Determination of chlorophenols in sediment using ultrasonic solvent extraction followed by solid-phase extraction, derivatization, and GC-MS analysis
Ming Xu, Lei Gui, Shu-Chuan Peng, Tian-Hu Chen and Ji-Zhong Wang

ABSTRACT
A method for determination of chlorophenols (CPs) in sediments was developed using ultrasonic solvent extraction followed by solid-phase extraction, derivatization, and gas chromatography–mass spectrometry (GC-MS) analysis. Some effective operational details and parameters on mixing of samples and extraction solvent, adsorption loss, and extraction cycles of the derivatives were studied and optimized. The calibration curves of standard solutions were observed in the range of 0.5–100 μg L⁻¹ and correlation coefficients ranged from 0.998 to 0.999. The limits of detection (LODs) for individual CPs are in the range of 0.026–0.072 ng g⁻¹. The method showed good performance with the recovery efficiencies of target CPs in spiked sediment at 73.2–105.6%. In addition, the feasibility of applying the proposed method to determine the concentration of CPs in field core sediment samples collected in three shallow lakes in Eastern China was examined. The obtained results show that the present method is a sensitive, simple, low cost and highly feasible method for determination of CPs in sediment samples.

INTRODUCTION
Chlorophenols (CPs), consisting of 19 congeners from monochlorophenols to fully chlorinated pentachlorophenol (PCP), are the widespread occurrence of synthetic organic contaminants in various environment media due to being greatly used for industrial, agricultural, and domestic purposes over more than 50 years (Czaplicka 2004). Many different types of evidence reported in previous studies also suggest that natural synthetic reactions are the important sources of CPs (Hodin et al. 1991; Hoekstra et al. 1999). CPs are well known to be toxic and carcinogenic at low levels (Chhabra et al. 1999), and they have been confirmed with high bioaccumulation and persistency (van Gestel & Ma 1988). Furthermore, CP congeners have been identified as the potential precursors of polychlorinated dibenzo-p-dioxins and dibenzofurans (Vollmuth et al. 1994; Xiao et al. 2015). As a result, both the US Environmental Protection Agency and the European Community (EC) have increasingly restricted the usage of some CP congeners as priority pollutants because of bioaccumulation and their negative effects on humans and animals (van Gestel & Ma 1988). In China, particularly the lower reaches of the Yangtze River, Eastern China, the primary purpose of using PCP and its sodium salt (Na-PCP) was to control schistosome intermediate host snails during the period between the early 1960s and early 1990s, accounting for approximately 60% of the national production (Zheng et al. 2012; Hu et al. 2016), while wood preservation and other uses accounts for the other 40% of demand (Zheng et al. 2012).

CPs are largely analyzed by gas chromatography–mass spectrometry (GC-MS) (Ribeiro et al. 2002; Czaplicka et al. 2005) and high-performance liquid chromatography (HPLC) (Peng et al. 2007; Gao et al. 2008). Derivatization is generally required prior to GC-MS analysis. Currently, CPs in various water bodies have been widely reported, in part, due to the relatively undemanding extraction and clean-up procedures such as solid-phase extraction (SPE) (Rodríguez et al. 1996; Fattahi et al. 2007a, 2007b), solid-phase microextraction (Ribeiro et al. 2002), and hollow fiber liquid phase micro-extraction (Peng et al. 2007). However, sediment and soil are significant sinks of CPs because of their relatively high hydrophobicity ($\text{log} K_{ow}$ ranged from 2.1 to 5.9) (Czaplicka 2004). Among various methods of determining CPs in soil and sediment (Wennrich et al. 2000; Wang et al. 2012), ultrasonic extraction combined with SPE, derivatization, and GC-MS are commonly accepted for their simplicity, operability, and repeatability; therefore, ultrasonic solvent extraction combined with SPE, derivatization, and GC-MS analysis was a feasible method for determination of CPs in sediments.

In the present study, the main objectives are to develop simple methods for determining the CPs in bottom sediment. Potential effect factors will be examined for optimization of the methods. Based upon the optimized method, the concentrations of 18 CPs in 163 sediment samples of four cores collected from three shallow lakes in the lower reaches of Yangtze River are quantified.

