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della Toscana

Società Chimica Italiana
Divisione di Spettrometria di Massa

Book of Abstracts

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The 2nd MS Sea- Day conference is organized by

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Agenda

Monday, July 10th, 2023	
13:00	Registration
14:00 - 14:30	Greetings from Authorities **Pietro Rubellini**: General manager Arpa Toscana **Francesca Piccioli**: OCFT president **Giuliana Bianco:** DSM president
14:30 - 15:10	**Fulvio Ferrara - Istituto Superiore di Sanità** European and Italian regulatory perspectives of protection and use of marine waters.
15:10 – 15:40	**Letizia Marsili - Università degli Studi Siena** Top marine predators and ocean heath: detection of persistent organic pollutants reveals ecosystems indicator species and their toxicological conservation risks.
15:40 - 16:10	**Vittorio Esposito - ARPA Puglia** Levels and kinetics of transfer processes of PCDD/Fs in a transitional waters ecosystem near a large industrial area in southern Italy.
16:10 - 16:40	**Fabio Stropeni – Shimadzu** An Automated Workflow for Quantitative Analysis of Microplastics in Environmental Samples via Pyrolysis-GC/MS.
16:40 - 17:10	**Gianluca Bartolucci - Università degli Studi Firenze** Tandem mass spectrometry approaches for recognition of isomeric compounds mixtures.
17:10 - 17:45	Question time
17:45 – 18:00	Poster Session - Flash presentation
18:00 - 19:30	Welcome cocktail
19:30 – 20:30	Social Event: "*Discover the Aquarium*"
Tuesday, July 11th, 2023	
9:30	Opening Works
10:00 - 10:40	**Joerg Feldmann - TESLA – Analytical Chemistry Institute of Chemistry University of Graz** Bioaccumulation of PFAS, arsenolipids and mercury nanoparticles in whales stranded in Scotland.
10:40 – 11:10	**Silvia Signorini - Università degli Studi di Milano** Investigating the mechanisms that promote tolerance to Ocean Acidification in different invertebrate species from the CO2 vent systems of Ischia Island (Italy) through metabolomics analysis.
11:10 - 11:50	Coffee Break
11:50 - 12:20	**Simone Moretti - IZS Umbria e Marche** Occurrence of legacy and emerging Poly and PerfluoroAlkyl Substances (PFASs) in turtle (Caretta Caretta) egg samples.
12:20 - 12:50	**Mattia Sibra – ARPAT** Speciation of organotins by Gas Chromatography – Inductively Coupled Plasma Spectrometry in marine samples.
12:50 - 13:20	**Emanuele Ceccon – Restek** Analysis of ultrashort-chain and short-chain (C1 to C4) per- and polyfluorinated substances in potable and non-potable waters.
13:20 - 14:30	Lunch
14:30 - 15:00	**Sara Valsecchi - IRSA – CNR** Target, non-target and suspect screening of PFAS in dolphins, marine turtles and sharks (Tuscany coast).
15:00 - 15:30	**Iacopo La Nasa - Università degli Studi di Pisa** Microwave-assisted sample pretreatments and analytical pyrolysis: a holistic approach for the study of microplastics and correlated pollutants.
15:30 - 16:00	**Paolo Scardina – Agilent** Rapid Identification and quantification of microplastics in sea water and sediment using the new Laser Direct Infrared Spectroscopy.
16:00 - 16:30	Question time
16:30 - 17:00	Coffee Break
17:00	Awards and closing remarks – DSM President

Scientific program

Plenary Lectures

European and Italian regulatory perspectives of protection and use of marine waters.

Fulvio Ferrara

Istituto Superiore di Sanità,

The sea and its coasts represent a complex environment characterized by a multiplicity of physical, chemical and biological elements that interact with each other and, above all, are influenced by neighboring territories and existing anthropogenic pressures.
The seas and oceans, in fact, represent the receptor body of all pollutants produced by human activities, and the study and assessment of the quality of these environments from both environmental and public health perspectives is of primary importance.
For this reason, policies and regulations to protect water and marine resources have developed at the International, European and National levels (through mainly transpositions of EU standards).
It is with the Water Framework Directive 2000/60/EC that the new framework for EU actions in the field of water was outlined from which a number of other specific directives were derived, including, for example, the Marine Strategy Directive 2008/56/EC, the Directive 2008/105/EC establishing environmental quality standards in the field of water policy or even the Directive 2013/39/EU updating it regarding priority substances. Not to forget Directive 2007/7/EC, which updates bathing water standards by introducing a completely new approach to protect the health of bathers.
In Italy over the years, all EU Directives have been transposed with the issuance of as many transposition decrees and implementing decrees, including Decree 152/2006 transposing Directive 2000/60, Decree 172/2015 transposing Directive 2013/39, or even Decree 116/2008 et seq. implementing Directive 2007/7 on bathing water.

Bioaccumulation of PFAS, arsenolipids and mercury nanoparticles in whales stranded in Scotland

Joerg Feldmann, Lhiam Paton, Amnah Al Zbedy, Andea Raab, Eva M Krupp

TESLA-Analytical Chemistry, University of Graz, Austria

To study environmental processes a multi-method approach is usually necessary. Sometimes common routine measurements do not give adequate answers to characterize a fate of POPs or toxic elements in the environment. The development of novel approaches to determine new bio- or environmental markers are necessary. This and its application to environmental samples is the core business of environmental analytical chemistry. In this lecture the bioaccumulation of PFAS (per and polyfluorinated alkylated substances) as well as mercury and arsenic in pilot and sperm whales stranded in Scotland will be described. Here speciation studies about mercury will feature besides HPLC-ICPMS, also XANES, Micro-XRF, MC-ICPMS, TEM, NanoSIMS, spICPMS for arsenic additional HPLC-ICPMS/ESIMS and for PFAS HPLC-ESIMS/MS and CIC.
The data show age and gender related behaviour for some but not for other toxic compounds.

Oral Presentations

Top Marine Predators and Ocean Health: Detection of persistent organic pollutants reveals ecosystem indicator species and their toxicological conservation risks

Letizia Marsili

Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente - Università di Siena

Ocean health is inextricably linked to human well-being and social development, as marine ecosystems provide a significant part of the services that humans need and use: food, livelihoods, and recreational opportunities to regulating the global climate. Unfortunately, human activities often have adverse effects on marine resources and the use of ecosystem services or benefits has resulted in rapid changes in coastal seas around the world. Defining the state of the oceans is a top priority to the point where, in 2012, an index to assess the health and benefits of the global ocean was published in Nature. This Ocean Health Index (OHI) consists of 10 goals based on extensive research by scientists, economists and sociologists, and one of the ten goals is Biodiversity. This goal assesses the conservation status of marine species based on the best available global data through two sub-goals: Species and Habitats. Pollution, accidental and deliberate, is one of the major threats to the marine species. Despite this, this risk is often underestimated when assessing the health and conservation status of different species. Similarly, the possibility of using the toxicological status of the different species to establish the good status of the marine environment in which they live is underestimated. In this context, the potential use of top marine predators (ranging from large pelagic fish, and cetaceans) in determining the environmental toxicological status linked to Persistent Organic Pollutants (POPs) of marine ecosystems, especially, but not exclusively, within the Mediterranean Sea, is presented. It is also emphasized that some large marine vertebrates are better indicators than others for the assessment of environmental contamination.

Levels and kinetics of transfer processes of PCDD/Fs in a transitional waters ecosystem near a large industrial area in southern Italy

V. Esposito, A. Maffei, M. Semeraro, D. Bruno, G. Battafarano, F. Massari, T. Pugliese, C. Giannotta, P. Milella, C. Pilolli, A. De Luca

ARPA Puglia Environment Agency of Apulia - Department of Taranto – POPs Laboratory

Summary: This paper presents the results of the investigations performed by the Apulian Environment Agency at a large remediation site in Taranto, Italy. The health of the marine ecosystem was assessed through the determination of the levels of Persistent Organic Pollutants (POPs) in filter-feeding organisms at direct contact with polluted sediments.

Keywords: bioaccumulation, marine environment, mussels

Introduction

The mainland used by Taranto industrial area and the bottom of two semi-enclosed transitional-water basins, Mar Grande and Mar Piccolo, are part of one of the largest italian remediation sites as listed by national legislation aimed at the identification and rehabilitation of polluted areas.

The area hosts several industrial facilities including thermal/combustion processes [1] with remarkably high raw materials and high energy demand and known potential sources of PCDD/Fs and PCBs release to air, land, and water [2]. These facilities include a large integrated steel plant, a medium-sized oil refinery, a large cement-works, two power plants, and three waste incinerators as well as a large naval base with military shipyards. Taranto is a relatively large southern Italian city, the capital of the Province of Taranto and an important commercial port, with a population of over two-hundred thousands inhabitants.

