



Original Article

# Contamination of Phthalic Acid Esters (PAEs) in Surface Sediment Samples Collected from Rao Cai River in Ha Tinh, Vietnam

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Received 12<sup>th</sup> April 2024

Revised 19<sup>th</sup> August 2024; Accepted 30<sup>th</sup> August 2024

**Abstract:** In this report, the solid phase extraction technique combined with the gas chromatography-mass spectrometry method (GC-MS) was optimized to determine the contamination of nine phthalic acid esters (PAEs) in the surface sediment samples collected from Rao Cai River in Ha Tinh, Vietnam. The method detection limits (MDLs) were from 2.0 to 6.0 ng/g-dry weight (ng/g-dw). The recoveries of surrogate standards (PAE-*d4*) in both blank and real samples ranged from 79.6 to 94.3% (RSD < 8.7%). The total concentration of PAEs in the surface sediment samples was in the range of 72.4-1390 ng/g-dw (mean/median: 561/552 ng/g-dw). Among PAEs, di-(2-ethyl)hexyl phthalate (DEHP) was detected at the highest level in all samples. In contrast, dimethyl phthalate (DMP), diethyl phthalate (DEP), and dipropyl phthalate (DPP) were found at low frequency and concentration. Moreover, the risk quotient of PAEs in sediments was estimated based on the measured concentrations. Diisobutyl phthalate (DiBP) posed a medium risk for fish.

**Keywords:** PAEs, DEHP, sediment, GC-MS, Rao Cai River.

## 1. Introduction

Phthalates or phthalic acid esters (PAEs) are a class of synthetic chemicals used in plasticizers in various commercial products such as cosmetics, personal care products, medicines, food products, construction materials, and so on [1, 2]. Paluselli et al., (2018) reported that the overall production of

PAEs globally was estimated at up to 8 million tons annually [3].

Besides their utility, PAEs have also been shown to be toxic to laboratory animals [1, 4]. Some toxicological studies have demonstrated that PAEs are ecotoxic, mutagenic, and carcinogenic and that their metabolic products can disrupt endocrine systems, adversely affecting the reproductive system, human health, and cell development [4, 5]. Therefore, several developed countries such as the United States, Europe, Japan, and South Korea have issued laws regulating the allowable limits of

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<https://doi.org/10.25073/2588-1140/vnunst.5665>

PAEs in commercial plastic products [5]. However, regulations/standards on the content of PAEs in environmental samples are still minimal. In Vietnam, the Ministry of Health has stipulated that the maximum allowable limit of DEHP in solid and liquid foodstuffs is 1.5 mg/kg and 1.5 mg/L, respectively [6].

Due to the massive production and extensive consumption, PAEs discharged throughout the surroundings [7]. Various studies showed the occurrence of PAE in micro-environments such as air [8, 9], dust [10], water [7], sediment [11], and food [12]. Recently, PAEs were found at high levels in sediment from three rivers in Northern Vietnam, including To Lich, Nhue, and Day rivers, with the respective ranges of 11,000-125,000, 2140-89,900 and 1140-43,100 ng/g-dw [11]. However, comprehensive PAE contamination in environmental samples in Vietnam is still very scarce.

This work focuses on determining nine PAEs in sediment samples collected from the Rao Cai River in Ha Tinh (Central Vietnam). Based on the measured concentration of PAEs in surface sediment samples, the ecological risk for aquatic animals was estimated.

## 2. Materials and Methods

### 2.1. Chemicals

Nine PAEs were purchased from Aldrich-Sigma with a purity of  $\geq 98\%$ , including dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-propyl phthalate (DPP), di-*n*-butyl phthalate (DBP), di-*isobutyl* phthalate (DiBP), di-*n*-hexyl phthalate (DnHP), di-cyclohexyl phthalate (DCHP), benzyl butyl phthalate (BzBP), di-(2-ethylhexyl) phthalate (DEHP). Seven *d4* (deuterated) standards purchased from Aldrich-Sigma with a purity of  $\geq 98\%$  were DMP-*d4*, DEP-*d4*, DPP-*d4*, DiBP-*d4*, DnHP-*d4*, BzBP-*d4*, and DEHP-*d4*. Each of the surrogate standards was used to calculate the concentrations of target compounds (except DBP and DCHP, which were calculated based on DiBP-*d4* and DnHP-*d4*, respectively).

