Assessment of stable carbon isotopes 13C/12C ratio in phthalates from surface waters using HPLC-HRMS-TOF approach

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Research Article

Keywords: phthalates, stable carbon isotopes ratio, HPLC, HRMS, surface water, Lake Baikal

Posted Date: March 15th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2559286/v1

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on July 11th, 2023. See the published version at https://doi.org/10.1007/s11356-023-28494-w.

Abstract

A method for estimating of the ratio of stable carbon isotopes $^{13}C/^{12}C$ in the composition of phthalates from surface water at a trace concentration level is proposed. It is based on the concentration of hydrophobic components of water using an analytical reversed phase HPLC column followed by their gradient separation and detection of eluted phthalates using a high-resolution time-of-flight mass spectrometry (HRMS-TOF) in the form of molecular ions. The ratio of stable carbon isotopes $^{13}C/^{12}C$ in phthalates is calculated as a ratio of the peak areas of the monoisotopic masses $[M + 1 + H]^+$ and $[M + H]^+$. Commercial phthalates, di-*n*-butyl phthalate (DnBP) and di-(2-ethylhexyl) phthalate (DEHP), were used as standards. The minimal concentration of D*n*BP and DEHP in water required for a reliable determination of $\delta^{13}C$ value is estimated by the level of ca. 0.2 μg L^{-1}. The technique has been verified during the monitoring of priority phthalates in the waters of Lake Baikal.

Introduction

Among organic micropollutants (OMPs), diesters of *o*-phthalic acid (phthalates) take a special place due to their biological properties and industrial application. As plasticizers, phthalates are introduced into polymeric materials for obtaining the required characteristics of plastics. Since phthalates are not chemically bounded to the polymer matrix, they are able to migrate into the environment during the use and recycling of polymer products. Traces of phthalates can be found in atmospheric aerosol and air, in solid household waste and in sludge from sewage treatment plants, in drinking, sea and river waters and bottom sediments (Mondal et al. 2022; Net et al. 2015). Phthalates manifest biological activity, their toxic effect was stated; they effect both human health and wildlife species such as mollusks, crustaceans, fishes and invertebrates (Rowdhwal and Chen 2018; Heudorf 2007; Benjamin 2015). Taking into account biological properties of phthalates and production of polymeric materials of up to 370 million tons per year (Annual production of plastics worldwide 2022), phthalates are included in the list of persistent organic pollutants (POPs) (Priority Pollutant List 2021).

Recent studies in the field of isotope analysis indicate the possibility of phthalates biosynthetic pathway in environmental objects (Namikoshi et al. 2006; Chen 2004; Babu and Wu 2010). The evidence of natural origin of phthalates were the content of natural carbon isotope ^{14}C in di-*n*-butyl phthalate (D*n*BP) isolated from the algae *Undaria pinnatifida*, *Laminaria japonica*, *Ulva sp.* as well as ^{13}C-labeled D*n*BP and monoethylhexyl phthalate from freshwater algae and cyanobacteria cell cultured on $NaH^{13}CO_3$ containing media as the sole carbon source. The isolation of only one from the two possible diastereomers of DEHP from brown sugar and *Aconitum baicalense* Turcz. ex Rapaics cultivated cells proves the biosynthesis of the phthalate (Chernykh and Semenov 1982; Semenov et al. 2016).

Apparently, phthalates found in surface waters at the trace level may not be solely anthropogenic pollutants but also come from biogenic sources, the contributions of both of them can be comparable in magnitude. In this case, a deliberate choice of methods for monitoring of phthalates in water bodies as

well as obtained results evaluation is of the utmost importance. The identification of dominant source of phthalates can be implemented by measuring the content of stable carbon isotopes ^{12}C and ^{13}C in phthalate composition. Taking into account the proceeding of biosynthesis under the kinetic isotope effect, which, in combination with the metabolic isotope effect, leads to the formation of secondary metabolites including phthalates in which a reduced content of carbon ^{13}C isotope is observed (Fiedziukiewicz et al. 2021; Blessing and Baran 2022). The isotopic fingerprint of a chemical compound allows to put forward an assumption on the natural/industrial origin of the detected phthalate.

The key method for solving the issue is compound-specific isotope analysis (CSIA), which determines isotopic composition at the molecular level (Wasylenko and Stephanopoulos 2013; Maberly et al. 1992). When OMPs are presented in a sample at the trace level, their analysis by CSIA requires the introduction of a preconcentration step using liquid-liquid (LLE), solid phase extraction (SPE), or similar procedures followed by subsequent separation of concentrated hydrophobic components by chromatographic methods. Thus, the accuracy of isotopic analysis using e.g. GC-IRMS or LC-IRMS methods will depend on the results of each additional step in the procedure of sample preparation (Schmidt et al. 2004; Blessing et al. 2008; Elsner et al. 2012). Despite the universality, both IRMS methods imply the conversion of total analyte carbon in on-line or off-line mode to carbon dioxide CO_2, for which a dozen isotopologues should be considered, among which the most intense are $^{12}C^{16}O_2$, $^{13}C^{16}O_2$, $^{12}C^{16}O^{18}O$ etc., which reduces the selectivity and, in the case of analyte contamination may lead to unreliable results. The LC-IRMS method is based on the use of water or aqueous salt solutions as eluents for the chromatographic separation stage (including ion exchange chromatography), in off-line mode the fractions of hydrophobic target compounds are derived without the use of organic solvents (Yun et al. 2022; Zhu et al. 2014). The GC-IRMS analysis of substances that do not have sufficient volatility implies a derivatization of analytes leading to an inevitable change in the carbon isotopes ratio $^{13}C/^{12}C$ ($\delta^{13}C$) of the components. In case of phthalates an increase in the stages of sample preparation results in secondary contamination of sample by phthalates from the laboratory background increasing the $\delta^{13}C$ value uncertainty.