The magnitude of the environmental pressure on the Taranto marine environment is known to

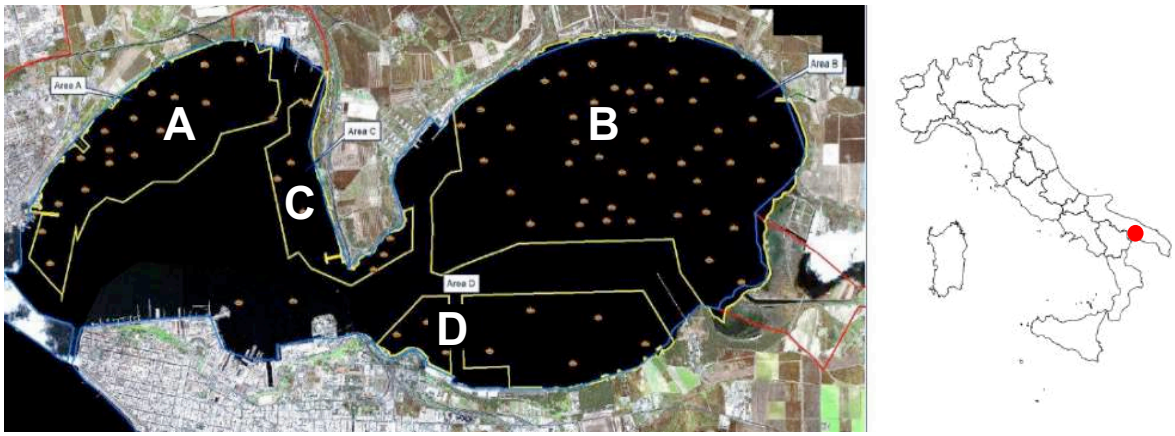

Figure 1a – Mar Piccolo, areas of rope-mussel

some extent [3]. Although there is some information on the potential impact of the measured environmental levels of heavy metals on the food chain through molluscs reared in Taranto coastal area [4], no data are available on presence of PCDD/F and dioxin-like PCBs in filter-feeding organisms at direct or close proximity with polluted sediments, like bottom-mussels, despite the presence of numerous rope-mussel culture installations.

Experimental

Mussels were collected according a pre-defined sampling protocol that is widely used in Italy at every marine remediation site. For every sampling station, a minimum of 30 molluscs of similar length, approximatively between 70% and 90% of the maximum length of the sampled population, were collected. Laboratory pre-analysis operations included biometric measurements (length) as well as the separation of the soft tissue from the shell and the forming of three pools that were homogenised and frozen for storage.

Tissue samples were freeze-dried under vacuum (lyophilised) prior to extraction by Accelerated Liquid Extraction with Hexane/Acetone 3:2. Fat content was determined although final concentrations are expressed on a wet basis according to EU Regulations. The extracts were percolated through diatomaceous earth treated with concentrated sulphuric acid for fat elimination and subsequently purified by means of an automated clean-up process. PCDD/Fs were separated by high resolution gas chromatography (HRGC) on a DB-5 MS capillary column (60 m x 0.25 mm, 0.25 µm film thickness). Isotope-dilution high-resolution mass-spectrometry determinations (HRMS) were carried out at a resolution of 10000 operating with electron ionisation (EI) at 45 eV in the selected ion monitoring (SIM) mode. PCBs and dl-PCBs were separated by HRGC on a DB-5 MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness) and determined by HRMS, in the same operating conditions used for PCDD/Fs. For each batch of seven samples a laboratory blank and a control sample were analysed. Toxic equivalent (TEQ) values were calculated using OMS Toxic Equivalency Factors (WHO-TEFs, 1998) and expressed as upper-bound concentrations.

Results

Results are expressed as wet weight concentrations as required by relevant legislation for fish food products in the EU. Average fat content was about 1.2%. PCDD/Fs concentrations ranged from 0.27 to 2.48 pgWHO-TE/g and the lowest values were found for mussels collected in Zone E (mean 0,46 pgWHO-TE/g) while highest values were found for mussels from Zones A (mean 2.21 pgWHO-TE/g) and C (mean 1.66 pgWHO-TE/g). Dioxin-like PCBs ranged from 1.55 to 12.59 pgWHO-TE/g with lowest values found for mussels collected in Zone B (mean 2.25 pgWHO-TE/g) while highest values were found for mussels from Zones A (mean 11.22 pgWHO-TE/g), C (mean 9.29 pgWHO-TE/g) and D (mean 7.11 pgWHO-TE/g). It has to be noted that the dl-PCBs level for the blank sampling station (Figure 1b) is quite high compared to the lowest values found in zone B, and are more similar to what is found for Zone E, to which the blank stations are actually not at very high distance.

The total WHO-TEQ values very much reflect the same spatial distribution of dl-PCB, since they are the main contributors to total toxicity. A similar pattern was found for terrestrial animals from farms close to the Taranto industrial area [5] and a general predominance of dl-PCB is actually a trend that has been often observed for the fish food group. Total WHO-TEQ ranged from 2.25 to 14.02 pgWHO-TE/g with lowest values in Zone B (mean 3.17 pgWHO-TE/g) while highest values were found for mussels from Zone A (mean 11.22 pgWHO-TE/g). Samples collected in Zones C and D appear to be somewhat less homogeneous. Congener profile for sample 5 Zone A, the most contaminated presents a PCDD/Fs profile with 2378-TCDF as the most abundant congener, that is consistent with the known more pronounced bioaccumulation for congeners with the least chlorine atoms[6].

Conclusions

POPs bioaccumulation is a useful tool in assessing the ecological health status of the marine ecosystem. For the area under investigation, the Gulf of Taranto, Zone A appears to be the most contaminated with highest values for both PCDD/Fs and PCBs and this appears to be consistent with the proximity of the 5-decades old industrial area, that operated well before the

ban on PCBs use entered into force (late 1970) and well before restrictions in the discharge of industrial waste to water basins were promulgated (mid 1970). Present releases to water could include washout of contaminated soil as well as continuing percolation from discharge to inadequate landfills. Actions to reduce those emissions to water should be undertaken in order to decrease PCDD/Fs and PCBs levels in the marine environment. As a continuing effort to monitor the insurgence of any potential risk related to the food-chain, the local Health Authority has communicated that all samples of reared rope-mussels collected during their Official Control activities have so far shown POPs levels within EU legal limits (Reg 1881/2006).

Acknowledgements
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References

1. Esposito V, Maffei A, Castellano G, Conversano M, Martinelli W, Assennato G. (2010); Organohalogen compounds 72: 736
2. Fiedler H. (1996); Chemosphere 32(1): 55
3. Buccolieri A, Buccolieri G, Cardellicchio N, Dell'Atti A, Di Leo A, Maci A. (2006); Marine Chem. 99(1): 227
4. Cardellicchio N, Buccolieri A, Di Leo A, Giandomenico S, Spada L. (2008); Food Chemistry 107(2): 890
5. Diletti G, Ceci R, Scortichini, G, Migliorati G.(2009) Organohalogen Compounds 71: 2359
6. Esposito V, Maffei A, Gigante L, Bruno D, Spartera M, Assennato G (2011); Organohalogen Compounds 73:1443

An Automated Workflow for Quantitative Analysis of Microplastics in Environmental Samples via Pyrolysis-GC/MS

Fabio Stropeni

Shimadzu Italia s.r.l.

Summary:. This work reports the quick, accurate, sensitive, and precise analysis of 12 selected MP polymers, chosen based on their prevalence in the environment , using the PY-GCMS technique . the experimental section of this work represent a reference for environmental scientists working on the analysis of MPs in waterbodies and environmental samples with some sample preparation tricks, like the use of CaCO3, to improve the performance of certain polymers.
In addition the method validation results in this work contribute as a guidance for the ongoing MPs studies being used by ASTM Committee D19 and ISO TC147 SC2 JWG to draft and validate new methods for the analysis of MPs in water.

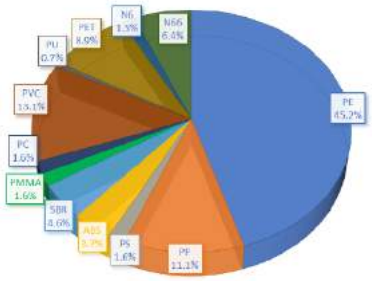
Keywords: PY-GCMS , microplastics, GCMS

Introduction

The ubiquitous occurrence and persistent nature of plastics in the environment may present potentially adverse issues that are not yet well understood. Large plastic debris not only clogs stormwater pipes but can be broken down into micro and nanoparticles that can be a sorption site for other pollutants, including chemicals such as pesticides and microorganisms [4]. These micro and nano-plastics can be ingested by water organisms and bioaccumulate up the food chain into humans. As a result of the need to mitigate plastic pollution, researchers are working to understand the effects of plastics in the environment and develop monitoring and mitigation programs.
Pyrolysis-GCMS (Py-GC/MS) is an innovative technique that is an accurate and precise alternative for the identification and quantitation of microplastics, and provides a fast workflow by shortening many conventional laboratories sample preparation steps, as highlighted by Pipkin et. al. (2021). Some traditional MPs methods require sample preparation, matrix isolation and solvent extraction [4]. Polymers such as polyethylene (PE) and polypropylene (PP) are not easily dissolved in solvents and recovery from initial material may be poor. Therefore, MP analytical challenges may occur with these traditional methods.
Since Py-GC/MS directly analyzes solid samples, sample preparation is easier than with traditional MP methods [4]. In addition, solvent extraction steps may be eliminated from the workflow, since solids can be taken as is and placed into a sample cup for analysis. Thus, using a Py-GC/MS may minimize solvent consumption, time for analysis and discrepancies in methods targeting a broad range of plastics. Prepared samples are placed in sample eco cups, analyzed, and reported in mass per volume, such as ug/L, unlike spectroscopic methods, which count particles and must infer mass concentration. The faster workflow of Py-GC/MS is further enhanced with specialized software, Frontier MP-search, that uses retention indices to readily identify characteristic pyrolyzates of the target compound.
This application note demonstrates the quick, accurate, sensitive, and precise analysis of 12 MPs using a Shimadzu GCMS-QP2020NX coupled to a Frontier PY-3030D pyrolyzer system.

