



Compound specific isotope analysis (CSIA) of phthalates and non-targeted isotope analysis (NTIA) of SPE-extractable organic carbon in dilute aquatic environments

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ABSTRACT

Isotope ratio analysis of total organic carbon (TOC) is well-established for the study of rivers, lakes, and oceans. Similarly, these aquatic systems are routinely surveyed to quantify natural and anthropogenic organic compounds and pollutants, including such things as pharmaceuticals, illicit drugs, and phthalates. We merged these two approaches to demonstrate compound specific isotope analysis (CSIA) on four phthalate esters and non-targeted isotope analysis (NTIA) on a gas chromatography isotope ratio mass spectrometry (GC-IRMS) platform. Water from the Rivers Foss and Trent and a waste-water treatment plant (WWTP) effluent was sampled, followed by solid phase extraction (SPE) and GC-IRMS. Di-ethyl hexyl phthalate (DEHP) concentration in the effluent was 0.20 µg/L while it was 0.22 µg/L and 0.14 µg/L in the Trent and Foss Rivers, respectively. CSIA $\delta^{13}\text{C}$ values for selected phthalates measured in river and effluent samples ranged from -24.3‰ to -28.8‰ . NTIA returned over 100 compounds which in aggregate aligned well with published values for isotope analysis of TOC. NTIA demonstrated that the volatile SPE-extractable fraction of TOC has high heterogeneity with $\delta^{13}\text{C}$ from -26.8‰ to -44.3‰ for matched compounds in both rivers. GC-IRMS can be applied to dilute environmental systems using both compound specific and non-targeted strategies.

Introduction

Anthropogenic compounds in rivers have been widely studied. Examples include illicit drugs, pharmaceuticals and everyday healthcare products (Boulard et al., 2020; Gómez et al., 2007; Hu et al., 2019; Loos et al., 2009). Phthalic acid esters (PAE) are commonly used plasticisers which find their way into wastewater treatment plant (WWTP) effluents and rivers via a range of sources including personal care products and human excretion after ingestion of food exposed to PAE present in the plastic packaging (Duty et al., 2005; Hsieh et al., 2019; Zolfaghari et al., 2014). Anthropogenic compounds are more easily found in WWTP effluent due to the higher concentrations present (Fono et al., 2006). Following WWTP treatment, the effluent is discharged into rivers with any remaining compounds mixing with a wide range of natural and man-made materials. The organic material in the river can be analysed using a range of targeted (Gas Chromatography – Mass Spectrometry (GC-MS), Liquid Chromatography – Mass Spectrometry (LC-MS), etc) approaches seeking to find particular compounds as well as assessed as total organic carbon (TOC).

TOC has been combined with Isotope Ratio Mass Spectrometry (IRMS) to study organic material in rivers with $\delta^{13}\text{C}$ values usually between -25‰ and -29‰ (Han et al., 2019; Liu et al., 2019; Peterson et al., 1994). Values of $\delta^{13}\text{C}$ from TOC present an aggregate of many individual compounds and little is known about $\delta^{13}\text{C}$ in the wide range of compounds found in rivers. Due to the rich array of compounds in rivers, non-targeted isotope analysis (NTIA) methods may be ideal tools for large scale studies of isotopic changes in individual compounds and distributions of δ values of compounds in surface waters.

Compound specific isotope analysis (CSIA) of river samples has not been done previously and few have been done on WWTP effluent (Bakkour et al., 2018). One of the barriers to CSIA in the environment is the many compounds in rivers leading to unresolved baselines in chromatograms. CSIA is commonly used in forensic and environmental studies but it also has application in sport, geochemistry, food and extra-terrestrial chemistry (Elsner and Imfeld, 2016; Lichtfouse, 2000; Pizzarello and Holmes, 2009; Yan et al., 2019). It incorporates a separation step followed by combustion, to produce CO, CO₂, N₂, or H₂ prior to measuring the isotope ratios (Sevastyanov, 2016).

Abbreviations: CSIA, Compound Specific Isotope Analysis; DEHP, di-(2-ethylhexyl) phthalate; DBP, di (n-butyl) phthalate; MEHP, mono-(2-ethylhexyl) phthalate; WWTP, Waste Water Treatment Plant.

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The major advantage of CSIA over elemental analysis isotope ratio mass spectrometry (EA – IRMS) is the application of chromatography to separate complex samples. It is already used for laboratory model studies of compounds (Elsner and Imfeld, 2016; Peng and Li, 2012; Spahr et al., 2015). CSIA was successfully applied to a selection of compounds in the range of 138 – 1389 ng/L using 10 – 12 litres of groundwater (Schreglmann et al., 2013). CSIA offers the possibility of assessing source and fractionation of anthropogenic material released into the environment (Maier et al., 2016; Taipale et al., 2019). Isotopic fractionation of carbon in the environment is affected by, but not limited to, factors such as humidity, temperature, atmospheric pressure and chemical reactions (Shibuya et al., 2007; Yeh and Wang, 2001; Zhang et al., 2018). These combined factors have made it possible to determine the origins of samples (Meier-Augenstein, 1999; Shibuya et al., 2007, 2006; Wu et al., 2015). Over the past decade, a number of studies have been published presenting model results of the analysis of emerging pollutants in spiked environmental samples using CSIA finding an inverse relationship between the size of the molecule and the level of isotopic fractionation (Maier et al., 2014; Peng and Li, 2012; Washington et al., 2019).

For some applications, the analysis may also be concerned with the concentration of the analyte as well as the isotopic composition. Analyses requiring both measures typically use two separate techniques to obtain the desired data. Traditionally those instruments would be gas chromatography (GC) or high performance liquid chromatography (HPLC) used for quantification and gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) used for isotope analysis (Piper et al., 2010; Saccon et al., 2013). Any isotope ratio mass spectrometer works on the same principle. The key element is the detector – faraday cup. Those are capable of highly precise measurement. This high precision makes it suitable for recording isotope ratios. However, the linear response of the type of detector used in an IRMS is well-known (Jochmann et al., 2006; Schmidt, 2003). The linearity of the continuous flow GC-C-IRMS system has been reported in the past and linear correlations have been demonstrated between GC-FID and GC-IRMS instruments (Piper et al., 2010; Thornton et al., 2011; Zhang et al., 2012). This means the signal recorded by GC-IRMS will be proportional to the concentration of the analyte in the sample. Therefore, allowing measurement of the concentration of the analyte using the GC-IRMS system. The difficulty of performing CSIA on any environmental sample is obtaining a complete separation of the analytes from other interfering compounds (Elsner and Imfeld, 2016), however, a range of published studies include incomplete separation sometimes following sample treatment to reduce these effects. Examples include, overlapping phenanthrene and anthracene following an enrichment procedure (Chen et al., 2017) and older environmental work on petroleum contamination in sediments (Rogers and Savard, 1999). Once obtained, the GC-IRMS signals can also provide quantitative information for selected compounds. Combining these, quantitative CSIA (qCSIA) can be applied to a low concentration environmental sample.