**MATERIALS AND METHODS**

**Reagents and standards**

HPLC-grade acetone, $n$-hexane, and methanol were purchased from Oceanpak Alexative Chemical Co., Ltd (Gothenburg, Sweden). Analytical reagent grade acetic anhydride and sulfuric acid were obtained from AccuStandard (New Haven, CT, USA). Anhydrous sodium sulfate and potassium carbonate provided by Aladdin (Shanghai, China) were pesticide quality. Ultrapurewater was obtained from a water purification system (Hokee, China).

A mixture standard containing 3-chlorophenol (3-CP), 4-chlorophenol (4-CP), 2,5-dichlorophenol (2,5-DCP), 2,6-dichlorophenol (2,6-DCP), 3,5-dichlorophenol (3,5-DCP), 2,4-dichlorophenol (2,4-DCP), 2,3-dichlorophenol (2,3-DCP), 3,4-dichlorophenol (3,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,5-trichlorophenol (2,3,5- TCP), 2,4,5-trichlorophenol (2,4,5- TCP), 2,3,6-trichlorophenol (2,3,6- TCP), 2,3,4-trichlorophenol (2,3,4- TCP), 3,4,5-trichlorophenol (3,4,5- TCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP), 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP), and PCP at 100 mg L$^{-1}$ for each was purchased from O2si Smart Solutions (USA). An isotope-labeled CP standard of 2,4-dichlorophenol-d$_3$ (2,4-DCP-d$_3$) was supplied by Dr. Ehrenstorfer (Augsburg, Germany) as surrogate standard. Stock solutions were prepared for each of the standards in methanol and the stock standard solution was stored at −4 °C.

**Sediment sample collection and preparation**

The four sediment cores were (24–56 cm in length) collected from three shallow lakes including Longgan Lake, Daguan Lake, and Chaohu Lake (Chaohu Lake #1 in the eastern lake and Chaohu Lake #2 in the western lake) along the lower reaches of the Yangtze River, Eastern China using a gravity core sampler during December 2015. As is well known, the sampling areas were severely affected by schistosomiasis japonica in the mid-1950s and a massive abundance of PCP was used for schistosomiasis control. A total of 163 samples were obtained by immediately sectioning sediment cores into 1 cm slices. A surface sediment sample with a depth of approximately 5 cm was collected from Longgan Lake using a stainless steel grab sampler. All samples were stored frozen (−20 °C) prior to laboratory processing, and they were applied to develop the analytical method for determination of CPs’ concentrations. Prior to extraction, the sediment was freeze-dried, sieved to a grain size of <0.150 mm, homogenized, and stored at −4 °C.

**Extraction procedure**

The methods for determination of CPs in water bodies by SPE enrichment and derivatization combining with GC-MS were previously described elsewhere (Rodríguez et al. 1996; Ben Hassine et al. 2015; Kartal et al. 2015). In these
reports, the factors including sample pH (Bagheri & Saraji 2001), extraction solvent (Wang et al. 2009), sorbent phase and volume of SPE column (Castillo et al. 1997), and derivatization time (Kartal et al. 2015) were greatly evaluated and optimized. In the present study, sediment-associated CPs were analyzed following these optimized methods with minor modification. Generally, CPs in sediment were ultrasonically extracted with mixture solvents of n-hexane and acetone, enriched with SPE cartridge, derivatized with acetic anhydride, and analyzed with GC-MS. Surface sediment from Longgan Lake was used for the method verification which included matrix spiked and duplicated samples. For matrix spiked samples, the surface sediment was previously extracted using the mixture solvent containing hexane and acetone three times for 30 min each in an ultrasonic bath and air dried.