Experimental

A Frontier lab multi-shot Pyrolyzer (Py) was interfaced with a Shimadzu GCMS (Figure 1). The system configuration for this application consisted of a Shimadzu GCMS, model QP2020 NX, Frontier multi-shot Pyrolyzer, model EGA/PY-3030D, an auto-shot sampler, model AS-1020E, a Frontier Lab ultra-alloy microplastics (UAMP) column, a vent-free GC/MS adapter, and a F-Search MP library software. Consumables consisted of a calibration standard mixture of 12 polymers, eco-cup LF and a packed inlet liner.
The polymers analyzed in this study were polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC) polycarbonate (PC), polyethylene terephthalate (PET), poly (methyl methacrylate) (PMMA), nylon-6 (N-6), polystyrene (PS), acrylonitrile-butadiene-styrene copolymer (ABS), styrene-butadiene rubber (SBR), nylon-6,6 (N-66) and MDI-polyurethane (PU).
A workflow to determine a characteristic pyrolyzate for seven of the above polymers is described in Shimadzu application news GCMS-2201 [5].
A five-point calibration curve was prepared using a Frontier MPs-CaCO3 standard [1]. The percentage distribution of polymers in the MPs-CaCO3 standard as well as the mass of each analyte in a 4 mg standard within a CaCO3 diluent is shown in Figure 2.



Polymer	Mass (µg) in 4 mg standard
PE	145.8
PP	35.7
PS	5.2
ABS	12.1
SBR	15.0
PMMA	5.3
PC	5.1
PVC	42.4
PU	2.1
PET	28.6
N6	4.9
N66	20.7

Figure 2: Percent polymer composition as well as mass of each analyte in 4 mg MPs-CaCO3 analytical standard with CaCO3 diluent.

Results

- Initial Demonstration of Low System Background

As a quality control measure, an initial demonstration of low system background was conducted before developing the calibration curve. Blank sample cups were analyzed, and the system was deemed to be free of contamination.

- Initial Calibration

A five-point initial external calibration plot was generated across a linear range for a mixed polymer analysis (Figure 4). Four replicates at each concentration level were analyzed and the average of the four points was calculated, plotted as a calibration curve, and the coefficient of determination was determined. Calibration results showed good linearity for all compounds. Figure 5 illustrates calibration curves for the least sensitive polymers, i.e., polymer signal with a S/N ratio > 10. Despite the lower sensitivity for these compounds, coefficient of determination (r2) for all 12 polymers was > 0.99

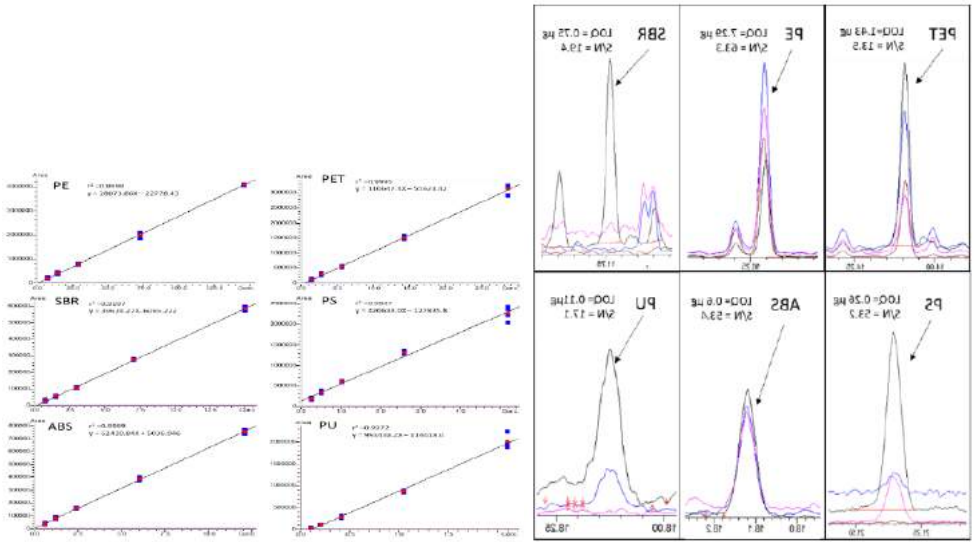


Figure 3: A Calibration curves for the least sensitive polymers. B. Limit of quantitation for the least sensitive polymers in this study.

- Initial Demonstration of Precision

A repeatability test was done on the lower and upper end of the calibration curve. Both 0.2 and 3 mg of MPs-CaCO3 standard mass were placed into seven individual cups and analyzed in order within the sequence, i.e., injection 1 through 7. The concentration of each analyte in each replicate was calculated using the initial calibration curve.

Percent RSD for the polymer replicates at 0.2 mg and 3 mg standard weights, respectively, ranged from 3.6-23.6 and 2.3-12.1 (Figure 3). Most compounds were within a RSD of 10%.

- Lower Limit of Quantification (LLOQ)

Various masses of the mix standards were weighted on a semi-micro balance and analyzed on the system until a S/N of > 10 was obtained for the least sensitive polymer. The LLOQ is arbitrarily defined as the lowest calibration point and, in this study, determined as 0.2 mg standard weight of the mix polymer. LLOQ for all polymers ranged from 0.1 - 7.3 µg (Figure 3b).

- Initial Demonstration of Accuracy

The mean concentration of each analyte in all replicates from the repeatability test was determined. This average concentration and the theoretical concentration were used to calculate the percent recovery at 0.2 and 3 mg standard mass. The 0.2 mg standard mass had percent recoveries of each polymer that ranged from 66.4 – 145.1 and at 3 mg recoveries ranged from 92.2 – 102.8.

- Continuing Calibration Check

A CCC was analyzed at the end of the batch (42 injections) to validate the use of the calibration used during the study. Percent drift of the CCC standard was used to evaluate the suitability of this calibration over the entire batch.

A mid-point calibration level (0.8 mg) was used to evaluate the percent drift. The peak area of the quantitation ion of the continuing calibration checks was monitored. A maximum drift of 20% was assigned to verify the use of the calibration curve. Results showed percent drift for all compounds in the CCC were less than 20%, satisfying the criteria of the test. This result indicates that the initial calibration curve was valid during the entire batch analysis.

Conclusion

The study demonstrated the satisfactory performance of the Shimadzu GCMS-QP2020NX coupled to a Frontier Py-3030D pyrolyzer for quantitation of selected plastics. The method validation results in this application note act as a Py-GC/MS method condition guidance for ongoing MPs studies.

In this application, a fast and precise workflow was developed for quantitation of twelve polymers. Calibration results showed linearity for all compounds and coefficient of determination (r2) > 0.99 were obtained.

Using seven replicates of standards at 0.2 and 3 mg, a precision experiment was conducted. Percent RSD for the polymer replicates at 0.2 mg and 3 mg, respectively, ranged from 3.6-23.6 and 2.3-12.1.

An accuracy evaluation showed that the 0.2 mg standard mass had percent recoveries of each polymer that ranged from 66.4 – 145.1 and at 3 mg recoveries ranged from 92.2 – 102.8. The LLOQ for all polymers ranged from 0.1 - 7.3 (0.2 mg).

In addition to the above, a CCC test was conducted. After 42 injections, the percent drift for all compounds was less than 20%.

References

1. Ishimura, T.; Iwai, I.; Matsui, K.; Mattonai, M.; Watanabe, A.; Robberson, W.; Cook, A.; Allen, H.L.; Pipkin, W.; Teramae, N.; Ohtani, H.; Watanabe, C. Qualitative and quantitative analysis of mixture of microplastics in the presence of calcium carbonate by Pyrolysis-GC/MS. Journal of Analytical and Applied Pyrolysis, 2021 (157): 105188.
2. WK67788 - New Test Method for Identification of Polymer Type and Quantity (Mass) Measurement of Microplastic Particles and Fibers in Waters with High-to-Low Suspended Solids Using Pyrolysis-Gas Chromatography/Mass Spectrometry: Py-GC/MS.
3. Joint ISO/TC 147/SC 2 - ISO/TC 61/SC 14 WG: Plastics (including microplastics) in waters and related matrices.
4. Pipkin, W.; Belganeh, R.; Robberson, W.; Allen, H.L.; Cook, A.; Watanabe, A. Identification of Microplastics in Environmental Monitoring Using Pyrolysis-GC-MS Analysis. LCGC North America. 2021 April 01; 39(4):179-186.
5. 5. How to Kickstart Your Micro- and Nanoplastic Py-GC/MS Analysis. Shimadzu Application News. GCMS-2201. is not requested.