Here we combined CSIA, qCSIA, and NTIA to investigate carbon isotope ratios in wastewater treatment effluent (WWTE) and river samples using GC-IRMS after solid phase extraction (SPE). This gave direct access to the volatile portion of SPE extractable organic carbon via NTIA and to CSIA and quantitative analysis of selected compounds. The predominant focus of this study was analysis of river water applying NTIA to unidentified compounds and CSIA and qCSIA to four phthalates. Of particular interest was investigating the extent that compounds composing TOC exhibit heterogeneity or whether the aggregate TOC measure is sufficient to characterise their isotopic behaviour. The goal was to perform GC-IRMS analysis of dilute wastewater and environmental samples in order to measure the distribution of carbon isotope ratios as comprehensively as possible.

Materials and Methods

Materials

Di-ethylhexyl phthalate (DEHP) (Tokyo Chemical Industries; GC grade), benzyl butyl phthalate (BBP) (Sigma; 98%), di-butyl phthalate (DBP) (Sigma; 99%) and bi-ethyl phthalate (DEP) (Sigma; 99.5%) and hexane (Arcos; Extra Pure 95+%) were used as received to prepare samples, standards and secondary reference materials. Two certified standards were used, caffeine (IAEA600) and glucose (BCR-657) to calibrate everyday working standards of the phthalates on the VPDB scale.

Sampling and sampling sites

The samples were collected in 2.5 L amber borosilicate glass bottles, using a stainless steel bucket for sampling. The bottles were cleaned with methanol then deionized water prior to sample collection. Effluent samples were collected on 27 Jan 2020, from the drain of the Stoke Bardolph Sewage Treatment Works (What3Words reference: conducted.reference.thigh; GPS: 52.973386, -1.037433) as it enters the River Trent. The river water samples were collected from the river banks. The sample from the River Trent was collected on 13 March 2020, ~750 m downstream from the WWTP drain on the same side (What3Words reference: croaking.traffic.comments; GPS: 52.980123, -1.038418). The sample from the River Foss was collected on 25 Feb 2020, on the east side of the Palmer Street footbridge ~1050 m from where it enters River Ouse (What3Words reference: engine.local.dose; GPS: 53.958455, -1.073318). The locations are presented in the Fig. 1.

Both rivers were at high flow (River Foss 4.5 m³/s and River Trent 190 m³/s) at the time of collection. The average flows of the Rivers Foss and Trent are 1 m³/s and 90 m³/s, respectively. Flows reported were based on the Huntington (station reference F2470) and North Muskham (station reference 4022) measuring stations as provided by the UK Environment Agency data services platform (environment.data.gov.uk/hydrology/explore, accessed 20/12/2020). The total rainfall recorded at the month of sampling was 266.8 mm in February for Bradford station and 27.2 mm in March recorded by Sutton Bonington station as provided by the UK MetOffice via their Historic Station Data provision (www.metoffice.gov.uk/research/climate/maps-and-data/historic-station-data, accessed 07/01/2021). These Bradford and Sutton Bonington stations were the nearest stations upstream to the sampling points in York and Nottingham, respectively.

Sample extraction - SPE

SPE extraction was completed within 6 hours on the day of collection. 2 L samples were extracted using a set of eight 6 mL Oasis MCX SPE cartridges (Waters, UK) containing 500 mg of sorbent. The extraction stages are presented in the Fig. 2 above. The cartridge was first conditioned with 6 mL of methanol (MeOH) and deionized (DI) water, then 250 mL of sample was loaded per cartridge with a flow rate of 3–5 mL/min. After all the sample was loaded cartridges were dried under vacuum for 10 min. Following loading stage, the cartridges were rehydrated with deionised water and eluted with 6 mL of pure MeOH. The aliquots were combined and dried on a hot plate set to <75 °C under a nitrogen stream. Once dry, the samples were transferred to GC vials with a conical insert using hexane and dried again. The samples were then resuspended in 5 µL of hexane for analysis. The vials were wrapped in Al foil during any storage to prevent sample degradation through the UV radiation (Peng et al., 2013b). No further extraction was done in order to avoid loss of compounds before non-targeted analysis.

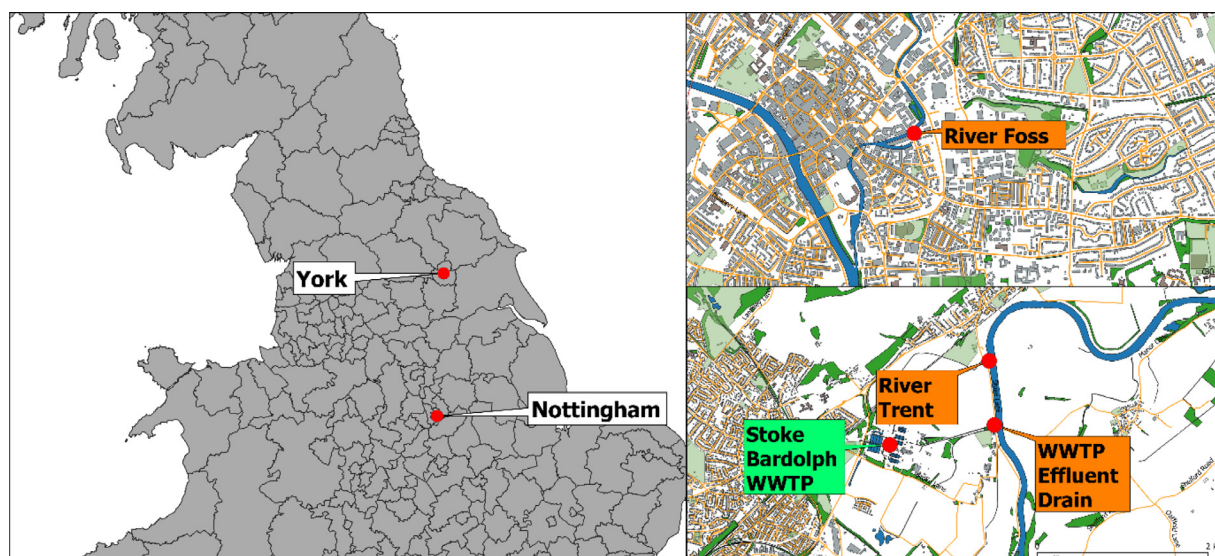


Fig. 1. Location of sampling points on the River Foss (York), the River Trent and the Stoke Bardolph WWTP drain (Nottingham). Maps were created with QGIS version 3.16.0 with data from OS OpenData licensed under the Open Government Licence. Contains OS data © Crown Copyright (2020).

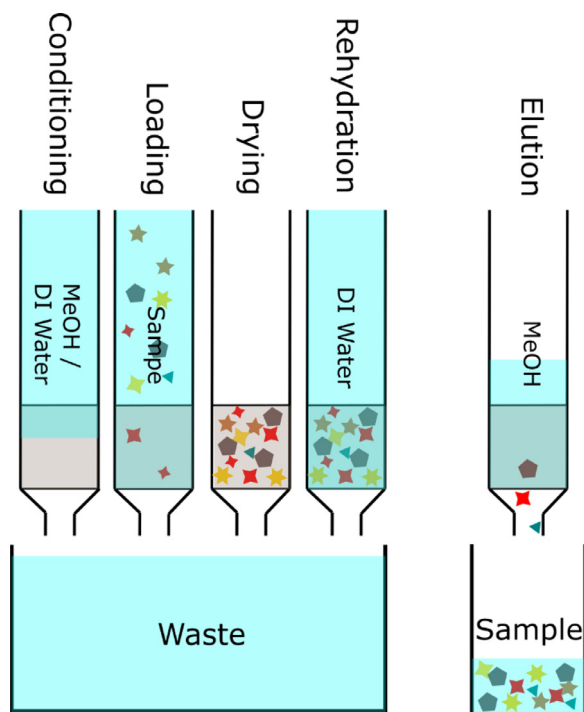


Fig. 2. Consecutive stages of SPE extraction for one cartridge. From left to right: conditioning, loading, drying, rehydration, elution.