In the present paper, we report on the technique of stable carbon isotopes $^{13}C/^{12}C$ ratio measurement in phthalates from surface waters at the trace level of the former by means of high performance liquid chromatography (HPLC) and high resolution time-of-flight mass spectrometry (ESI-HRMS). The proposed method differs from the known ones by using of analysis a direct concentration of hydrophobic components of water, including phthalates (lg k_{ow} from 1.47 to 8.06, Cousins and Macka 2002) at the first stage, on a short reversed phase analytical column in on-line mode followed by gradient elution (separation), and detection of phthalates by high-resolution time-of-flight mass spectrometric detector in the form of molecular $[M + H]^+$ ions. Due to the content of priority phthalates: DEHP, D*n*BP, diethyl phthalate (DEP), dimethyl phthalate (DMP), dioctyl phthalate (DOP) and benzyl butyl phthalate (BBP) in surface waters is decreased in line (maximum level, μg L^{-1}, Mondal et al. 2022):

200 (DEHP) > 120 (D*n*BP) > 33 (DEP) ~ 32 (DMP) 6.1 (DOP) ~ 4.4 (BBP),

the proposed method was focused on DEHP and D*n*BP as the dominants in the series of POPs found in surface waters.

Materials And Methods

Reagents And Standards

For HPLC analysis acetonitrile (HPLC grade, ChimMed, Russia), freshly distilled low-mineralized artesian water (Baikal-Incom Ltd., Russia), methanol (HPLC grade, Vecton, Russia) and formic acid (98%, Panreac, AppliChem, Germany) were used. Extraction of phtalates from samples and preparation of standard solutions for GC-MS were done using *n*-hexane (HPLC grade, Cryochrom, Russia) and acetone (reagent grade, EKOS-1, Russia). The content of phthalates in organic solvents was controlled by GC-MS prior analysis according to the signal-to-noise ratio (S/N) of the analytes, when $S/N \geq 3$, the solvents were distilled before use. Glassware was sequentially washed with a solution of $K_2Cr_2O_7$ in anhydrous sulfuric acid, then with distilled water and acetone, hermetically sealed with aluminum foil stoppers. Sodium sulfate (NevaReaktiv, Russia) calcined at 300°C for 6 h. Reference samples of D*n*BP (Lot# BCBS3644V) and DEHP (Lot# BCBR8079V) were purchased from Sigma Aldrich (Germany).

Water Sampling

Water samples were taken during expedition work (June 2022): from the upper water layer of the pelagial of Lake Baikal with an SBE-32 cassette sampler (CarouselWaterSampler, Sea-BirdElectronics, USA) at a depth of 5 m, surface water samples were taken from the coastal zone at a distance of up to 100 m from the coast. In March 2022, water samples were taken from under the ice of the lake using a steel bathometer. Sampling stations are shown on the map (refer to Fig. 3). Sample volume was ca. 1 L. An aqueous solution of 0.5 mL 1 M sodium azide (MERCK, Germany) was added as a preservative. The each glass bottle containing water sample were capped with aluminum foil and stored at + 5°C before analysis. Phytoplankton was sampled at stations No. 5, 7, 10, 11 from 0–15 m using an Apstein net, 30 µm cell size. The samples were settled and filtered, and the biomass was transferred into aluminium foil envelope and stored at -15°C.

Hplc-hrms-tof Analysis

Methanol in the amount of 0.75 mL was added to a 14.25 mL water sample, and the resulting solution was placed into the loop inlet of HPLC device (Agilent 1200, Agilent, USA) with a maximum volume of 10 mL. The sample was concentrated on a reversed phase column (75 · 2 mm, Nucleosil 100-5-C18 sorbent, Macherey-Nagel, Germany) at a flow rate of 0.2 mL min^{-1} for 50 min. Concentrated hydrophobic components of water were further eluted under the following conditions: eluent A – 0.1% formic acid in water; eluent B – 0.1% formic acid in acetonitrile; flow 0.12 mL min^{-1}; gradient B from 30–100% for 20 min, then 15 min 100% B. The phthalates were detected using high-resolution time-of-flight mass

spectrometer (Agilent 6210, Agilent, USA) in the ESI + mode, the mass range *m/z* from 60 to 600; carrier gas (nitrogen, 99.8%) flow and temperature were set at 5 L min^{-1} and 325°C, respectively, nozzle pressure – 45 psi, capillary voltage – 3.5 kV. The device calibration was performed using the ESI-L Low Concentration Tuning Mix (P/N G1969-85000, Agilent, USA) once in two months.