Solvents, including *n*-hexane, acetone, and dichloromethane (DCM), were purchased from Merck KGaA (Darmstadt, Germany). All of the target and surrogate chemicals were dissolved in *n*-hexane. The solid-phase extraction (SPE) cartridges (CNWBond HC-C18, 500 mg/6 mL) were received from ANPEL Laboratory Technologies Inc. (Shanghai, China).

### 2.2. Sampling Collection

Rao Cai River, one of the large rivers flowing in Ha Tinh province, Vietnam, is 74 km long. In this study, 10 surface sediment samples (S1 to S10) were collected from Rao Cai River by a stainless-steel grab sampler at 10 sampling locations (S1 to S10) with a depth range of 0 to 20 cm in October 2023 (Figure 1). The sediment samples were covered by aluminum foil, stored in an icebox, and immediately delivered to the laboratory. All samples were freeze-dried, powdered, sieved, and then stored at 4 °C in dark glass bottles before being analyzed.

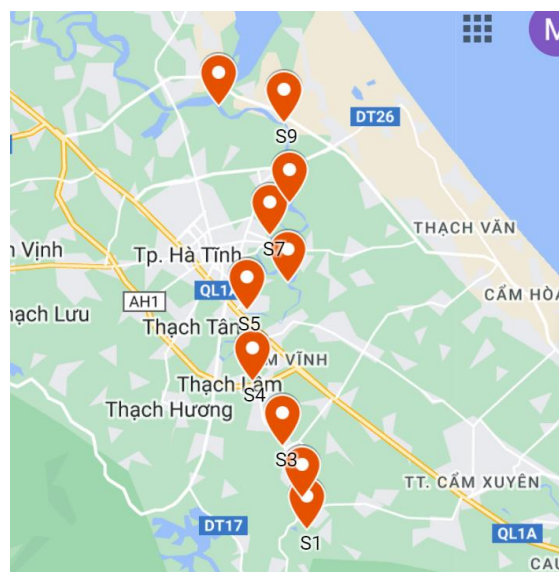


Figure 1. The sampling map.

### 2.3. Sample Preparation

In this study, 200 ng of surrogate standards (PAE-*d4*) were spiked into 0.5 g of samples after the progress of freeze-dried sediment. The

sample was shaken by an orbital shaker (Eberbach Corp., Ann Arbor, MI, USA) with 5 mL of DCM/*n*-hexane (2:1, v:v) for 15 min at 300 rpm. Then, the sample was centrifuged by an Eppendorf Centrifuge 5804 machine (Hamburg, Germany) at 3000 rpm centrifugation for 10 min. The supernatant was transferred into a 15 mL glass tube. The extraction process was repeated twice. Next, the combination of extracts was concentrated to approximately 2 mL under a gentle stream of nitrogen. After that, the concentrated samples were cleaned up by C18 solid-phase extraction (SPE) cartridges (500 mg/6 mL, Macherey-Nagel, Thermo Fisher Scientific Inc., USA) and were conditioned with 5 mL of *n*-hexane. The target compounds were eluted using a 15 mL combination of DCM/*n*-hexane (1:1/ v:v). Lastly, the eluted solution was concentrated to 1 mL and transferred into a 1.5 mL vial for GC-MS analysis.

#### 2.4. GC-MS Analysis

Gas chromatography (GC-7890B) and mass spectrometry (MS-5977A) from Agilent Technologies, USA, were applied to determine PAEs. To separate target chemicals, 2  $\mu$ L of the standard solution and samples were injected into a capillary column (DB-5MS: 30 m  $\times$  0.25 mm I.D.  $\times$  0.25  $\mu$ m) at a constant 1.0 mL/min flow rate. The chromatographic analysis was indicated in a prior study [8]. Sample solutions were vaporized at 280  $^{\circ}$ C after being injected in splitless mode. The oven temperature initially started at 80  $^{\circ}$ C (held for 1.0 min), followed by increasing linearly to 180  $^{\circ}$ C (12  $^{\circ}$ C/min, held for 1.0 min), then kept rising to 230  $^{\circ}$ C (6  $^{\circ}$ C/min, held for 2.0 min) before increasing to 270  $^{\circ}$ C (8  $^{\circ}$ C/min, held for 2.0 min) and lastly went up to 270  $^{\circ}$ C (8  $^{\circ}$ C/min) and held for 10 minutes. PAEs were qualified and quantified in samples by selected ion monitoring (SIM) mode. The ion fragment (*m/z*) of target compounds and surrogate standards are shown in Table 1.