In brief, prior to sediment analysis, standard solutions in 0.5–100 (0.5, 5, 10, 20, 50, and 100) ng mL⁻¹ were derivatized with 1 mL acetic anhydride in 5 mL of methanol and 20 mL K₂CO₃ (0.1 mol L⁻¹) for 5 min by shaking to assess the performance of derivatization. The derivatives in the aqueous phase further were extracted with 5 mL of n-hexane and the organic phase was dried over anhydrous sodium sulfate. Herein, the standard solutions at 20 ng mL⁻¹ were derivatized in triplicate and two successive 5 mL hexane extractions of the derivatives were conducted to assess the extraction efficiency. The final extract was concentrated to 1 mL under a gentle stream of nitrogen and analyzed by GC-MS. Before ultrasonic extraction, the sediment was acidified with sulfuric acid, followed by solvent extraction. As sulfuric acid is insusceptible to dissolving in organic solvents, shaking the tube is necessary to avoid insufficient contact between the solvents and sediment sample. Herein, two batches of experiments with different shaking times (1 min and 5 min) were conducted using matrix spiked samples (the addition at 50 ng for each compound). In order to assess adsorption loss of the test container surface, a suitable rinse solvent should be considered with care. Throughout all the analytical procedures, this loss could happen after ultrasonic extraction and concentration because the CPs would be loaded to SPE cartridge for the clean-up using 20 mL ultrapure water. Ultrapure water (4 mL) and methanol (4 mL) were used as the rinse solvent to transfer CPs from tube to SPE cartridge in two batch experiments. Furthermore, in order to assess the repeatability of the method, surface sediment from Longgan Lake was analyzed for evaluating recoveries of CPs.

Finally, the core sediment sample (approximately 5 g) was analyzed by the optimized method described above. Briefly, the sample was acidified with 3 mL of sulfuric acid (9 mol L⁻¹) into a centrifuge tube. After addition of 50 ng of internal standard (2,4-DCP-d₅) and 15 mL of solvent mixture of n-hexane and acetone (1:1 in volume), the centrifuge tube was closed and shaken for mixing. The samples were ultrasonically extracted for 30 min each. Extract was separated by centrifugation and transferred to a glass tube. The extraction was repeated with another 15 mL of hexane and acetone for 30 min. The two extract portions were merged, evaporated to dryness by a rotary evaporator and redissolved in 20 mL ultrapure water. The extracts in water were enriched and cleaned up using SPE cartridges (Envi-18, 500 mg) from Supelco (USA) which were previously conditioned with methanol and ultrapure water. Analytes in SPE cartridges were desorbed with 5 mL of methanol at the flow-rate of 1 mL min⁻¹. The elution was derivatized following the method described above.

Equipment and chromatographic analysis

Agilent 7890A gas chromatography–5975C mass spectrometry (Agilent Technologies, Palo Alto, CA, USA) with a 30 m HP-5MS (inner diameter = 0.25 mm, film thickness = 0.25 μm; Agilent Technologies) capillary column was employed for the qualitative and quantitative analyses of CPs. Injections (1 μL volume) were made in splitless mode and injector temperature was set at 250 °C. Helium (99.999%) was used as carrier gas at a constant flow rate of 1.0 mL min⁻¹. The initial GC oven temperature was held at 60 °C for 1 min before the temperature was increased to 245 °C at a rate of 10 °C min⁻¹ and held for 5 min. Finally, the temperature was heated to 300 °C at a rate of 10 °C min⁻¹ and then held for 1 min. The temperatures of ion source and interface were maintained at 230 and 280 °C, respectively. The ion source was performed with electron ionization at 70 eV and mass spectrometric detection was performed using the selected ion monitoring (SIM) mode.
RESULTS AND DISCUSSION