The chromatograms were recorded in the following order: three parallel water samples (the first one was excluded) and two parallel sets of standard mixture of phthalates (DEHP and D*n*BP, each 10 μg mL^{-1} in MeOH, 2 μL) and a blank sample using one set of reagents, eluents, standard solutions, and glassware without the concentration. To measure the $^{13}C/^{12}C$ ratio in phthalates, the selected mass range chromatograms (EIC) at *m/z* 279.16 ± 0.05 for D*n*BP and *m/z* 391.28 ± 0.05 for DEHP (molecular ions in form of $[M + H]^+$) were extracted from total ion current (TIC) chromatogram. Mass spectra (scans) of each phthalate were averaged within their chromatographic peak on the EIC chromatograms. In the averaged mass spectrum of both D*n*BP and DEHP, the peaks of $[M + H]^+$ and $[M + 1 + H]^+$ corresponding to the content of ^{12}C and ^{13}C, respectively, were integrated and the arithmetic mean area of the same peaks from blank experiments were subtracted. The ratio of stable carbon isotopes $^{13}C/^{12}C$ in the composition of phthalates ($\delta^{13}C$, ‰) was calculated relative to that of standard samples of commercial phthalates D*n*BP and DEHP using the formula:

$$\delta^{13}\ ,‰ = \left(\frac{\left(^{13}\ /^{12}\ \right)_{s}}{\left(^{13}\ /^{12}\ \right)_{st}} - 1 \right) \times 1000$$

,

where $(^{13}C/^{12}C)_S$ is the ratio of stable carbon isotopes in phthalate from sample, $(^{13}C/^{12}C)_{St}$ is the ratio of stable carbon isotopes in phthalate from standard sample. The effect of phthalate's concentration on the $^{13}C/^{12}C$ ratio was carried out on standard solutions of D*n*BP and DEHP (10 μg mL^{-1} in MeOH) by its sequential dilution. The root-mean-square error (RMSE) of carbon isotopes $^{13}C/^{12}C$ ratio measurements in phthalates of standard solution does not exceed ± 0.47% и ±0.55% (N = 24 for the period of February – December 2022), daily average is ± 0.14% (N = 8) for both D*n*BP и DEHP, respectively.

Gc-ms Analysis

The content of phthalates in water samples was estimated by a method (Gorshkov et al. 2017) including LLE of phthalates into *n*-hexane and direct analysis of extract aliquots by GC-MS. Deuterated phthalates: dipropyl phthalate (DPP-d_4) and dihexyl phthalate (DHP-d_4) (Witega, Germany) were added as internal standards for quantitation. Peaks of phthalates and standards were recorded in the selected ion monitoring mode (SIM, *m/z* 149 and 153) and identified on the chromatograms by comparing their relative retention times. The concentration of phthalates in water samples was measured as the average value of the results from two parallel samples. The secondary contamination of the analyzed samples by

phthalates from the laboratory background was evaluated by the procedure of a blank experiment, the obtained concentrations were subtracted from the results of analyses. The limit of D*n*BP and DEHP detection estimated as 0.1 and 2.0 ng L^{-1}, RMSD are 0.24 and 0.14, respectively.

Determination of D n BP and DEHP in water with various sample preparation methods

On-line HPLC. The hydrophobic components of bottled water (10 mL) were concentrated on HPLC column, and then the concentrated substances were separated in gradient elution mode. The $^{13}C/^{12}C$ ratio in phthalates was measured further according to the abovementioned procedure. In separate experiment the fraction containing both D*n*BP and DEHP was collected, and the content of phthalates was measured by means of GC-MS.

LLE method. An aliquot of 200 mL water sample was added to a 500 mL separating funnel, extracted with 10 mL of *n*-hexane for 3 min and left until the phases were completely separated. The organic layer (*n*-hexane) was sampled into a 50 mL flask, the extraction was repeated twice. The extracts were combined and concentrated on a rotary evaporator to a volume of ca. 0.1 mL, the concentrate was transferred to an autosampler vial and 0.5 mL of methanol was added, the volume was adjusted to 0.1 ml in slow nitrogen stream. The $^{13}C/^{12}C$ ratio of phthalates was measured in the resulting sample by HPLC-HRMS-TOF and the amount by GC-MS.

SPE method. The phthalates concentrated using Discovery DSC SPE cartridge (500 mg, Supelco) preconditioned with 6 mL of methanol and 6 mL of bottled water. An aliquot of 200 mL water sample passed through the cartridge at a rate of 3–5 mL min^{-1}, then the cartridge was dried under vacuum for 10 min, phthalates were eluted from the cartridge with 6 mL of methanol. The eluate was concentrated on a rotary evaporator to a volume of ca. 1 mL and further adjusted to 0.1 mL by slow nitrogen stream. The $^{13}C/^{12}C$ ratio and content of phthalates were measured accordingly.

Determination of D n BP and DEHP in phytoplankton biomass

Two phytoplankton samples of ~ 0.5 g and one with a mass of ~ 0.1 g were taken (accurate weight up to 0.1 mg), thawed and homogenized. The low weight sample was dried up to a constant weight and the moisture content was determined by the gravimetric method. Mixtures of DPP-d_4 and DHP-d_4 standards (50 µl, 0.1 µg $µL^{-1}$ each) were added to the first two samples and triturated in presence of sodium sulfate until a homogeneous powder. The latter was extracted twice by *n*-hexane-acetone (10 mL, 1:1, v./v.) in an ultrasonic bath for 10 min. The aliquot of 1 mL is taken from combined extracts and analyzed by GC-MS. To measure the $^{13}C/^{12}C$ ratio, the same protocol was applied excluding the addition of deuterated standars, the extracts after solvent evaporation were dissolved in 100 µl methanol.