Table 1. Ion fragments of target compounds and surrogate standards

Target compounds	<i>m/z</i>	Surrogate standards	<i>m/z</i>
DMP	<i>163</i>	DMP- <i>d4</i>	<i>153</i>
DEP	<i>149</i> (177)	DEP- <i>d4</i>	<i>153</i>
DPP	<i>149</i>	DPP- <i>d4</i>	<i>153</i>
DBP	<i>149</i> (233)	DiBP- <i>d4</i>	<i>153</i>
DiBP	<i>149</i> (233)		
DnHP	<i>149</i> (279)	DnHP- <i>d4</i>	<i>153</i>
DCHP	<i>149</i>		
BzBP	<i>149</i> (206, 233)	BzBP- <i>d4</i>	<i>153</i>
DEHP	<i>149</i> (167, 279)	DEHP- <i>d4</i>	<i>153</i>
Italic numbers of <i>m/z</i> indicate quantification ions and numbers in parentheses indicate confirmation ions.			

### 3. Result and Discussion

#### 3.1. Optimization of Sample Preparation

During sampling, sample preparation, and experiment, minimizing the impact of PAE trace levels is a crucial issue. Therefore, the samples were collected using a stainless steel grab and wrapped with aluminum foil. In addition, all glassware was heated at 450  $^{\circ}$ C for 20 h and then stored at 100  $^{\circ}$ C before being used in the experimental process. Moreover, the sediments were freeze-dried to eliminate any volatile target components, and then they were ground to have more surface area. For every batch of samples, procedural blanks were analyzed with the trace levels of DEP (0.25-0.43), DiBP (2.23-4.81), DMP (1.2-3.21), DEHP (1.08-1.35), and DBP (0.89-1.27) ng/g-dwt. All reported amounts in actual samples were deducted from the values discovered in the procedural blank.

The results of the surrogate standards recoveries in blank samples (n=7) are shown in Table 2.

Table 2. The recoveries of surrogate standards

Surrogate standards	Recoveries (%) ± rsd (%)
DMP- <i>d4</i>	90.5 ± 5.6
DEP- <i>d4</i>	84.6 ± 8.6
DPP- <i>d4</i>	94.3 ± 4.4
DiBP- <i>d4</i>	88.7 ± 6.3
DnHP- <i>d4</i>	79.6 ± 5.6
BzBP- <i>d4</i>	89.8 ± 4.5
DEHP- <i>d4</i>	92.9 ± 8.7

The method detection limits (MDLs) were calculated according to the instrument detection limits (IDLs, signal-to-noise: S/N ≈ 3), the mean weight of the sample (0.5 g), the final concentrated solution (1 mL), and the mean recoveries of PAEs. The method quantification limit (MQL) was assigned by the value of MDL\*3. The MQLs of PAEs varied from 2.0 to 6.0 ng/g-dw. The standard solution ranges of 1.0 to 1000 ng/mL (8 points, with R<sup>2</sup> ≥ 0.997) were the settings for the calibration curves. The analytical procedure of PAEs in the sediment sample is shown in Figure 2.

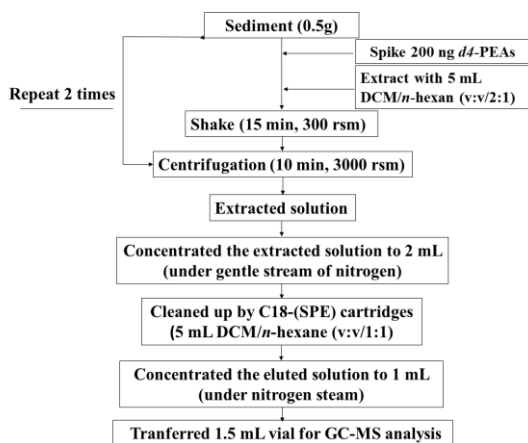


Figure 2. The analytical procedure of PAEs in sediment.

### 3.2. Total Concentration of PAEs in Sediment Sample

Figure 3 shows the total concentrations of PAEs in sediments that were taken from the Rao Cai River in Ha Tinh province, Vietnam.