Performance of derivation and GC-MS analysis

Considering the high polarity, CPs tend to appear as broad and tailed peaks in the chromatographic column. An effective choice to overcome these challenges is to transform CPs to less polar products with high volatility, thermal stability, and sensitivity on the chromatographic separation by derivation (Rodríguez et al. 1996). Various derivatization reagents including acylation (Fattahi et al. 2007a, 2007b), silylation (Basheer & Lee 2004), and alkylation (Fiamegos et al. 2003) are commonly used, and acetylating agents have been proved as the most efficient, simplest, and fastest reactions for CP congeners (Rodríguez et al. 1996). In the present study, CP compounds in 0.5–100 ng mL⁻¹ standard solution were derivatized with 1 mL acetic anhydride and 20 mL K₂CO₃ (0.1 mol L⁻¹) in 5 mL methanol for 5 min, and the GC-MS chromatogram is shown in Figure 1. All target compounds were identified with their retention time and characteristic ions (Table 1). Due to chromatographic coelution, the data of 2,4-DCP and 2,5-DCP were reported as their sum. The correlation coefficients ($R^2$) of six-point calibration curves (0.5, 5, 10, 20, 50 and 100 ng mL⁻¹) are higher than 0.998 for each CP congener (Table 1). On the other hand, after derivation, the derivatives in the aqueous phase were extracted with n-hexane. It was found that the extraction efficiency is dependent on the volume of solvent and number of extraction cycles. In the present study, 5 mL hexane was employed to extract the derivatives with two extraction cycles. The results indicate most of the target compounds were in the first extract, accounting for more than 99% of total measured abundance, while the percentages in the second extract were ignorable (generally less than <1%). Therefore, the extraction of derivatives was conducted by one extraction cycle with 5 mL n-hexane in the subsequent measurement.

Ultrasonic extraction

Before ultrasonic extraction, sulfuric acid was added into sediment samples, and then the extraction solvent was added into the centrifuge tube. The centrifuge tube was closed and shaken to mix samples and the solvent thoroughly. At low pH, the acid–base equilibrium for the CPs shifts significantly toward the neutral forms (Bagheri & Saraji 2001). Therefore, acidification of the sample prior to the extraction and SPE concentration can increase the extraction efficiency. Due to higher density of sulfuric

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT (min)</th>
<th>Selection ions (m/z)</th>
<th>$R^2$</th>
<th>LODs (ng g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-CP</td>
<td>14.246</td>
<td>128/130</td>
<td>0.998</td>
<td>0.072</td>
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<tr>
<td>4-CP</td>
<td>14.349</td>
<td>128/130</td>
<td>0.998</td>
<td>0.050</td>
</tr>
<tr>
<td>2,6-DCP</td>
<td>15.801</td>
<td>162/164</td>
<td>0.998</td>
<td>0.027</td>
</tr>
<tr>
<td>2,4/2,5-DCP</td>
<td>16.049</td>
<td>162/164</td>
<td>0.998</td>
<td>0.030</td>
</tr>
<tr>
<td>2,3-DCP</td>
<td>16.215</td>
<td>162/164</td>
<td>0.998</td>
<td>0.057</td>
</tr>
<tr>
<td>3,5-DCP</td>
<td>16.538</td>
<td>162/164</td>
<td>0.998</td>
<td>0.063</td>
</tr>
<tr>
<td>3,4-DCP</td>
<td>16.871</td>
<td>162/164</td>
<td>0.998</td>
<td>0.039</td>
</tr>
<tr>
<td>2,4,6-TCP</td>
<td>17.423</td>
<td>196, 198</td>
<td>0.998</td>
<td>0.070</td>
</tr>
<tr>
<td>2,3,4-TCP</td>
<td>18.04</td>
<td>196, 198</td>
<td>0.998</td>
<td>0.068</td>
</tr>
<tr>
<td>2,3,5-TCP</td>
<td>18.118</td>
<td>196, 198</td>
<td>0.998</td>
<td>0.069</td>
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<tr>
<td>2,4,5-TCP</td>
<td>18.188</td>
<td>196, 198</td>
<td>0.998</td>
<td>0.057</td>
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<tr>
<td>2,3,4-TCP</td>
<td>18.787</td>
<td>196, 198</td>
<td>0.998</td>
<td>0.060</td>
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<tr>
<td>2,3,5-TCP</td>
<td>18.942</td>
<td>196, 198</td>
<td>0.998</td>
<td>0.032</td>
</tr>
<tr>
<td>2,3,4,6-TeCP</td>
<td>19.791</td>
<td>232, 230</td>
<td>0.999</td>
<td>0.035</td>
</tr>
<tr>
<td>2,3,4,5-TeCP</td>
<td>19.854</td>
<td>232, 230</td>
<td>0.999</td>
<td>0.031</td>
</tr>
<tr>
<td>2,3,4,5-TeCP</td>
<td>20.585</td>
<td>232, 230</td>
<td>0.998</td>
<td>0.026</td>
</tr>
<tr>
<td>2,3,4,5-TeCP</td>
<td>22.241</td>
<td>266, 264</td>
<td>0.998</td>
<td>0.032</td>
</tr>
</tbody>
</table>
acid, the solvent and samples were insusceptible to sufficient contact, resulting in low extraction efficiency. Therefore, the samples must be adequately shaken prior to ultrasonic extraction. In the present study, two batch experiments were conducted by spiking 50 ng mL$^{-1}$ of CPs standard to investigate the effect of shaking time (1 min and 5 min) on the extraction efficiency. Results clearly show recovery efficiencies for all target CP congeners with 5 min of shaking time were much higher than those with 1 min (Figure 2). The recovery efficiency ranged from 73.2% for 2,6-DCP to 105.6% for PCP for the batch with 5 min shaking, but less than half of spiked CPs were not recovered with 1 min shaking. Therefore, sufficient contact between CPs and extraction solvent was the important factor controlling the extraction efficiency. In addition, the relative standard deviations of recovery efficiency for all CP compounds with 1 min shaking (from 2.4% for 2,4,5-TCP to 22.9% for 2,4,6-TCP) were much higher than those with 5 min shaking (from 1.30% for 2,3-DCP to 11.9% for 2,4,5-TCP), suggesting insufficient contact would result in large quantifying uncertainties in CPs’ concentrations.