Results And Discussion

Concentration and separation of phthalates

The proposed method for carbon isotopes $^{13}C/^{12}C$ ratio measurement in phthalates was designed for the analysis of surface waters where the content of analytes is at the level of traces. Taking into account a low concentration of target compounds and possible contamination of sample with phthalates from the laboratory background, an on-line HPLC was proposed for concentration of hydrophobic components of sample on the analytical reversed phase column. The use of a short small-diameter HPLC column (75 · 2 mm) allows to concentrate phthalates from the sample of up to 10 mL in 50 min. At the next analysis stage, the concentrated hydrophobic components of water sample are separated by gradient elution. The peak capacity (Z = 30–50) and efficiency (N ≥ 4000 t.t.) of the HPLC column ensure the elution of both D*n*BP and DEHP in chromatographic peaks within a volume of < 90 µL. As a result, when the content of phthalates in a water sample is not less than 0.2 µg L^{-1}, the concentration of water sample by k ≥ 660 times is sufficient for measuring of stable carbon isotopes $^{13}C/^{12}C$ ratio.

On-line concentration procedure with a reduced number of sample preparation steps results in decrease of phthalate contribution from the laboratory background and hence in making the $^{13}C/^{12}C$ ratio measurements authentic. The presence of phthalates in solvents (< 2 ng L^{-1} for organic solvents and 0.3–1.6 µg L^{-1} in eluent A used for HPLC) as well as in chromatographic equipment determines the occurrence of background response of these substances in blank experiments. It should be noted that a relatively high concentration of background phthalates in the eluent A does not have a significant influence onto the $^{13}C/^{12}C$ ratio measurements because its consumption in a single analysis does not exceed 2 mL (taking into account the column regeneration) or a few ng of phtalates. The recorded peaks of laboratory background phthalates were considered as a systematic error, and were subtracted from the corresponding analyte peaks while processing chromatograms.

Keeping in mind the trace level of target compounds, a volume of one to several liters of sample is required for OMPs analysis in water using the CSIA approach as well as an introduction of additional concentration step into the preparation procedure, e. g. LLE or SPE. The need of concentration of the sample of augmented volume is accompanied by an increase not only in analysis time but also by undesirable intake of accompanying components form the matrix. Since the CSIA method is less sensitive and not mass-selective for analysis of multicomponent samples, the necessary of target compound separation from other interfering substances in some cases cannot be achieved, in particular, when the concentration of POPs in surface waters is at the sub-µg L^{-1} level (Blessing Baran 2022). The application of ESI-HRMS-TOF at the final stage of analysis is characterized by greater selectivity and reliability of analytical peaks identification. An addition of formic acid into the eluents for the purpose of protonation of phthalates (the detection of compounds in $[M + H]^+$ form) has virtually no effect on the shape of phthalate peaks, only slight reduction of their retention time occurs. Depending on hydrophobic properties of sample components, the acid addition allows reducing the background accompanying and interfering substances that are eluted under the same conditions as phthalates at the first stage of separation, Fig. 1.

It should be noted that, depending on the concentration method, not only low recovery of phthalates may occur, but also the accumulation of phthalates from the laboratory background in the samples during the LLE or SPE procedure. The contribution of the laboratory background (abiotic source) to the carbon isotopes $^{13}C/^{12}C$ ratio will thus be critical in source identification, in particular for potentially biogenic phthalates analysis, Table 1.

Table 1
The content and $^{13}C/^{12}C$ ratio in phthalates from bottled water sample

Sample preparation procedure	D*n*BP		DEHP	
	µg L^{-1}	$^{13}C/^{12}C$, ‰	µg L^{-1}	$^{13}C/^{12}C$, ‰
On-line HPLC, n = 5	0.44	13.56 ± 0.24	0.57	21.25 ± 0.08
LLE, n = 5	0.42	14.27 ± 0.21	0.78	20.89 ± 0.22
	0.31[a]	-	1.6[a]	-
SPE, n = 5	0.54[b]	15.36 ± 0.48	0.26[b]	23.58 ± 0.13
	0.15[c]	-	0.08[c]	-

[a] Water after distillation; [b] Eluted with *n*-hexane; [c] Eluted with methanol; n – repetition count.

The $^{13}C/^{12}C$ ratio determination

The $^{13}C/^{12}C$ ratio of stable carbon isotopes in the composition of phthalates found in surface water samples is estimated from the ratio of the peak area of monoisotopic molecular ions $[M + H]^+$ and $[M + 1 + H]^+$, corresponding to the content of stable carbon isotopes ^{12}C and ^{13}C, respectively, Fig. 1. The $\delta^{13}C$ value is calculated relatively to the $^{13}C/^{12}C$ ratio in commercial D*n*BP and DEHP phthalates used as standards. The mean $^{13}C/^{12}C$ value (%) in D*n*BP and DEHP is 14.01 ± 0.47 and 21.33 ± 0.55*, respectively. According to Fiedziukiewicz and Hanley (2021) the $\delta^{13}C$ value for D*n*BP and DEHP are − 29.9‰ and − 26.5‰ on the VPDB scale, respectively. The calculation of $\delta^{13}C$ value for phthalates according to the VPDB scale using the HPLC-ESI-HRMS-TOF approach seems impossible because on the stage of liquid chromatography separation, a carbon-containing eluent, acetonitrile, is used. The presence of the latter does not interfere with the generation of phthalate ions. In contrast, the use of conventional CSIA methods assumes transferring a high purity sample into the gaseous state by its combustion, oxidation, or pyrolysis prior to mass spectrometric analysis.