GC-C-IRMS

The analysis was performed using an Agilent GC 7890 coupled to a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer fitted with a Gerstel CIS 4 autosampler. The depth of a needle for sampling was set up to be less than a 1 mm from the bottom of the vial insert. GC parameters were: injector port: 280 °C; oven: 50 °C; ramp: 3 °C/min to 300 °C and hold time of 8 min; column stationary phase (5%-Phenyl)-methylpolysiloxane (Agilent DB-5); column length: 30 m; internal diameter 0.250 mm; film thickness 0.25 µm. The GC-C-IRMS combustion reactor was preconditioned at 1000 °C for 1 h before the analysis. Sys-

tem contamination was monitored by injecting blanks. Any peaks in the blank were small in comparison with the samples and standards.

Results and discussion

Compound specific isotope analysis

To evaluate the relationship between signal level and $\delta^{13}\text{C}$ values in phthalates, a range of concentration standards were run at the same time as the isotope analysis. The range of concentrations evaluated was 5 – 100 µg/mL. With 1 µL injections this is equivalent to 5–100 ng of compound being introduced into the system. GC-C-IRMS is reported to require 10–100 ng of an analyte for reproducible results (Elsner and Imfeld, 2016). The standard deviation (SD) for $\delta^{13}\text{C}$ was $\pm 0.4\text{‰}$ across the full range (Figure 3) including the lowest standard concentration (5 µg/mL). Standard error values at separate concentrations ranged between $\pm 0.1\text{‰}$ and $\pm 0.2\text{‰}$ for a set of 30 µg/mL ($n = 5$) injections. The average $\delta^{13}\text{C}$ value at each concentration ranged from -26.6‰ to -26.4‰ (see Figure 3). No significant trends were observed in either the mean values or precision throughout the range of concentrations. The minimum concentration considered was 5 µg/mL which corresponds to 0.013 µg/L in a field sample prior to the extraction procedure. The 5 µg/mL DEHP reference (Figure 3) gave a signal of 479 unprocessed units for $m/z = 44$.

Phthalate esters: DEHP, BBP, DBP and DEP, were detected and characterised in waters from WWTP effluent discharge and the two rivers. Figure 4 provides representative GC-IRMS chromatograms. DEHP was found in all samples analysed consistent with this ester being the most widely produced plasticiser in the world according to previous reports (Zolfaghari et al., 2014). Figure 4 makes clear that a rising background exists in the river extracts, however this is not outside the range seen in previous studies using GC-IRMS (Chen et al., 2017; Rogers and Savard, 1999). The figure also makes clear the richness of the volatile SPE-extractable fraction of organic carbon in rivers. Studying these compounds one-by-one after perfect extraction and separation is unrealistic in these samples.

The trend from WWTP to River Trent for BBP was consistent with environmental fractionation or a source having a lighter isotope signature in the river. DEHP showed only a small isotope shift between WWTP and the River Trent. This difference is greater for BBP. These observations are in line with previously reported smaller fractionation during a chemical oxidation reaction where phthalates had longer ester chains

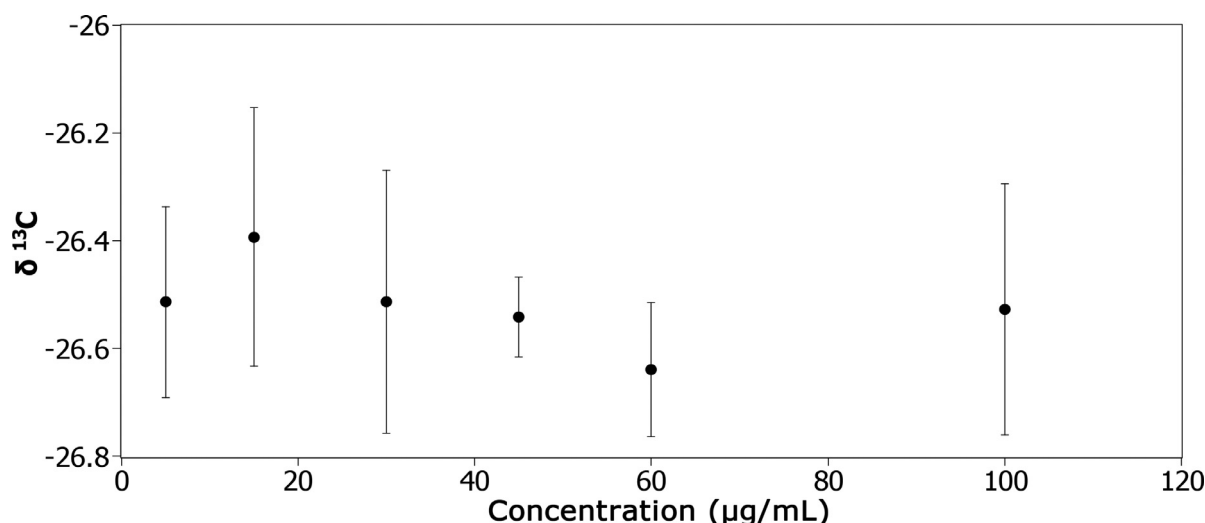


Fig. 3. DEHP $\delta^{13}\text{C}$ values across a range of concentrations with standard error represented by error bars.

Table 1

Phthalic acid esters and their carbon isotope values. N/D - not detected; N/R - not resolved.

Phthalic Esters	Abbreviation	Molecular Weight (g/mol)	CAS No.	Retention Time (s)/Relative Retention Time	$\delta^{13}\text{C}$ Standards	WWTP Effluent	River Trent	River Foss
Diethyl phthalate	DEP	222	84-66-2	1653/0.6127	-25.6	-25.8	N/R	N/D
Dibutyl phthalate	DBP	278	84-74-2	2109/0.7817	-29.9	N/R	N/D	-26.3
Benzyl butyl phthalate	BBP	312	85-68-7	2517/0.9329	-27.7	-26.9	-24.3	N/D
Di-2-ethylhexyl phthalate	DEHP	391	117-81-7	2698/1.0000	-26.5	-28.8	-28.4	-27.1

(Zhang et al., 2018). They are also consistent with a laboratory study which reported DEHP fractionated in an aerobic environment (Peng and Li, 2012).