The mean/median of the total concentrations of PAEs was 561/552 ng/g-dw, ranging from 72.4 to 1390 ng/g-dw. Furthermore, the total concentration of PAEs varies throughout the river; the maximum concentration was found at S6 (1390 ng/g-dw), and the lowest concentration was found at S1 (72.4 ng/g-dw). The highest concentration was found at location S6, which begins to receive wastewater from the market and the city’s residential areas. Meanwhile, at location S1, the PAE concentration was found to be the lowest. This is a less polluted location due to its proximity to the river's source.

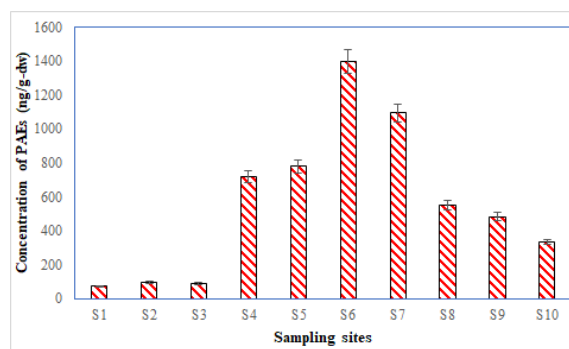


Figure 3. The concentrations (ng/g-dw) of PAEs in sediment.

A comparison was conducted between this result via prior studies [11, 13]. For instance, the Rao Cai River had a more minor total concentration of PAEs than three different rivers in Vietnam such as the To Lich (50,000/42,200 ng/g), Day (13,800/10,400 ng/g), and Nhue rivers (29,300/20,700 ng/g) [11]. However, the total concentration of PAEs in Rao Cai sediment from this study was also slightly larger than that in the Yangtze River, China [13]. These results suggested that the sediment from the Rao Cai River was significantly contaminated with PAEs.

### 3.3. Distribution of PAEs in Sediment Samples

Concentration profiles of PAEs were determined in the sediment from the Rao Cai River (Figure 4). Four PAEs, including DEP, DiBP, DBP, and DEHP, were detected in all samples under investigation. Furthermore, the

most common PAE was DEHP (38.5%), which was followed by DBP (21.3%) and DiBP (20.1%). In contrast, a small percentage of PAEs in the sample were DnHP (0.04%), DPP (0.08%), and BBP (0.22%).

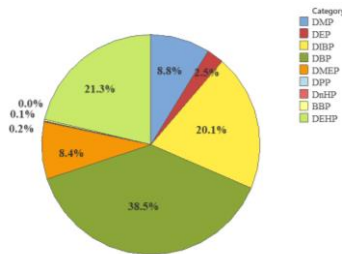


Figure 4. The distributions (%) of PAEs in sediment.

Similar to the findings of this study, the PAE patterns in sediments were taken from three different Vietnamese rivers [11]. The distribution of DBP and DEHP was generally much higher than that of other PAEs. This could be related to how PAEs are contaminated in these rivers differently [11].

### 3.4. Ecological Risk Assessment

In this work, the European Commission's technical guideline document on risk assessment [14] was used for the calculation of risk quotient (RQ) in sediments from Rao Cao River, similar to previous studies [11, 13], as equation below:

$$RQ = \frac{MEC}{PNEC_{\text{sediment}}} \quad (*)$$

Where MEC, and  $PNEC_{\text{sediment}}$  were the measured concentrations of PAEs, predicted no effect concentration of PAEs in sediment, respectively. The  $PNEC_{\text{sediment}}$  value was determined using the following formula:

$$PNEC_{\text{sediment}} = (LC50, EC50, \text{ or } NOEC) / AF$$

Where LC50, EC50, NOEC, and AF are the median lethal dose, half-maximal effective concentration, no observed effect concentration, and assessment factor, respectively. Alternatively, the value of AF was 100, 50, or 10, corresponding to long-term/chronic no observed effect concentration (NOEC) values for different trophic levels. Data on the acute or long-term toxicity of PAEs to fish, algae, and crustaceans in aquatic environments has been obtained from Li et al., (2017) [1]. In sediment, PAEs (DMP, DEP, DIBP, DBP) had  $\log K_{ow}$  from 3-5, RQ value >1 posed a high risk, when PAEs (DEHP) had  $\log K_{ow} > 5$ , RQ value >10 posed a high risk.