Tandem mass spectrometry approaches for recognition of isomeric compounds mixtures

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Summary: The proposed MS/MS approach allows the isomer recognition by playing in the "energetic dimension" of the experiment. The chromatographic set up was tuned to minimize the run time, without requiring high efficiency or resolution between the isomers. Then, the MS/MS properties were explored to solve the signal assignment.

Keywords: isomers, MS/MS, LEDA

Introduction

Mass spectrometry (MS) is an attractive technology in the chemical analysis thanks to its high selectivity, wide dynamic range and high throughput capabilities. Indeed, it is widely used in the analytical methods to obtain quali-quantitative information on the analytes. However, the specificity of the mass spectrometry signal may be compromised when the compounds show both common fragment ions and similar chromatographic retention properties, as in the case of isomers analysis. To expand the MS application, some authors explored the possibility of recognizing isomers by tandem mass spectrometry (MS/MS) experiments based on the intensity of "diagnostic fragments". Unfortunately, these cases rarely happen while it is common to obtain MS or MS/MS spectra of isomers with the same ion signals and, even if different branching ratios are present, it is hard to distinguish them, mostly if simultaneously present in the sample. In the simplest scenario, when similarly fragmenting isomers are present in a sample, it is common practice to separate them chromatographically to allow their quali-quantitative characterization. Generally, this approach may require a longer time to set up the separation method with the evaluation of different chromatographic columns, mobile phases and elution programs to obtain adequate analytes separation. All these procedures are usually molecule-specific and rarely can be extended to other compounds. Nevertheless, in the last two decades, many MS/MS strategies were developed to solve this problem by allowing the characterization and quantification of isomers and/or isobars in mixtures via a standardized approach, applicable to different compounds. Among of these strategies, the most promising and interesting for a widespread application in isomers recognition are based on (1) energy-resolved tandem mass experiments and (2) kinetics of the ion-molecule interaction, even if the latter are limited to the use of the ion trap [1]. Summarizing the reported results, the discrimination between the isomers was achieved by optimizing the selection of precursor ion, its fragmentation through collision induced dissociation (CID) mechanism and the analysis of fragmented ions produced. Each of these phases was explored, developed and tuned to carry out an adequate specificity to distinguish the isomers in the sample without the support of any structural manipulation (i.e. derivatization and isotopic enrichment) or chromatographic separation. The use of the right approach provides many analytical advantages, among which the most important are sensitivity, reliability and faster analysis. As regards the topic of isomers recognition, our group proposed and developed an MS/MS post-processing mathematical algorithm named LEDA (Linear Equation of Deconvolution Analysis) that allows the recognition of isomer compounds without their chromatographic separation [2-5]. Profiting by LEDA features, recently we introduced a methodological approach that simplifies the liquid chromatography (HPLC) parameters, allowing the use of a short column and a fast elution gradient, leading to an increased productivity without losing determination specificity. With this approach, the chromatographic column was used only to avoid or limit the interference of the sample matrix towards the analyte ionization process (matrix effects).Thanks to this experience, the logical trend suggests the evaluation of the hydrolytic kinetics of plasma enzyme systems when spiking a pair of isomers in the sample. In detail, we propose the case where only one of the isomers is hydrolyzed; in this way, adding the sample with the mixture of isomers, we can verify the influence of the stable isomer on the degradation rate of the other. To carry out this evaluation, the LEDA approach was applied to the plasma stability experiments on a pair of isomers.

Results

The investigation was devoted to monitoring the hydrolytic activity of human plasma enzymes towards selected couples of isomers simultaneously added to the samples. The description of the experimental results of the proposed study was presented in the following steps:

- the check of the achieved chromatographic separation;
- the exploration of the MS/MS features in the isomers distinction;

- the evaluation of a mathematical device (LEDA) that allows the conversion of the common MS/MS signals in specific isomer abundances, related to their relative concentration in the sample;
- the assessment of the LEDA quantitative performances;
- the application of the optimized conditions of the LEDA approach in plasma stability experiments.

The proposed mathematical device LEDA is a matrix of linear equations (1) that elaborates each MS/MS signal, distinguishes the possible components and assigns the correct abundance to the isomers present.

$$\left(\frac{Pi}{Ri}\right)_m = \sum_{x=1}^{n} \left(\frac{Pi}{Ri}\right)_x * [\%]_x \quad (1)$$

(Pi/Ri)m is the abundance ratio between the product ion (Pi) vs reference ion (Ri) measured (m) in the sample. (Pi/Ri)x is the characteristic abundance ratio between the Pi vs Ri of pure isomer. [%]x is the concentration (%) of the isomer in the sample.

Conclusion

The LEDA "mathematical device", introduced to process the acquired MS/MS data, demonstrated to be reliable in recognizing and separating the possible components present in the sample signals (Fig. 1). Concerning its effectiveness, checked by processing the HPLC-MS/MS analysis of a series of known mixture solutions, we introduced the "validation plot" that, through the evaluation of the characteristic parameters, described the accuracy and precision of the quantitative ability of the LEDA elaboration. Therefore, the general procedure proposed was found adequate to study the selected of isomer compounds without their chromatographic separation but applying and developing the MS/MS features.

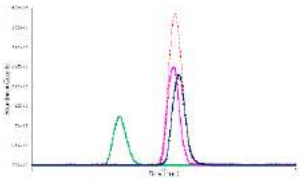

Figure 1. The LEDA reconstructed chromatographic profiles of mixture of isomers spiked in human plasma sample

References

1. Crotti, S.; Menicatti, M.; Pallecchi, M.; Bartolucci G. Tandem mass spectrometry approaches for recognition of isomeric compounds mixtures. *Mass Spectrom. Rev.* **2021**, e21757.
2. Menicatti, M.; Guandalini, L.; Dei, S.; Floriddia, E.; Teodori, E.; Traldi, P.; Bartolucci G. The power of energy resolved tandem mass spectrometry experiments for resolution of isomers: the case of drug plasma stability investigation of multidrug resistance inhibitors. Rapid Commun. Mass Spectrom. 2016, 30, 423–432.
3. Menicatti, M.; Guandalini, L.; Dei, S.; Floriddia, E.; Teodori, E.; Traldi, P.; Bartolucci G. Energy resolved tandem mass spectrometry experiments for resolution of isobaric compounds: a case of cis/trans isomerism. Eur. J. Mass Spectrom. 2016, 22, 235-243.
4. Menicatti, M.; Pallecchi, M.; Bua, S.; Vullo, D.; Di Cesare Mannelli, L.; Ghelardini, C.; Carta, F.; Supuran, C. T.; Bartolucci G. Resolution of co-eluting isomers of anti-inflammatory drugs conjugated to carbonic anhydrase inhibitors from plasma in liquid chromatography by energy-resolved tandem mass spectrometry. J. Enzyme Inhib. Med. Chem. 2018, 33, 671-679.
5. Menicatti, M.; Pallecchi, M.; Ricciutelli, M.; Galarini, R.; Moretti, S.; Sagratini, G.; Vittori, S.; Lucarini, S.; Caprioli, G.; Bartolucci G. Determination of coeluted isomers in wine samples by application of MS/MS deconvolution analysis. J. Mass Spectrom. 2020, 55, e4607.
6. Pallecchi, M.; Menicatti, M.; Braconi, L.; Supuran, C. T.; Dei, S.; Teodori, E.; Bartolucci G. Application of LEDA algorithm for the recognition of P-glycoprotein and Carbonic Anhydrase hybrid inhibitors and evaluation of their plasma stability by HPLC-MS/MS analysis. J. Pharm. Biomed. 2022, 219, 114887.

Investigating the mechanisms that promote tolerance to Ocean Acidification in different invertebrate species from the CO_2 vent systems of Ischia Island (Italy) through metabolomics analysis

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CO_2 vents of Ischia Island represent natural laboratories to study the effects of Ocean Acidification on benthic marine invertebrate species. Pioneering research conducted in these systems, demonstrated that not-calcifying organisms, like some mollusks and polychaetes, thrive under acidified conditions, whereas many calcifying species disappear along the reducing pH gradient. However, the molecular and cellular mechanisms underlying tolerance or sensitivity to OA are still poorly investigated.
Therefore, our research aimed to evaluate the potential changes in the metabolome profile in two different species living at the 'Castello Aragonese' CO_2 vent of Ischia Island.
The not-calcifying polychaetes, *Platynereis* spp. and the calcifying mollusks, *Mytilus galloprovincialis* were collected from the southern part of the Castello Aragonese, which is characterized by a gradient of different pH conditions (from 8.1 to 7.4). Untargeted metabolomics was carried out using TripleTOF™ 6600 System.
The analysis confirmed an energetic cost related to living under OA for both species. Moreover, results suggest that the effects of OA are dependent on the metabolic and physiological performances of the individual species, an essential aspect to predict the vulnerability of organisms to face multiple challenges occurring in the future oceans of the Anthropocene.