The measurements presented in Table 1 demonstrate that SPE methods followed by GC-IRMS allow $\delta^{13}\text{C}$ values to be obtained in dilute environmental samples using compound specific strategies. Degradation of phthalates in the river is expected based on the slight enrichment in heavy carbon isotope of compounds in a model of a river (Schreglmann et al., 2013). With constant discharge of those pollutants this information would not have been available from the concentration. The accuracy of current CSIA results can be improved further by reducing high background observed due to the complexity of the sample (Blessing et al., 2008). CSIA provides valuable additional information unavailable by traditional methods via a simple and direct method. This can be applied to study inflows into the river to understand sources and fractionation in river systems.

Quantification

Quantification of DEHP was performed by GC-C-IRMS on samples from the WWTP effluent and samples from the Trent and Foss rivers. DEHP concentration in effluent and river water downstream from the WWTP drain were approximately 0.20 µg/L. As per EU Environmental Quality Standard directive 2008/105/EC the DEHP limit in surface waters is ≤1.3 µg/L for annual average. No maximum allowed concentration is specified for DEHP by this directive. Concentration values as described correspond to 3.7 kg of DEHP passing through the River Trent every day while only 53.1 g of DEHP passes through the River Foss every day. Slower river velocity and therefore daily DEHP residence time would be expected to correlate with observed enrichment of ^{13}C . Slower moving rivers increase the residence time of pollutants thus lengthening the time for decomposition and any associated fractionation. Combining isotopic data and the concentration can confirm fractionating degra-

dation instead of dilution or other non-fractionating processes. Future work will be able to confirm this.

The power of GC-C-IRMS can be extended. For example, it could be combined with relative response factors to allow a well-designed set of standards to assess multiple targeted compounds. Similarly, hybrid GC-MS/GC-IRMS systems (Muccio and Jackson, 2011) could be extremely powerful for future studies of aquatic environments.

NTIA - non-targeted isotope analysis

The GC-IRMS runs (c.f. Fig. 4) produced a large number of peaks corresponding to unknown compounds. The signals from these non-targeted unknown compounds were used to obtain $\delta^{13}\text{C}$ values with the phthalate standards as references. The WWTP, Trent, and Foss samples provided 117, 127, and 121 peaks, respectively. Most of the 100+ peaks in these chromatograms were unidentified. It is unrealistic to identify all, prove with authentic standards, and generate appropriate isotope standards before examining isotope ratios. A non-targeted approach allows comparison of compounds between samples (e.g. different rivers) and the identification of exceptional compounds for subsequent CSIA. Standards ideally should be chemically close to the sample (Dunn and Carter, 2018); however, to make non-targeted comparisons the working phthalate standards were used. Future studies can examine the amount of bias introduced by this standardisation, but the NTIA results presented here will reflect the use of these standards.

The $\delta^{13}\text{C}$ values from all peaks were plotted against the raw m/z 44 signal (Figure 5) which indicated low intensity signal correlated with a bigger spread of measured $\delta^{13}\text{C}$. If the variability of the 5 µg/mL DEHP standard is considered as a cut off, values can be used down to ~479 intensity units. After imposing the intensity threshold from the 5 µg/mL DEHP standard 44, 26, and 31 peaks remained providing best quality $\delta^{13}\text{C}$ values for the WWTP, Trent, and Foss samples, respectively. Although we used this threshold, the $\delta^{13}\text{C}$ and intensity plot suggests that a lower threshold (~150) could be used. The initial wide spread of the

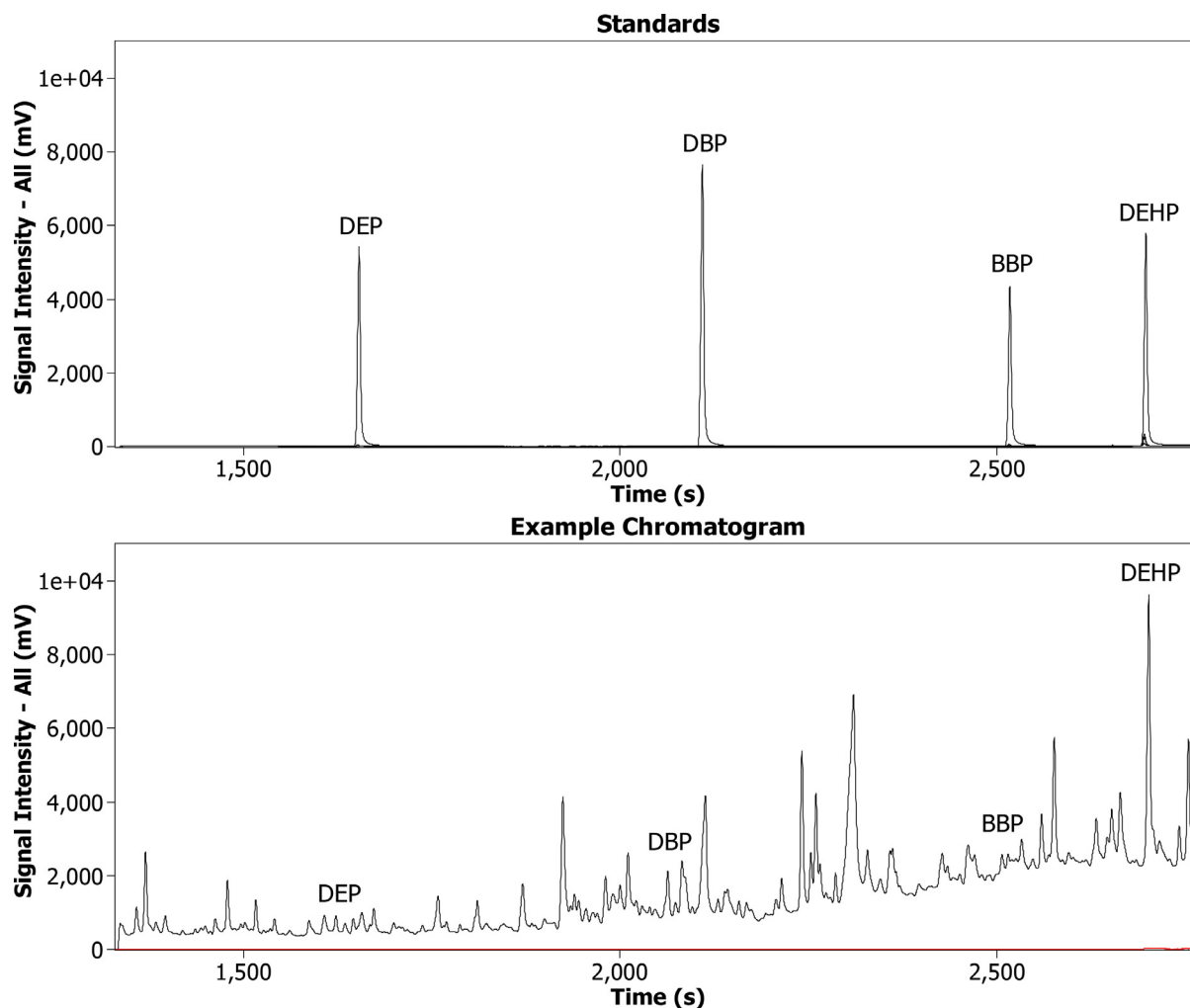


Fig. 4. Example chromatograms. Upper panel: overlay of phthalate standards; Lower panel: chromatogram of the effluent sample with blank superimposed.

