4-tert-octyl-phenoxy silane and phenanthrene-d10 were plotted against the concentrations of OP. The calibration curve was obtained with the range

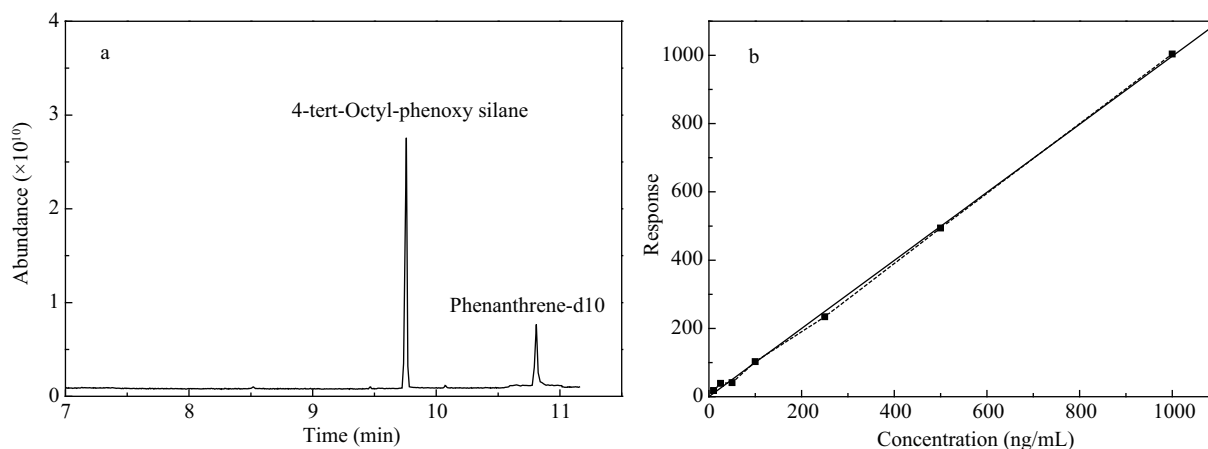


Fig. 2 (a) Total ion chromatogram of the OP derivative and the internal standard; (b) calibration curve for OP from 10 to 1000 ng/mL.

of 10–1000 ng/mL. As calculated from **Fig. 2b**, the regression equation was $y = 0.9972x + 0.3459$ with its correlation coefficient higher than 0.9996. Therefore, the calibration curve can be used for sample OP detection.

2.1.2 Detection limits

Due to the application of MS, the sensitivity of detection was significantly increased. The standard stock solution was diluted to 0.05, 0.06, 0.08, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 ng/mL with acetone and then analyzed by GC-MS after derivatization and internal standard addition. The sample concentration detection limit of OP was 0.06 ng/mL, wherein the peak of the OP derivative was three times as high as the noise peak ($S/N = 3$).

2.1.3 Recovery and precision

Three different concentration levels of OP standard solutions were added to ultra-purity water according to the above method. The tests for a certain concentration of OP were repeated five times in parallel operation to determine the precision. The recoveries were calculated by subtracting the results for the non-spiked samples from those for the spiked sample. As shown in **Table 1**, the average recovery was between 84.67%–109.7% with the determination precision in the range of 6.240%–12.96%.

2.2 OP analysis of surface water samples from Jinan

Concentrations of OP in surface water samples are shown in **Fig. 3**. OP concentrations in **Table 2** are the average values of three tests.

Among the six target sites, Daming Lake and the city moat had relatively low OP concentrations of 23.18 ng/L and 17.62 ng/L (**Fig. 3**), respectively. This is because the water (**Fig. S1B**) from two springs (Baotu Spring in site 1 and Heihu Spring in site 3), which are rarely contaminated, flows into the city moat and then enters Daming Lake. The flowing water will not induce OP deposition and accumu-

lation. Furthermore, there is no industrial wastewater and domestic sewage discharge nearby, resulting in a relatively low OP concentration.

In the urban area of Jinan, the Xiaoqing River flows from west to east with a length of 12.8 kilometers and a total area of 2.68 square kilometers. Since all the domestic water enters into the Xiaoqing River finally, a higher average OP content of 214.1 ng/L was determined in the Xiaoqing River. As shown in **Fig. S1C**, site 1 showed the highest OP content because when sampling, river dredging was in operation and the pollutants in the sludge returned into the water. The higher OP concentration of site 4 is mainly because the Xiaoqing River receives large amounts of wastewater from industries such as textile and paper mills, which potentially discharge more OP than other sites whose drainage areas are mainly residential.

Jinxiuchuan Reservoir lies in the southern mountain area of Jinan, with a total designed capacity of 41.5 million cubic meters. It is mainly used for flood control, irrigation and urban water supply. As shown in **Fig. S1D**, the OP concentrations became higher from the center to the edges, with the lowest concentration of 15.88 ng/L at site 4 and the highest of 71.24 ng/L at site 1 (**Fig. 3**). This may be related to the fact that nearby residents and restaurants discharged washing water into the reservoir. In addition, the poor mobility of water in the reservoir leads to the slow dispersion of target pollutants, causing the asymmetric OP distribution in the water.

As the second longest river in China, the Yellow River flows through the north of Jinan. Queshan Reservoir, the most important reservoir in the north of Jinan, plays a key role in water supply for the city. All water in the Queshan Reservoir comes from the Yellow River. OP concentrations of 14.66–17.72 ng/L in the Yellow River and 10.60–26.43 ng/L in the Queshan Reservoir (**Table 2**) were detected. There are fewer residents and industries around the river than in the urban area, resulting in less OP pollution. As for Queshan Reservoir, it is an absolutely closed surrounding without outside interference. Lastly, the water flow in the Yellow River is very large. Thus, the target organic pollutant could be greatly diluted with its flow.