Five PAEs, such as DMP, DEP, DIBP, DBP, and DEHP, were chosen to assess the possible environmental impact of PAEs in the Rao Cai River. The value of RQ for DMP, DEP, DiBP, DBP, and DEHP with different trophic levels in different sampling sites is shown in Table 3.

Table 3. The value of RQ for DMP, DEP, DiBP, DBP, and DEHP with different trophic levels in sampling sites

PAEs	Tropical level	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
DMP	Algae	0.0005	0.0103	0.0076	0.0214	0.0429	0.0795	0.0957	0.0514	0.0381	0.0238
	Crustaceans	0.0005	0.0107	0.0079	0.0223	0.0446	0.0826	0.0994	0.0534	0.0396	0.0248
	Fish	0.0004	0.0093	0.0069	0.0194	0.0388	0.0719	0.0866	0.0465	0.0345	0.0216
DEP	Algae	0.0011	0.0011	0.0017	0.0051	0.0069	0.0139	0.0069	0.0057	0.0084	0.0055
	Crustaceans	0.0033	0.0034	0.0050	0.0152	0.0205	0.0417	0.0207	0.0169	0.0250	0.0165
	Fish	0.0055	0.0056	0.0082	0.0251	0.0338	0.0686	0.0340	0.0278	0.0412	0.0273
DIBP	Algae	-	-	-	-	-	-	-	-	-	-
	Crustaceans	-	-	-	-	-	-	-	-	-	-
	Fish	0.361	0.483	0.447	<b>3.97</b>	<b>1.76</b>	<b>4.26</b>	<b>5.30</b>	<b>2.42</b>	<b>2.23</b>	<b>1.39</b>

DBP	Algae	0.125	0.0834	0.102	0.753	<b>1.05</b>	<b>1.19</b>	0.581	0.417	0.510	0.327
	Crustaceans	0.101	0.0675	0.0826	0.610	0.850	0.958	0.4701	0.337	0.413	0.264
	Fish	0.0263	0.0175	0.0214	0.158	0.220	0.248	0.122	0.0874	0.107	0.0685
DEHP	Algae	0.0657	0.113	0.0842	0.9175	1.13	1.60	1.41	0.565	0.421	0.335
	Crustaceans	0.0016	0.0027	0.002	0.0219	0.0270	0.0383	0.0337	0.0135	0.0101	0.008
	Fish	0.0001	0.0001	0.0001	0.0009	0.0011	0.0016	0.0014	0.0006	0.0004	0.0003
“-” no information about the value of LC50, EC50, NOEC											

From Table 3, RQ values for DMP and DEP ranged from 0.0005 to 0.0957, and RQ for DEHP ranged from 0.0001 to 1.60 that there was no risk to fish, crustaceans, or algae. Similarly, the value of RQ for DBP did not pose a risk to fish or crustaceans. However, 20% of sampling sites pose a high risk to algae. At 70% of locations, the RQ value for DiBP posed a high risk for fish (*Pimephales promelas*). These findings are comparable to those obtained in sediments from the Yangtze River, China [13], and Hangzhou Bay, China [15].

#### 4. Conclusion

This study reported the occurrence and distribution of PAEs in the surface sediments from Rao Cai rivers, Ha Tinh, Vietnam. The method was optimized with high recovery (79.6-94.3%) and low MDL (2.0-6.0 ng/g). The method was applied to calculate the presence of PAE in sediment collected at Rao Cai River, Ha Tinh, Vietnam. The total concentrations of PAEs were found in the sediment samples in the ranges of 72.4 to 1390 ng/g-dw (mean/median: 561/552 ng/g-dw). Similar to other micro-environments, DEHP accounted for the highest distribution of nearly 40%, and the lowest was DnHP at 0.04%. The ecological risks of DiBP, DBP, and DEHP were shown for aquatic organisms, and the results showed that there is no risk to marine algae, fish, and crustaceans with DMP, DEP, and DEHP compounds. However, the RQ value for DPP indicates a high risk to fish and DiBP to algae. Overall, the results of this study may help the authorities issue regulations associated with PAEs in the years to come.

#### Acknowledgements

Nguyen Nu My Ha was funded by the Postdoctoral Scholarship Programme of Vingroup Innovation Foundation (VINIF), code VINIF.2023.STS.27.

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