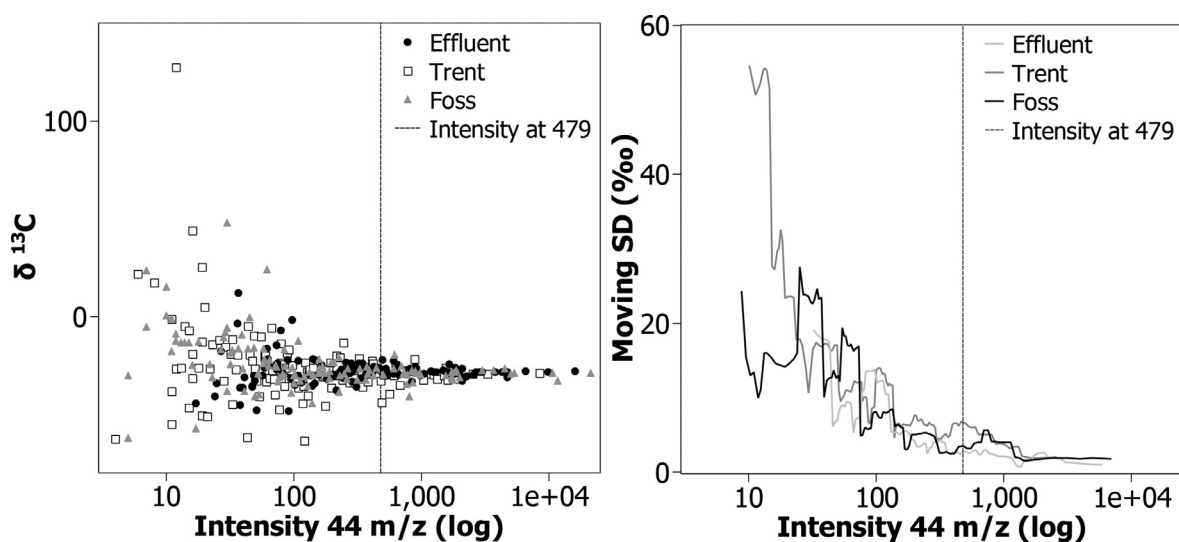


Fig. 5. Behaviour of $\delta^{13}\text{C}$ with $m/z = 44$ intensity. Average $\delta^{13}\text{C}$ values vs intensity (left) and moving standard deviation of $\delta^{13}\text{C}$ ($n = 10$) vs intensity (right). The vertical line indicates the position of the intensity cut off (479) based on the 5 $\mu\text{g/mL}$ DEHP standard.

Table 2

Average $\delta^{13}\text{C}$ of the total volatile organic carbon based on individual values measured. Slope (significance) between samples. S: slope; C: correlation coefficient. Values were considered significant if $p < 0.05/6$ to adjust for multiple comparisons. These are indicated with a *.

		Average $\delta^{13}\text{C} \pm \text{SD}$	WWTP	River Trent
WWTP Effluent	Full intensity range	$-28.4 \pm 7.6\text{‰}$ (n=117)		
River Trent		$-25.7 \pm 20.3\text{‰}$ (n=127)	S: 0.20 (0.013) C: 0.54 (1.1E-13)*	
River Foss		$-24.6 \pm 14.0\text{‰}$ (n=121)	S: -0.062 (0.46) C: -0.093 (0.27)	S: 0.066 (0.37) C: 0.060 (0.42)
WWTP Effluent	Intensity range >479 mV	$-28.6 \pm 2.2\text{‰}$ (n=44)		
River Trent		$-30.8 \pm 4.4\text{‰}$ (n=26)	S: 0.19 (0.38) C: 0.18 (0.39)	
River Foss		$-29.7 \pm 3.4\text{‰}$ (n=31)	S: 0.041 (0.85) C: 0.046 (0.83)	S: 1.1 (1.4E-10)* C: 0.96 (2.1E-12)*

data becomes more stable within the range of 80 – 100 intensity units and improves further as intensity rises. For this work, we set the threshold at the more stringent intensity threshold; however, future users of NTIA may consider relaxing this as long as the moving standard deviation, standard signal levels and well-resolved peaks justify it. The moving standard deviation is the standard deviation of the moving average ($n = 10$) computed on the intensity rank sorted arrays of $\delta^{13}\text{C}$ values for the three sample locations. Peak resolution is known to influence isotopic analysis (Meier-Augenstein, 1999) and a valley between two peaks of up to 10% is considered sufficient for GC-IRMS (Zhang et al., 2012). We required peak resolution ≥ 1.0 which gives 2.3% overlap of adjacent peaks.

The average $\delta^{13}\text{C}$ of compounds in both rivers was slightly enriched relative to the effluent based on all data prior to application of minimum intensity, an effect which inverted after application of the intensity threshold (Table 2, column 3). The averages were similar to dissolved organic carbon reported in the literature of -26‰ to -27‰ (Hélie and Hillaire-Marcel, 2006). Average values of soil organic matter of C_3 type plants are also close with values close to $\delta^{13}\text{C}$ -26‰ (Connin et al., 1997). Dissolved inorganic carbon found in rivers is reported to be in a range of -10‰ to -12‰ (Lee et al., 1987). Our data proves that $\delta^{13}\text{C}$ can be measured on individual compounds composing volatile organic carbon from WWTP and river samples.

More important than the average is the relationship between matched peaks appearing in samples from the two rivers. To investigate this, the $\delta^{13}\text{C}$ values were aligned by retention time (Figure 6) for peaks with resolution ≥ 1.0 , retention time within ± 3 s, and max intensity ≥ 479 at 44 m/z. The $\delta^{13}\text{C}$ values were significantly correlated between the two rivers with a slope of 1.1 between these two rivers (Table 2, column 5). Similar correlations were not observed between either river and the WWTP, although matched compounds between the WWTP and the Trent river came close to meeting significance criteria.

With one exception, all $\delta^{13}\text{C}$ values from the Trent river were more negative than the river Foss indicative of a greater proportion of the lighter isotope ^{12}C in the Trent. This suggests that compounds with initially similar $\delta^{13}\text{C}$ undergo greater fractionation in the slower moving Foss river than in the Trent. This hypothesis could be tested with future studies having more intensive sampling over time and with greater sampling along the rivers and tributaries of these rivers. NTIA has provided an intriguing view of isotope behaviour in a range of matched but unknown compounds found in both rivers.

Two observations were striking in the matched compounds. The first was the magnitude of differences between WWTP and the sample taken from the Trent a short distance downstream from the WWTP drain. The differences ranged from -3.4‰ to 5.6‰ for a distance of 9 % units (Fig. 6, lower). Most (10 out of 12) were enriched in ^{13}C in the WWTP effluent. Although most compounds exhibited only modest differences, 5 were more extreme. NTIA made clear there is considerable variability hidden in aggregate values such as those represented by TOC. It also gives an indication of which compounds might be most sensitive indicators of anthropogenic input to a river.