**Adsorption loss**

Considering the relative high octanol–water coefficient for CPs ($K_{ow}$ values from 2.48 to 2.50 for 3-CP and from 5.02 to 5.86 for PCP) (Czaplicka 2004), adsorption loss to the test container surface could reduce the recovery efficiency (Wheelock et al. 2005). Throughout the whole analytical procedures, this loss would happen after ultrasonic extraction and concentration because the CPs would be loaded to SPE cartridges for the clean-up using 20 mL ultrapure water. To avoid the adsorption loss, the vials should be completely washed with effective solvent. In the present study, two batches of experiments (4 mL ultrapure water and 4 mL methanol) were carried out to investigate the effect of different solvent on the recovery. The results show there is no significant difference on the recoveries in the two batches for monochlorophenols and dichlorophenols, but the recoveries of the highly chlorinated phenols (trichlorophenols, tetrachlorophenols, and PCP) in the samples treated with 4 mL methanol as wash solvent were clearly higher than those in the samples without being washed (Figure 3). One of the most important reasons is due to the fact that low chlorinated phenols with low $K_{ow}$ values had low affinity to the vial well compared with those highly chlorinated CPs. Therefore, after the aqueous solvent was loaded to SPE cartridges and glass vials were further rinsed with 4 mL methanol, considerable recovery efficiencies for all target CPs were obtained (from 73.2% for 2,6-DCP to 106% for PCP).

![Figure 2](http://example.com/figure2.png) **Figure 2** | Variation in the recovery (%) of CPs for different mixing times.

![Figure 3](http://example.com/figure3.png) **Figure 3** | Variation in the recovery (%) of CPs: the glass vials were rinsed with different solvents.
Performance of the method

The limits of detection (LODs) of individual CP compounds were evaluated by the ratio of signal to noise (S/N = 3), corresponding to 0.026–0.072 ng g⁻¹ for individual CP congeners for sediment samples (Table 1). Aliquots of 5, 10, and 50 ng of 18 CPs standards were spiked to surface sediments collected from Longgan Lake as matrix spiked samples; the recoveries of the 18 CPs varied from 46% to 101%, 50% to 114%, and 73.2% to 105.6%, respectively (Table 2). Generally, good recoveries were obtained for all target compounds despite relatively low recovery efficiencies of highly chlorinated CPs being observed in the samples with low spiked concentration. It is shown that the derivatization process has a greater effect on the determination of low concentration of CPs, so that better control of the derivatization conditions can improve the precision and recovery of the low concentration. In addition, to verify the duplication of the method, four surface sediment samples collected from Longgan Lake were treated and the results show that all CP homologues were detectable with the average concentration and relative standard deviation varying from 0.28 to 2.02 ng g⁻¹ and 0.90 to 14.75%, respectively (Table 3).