Occurrence of legacy and emerging Poly and PerfluoroAlkyl Substances (PFASs) in loggerhead turtle (*Caretta caretta*) egg

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Summary: Poly and PerFluoroAlkyl Substances (PFASs) are classified as POPs and related to a series of diseases. Both legacy and emerging PFASs were detected in loggerhead turtle (Caretta caretta) egg samples. Obtained results and preliminary considerations are shown.

Keywords: legacy and emerging PFASs, loggerhead turtle (Caretta Caretta) egg samples, LC-Q Exactive.

Introduction

Poly- and PerFluoroAlkyl Substances (PFASs) are a wide group of synthetic compounds largely used in consumer products due to their both hydrophobic and lipophobic behavior. Unfortunately, such class of substances is now ranked as high concern due to the environmental persistence, mobility, and ability to bioaccumulate, leading to adverse effects in exposed biota and humans. Freshwater and marine species are considered sentinels to monitor the overall PFAS pollution in the food webs. In this work, we investigated unhatched loggerhead turtle (*Caretta caretta*) egg pools collected from forty-one nests laid in 2021along the coast of Campania region to evaluate occurrence and concentrations of both legacy and emerging PFASs. Emerging PFASs are compounds recently found in environmental and biota samples. Among emerging PFASs, ChloroPerFluoroPolyEtherCarboxylic Acids (ClPFPECAs) have been recently reported in water, soil and biota. [1,2] . These compounds are complex mixtures of isomeric congeners only partially characterized, having ethoxy (e) and propoxy (p) groups as repetitive units with a difluoroethanoic acid and a hexafluorochloro propoxy moiety as common functional groups (CAS 329238-24-6). Their generic names are ClPFPECA-n(e),n(p) (e.g. ClPFPECA-1,1) and their general structure is shown below (fig.1).

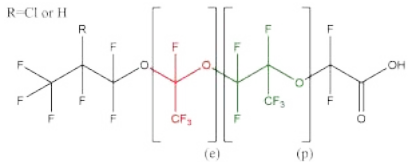

Figure 1 -General structure of perfluoroethercarboxylic acid oligomeric series (R=Cl: ClPFPECA; R = H: HPFECA). The R group position is likely variable

Legacy PFASs were detected in all analyzed samples with PFOS as the most abundant analyte. On the other hand, ClPFPECAs were detected in only 22 samples.

Experimental

Unhatched eggs were collected at the end of the emergence phase from 41 loggerhead turtle nests. For each nest, three eggs, in which embryonic development had stopped at very early stages (<stage 16), were selected and polled together for *PFASs* analysis. Egg pools were analyzed using the method reported by Moretti et al. [2] with slight modifications. Using a Q Exactive® mass spectrometry coupled to an UHPLC system (Thermo Fisher Scientific), different experiments were performed in order to obtain the best possible sensitivity for targeted determination of legacy and emerging PFASs. Moreover, suspect screening analysis was done for the determination of HPFPECAs and PerFluoroPolyEther diCarboxylic Acids (PFPEdCAs).

Results

The concentration of legacy PFASs expressed as sum of compounds ranged from 938 to 7,951 ng/kg (median 3339 ng/kg) in accordance to the value reported for hawksbills turtle eggs [3]. L-PFOS was the most concentrated analyte ranged from 421 to 4,260 ng/kg (median 1,718 ng/kg). A good correlation (r2 = 0.975) was observed between the sum of legacy PFASs (ng) and PFOS (ng) in each nest (Fig. 2). Moreover, the verified log-normal distribution of legacy PFASs results suggested that this may represent the baseline contamination for loggerhead turtle eggs in Mediterranean Sea. About emerging PFASs, only those compounds belonging to the ClPFPECA class were detected in the samples with ClPFPECA-0,1 and ClPFPECA-0,2 as the most represented and concentrated (from 10 to 1588 ng/kg) compounds. The total concentration of ClPFPECAs ranged from <LOD to 1,588 ng/kg (median 10 ng/kg). Four samples (different nests and turtles) had concentrations of ClPFPECA higher than that of other eggs (ranging from 298 to 1,588 ng/kg against an average of 32 ng/kg). ClPFPECAs were not correlated to the PFOS amount (r2 = 0.08) so it is reasonable to assume the presence of a localized contamination site(s) that cannot be referred either to a diffuse or a baseline level of contamination.

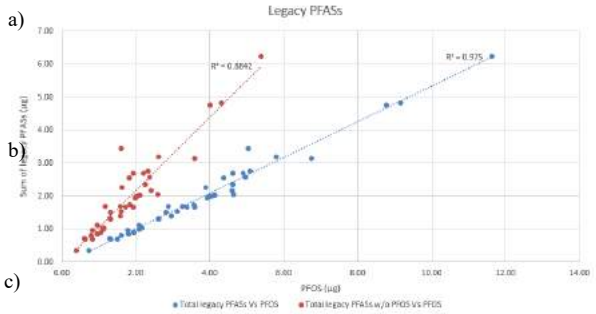

Figure 2 – Correlation between the sum of legacy PFASs (ng) and PFOS (ng) in each nest

Conclusion

Legacy PFASs were found in all analyzed samples with PFOS as the most concentrated compound. Their occurrence likely represents the baseline contamination in loggerhead turtle eggs in Mediterranean Sea. Moreover, the presence of emerging PFPECAs was identified in loggerhead turtle egg samples. Their occurrence, not recorded in all the samples, however, suggests the presence of hot spot areas, rather than a generalized baseline contamination in the Mediterranean Sea.

Acknowledgments

The authors gratefully acknowledge all the NGOs and volunteers that contributed to loggerhead turtle nesting monitoring in Campania. This activity was supported by the UOD 08 of the Regione Campania and Italian Health Ministry (Ricerca Corrente IZS ME 07-22 “La tartaruga Caretta caretta sentinella della salute degli ecosistemi marini: studio sulle correlazioni tra fattori di rischio ambientali, microbiologici e chimici.”).

References

1. J. W. Washington, C. G. Rosal, J. P. McCord, M. J. Strynar, A. B. Lindstrom, E. L. Bergman, S. M. Goodrow, H. K. Tadesse, A. N. Pilant, B. J. Washington, M. J. Davis, B. G. Stuart, T. M. Jenkins. *Science,* 368 (2020), pp 1103–1107.
2. S. Moretti, C. Barola, D. Giusepponi, F. Paoletti, A.Piersanti, O. Tcheremenskaia, G. Brambilla, R. Galarini. *Chemosphere*, 312 (2023), 137214.
3. C. Wood, G.H. Balazs, m. Rice, T.M. Work, T.T. jones, E. Sterling, T.M. Summers, J. Brooker, l. Kurpita, C.S. King, J.M. Lynch. *Environmental Pollution*, 279 (2021), 1168

Speciation of organotins by Gas Chromatography – Inductively Coupled PlasmaSpectrometry in marine samples

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Summary: The aim of this work is the development of an analytical method suitable for the determination and speciation of organotin compounds in marine samples. The method is based on extraction step as reported in EPA 8323/2003, and GC-ICP/MS determination.

Keywords: *Organotins, GC-ICP/MS, marine samples*

Introduction

Organotin compounds are a class of organometallic compounds that include mono-, di-, tri-, and tetrabutyl aswell as triphenyl tin compounds. Organotins have been widely used in various industries for applications such as antifouling agents for underwater structures, stabilizers in plastics, and catalysts in soft foamproduction.

Due to their extensive use, these compounds have become prevalent in the environment, with concentrations typically ranging from parts per trillion (ppt) to parts per billion (ppb) in seawater and from ppb to parts per million (ppm) in sediments and biota.

From an ecotoxicological perspective, tributyl tin (TBT) is considered the most hazardous substance within this chemical class.

Several studies have shown a direct correlation between exposure to this compound and shell malformationin oysters, imposex, and reduced resistance to infections in marine snails. Furthermore, TBT is relevant to human health, as exposure to this compound can cause acute kidney and central nervous disorders [1], [2], [3].

Currently, organotin compounds are monitored in environmental matrices such as water, sediment, and shellfish to assess their distribution and compliance with legal regulations [4].

Numerous analytical methods have been developed for these purposes and most of these methods involve a derivatization step to enhance the volatility of these compounds, thereby improving their gas chromatographic behavior.

In 2016, the AVL laboratory of ARPAT developed an high sensitive method for determining TBT in waters and clean sediments based on derivatization and GC-MS/MS analysis[5]. However, this procedure is not applicable to harbour sediments and biota due to the high levels of organotins and the presence of coextractives, which can cause issues with derivatization and instrumental contamination.

Consequently, in 2022, we performed an alternative method for the determination of total organotin in biota using an extraction method based on EPA 8323/2003[6], employing hexane/tropolone solutions, sonication in a water bath sonicator, micro-concentration by a nitrogen evaporator, acid mineralization in an oven, and ICP-MS determination[7]. Although this procedure had the advantage, compared to previously reportedmethods, of avoiding derivatization and clean-up steps, it did not allow for the chemical speciation of organotin compounds.

Therefore, we have developed an analytical method for speciating and evaluating the concentration of organotins in marine samples based on extraction and GC-ICP/MS detection without derivatizing the sample after extraction.