The second striking observation was a pair of compounds with extreme $\delta^{13}\text{C}$ relative to expectations for C_3 or C_4 plants in environments like the UK (Fig. 6, upper) (Kohn, 2010). NTIA highlighted a pair of compounds with $\delta^{13}\text{C}$ near -35‰ and -43‰. Values in this range have been observed in algae and the herbivores that feed on them in small unproductive tributary streams and sites downstream (Finlay, 2004). These strongly negative $\delta^{13}\text{C}$ values are consistent with biogenic methane as an input carbon source (Filippini et al., 2018). As noted previously, NTIA allows such distinct initially unidentified compounds to be highlighted by NTIA and subsequently followed up with identification, purification (Bakkour et al., 2018) and CSIA to obtain more detailed understanding of these markers. Both compounds would be completely masked in aggregate metrics like TOC. Without this view into the hidden richness in SPE-extractable volatile carbon provided by NTIA finding and selecting such markers for CSIA would be difficult, if not impossible.

Conclusion

This work demonstrated a set of interlocking and complementary techniques combining traditional quantitative measurements, CSIA, and NTIA applied to dilute aquatic samples. This work was done on the volatile SPE extractable portion of total organic carbon. The approach could also be combined with LC-IRMS to give a more complete picture of isoscapes and fractionation in the environment. The work provides an extension to existing methods for the analysis of river systems (Davoli et al., 2019; Ehleringer et al., 1999; Meikle et al., 2019; Mercan et al., 2019; Zuccato et al., 2005) allowing compounds to be better understood.

Although phthalates have been studied in model experiments and spiked samples (Liu et al., 2016, 2015; Peng et al., 2013a; Peng and Li, 2012), to our knowledge this is the first report of CSIA done on these pollutants extracted directly from river water samples. The four phthalates targeted for CSIA analysis here may be of interest to researchers concerned with this group of pollutants. Here, they have an additional importance. The standardisation required for CSIA is a necessary prerequisite for subsequent NTIA.

NTIA demonstrated that the volatile SPE-extractable portion of total organic carbon exhibits remarkable heterogeneity in isotope ratios. This heterogeneity is invisible to existing TOC isotope ratio analysis. NTIA was able to demonstrate the heterogeneity was largely preserved in two different rivers with significant correlation between non-targeted compounds found in both rivers.

Both approaches raise important issues of the effects of background in such samples. The existing state-of-the-art in GC-IRMS ranges from stringent isolation and separation (Bakkour et al., 2018) to chromatograms more similar to Fig. 4 (Chen et al., 2017; Rogers and Savard, 1999). In our review of the literature, clear studies establishing the necessity of the former or the impact of the latter are rare. This lack of information needs to be addressed in future studies.

The methods described apply to any water sample having dilute analytes amenable to SPE methods, allowing the individual compounds

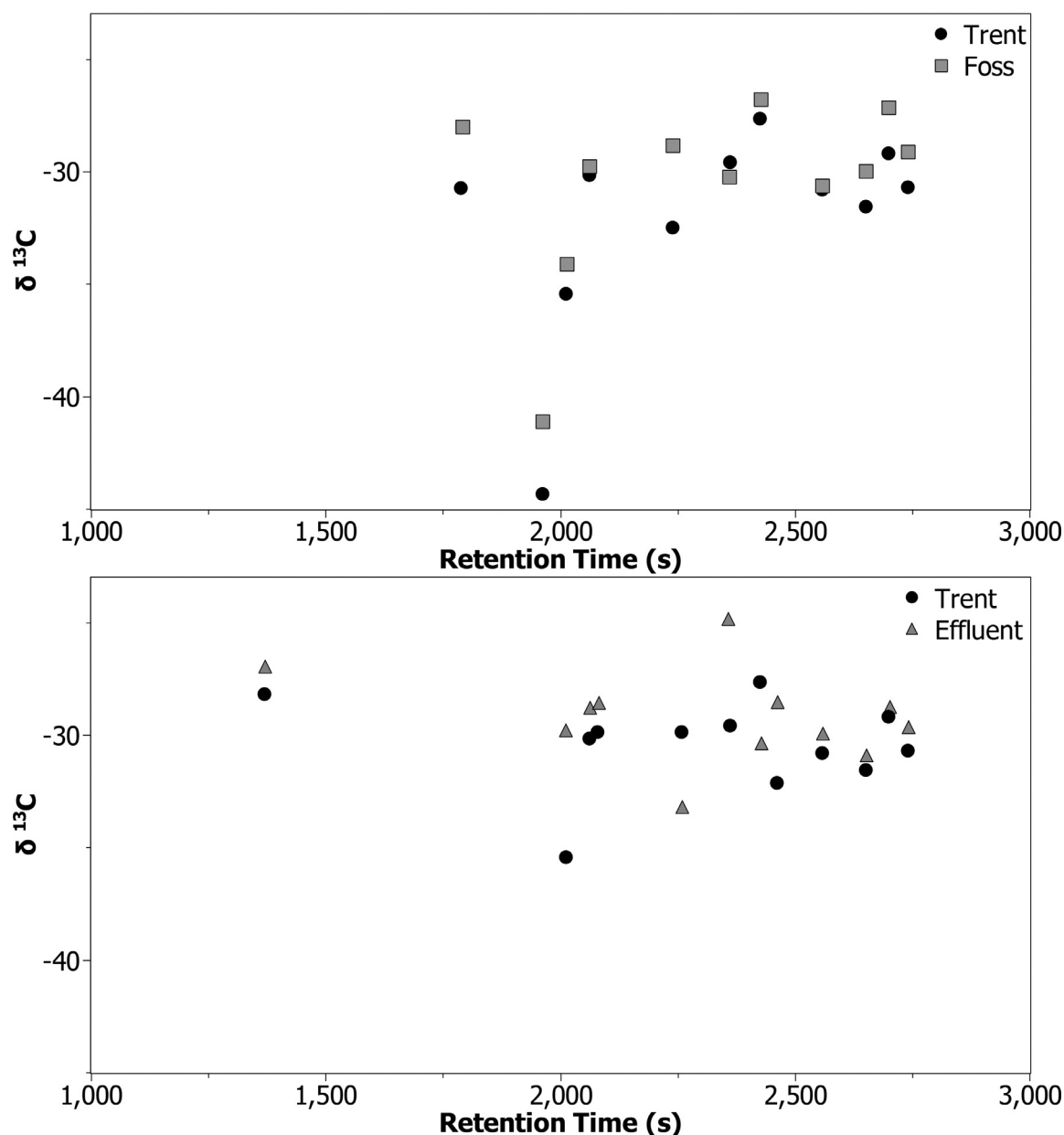


Fig. 6. $\delta^{13}\text{C}$ comparison of distribution of individual compounds in WWTP effluent and rivers. The upper panel presents a comparison between the River Foss and River Trent. The lower panel compares the WWTP effluent and the River Trent.

to be monitored as they move through the ecosystem. Application to a broader spectrum of pollutants and environmental matrices will contribute to understanding pollutant fates in the environment.