The measurement of $^{13}C/^{12}C$ ratio in the composition of phthalates by the ESI-HRMS-TOF method gives a qualitative characteristic of detected phthalates, while their concentrations are assessed by GC-MS. It is shown that at the content of phthalates in water sample in the range from ca. 0.2 to 2 µg L^{-1}, the RMSE of

the $^{13}C/^{12}C$ value (%) does not exceed ± 0.32 and ± 0.68 for D*n*BP and DEHP, respectively, for a single series of experiments, Fig. 2. The analysis of samples, in which the content of phthalates is less than 0.2 μg L^{-1} is characterized by a significant scattering of the $^{13}C/^{12}C$ value, the RMSE are from ± 2 to ± 40 as a result of uncontrolled contribution of laboratory background phthalates.

The $^{13}C/^{12}C$ ratio in DnBP and DEHP from surface waters

The measurement of stable carbon isotopes $^{13}C/^{12}C$ ratio in the composition of phthalates according to the proposed method was tested on POPs monitoring at the trace level in Lake Baikal, waters of which are notable by their purity compared with other freshwater sources (Khodzher et al. 2017). The content of D*n*BP and DEHP in the waters of the lake during the spring season of 2022 was estimated from 0.01 to 0.18 μg L^{-1} and 0.06 to 0.48 μg L^{-1}, respectively, Table 2. Since the evaluation of $\delta^{13}C$ is possible for surface water samples where the content of D*n*BP or DEHP is ≥ 0.2 μg L^{-1}, the analysis was carried out mainly on the example of DEHP, the concentration of which was sufficient for that in major part of samples. It was found that in all analyzed samples, the $\delta^{13}C$ value for DEHP, measured relative to commercial DEHP, are below zero indicating the contribution of a biotic source to the total content of the phthalate in upper water layer of the lake. A potential biogenic source of phthalates during the spring season can be attributed to phytoplankton. The DEHP content in the latter estimated at the level of 170 μg g^{-1}, while the $^{13}C/^{12}C$ ratio (%) was 20.34 ± 0.06, and the $\delta^{13}C$ value is equal to − 46.4 ± 2.8‰. It should be noted that phytoplankton's $\delta^{13}C$ value is the lowest one among all studied samples, and hence phytoplankton as a DEHP producer contains the minimum amount of the phthalate from an abiotic source.

Table 2 Stable carbon isotopes $^{12}C/^{13}C$ ratio in the composition of phthalates from upper water layer of Lake Baikal

Station, No	D*n*BP, n = 2			DEHP, n = 2		
	Concentration, μg L^{-1}	$^{13}C/^{12}C$	$\delta^{13}C$, ‰	Concentration, μg L^{-1}	$^{13}C/^{12}C$	$\delta^{13}C$, ‰
Upper water layer, depth 5 m, pelagial of the lake						
2	0.18	-	-	0.31	20.89±0.11	–20.6
3	0.06	-	-	0.21	20.99±0.42	–
7	0.11	-	-	0.06	-	15.9
8	0.09	-	-	0.48	20.92±0.11	-
9	0.01	-	-	0.17	20.89±0.03	–19.2
10	0.10	-	-	0.09	-	–
11	0.01	-	-	0.21	20.69±0.09	20.6 - – 30.0
Coastal zone						
4	0.03	-	-	0.26	20.54±0.12	–37.0
6	0.07	-	-	0.63	21.11±0.44	– 10.3
Upper water layer during the ice cover period						
1*	1.52	14.49±0.13	34.3	0.68	21.04±0.28	–13.6
1**	0.16	-	-	5.78	20.82±0.03	–
3*	0.31	14.78±0.16	55.0	0.34	21.06±0.02	23.9
3**	0.11	-	-	5.04	20.85±0.07	–
5*	0.56	14.44±0.01	30.7	0.91	20.88±0.02	12.7 –
5**	0.06	-	-	1.69	20.91±0.14	22.5 – 21.1 – 19.7

* Under ice water, depth 0 m. ** Under ice water, depth 5 m.

At the reference central stations Nos. 3, 7, 10, and 11, a relatively high concentration of DEHP was found in samples collected from southern and northern basins. The dominant contribution of a biogenic source

to the composition of DEHP in the water sample from the northern part of the lake ($\delta^{13}C = -30‰$) is observed. Water samples (Nos. 2 and 3) collected from the southern basin contained a comparable amount of phthalates while a greater contribution of an abiotic source ($\delta^{13}C = -15.9‰, -20.6‰$) occurs. In samples from the coastal zone, a relatively high DEHP content from 0.26 to 0.63 μg L^{-1} was found, the dominant source of the phthalate at station No. 6 was an abiogenic, and at station No. 4 – a biogenic one.