Experimental

In this study, we conducted a speciation analysis of organotins, specifically Dibutyltin, Tributyltin, Tripropyltin, and Triphenyltin, in marine sediments. The analytical instruments used in this research were a Gas Chromatography Trace 1310, a transfer line GCI 100 for coupling GC with an ICP, and an Inductively

Coupled Plasma (ICP) ICapQ. To inject the sample was used a PTV module with a 2mm ID baffled inletliner in splitless mode. The column utilized was a 5% Phenyl-Methylpolysiloxane column with dimensionsof 30m length, 0.250mm diameter, and 0.25um particle size.
The method development involved the use of single chloride standard solutions at a concentration of 1000 mg/L, which were subsequently diluted to different levels for calibration purposes. As a control, a Certified Reference Material BCR-646, consisting of freshwater sediment, was employed. This reference material had been previously used in a preliminary study for the determination of total organotins[7].
The extraction procedure was based on EPA Method 8323/2003[6]. It involved the use of a hexane/tropolone solution, followed by sonication in a water bath sonicator and microconcentration using a nitrogen evaporator. The resulting extract was dissolved in hexane and subjected to separation via Gas Chromatography. The tin content was determined using ICP/MS.
All calibration solutions were prepared by dissolving certified reference materials in hexane, utilizing chloride derivates. The driving force of this choice is based on the fact that chloride salts are the main components of marine samples.
Furthermore, each sample was analyzed using ICP/MS to determine the total organotin content, serving as a control for the yield ratio. The sample preparation procedure followed the same approach as the previous work[7].

Results and conclusions

In this study, we have successfully developed a method for the separation of organotins without the need for derivatization after extraction. The conditions of the gas chromatography and ICP/MS analysis enabled us to achieve a high sensitivity for tin detection. Our method demonstrated the capability to detect organotin concentrations as low as 1 µg/kg of TBT (as TBT chloride) (refer to Fig. 1), highlighting its effectiveness for trace-level analysis.

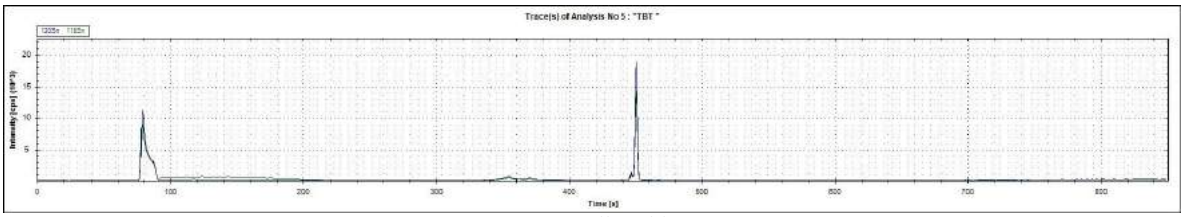

Figure 1. Tributyltin

To ensure accurate quantification, we employed Tripropyltin (TPrT) (refer to Fig. 2) as an internal standard. The choice of TPrT as the internal standard was based on its absence in antifouling or stabilizing production, minimizing potential interference from external sources.

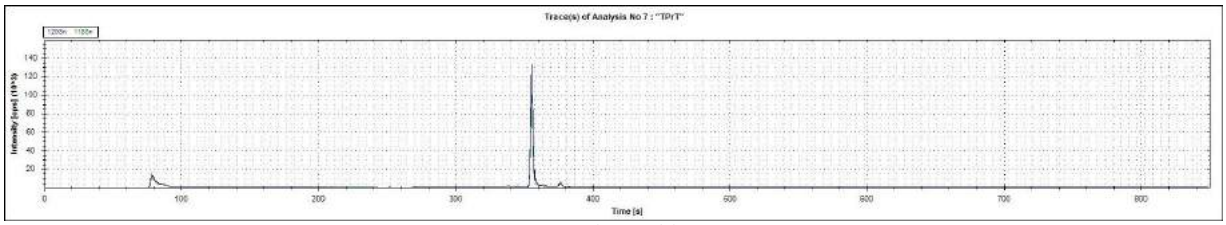

Figure 2. Tripropyltin

For Mass Spectrometry analysis, we set the instrument to reveal the two isotopes of tin, specifically 118 m/z and 120 m/z. The determination of the correct ratio between these isotopes allowed us to establish with high certainty that the chromatogram corresponds precisely to tin, further enhancing the reliability of our results.
Overall, our developed method offers a valuable approach for the speciation and quantification of organotins in marine sediments. The absence of derivatization simplifies the analysis process, while the high sensitivity of the gas chromatography and ICP/MS facilitates accurate detection even at low concentrations.

These findings contribute to the understanding of organotin distribution in marine environments and provide a robust analytical tool for environmental monitoring and assessment.

References

1. M. Hoch, Organotin compounds in the environment - an overview, Appl. Geochem. 16 (2001) 719-743.
2. B. Antizar-Ladislao, *Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review*, Environ. Int.34 (2008) 292-308
3. Gunnar Nordberg, *Handbook on the Toxicology of metals*, third edition p. 854
4. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmentalquality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council
5. F. Bellandi, V. Filippi, M. Mazzetti, *"Analysis of TBT in superficial and sea waters by solid-phase derivatization and GC-MS/MS"* 3rd MS ENVI Day September 26-28, 2016 Livorno (Italy) – ATTI DEL CONVEGNO pg. 35
6. Method EPA 8323 *"Determination of organotins by Micro-Liquid Chromatography-Electrospray Ion Trap Mass Spectrometry"*
7. E. Di Alessandro, C. Cini, F. Castellani Tarabini, V. Filippi, M. Sibra, P. Altemura, *"Determination of organotin compounds by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in biota and sediments"* 5th MSENVI Day November 28-29, 2022 Milano (Italy).

Analysis of ultrashort-chain and short-chain (c1 to c4) per-and plyfluorinatedsubstances in potable and non-potable waters

Jamie York, Shun-Hsin Liang, Justin Steimling

Restek S.p.A. Restek S.r.L.

Introduction

Perfluoroalkyl substances are a group of man- made chemicals widely used in industrial applications and consumer products. Their widespread usage and resistance to degradation has resulted in PFAS being a ubiquitous environmental contaminant and the potential health effects is of growing concern. While many of the long-chain PFASHave been recognized as harmful, alternative compounds have emerged in their place.

Short-chain PFAS compounds are consideredto be less bio accumulative and toxic than long-chain, but their widespread use has resulted in their increased environmental accumulation. In this work, the analysis of ultrashort-chain and short-chain (C1 to C4) per- and polyfluorinated substances is outlined and applied to potable and non- potable waters by LC-MS/MS.

Materials and methods

Reverse osmosis water was collected from thefacility of Restek Corp. (Bellefonte, PA). Wastewater samples were gifts from GDIT (Falls Church, VA). Bottled waters were obtained from local grocery stores. Norm-Jectsyringe (10 mL Luer Lock Tip) and syringe filter (30mm, 0.45µm Nylon) were obtained from Restek Corp. (Bellefonte, PA) and used for wastewater filtration. Polypropylene vials (700 µL) and polyethylene caps were purchased from Waters Corp. (Milford, MA) and used for the preparation of standard and sample solution. The analysis was performed on the Waters Acquity I-class UPLC system coupled to a Xevo TQ-S triple quadrupole mass spectrometer. Compound tuning was conducted using negative mode of electrospray ionization to determine precursorand product ions. A Raptor Polar X column (50 x 2.1mm, 2.7 µm) from Restek Corp. (Bellefonte, PA) was used for chromatographic analysis of seven C1 – C4 PFAS analytes. The aqueous mobile phase (Mobile Phase A) was a mixture of 10 mM ammonium formiate and 0.1% formic acid in water and the organic mobile phase (Mobile Phase B) was 0.1% formic acid in 95:5 acetonitrile:isopropanol. Separation was performed with the isocratic elution under 85% B for 7 minutes of each injection of 10 µL of standard and sample solutions. The flow rate was 0.3 mL/minute, and the columntemperature was controlled at 40° C.

$$x(t)=\left[s(t)\;\; x(t+\tau)\;\; \ldots\; s\left(t+(k-1)\tau\right)\right] \qquad (1)$$

Results

A direct injection workflow was establishedto provide a unique solution for the determination of ultrashort-chain and short-chain PFAS in various water matrices. The reported method was rugged, accurate, and precise implementing a fast 7-minute chromatographic analysis. Most importantly,this solution can offer a great tool for the monitoring of these emergent PFAS in environmental water system and assist in generating a guideline for future regulatory references.

Discussion and conclusion

An LC method was established with the aim to obtain the best MS detection sensitivity andyet could mitigate the matrix interference.