We have shown that with SPE pre-treatment it is possible to measure the isotopic ratio of specific compounds in the ng/L range extracted from a relatively small sample (2 L) as opposed to 10 - 12 L (Schreglmann et al., 2013). This study used GC-IRMS in both CSIA and NTIA of compounds extracted directly from WWTP post-treatment effluent water and river water samples. GC-IRMS provided insight into the relationship between rivers and WWTP effluent. In the future, combining a traditional mass spectrometer (MS or MS/MS) together with the GC-IRMS system (Muccio and Jackson, 2011) could provide simultaneous provisional identification, quantification, and isotopic data. This in turn will provide improved understanding of the biogeochemistry of specific compounds and classes of compounds in aquatic environments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Bakkour, R., Bolotin, J., Sellergren, B., Hofstetter, T.B., 2018. Molecularly imprinted polymers for compound-specific isotope analysis of polar organic micropollutants in aquatic environments. *Anal. Chem.* 90, 7292–7301.
- Blessing, M., Jochmann, M.A., Schmidt, T.C., 2008. Pitfalls in compound-specific isotope analysis of environmental samples. *Anal. Bioanal. Chem.* 390, 591–603.
- Boulard, L., Dierkes, G., Schlüsener, M.P., Wick, A., Koschorreck, J., Ternes, T.A., 2020. Spatial distribution and temporal trends of pharmaceuticals sorbed to suspended particulate matter of German rivers. *Water Res* 171, 115366.