Notable are the extreme amount up to 5.8 μg L^{-1} of DEHP[†] found in samples from the upper water layer (Nos. 1** and 3**, depth 5 m) during the ice cover period on the lake at the end of the winter season. Based on the analysis of $\delta^{13}C$ values, the biogenic source of DEHP was suggested as dominant one due to the absence of an atmospheric channel for the entry of POPs into the lake waters. In samples taken under the ice (stations No: 1* and 3*; depth 0 m), a significant decrease in the DEHP concentration by an order of magnitude was observed. The high content of D*n*BP in these samples of probably anthropogenic origin ($\delta^{13}C = 30–55‰$) confirms the presence of their source as a result of phthalate migration into the under ice water layer being accumulated in the ice and snow during the ice cover period. The $^{13}C/^{12}C$ ratio (%) in the composition of D*n*BP (0.46 μg L^{-1}) from the snow sample was estimated as 14.24 ± 0.02 ($\delta^{13}C = 16.4‰$). The magnitude of $\delta^{13}C$ indicates the dominance of the phthalate from an anthropogenic source. Interestingly, the $\delta^{13}C$ value for DEHP at St. 5* and 5** are identical representing equal biotic and anthropogenic source contributions. In the snow, which has just fallen, the DEHP was found at a concentration of 0.67 μg L^{-1}, the $^{13}C/^{12}C$ ratio (%) was 21.45 ± 0.11, calculated $\delta^{13}C$ value is + 5.7‰. The latter value is accepted as a conditionally maximum level of DEHP accumulation from an anthropogenic source, Fig. 3.

[*] The $^{13}C/^{12}C$ value (%) was also measured for phthalates from commercial manufacturers, where it was estimated to be in the range from 13.88±0.03 to 14.51±0.05 for D*n*BP (Polymer) and from 21.67±0.01 (Sigma Aldrich) to 24.46±0.12 for DOP (Polymer).

[†] The World Health Organization (WHO) set a recommended value < 8 μg L^{-1} for DEHP in drinking water (International Programme on Chemical Safety 2002). According to U.S. EPA recommendations, DEHP levels below 6 μg L^{-1} are considered safe. The approved MACs for DEHP in Russia is 8 μg L^{-1} (SanPiN 2007).

Conclusion

Thus, the ratio of stable carbon isotopes in the composition of phthalates from surface waters on the sub-μg scale has been established by means of an alternative to the known methods, the HPLC and ESI-HRMS-TOF combination. The method includes initial concentration of hydrophobic components of water sample using the short reversed phase analytical HPLC column followed by subsequent water – acetonitrile gradient elution of analytes. The ESI-HRMS-TOF at the final stage provides high selectivity and reliability for phthalate peak identification and, most importantly, the possibility for direct detection of

^{12}C and ^{13}C-containing analytical responses, hence the $^{13}C/^{12}C$ value becomes readily accessible. The established statistically reliable measurement of the $^{13}C/^{12}C$ ratio in the composition of phthalates from surface waters is possible when the content is at least 0.2 μg L^{-1}. The proposed method for measuring the ratio of stable carbon isotopes is capable to shed light upon the dominant biogenic/abiogenic source of phthalates when monitoring POPs in the surface waters from Lake Baikal. It has been shown for the first time that relatively high levels of DEHP concentration in spring season, including extreme concentrations of this pollutant during the period of ice cover on the lake, are associated with the dominance of a biogenic source or phytoplankton. The proposed method for measuring of stable carbon isotopes ratio on the example of phthalates, D*n*BP and DEHP as key POPs in surface waters can be easily optimized for other dichotomous origin compounds at a sufficient concentration level.

Declarations

Author Contribution Anton V. Kuzmin performed HPLC-HRMS-TOF analysis and data collection, analyzed the results (equal) and finalized the manuscript. Tatyana A. Grigorieva performed water sampling, GC-MS and statistical analysis. Alexander G. Gorshkov as supervisor and principle investigator generated original idea, wrote and edited the manuscript, analyzed the results (equal). All authors read and approved the final manuscript.

Funding The study was carried out within the framework of State project 0279-2021-0005 (State reg. no. 121032300224-8).

Acknowledgements The study was carried out using the equipment of The Shared Research Facilities for Physical and Chemical Ultramicroanalysis LIN SB RAS. The authors thank I. V. Hanaev and E. D. Bedoshvili for water and phytoplankton sampling, O. V. Kustova and O. N. Izosimova for assistance with GC-MS analysis, and Prof. A. A. Semenov (SIFIBR SB RAS) for fruitful discussions of the results.

Data Availability The datasets generated and/or analyzed that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Competing interests The authors declare no competing interests.