With linear regression (1/x weighted), all analytes showed acceptable linearities with r2 >0.995 and deviations <20% at the range of2.5 – 800 ppt for C1 – C4 PFSA, 5.0 – 800 ppt for PFBA and PFPrS, and 20 – 800 ppt forTFA. The tap water, spring bottled water, and potable water (POTW) water were fortified at 25, 50, and 175 ppt for all analytes. Three batches of analyses were performed on different days for a total of nine repetitions at each fortified level. There was differential amount of TFA in all 3 water samples. The tap water had incurred TFMS as well. In addition to TFA and TFMS, the POTW water also contained PFBS and PFPrA. These incurred concentrations were subtracted from the calculated concentrations of fortified samples to determine the recovery. Due to a much higher TFA concentration in the POTWwater, the fortification was performed on a 5-fold diluted (in RO water) POTW water for the accuracy and precision analysis of TFA. There was no data to be collected for TFA at 25 ppt fortified concentration as it was unableto obtain accurate quantification by concentration subtraction of incurred TFA. All analytes had recovery values of 86.6 - 107% across three fortification levels among three different types of waters. Satisfactory method precision was demonstrated with

%RSD values within 1.62 to 10.7%. This direct injection workflow was applied to the determination of C1 to C4 PFAS in a variety of tap waters, bottled waters, natural spring water, well water, and wastewaters from different sources. Three preparations of blankand fortified (50 ppt) samples were injected for the analysis. It was shown that the averaged recoveries of fortified QC samples were all within 75 to 120%. This demonstrated that the established method wassuitable for accurate measurement of C1 to C4 PFAS in both potable and non-potable waters. The data indicated that TFA was ubiquitously present in tap waters at the rangefrom ~120 to 500 ppt. TFMS was present andquantifiable in most tap waters and PFPrA was detectable in several tap waters. The tested spring bottled waters contained TFA aswell. It was clean of C1 to C4 PFAS for an RO purified bottled water and an RO filtrated tap water. The well water tested had a relatively higher amount of TFMS. The wastewaters originated from POTW, hospital,metal finisher, and chemical manufacturer all had higher levels of TFA and PFPrA. The wastewater effluent collected from a chemicalmanufacturer had significantly elevated levelsTFA, PFPrA, and PFBA contamination.

Target, non-target and suspect screening of PFAS in dolphins, marine turtles and sharks (Tuscany coast, Mediterranean Sea)

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Summary: In this study, we investigated the PFAS occurrence in tissues of striped dolphins (*Stenella coeruleoalba*), sea turtles (*Caretta caretta*) and different shark species (*Prionace glauca, Carcharinus plumbeus* and *Isurus oxhyrinchus*) stranded or accidentally caught along Tuscany coast between 2020 and 2022.

Keywords: PFAS, Marine organisms, Mediterranean Sea

Introduction

Sampling stranded specimens allows investigating levels of diverse contaminants and to carry out studies to assess health status of marine organisms. Global distribution of some per- and polyfluoroalkyl substances (PFAS) in waters and in aquatic organisms, has been documented in many studies, demonstrating their persistence in the environment and their bioaccumulation and biomagnification through the trophic chain. Thus, PFAS represent emerging chemicals that are of environmental concern for marine organisms.

Materials and Methods

Samples of blood, muscles, liver and brain were extracted by QuEChERS protocol and the extracts were analysed by ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-HRMS Thermo Fisher Scientific Q-Orbitrap, Waltham, MS, USA). 11 perfluorocarboxylic acids, (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA), 6 perflurosulphonic acids (PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFDS) and the perfluorooctane sulfonamide (FOSA) were quantified by isotopic dilution. Moreover, non-targeted analysis was carried out on HRMS raw files using Compound Discoverer 3.1 (Thermo Scientific, USA) by comparison with the PFAS lists submitted to the NORMAN Suspect List Exchange Database and USEPA CompTox Chemical.

Results and Discussion

PFBA, PFPeA, PFHxA, PFBS, PFPeS and PFDS always resulted as below detection limits and were excluded from any further discussion. The other target analytes were measured at high concentrations in the tissues of dolphins (i.e. blood, brain, liver and muscle; Figure 1) whereas only the PFOS was detected above detection limits in blood, liver and muscle samples of turtles and sharks. Liver tissue showed the highest PFAS levels, whereby PFOS was the dominant compound of the fingerprint. PFOS levels in dolphin liver (148 ± 104 ng/g ww; N = 23) were 10 fold higher than in the liver of the other marine species (1.12 ± 1.09 ng/g ww; N=9 and 1.22 ng/g ww; N=2 for sharks and sea turtles, respectively).

Suspect screening analysis allowed identifying 2 novel perfluorosulfonamides (FBSA(C4) and FHxSA(C6)) in all the analysed tissues of dolphin, as well as a series of n:3 fluorotelomer carboxylic acid (FTCA) in liver tissue only (Figure 2). In dolphins, the muscle to blood ratios were generally below the unit for most of the PFAS indicating a low affinity of all the PFAS for the muscle tissue. Actually, part of the PFAS detected in muscle can be due to the blood embedded into the muscle fibres but there is a slightly higher muscle accumulation of the long-chain PFAS than of the short-chain ones, probably

due to the affinity of the fluorinated tail with the membrane phospholipid bilayer. The PFAS brain to blood ratios increased with fluorinated chain length. This trend is consistent with most of the published data for wildlife species: the long-chain PFAS may cross cerebral barriers more effectively than short-chain PFAS and they have stronger association with phospholipids that can enhance the bioaccumulation in phospholipid rich tissue, such as the brain [1].

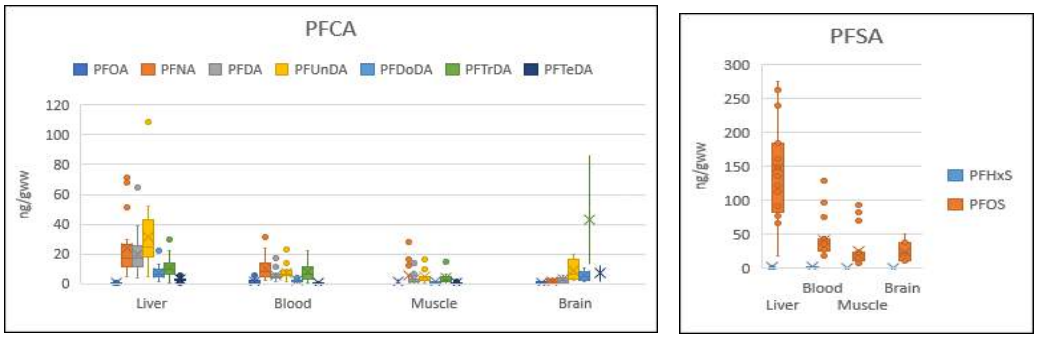

Figure 2a. Concentration of the PFAS detected above the limit of quantification (LOQ) in the tissues of stranded dolphins.

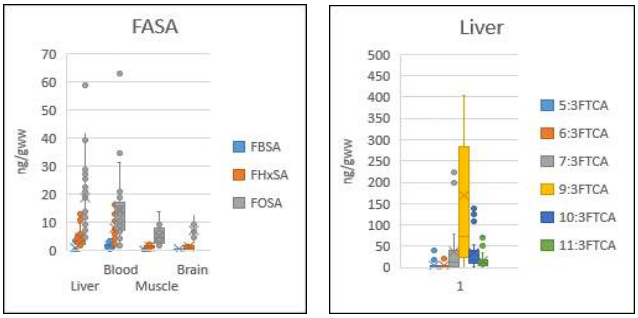

Figure 2b. Concentration of the suspected PFAS (n:3 FTCA were semi-quantified by PFOA).

Unlike muscle and brain, the liver to blood ratios showed an increase according to the number of CF2 for perfluorosulphonic acids (PFSA) and perfluorosulphonamides while they were rather variable for perfluorocarboxylic acids (PFCA). The liver is the main site of metabolism, where the transformation of the PFAS precursors occurs. The lack of accumulation pattern and elevate levels of PFCA in liver may suggest a high level of metabolisation of PFCA precursor compounds. This hypothesis was supported by the presence in liver of high levels of n:3 FTCA - stable intermediates of the metabolisation of perfluorotelomers, such as fluorotelomer alcohols (FTOH) and fluorotelomer sulfonates (FTS) [2]. They were mainly detected at high levels in the liver, while only in traces in some blood samples. These findings suggest that they are generated from precursors present at high concentration in liver cells but they are not displaced to different tissues through the bloodstream. In contrast, the striped dolphins probably lack, totally or partially, the ability of transforming perfluorosulphonamides to PFSA within the liver [3] and these semi-neutral compounds accumulate in the tissues according to their affinity.

Conclusions

Our findings demonstrated that PFAA and perfluorosulphonamides accumulate in the marine cetacean species, whereas in sea turtles and sharks living in the same area accumulation does not occur. Metabolisation in liver of fluorotelomer precursors seems to be the main source of PFCA in dolphins. Further studies should be necessary to disentangle if the main pathway of exposure to precursors is the breathing or the diet.

References

1. Dassuncao C, et al. *Environ Sci Technol Lett.*;6(3) (2019), 119-25. [doi:10.1021/acs.estlett.9b00031]
2. Xie S, et al. *Chemosphere*. 249 (2020), 126016. [doi:10.1016/j.chemosphere.2020.126016]
3. Lopez-Berenguer G, et al. *Environ Pollut.*267 (2020), 115367. [doi:10.1016/j.envpol.2020.115367]

Microwave-assisted sample pretreatments and analytical pyrolysis: a holisticapproach for the study of microplastics and correlated pollutants

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Summary: Assessing micro- and nano-plastic pollutants is crucial but challenging. While many studies determine their distribution and concentration, understanding their interaction with the environment and organisms remains limited. Recent research focuses on identifying chemical species associated with microplastics, including plasticizers and organic compounds. This study employs microwave-assisted extraction, analytical pyrolysis, gas chromatography, and mass spectrometry to quantify microplastics andassociated pollutants in various samples, offering potential for characterizing soluble oxidation products, polymer additives, and toxic compounds.