Based on the level of OP concentrations, no evident correlations were found between the two water paths for surface water in Jinan, as indicated in the Introduction section. It is confirmed that industrial discharge and human activity are two factors that would bring OP pollution problems.

3 Conclusions

In summary, we have determined OP concentrations in surface water samples of Jinan using GC-MS coupled with SPE, derivatization and an internal standard. The general distribution of OP in different types of surface water samples of Jinan was obtained. The Daming Lake and the

Table 1 Average recovery and precision of OP measurements

Additive	Additive amount (ng/L)	Average recovery ($n = 5$) (%)	Precision (RSD %)
OP standards	100.0	84.67	6.990
	250.0	109.7	6.240
	500.0	90.20	12.96

Table 2 OP concentrations in water samples in different sampling sites

Sampling sites	The highest concentration (ng/L)	The lowest concentration (ng/L)	Average concentration (ng/L)
Daming Lake	41.56	6.930	23.18
The city moat	26.68	3.850	17.62
Xiaoqing River	474.2	66.03	214.1
Jinxiuchuan Reservoir	71.24	15.88	51.14
The Yellow River	17.72	14.66	15.68
Queshan Reservoir	26.43	10.60	15.96

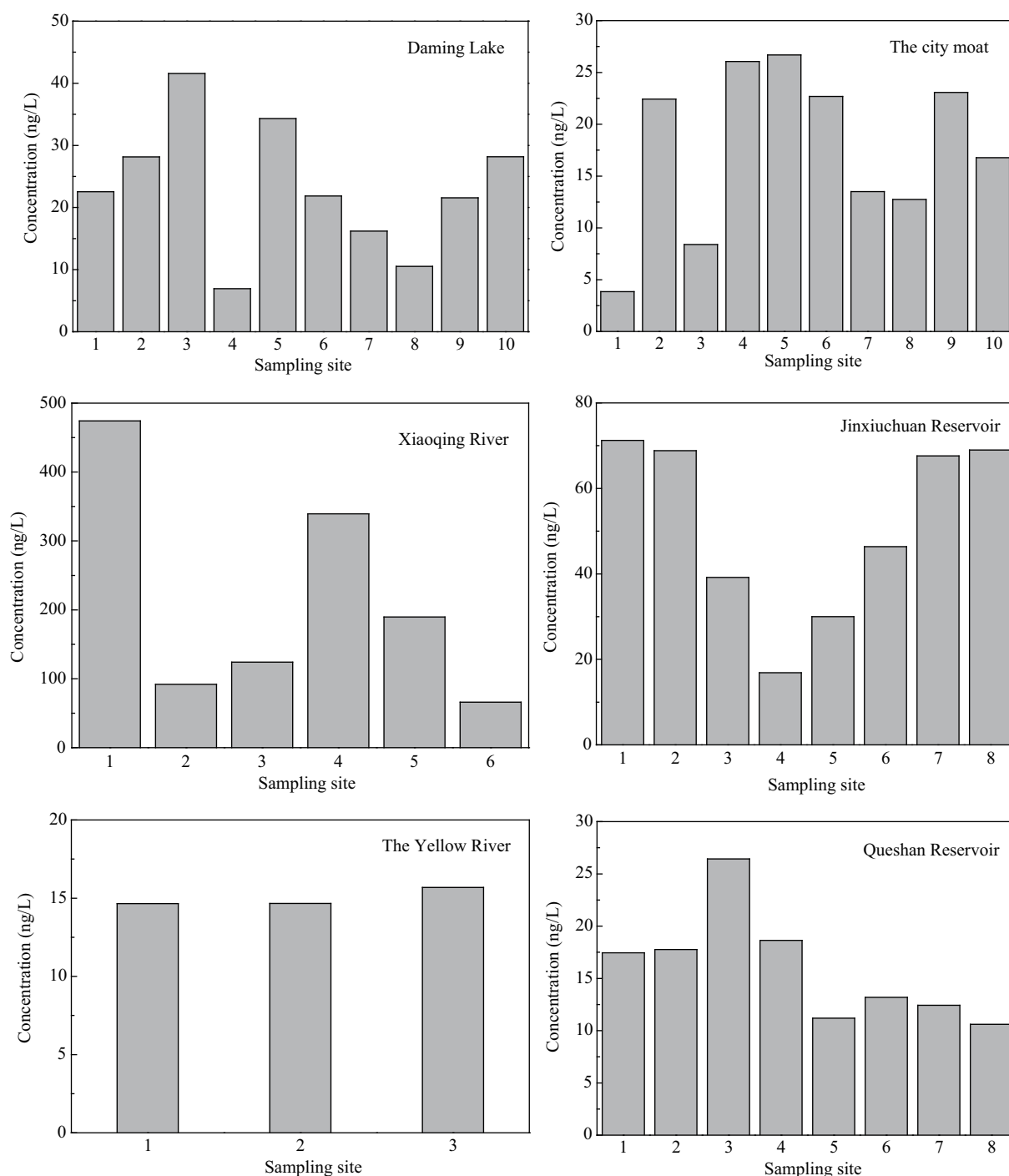


Fig. 3 OP concentrations in different sampling sites of Daming Lake, the city moat, Xiaoqing River, Jinxiuchuan Reservoir, the Yellow River and Queshan Reservoir.

city moat were less contaminated, with low OP contents. However, the Xiaoqing River was highly polluted. Due to human activities, Jinxiuchuan Reservoir had a higher concentration of OP compared with the Yellow River and Queshan Reservoir. The presented data can be a reference for the government to establish environmental protection policies related to OP.

Acknowledgments

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Supporting materials

Supplementary data associated with this article can be found in the online version.

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