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Figures

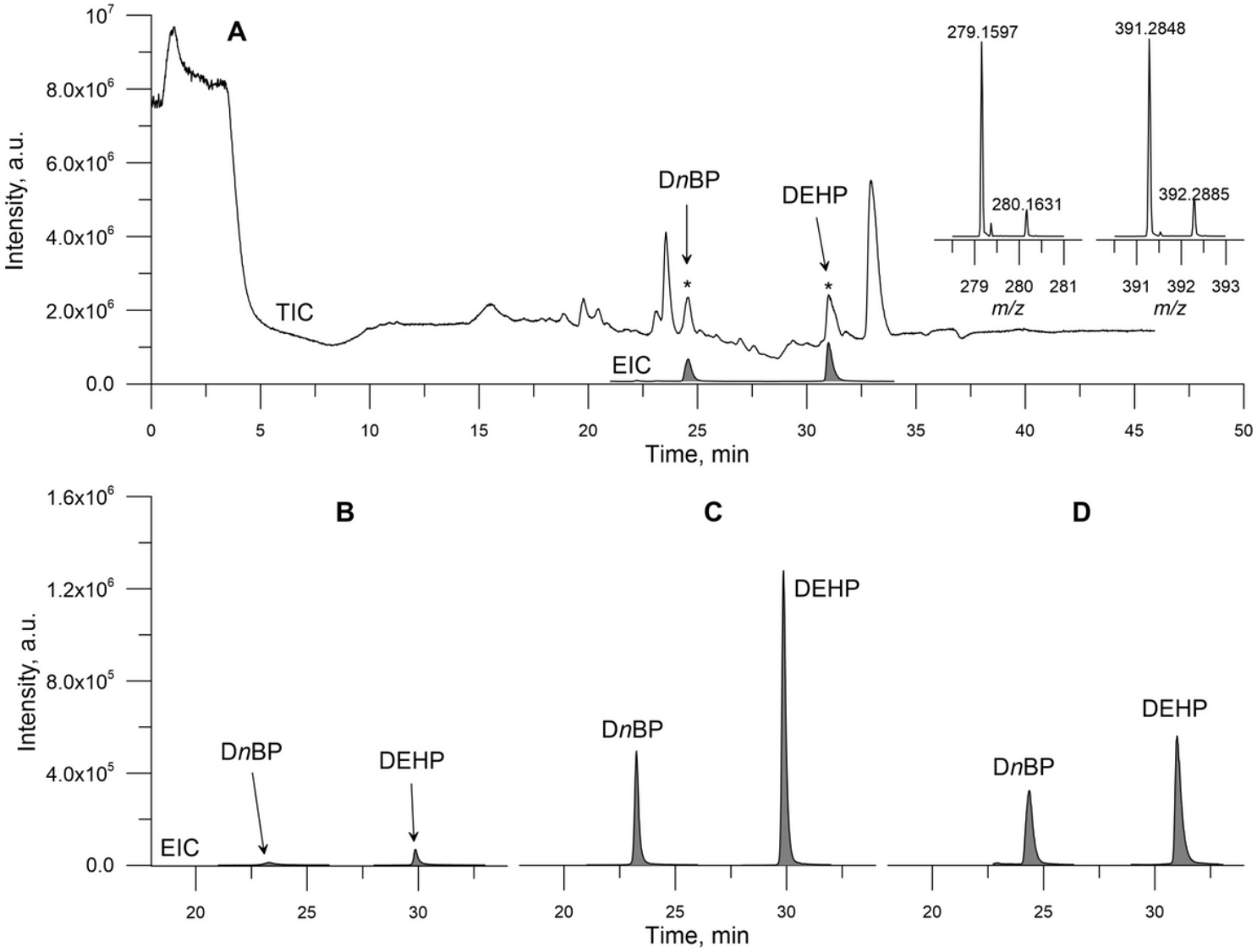

Figure 1

HPLC-HRMS-TOF chromatogram of water sample 1* collected during the period of ice cover on Lake Baikal, the concentrations of D*n*BP and DEHP are 1.52 и 5.8 μg L^{-1}, respectively. **A –** total ion current (TIC) and extracted ion current (EIC) chromatograms as well as high resolution mass spectra of D*n*BP and DEHP, **B** – EIC chromatogram of blank sample, **C** – EIC chromatogram of standard solution of D*n*BP and DEHP, **D** – EIC chromatogram of the water sample