- Chen, Y., Zhang, X., Chang, J., Ma, S., 2017. Development of a novel method for the enrichment of unsubstituted naphthalene and phenanthrene from crude oils for compound-specific stable isotope analysis. *Anal. Methods* 9 (35), 5135–5139. doi:10.1039/c7ay01257h.
- Connin, S.L., Virginia, R.A., Chamberlain, C.P., 1997. Carbon isotopes reveal soil organic matter dynamics following arid land shrub expansion. *Oecologia* 110, 374–386.
- Davoli, E., Zuccato, E., Castiglioni, S., 2019. Illicit drugs in drinking water. *Curr. Opin. Environ. Sci. Heal.* 7, 92–97.
- Dunn, P., Carter, J.F., 2018. Good Practice Guide for Isotope Ratio Mass Spectrometry, Second Edition, 2018. FIRMS stands for the Forensic Isotope Ratio Mass Spectrometry Network. FIRMS <https://www.forensic-isotopes.org/gpg.html>.
- Duty, S.M., Ackerman, R.M., Calafat, A.M., Hauser, R., 2005. Personal care product use predicts urinary concentrations of some phthalate monoesters. *Environ. Health Perspect.* 113, 1530–1535.
- Ehleringer, J.R., Cooper, D.A., Lott, M.J., Cook, C.S., 1999. Geo-location of heroin and cocaine by stable isotope ratios. *Forensic Sci. Int.* 106, 27–35.
- Elsner, M., Imfeld, G., 2016. Compound-specific isotope analysis (CSIA) of micropollutants in the environment — current developments and future challenges. *Curr. Opin. Biotechnol.* 41, 60–72.
- Filippini, M., Nijenhuis, I., Kimmel, S., Chiarini, V., Crosta, G., Richnow, H.H., Gargini, A., 2018. Multi-element compound specific stable isotope analysis of chlorinated aliphatic contaminants derived from chlorinated pitches. *Sci. Total Environ.* 640–641, 153–162.
- Finlay, J.C., 2004. Patterns and controls of lotic algal stable carbon isotope ratios. *Limnol. Oceanogr.* 49, 850–861.
- Fono, L.J., Kolodziej, E.P., Sedlak, D.L., 2006. Attenuation of wastewater-derived contaminants in an effluent-dominated river. *Environ. Sci. Technol.* 40, 7257–7262.
- Gómez, M.J., Martínez Bueno, M.J., Lacorte, S., Fernández-Alba, A.R., Agüera, A., 2007. Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. *Chemosphere* 66, 993–1002.
- Han, X., Cheng, X., Li, S., Yuan, J., Zhang, Q., 2019. Carbon concentrations and their stable isotopic signatures in the upper Han River. *China. Environ. Sci. Pollut. Res.* 26, 14116–14127.
- Hélie, J.-F., Hillaire-Marcel, C., 2006. Sources of particulate and dissolved organic carbon in the St Lawrence River: isotopic approach. *Hydro. Process.* 20, 1945–1959.
- Hsieh, C.-J., Chang, Y.-H., Hu, A., Chen, M.-L., Sun, C.-W., Situmorang, R.F., Wu, M.-T., Wang, S.-L., 2019. Personal care products use and phthalate exposure levels among pregnant women. *Sci. Total Environ.* 648, 135–143.
- Hu, P., Guo, C., Zhang, Yan, Lv, J., Zhang, Yuan, Xu, J., 2019. Occurrence, distribution and risk assessment of abused drugs and their metabolites in a typical urban river in north China. *Front. Environ. Sci. Eng.* 13, 56.
- Jochmann, M., Blessing, M., Haderlein, S., Schmidt, T., 2006. A new approach to determine method detection limits for compound-specific isotope analysis of volatile organic compounds. *Rapid Commun. Mass Spectrom.* 20, 3639–3648.
- Kohn, M.J., 2010. Carbon isotope compositions of terrestrial C3 plants as indicators of (paleo)ecology and (paleo)climate. *Proc. Natl. Acad. Sci.* 107, 19691–19695.
- Lee, C., McKenzie, J.A., Sturm, Z.M., 1987. Carbon isotope fractionation and changes in the flux and composition of particulate matter resulting from biological activity during a sediment trap experiment in Lake Greifen. Switzerland. *Limnol. Oceanogr.* 32, 83–96.
- Lichtfouse, E., 2000. Compound-specific isotope analysis. Application to archaeology, biomedical sciences, biosynthesis, environment, extraterrestrial chemistry, food science, forensic science, humic substances, microbiology, organic geochemistry, soil science and sport. *Rapid Commun. Mass Spectrom.* 14, 1337–1344.
- Liu, H., Li, Y., He, X., Sissou, Z., Tong, L., Yarnes, C., Huang, X., 2016. Compound-specific carbon isotopic fractionation during transport of phthalate esters in sandy aquifer. *Chemosphere* 144, 1831–1836.
- Liu, H., Wu, Z., Huang, X., Yarnes, C., Li, M., Tong, L., 2015. Carbon isotopic fractionation during biodegradation of phthalate esters in anoxic condition. *Chemosphere* 138, 1021–1027.
- Liu, Y., Wang, X., Wen, Q., Zhu, N., 2019. Identifying sources and variations of organic matter in an urban river in Beijing, China using stable isotope analysis. *Ecol. Indic.* 102, 783–790.
- Loos, R., Gawlik, B.M., Locoro, G., Rimaviciute, E., Contini, S., Bidoglio, G., 2009. EU-wide survey of polar organic persistent pollutants in European river waters. *Environ. Pollut.* 157, 561–568.
- Maier, M.P., De Corte, S., Nitsche, S., Spaett, T., Boon, N., Elsner, M., 2014. C & N isotope analysis of diclofenac to distinguish oxidative and reductive transformation and to track commercial products. *Environ. Sci. Technol.* 48, 2312–2320.
- Maier, M.P., Prasse, C., Pati, S.G., Nitsche, S., Li, Z., Radke, M., Meyer, A., Hofstetter, T.B., Ternes, T.A., Elsner, M., 2016. Exploring trends of C and N isotope fractionation to trace transformation reactions of Diclofenac in natural and engineered systems. *Environ. Sci. Technol.* 50, 10933–10942.
- Meier-Augenstein, W., 1999. Applied gas chromatography coupled to isotope ratio mass spectrometry. *J. Chromatogr. A* 842, 351–371.
- Meikle, J., Cresswell, S.L., Boyd, S.E., Carter, J.F., Collett, S.P., 2019. A stable isotope ratio approach to investigate the origins of illicit methylamphetamine in Queensland. *Australia. Forensic Chem.* 16, 100174.
- Mercan, S., Kuloglu, M., Tekin, T., Turkmen, Z., Dogru, A.O., Safran, A.N., Acikol, M., Ascioglu, P., 2019. Wastewater-based monitoring of illicit drug consumption in Istanbul: Preliminary results from two districts. *Sci. Total Environ.* 656, 231–238.
- Muccio, Z., Jackson, G.P., 2011. Simultaneous Identification and $\delta^{13}\text{C}$ classification of Drugs Using GC with concurrent single quadrupole and isotope ratio mass spectrometers*. *J. Forensic Sci.* 56, S203–S209.
- Peng, X., Feng, L., Li, X., 2013a. Pathway of diethyl phthalate photolysis in sea-water determined by gas chromatography–mass spectrometry and compound-specific isotope analysis. *Chemosphere* 90, 220–226.
- Peng, X., Li, X., 2012. Compound-specific isotope analysis for aerobic biodegradation of phthalate acid esters. *Talanta* 97, 445–449.
- Peng, X., Li, X., Feng, L., 2013b. Behavior of stable carbon isotope of phthalate acid esters during photolysis under ultraviolet irradiation. *Chemosphere* 92, 1557–1562.
- Peterson, B., Fry, B., Hullar, M., Saupe, S., Wright, R., 1994. The distribution and stable carbon isotopic composition of dissolved organic carbon in estuaries. *Estuaries* 17, 111–121.
- Piper, T., Geyer, H., Gougoulidis, V., Flenker, U., Schänzer, W., 2010. Determination of $^{13}\text{C}/^{12}\text{C}$ ratios of urinary excreted boldenone and its main metabolite 5 β -androst-1-en-17 β -ol-3-one. *Drug Test. Anal.* 2, 217–224.
- Pizzarello, S., Holmes, W., 2009. Nitrogen-containing compounds in two CR2 meteorites: ^{15}N composition, molecular distribution and precursor molecules. *Geochim. Cosmochim. Acta* 73, 2150–2162.
- Rogers, K., Savard, M., 1999. Detection of petroleum contamination in river sediments from Quebec City region using GC-IRMS. *Org. Geochem.* 30 (12), 1559–1569. doi:10.1016/S0146-6380(99)00132-1.
- Saccon, M., Facca, C., Huang, L., Irei, S., Kornilova, A., Lane, D., Rudolph, J., 2013. Method for the determination of concentration and stable carbon isotope ratios of atmospheric phenols. *Atmos. Meas. Tech.* 6, 2965–2974.
- Schmidt, T.C., 2003. Analysis of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA) in ground and surface water. *TrAC Trends Anal. Chem.* 22, 776–784.
- Schreglmann, K., Hoeche, M., Steinbeiss, S., Reinicke, S., Elsner, M., 2013. Carbon and nitrogen isotope analysis of atrazine and desethylatrazine at sub-microgram per liter concentrations in groundwater. *Anal. Bioanal. Chem.* 405, 2857–2867.
- Sestavtsov, V.S., 2016. Isotope Ratio Mass Spectrometry of Light Gas-Forming Elements. CRC Press.
- Shibuya, E.K., Sarkis, J.E.S., Negrini-Neto, O., Martinelli, L.A., 2007. Carbon and nitrogen stable isotopes as indicative of geographical origin of marijuana samples seized in the city of São Paulo (Brazil). *Forensic Sci. Int.* 167, 8–15.
- Shibuya, E.K., Souza Sarkis, J.E., Neto, O.N., Moreira, M.Z., Victoria, R.L., 2006. Sourcing Brazilian marijuana by applying IRMS analysis to seized samples. *Forensic Sci. Int.* 160, 35–43.
- Spahr, S., Bolotin, J., Schleucher, J., Ehlers, I., von Gunten, U., Hofstetter, T.B., 2015. Compound-specific carbon, nitrogen, and hydrogen isotope analysis of N-nitrosodimethylamine in aqueous solutions. *Anal. Chem.* 87, 2916–2924.
- Taipale, S.J., Peltomaa, E., Kukkonen, J.V.K., Kainz, M.J., Kautonen, P., Tirola, M., 2019. Tracing the fate of microplastic carbon in the aquatic food web by compound-specific isotope analysis. *Sci. Rep.* 9, 19894.
- Thornton, B., Zhang, Z., Mayes, R.W., Höglberg, M.N., Midwood, A.J., 2011. Can gas chromatography combustion isotope ratio mass spectrometry be used to quantify organic compound abundance? *Rapid Commun. Mass Spectrom.* 25, 2433–2438.
- Washington, J.W., Rosal, C.G., Ulrich, E.M., Jenkins, T.M., 2019. Use of carbon isotopic ratios in nontargeted analysis to screen for anthropogenic compounds in complex environmental matrices. *J. Chromatogr. A* 1583, 73–79.
- Wu, Y., Luo, D., Dong, H., Wan, J., Luo, H., Xian, Y., Guo, X., Qin, F., Han, W., Wang, L., Wang, B., 2015. Geographical origin of cereal grains based on element analyser-stable isotope ratio mass spectrometry (EA-SIRMS). *Food Chem.* 174, 553–557.
- Yan, C., Li, Z., Li, S., Wang, Z., Xing, L., Liu, Y., Mou, W., Wang, K., 2019. Determination of $\delta^{13}\text{C}$ of trace hydrocarbons in natural gas using syringe solid phase extraction (SSPE) coupled with gas chromatography/Isotope ratio mass spectrometry (GC/IRMS). *Geofluids* 19, 6352378.
- Yeh, H.-W., Wang, W.-M., 2001. Factors affecting the isotopic composition of organic matter. (1) Carbon isotopic composition of terrestrial plant materials. *Proc. Natl. Sci. Coun. Rep. China. B.* 25, 137–147.
- Zhang, D., Wu, L., Yao, J., Herrmann, H., Richnow, H.H., 2018. Carbon and hydrogen isotope fractionation of phthalate esters during degradation by sulfate and hydroxyl radicals. *Chem. Eng. J.* 347, 111–118.
- Zhang, Y., Tobias, H.J., Sacks, G.L., Brenna, J.T., 2012. Calibration and data processing in gas chromatography combustion isotope ratio mass spectrometry. *Drug Test. Anal.* 4, 912–922.
- Zolfaghari, M., Drogui, P., Seyhi, B., Brar, S.K., Buelna, G., Dubé, R., 2014. Occurrence, fate and effects of Di (2-ethylhexyl) phthalate in wastewater treatment plants: A review. *Environ. Pollut.* 194, 281–293.
- Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S., Fanelli, R., 2005. Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. *Environ. Heal.* 4, 1–7.