CPs in the lakes of lower reaches of Yangtze River, Eastern China

The recovery efficiency of 2,4-DCP-d₃ varied from 56.1 to 127% with an average of 79.3 ± 17.9% for all sediment samples. All CPs were detectable (Table 3) and the core sediment contained a wide variety of CPs. Generally, PCP was the most frequently quantified, with the concentration varying from 0.06 to 2.71 ng g⁻¹ for Longgan Lake, from ND to 1.90 ng g⁻¹ for Daguan Lake, from ND to 5.25 ng g⁻¹ for Chaohu Lake #1, and from ND to 1.50 ng g⁻¹ for Chaohu Lake #2, respectively. Additionally, 4-CP, 2,3,5-TeCP and 2,3,5,6-TeCP were predominant contaminants for four lakes, and 2,3,5-TCP was also observed with high concentration for Longgan Lake and Daguan Lake. For Longgan Lake, 2,6-DCP was undetectable for all samples; the remaining CPs were found with high detection frequency (>40%). 2,6-DCP, 3,4-DCP, and 2,4,6-TCP were barely observed in Daguan Lake, but 3,5-DCP was detected in a few samples in Chaohu Lake.

The total concentrations of Σ₁₈CP ranged from 1.17 to 20.3 ng g⁻¹ for Longgan Lake, from 0.94 to 50.7 ng g⁻¹ for Daguan Lake, from 4.12 to 41.0 ng g⁻¹ for Chaohu Lake #1 in the eastern lake, and from 0.27 to 73.2 ng g⁻¹ for Chaohu Lake #2 in the western lake, respectively. Thus the total concentrations of Σ₁₈CP were generally lower in Longgan Lake than in Daguan Lake and Chaohu Lake, reflecting elevated CPs’ contamination in the aquatic ecosystem of the lower reaches of Yangtze River. The core of Chaohu Lake #2 contained the highest concentrations of Σ₁₈CP, possibly reflecting the influence of municipal and industrial effluent from Hefei city. The vertical distribution of Σ₁₈CP shows that two concentration peaks for cores in Chaohu Lake were investigated with 12 and 22 cm in depth for Chaohu Lake #1 and 13 and 36 cm in depth for Chaohu Lake #2, respectively (Figure 4). Unimodal distributions of Σ₁₈CP for Longgan Lake and Daguan Lake with the peak concentration at 37 and 13 cm in depth,
respectively, were observed. Furthermore, relatively low concentrations of $\Sigma_{18}$CP in the surface and subsurface sediment samples indicated a decrease in the usage of CPs in the Yangtze River Basin currently. Overall, there was a widespread occurrence of CPs in the sediment of the lakes in the lower reaches of the Yangtze River, Eastern China.

**CONCLUSION**

The present study indicates that ultrasonic solvent extraction followed by SPE, derivatization, and GC-MS analysis is a suitable method for quantification of CPs from sediment samples. The key factors which possibly impact the recovery were optimized. The performance of the proposed method was evaluated by the recovery of matrix spiked samples and duplicate field samples. A total of 163 samples from four sediment cores collected in Longgan Lake, Daguan Lake, and Chaohu Lake in the lower reaches of the Yangtze River, Eastern China were further treated to quantify the concentration of 18 CPs. In conclusion, this method is simple, rapid, sensitive, and reproducible to determine CPs in sediment.

**ACKNOWLEDGEMENTS**

The present study was financially supported by the Fundamental Research Funds for the Central Universities.
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First received 10 December 2016; accepted in revised form 27 March 2017. Available online 22 April 2017