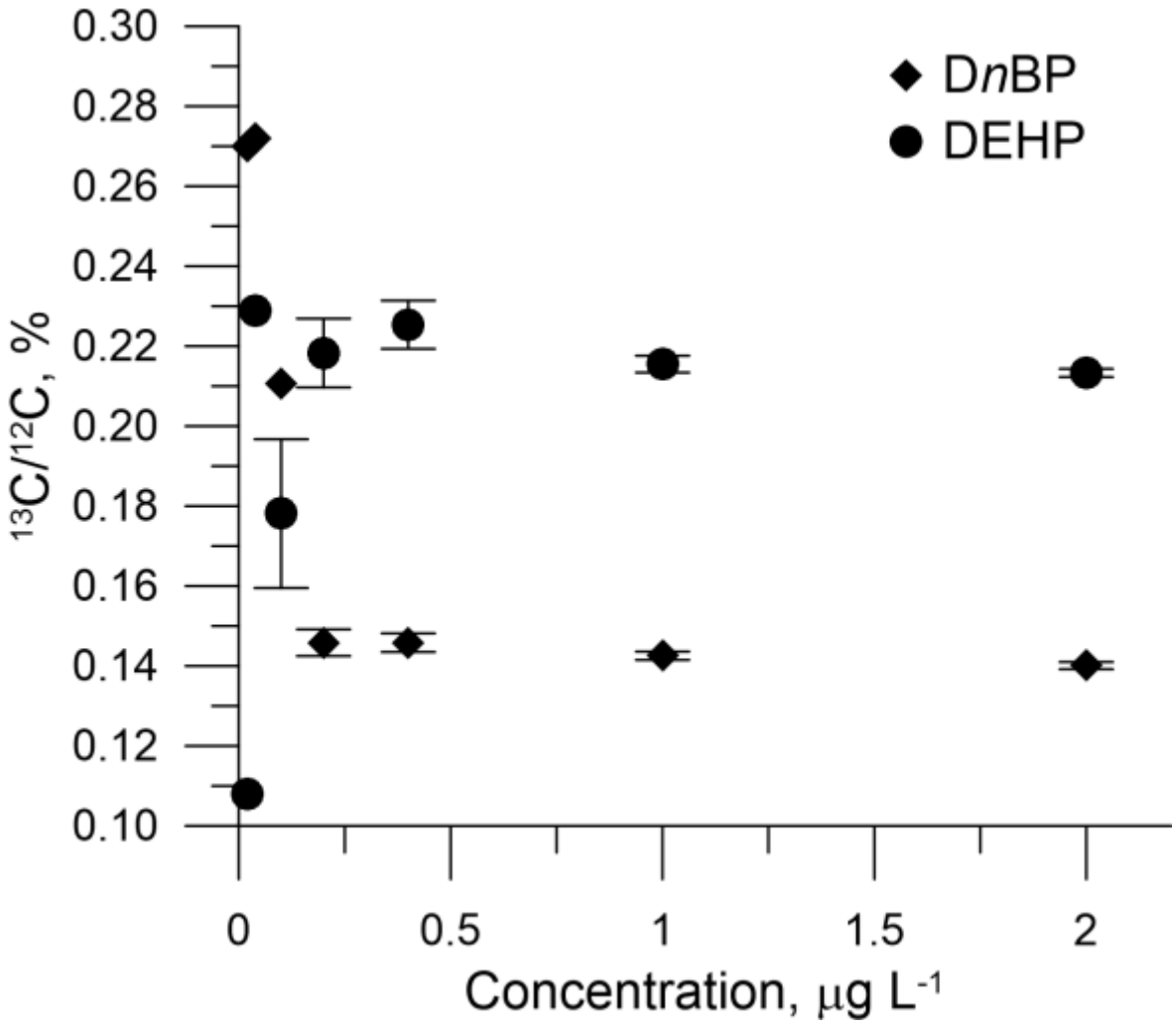

Figure 2

The effect of phthalate concentration on stable carbon isotopes ratio

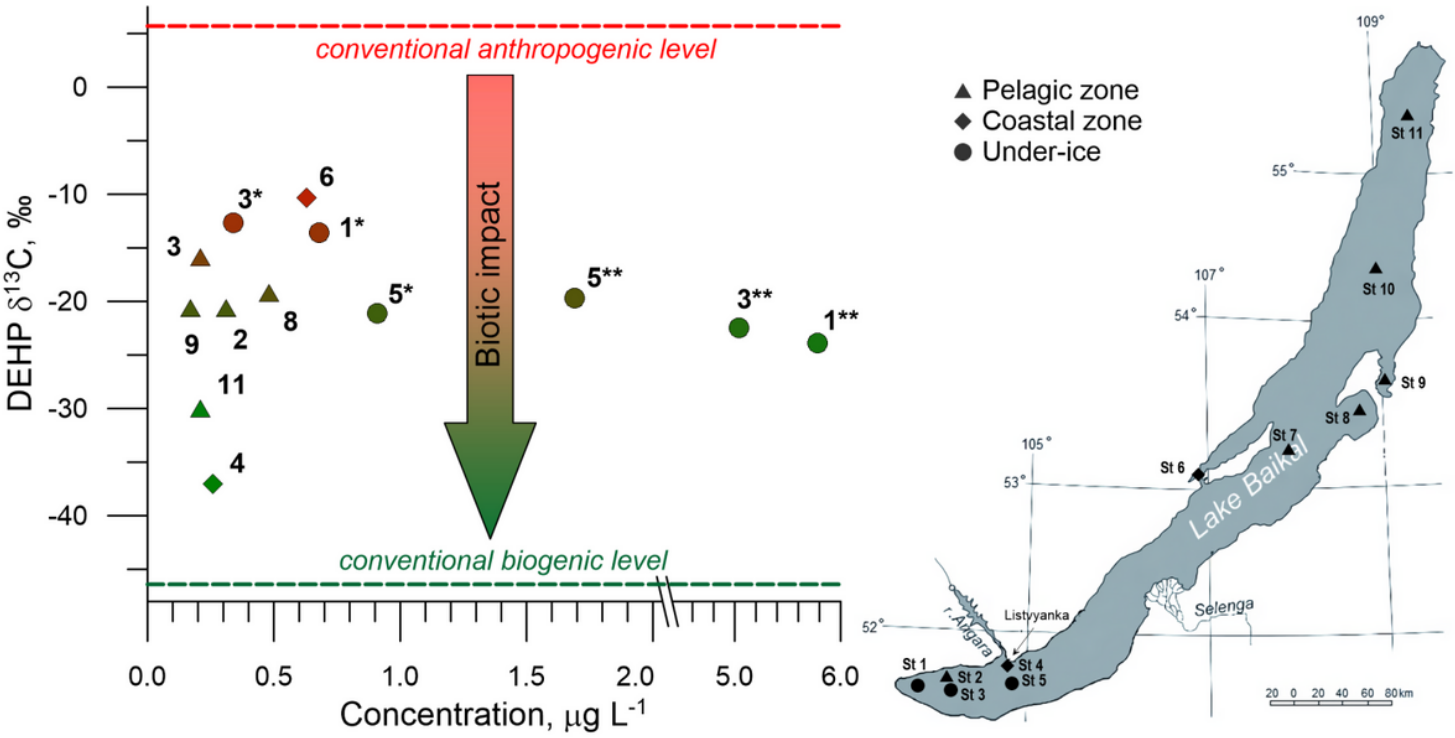

Figure 3

The distribution of $\delta^{13}C$ values of DEHP found in the surface waters from Lake Baikal between conventional biogenic and anthropogenic frontiers. Map of Lake Baikal and sampling sites. At stations **1** and **2** sampling was carried out at a distance of up to 3 km from the coast, at station **3**, **5**, **7**, **10** and **11** at the reference central stations; **4** – Listvenichnyy Bay, **6** – Mukhor Bay, **8** – Barguzin Bay, **9** – Chivyrkuy Bay

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