Keywords: mw-assisted extraction, MW-assisted digestion, persistent organic pollutants, microplastics,analytical pyrolysis

Introduction

Sampling, separation, detection and characterization of micro- and nano-plastic pollutants is a challenging and critical goal, fundamental to assess their amount, fate, and the related hazards for ecosystems. While an increasingly large number of studies are focused on determining the distribution and concentration of plastic microparticles in different environmental compartments, there is still a major lack of understanding of the most relevant mechanisms of interaction and exchange of this class of pollutants with the environment and with organisms. In the last years we observed an increase of the studies focused on the evaluation of the chemical species associated with the presence of microplastics in the environment, such as plasticizers, low molecular weight degradation products, adsorbed persistent organic pollutants, aliphatic aromatic hydrocarbons, and volatile organic compounds.

Results

In this work we combined microwave-assisted extraction and digestion, together with analytical pyrolysis coupled with gas chromatography and mass spectrometry (Py-GC-MS), to study and quantify microplastics together with different classes of associated pollutants, in different environmental and biological samples [1].This approach can be potentially used to characterize and quantify together with microplastics, the presence of soluble oxidation products from polymers, polymer additives (phthalate plasticizers), polycyclic aromatic hydrocarbons, and polychlorinated biphenyls.

The system used for the extractions was an ETHOS X Advanced Microwave Extraction System quippedwith a SK-15 high-pressure rotor (Milestone Srl, Italy), while the quantification was performed using a multi-shot pyrolyzer EGA/PY-3030D coupled with an Auto-shot sampler AS-1020E autosampler (Frontier Lab., Japan). The system was interfaced with an Agilent Technologies 8890 gas chromatograph, which was combined with a 5977B mass selective single quadrupole mass spectrometer detector (Agilent Technologies, USA). The limits of detection for pollutant extraction and analysis ranged from 0.001 to 0.441 ng, while the limits of quantification ranged from 0.002 to 1.360 ng. For microplastics, the limits of detection and quantification were lower than 0.13 µg and 0.4 µg, respectively.

Conclusions

The combination of the microwave-assisted treatment and the combined analytical approaches based on pyrolysis achieved significant information for a better understanding of the chemical nature of degradation products potentially released by the different polymers present as microplastics in the environment, and ofthe possible presence of other chemical species that are generally neglected in the study of microplastics.

References

1. 1. J. La Nasa, G. Biale, L. Fiorentini, A. Ceccarini, D. Carnaroglio, M. Mattonai, F. Modugno, Characterization and quantification of microplastics and organic pollutants in mussels by microwave-assisted sample preparation and analytical pyrolysis, 2023, submission in progress

Rapid Identification and quantification of microplastics in sea water and sediment using the new Laser Direct Infrared Spectroscopy

Paolo Scardina

Agilent Technologies Italia

Summary: Microplastics are emerging pollutants in sea water and sediment. Dimensional characterization and identification require very long measurement time using classical spectroscopy techniques like FT-IR Imaging or Micro- Raman. New Laser Direct Infrared Spectroscopy (LDIR) allow to count, characterize, and quantify microplastics in few minutes.

Keywords: Microplastics, Infrared Microscopy, Laser Direct Infrared

Introduction

Microplastics are emerging pollutants in sea water and sediment, due to the accumulation in the environmentthe amount of microplastic in sea water and sediment is continuously growing.

Sample preparation

There are many sample preparation methods available for microplastics characterization depending by the matrix, complex matrix like sea water and sediment requires 3 main steps: organic matter removal, separationfrom the sediment and filtering.

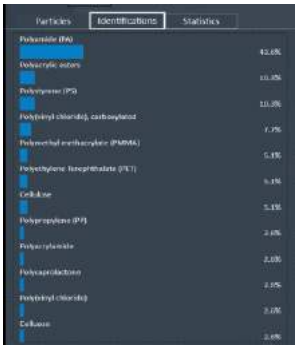
Spectroscopic techniques commonly used for microplastics characterization

FTIR Imaging and Micro- Raman are spectroscopic techniques widely used for microplastics characterization, they both requires long time for a full automated dimensional and characterization and identification, in addition Micro-Raman is affected by potential fluorescence interference. Focal plane array FTIR imagingother than long time for measuring requires many hours for data processing.

Laser Direct Infrared Spectroscopy for microplastics characterization

The recently introduced Laser Direct Infrared Spectroscopy (LDIR) allow the characterization of microplastics samples in few minutes, thanks to the capability to execute a scan on the full sample at a specificwavenumber to identify C-H bending absorption, coupling the absorbing areas with high-definition visible image, and then operating a full scan in each area of interest to identify the particles.

This process is very fast and common samples can be fully characterized in less than 10 minutes.



LDIR can be used on samples prepared on Kevley slides or directly on gold coated filters, a very good reproducibility on particle number measured and on size accuracy has been tested.

Conclusions

Laser Direct Infrared Spectroscopy (LDIR) is able to characterize microplastics samples fully automatically with the highest accuracy and reproducibility in times from 100 to 1000 times lower than conventional spectroscopic methods and proves to be ideal for research and routine microplastics analysis.

Poster Presentation

Cross contamination effects from vial septa used in soil and sediment samplingfor Volatile Organic Compounds (VOC) analyses. A GC-MS study.

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Summary: VOC release effect from contaminated septa have been estimate with the aid of head space andP&T GC/MS techniques

Keywords: VOC, cross-contamination, GCMS

Introduction

Sampling soils or lake and sea sediments for VOCanalyses requires appropriate containers to obtain reliable results and to avoid both losses of thevolatile analytes and contamination from alien sources. Recent papers provide detailed reviewingabout sampling and quality control practicesrecommended by regulatory agencies and scientific organizations for acquiring reliable,defensible sediment samples in marine environments [1]. In marine sediments the VOC amounts to be measured could be very small, at magnitude orders of pg/kg [2], so that glassware and sampling vial cleaning becomes of utmost importance. Reference documents providing instructions for an appropriate glassware, container cleaning also exist [3].

Several papers studied cross-contamination phenomena occurring in water or soil sampling for VOC analyses [4,5]. Other research considered

-more in general- cross-contamination phenomena occurring at various levels for inorganic and/or organic analytes [6-8] in different cases. Ulanowska et al. [9] studied the contamination by VOC residues released from vial septa andinterfering with the searched VOCs in trace analysis on breath samples. In this contribution weconsidered contaminated septa from sealing caps of vials containing strong polluted samples and their potential contamination impact if used for taking successive samples.

Experimental

Butyl/PTFE or silicone/PTFE septa were removedfrom vials of three contaminated soil samples and rinsed with ultrapure water in order to only remove soil residues and particles. Soil samples were analysed in the routine analysis with EPA 5021A-EPA 8260D head space-GC/MS method. The three septa have deliberately not been subjected to laboratory washing procedures in order to maximize the potential cross-contamination phenomena as much as possible.

Figure 1. Septa taken from sealing caps, on vialscontaining contaminated soil samples.

The septa were then individually immersed into a 40 ml glass vial filled with ultra-pure water and analysed as being water samples with EPA5030C-EPA8260D purge & trap-GC/MS method.

Results

Rubber and PTFE septa were chosen for thesetests due to a stronger affinity between plasticmaterials and organic substances. Plastic materialsin sample containers are known to capture organicsubstance residues and to be prone to cross- contaminations. Table 1 summarizes the concentration found inwater after release from the contaminated and un- cleaned septa for each substance found, and compares with the corresponding concentration found on soil, for each sample a, b or c ("Id" = sample identifier). Total

C5-C12 hydrocarbons found on soils were compared with the sum of all VOCs found released in water. Concentrations <10 µg/Kg (LOQ) on soils have arbitrarily beenset equal to ½ LOQ, that is, 5 µg/Kg. In Figure 2a and 2b the concentration levels found in water in the list are plotted versus original concentrations on soils, for each substance identified in both cases. If the concentrations of the VOCs released in water are plotted as a function of the respective concentrations originally found in the soil samplesthat were in contact, the occurrence of cross- contamination effects at some extent, can be evidenced; however, these can be approximately estimated to be very small: from one to three orders of magnitude lower. It should be remarked however that septa were not cleaned with any washing procedure expressly to highlight anyoccurrence of potential contamination effects.

Table 2. VOC amounts released in water from contaminated septa and original VOC concentrations found on soils

.

Id	Substance identified	Soil original sample content,µg/Kg	Release from vialseptum in water, µg/l	Id	Substance identified	Soil original sample content,µg/Kg	Release from vialseptum in water, µg/l
a	MTBE	10	0,01	b	Styrene	600	2,4
a	Benzene	*<10**	0,1	b	1,3,5 Trimethylbenzene	5200	9,4
a	Toluene	*<10**	0,6	b	1,2,4 Trimethylbenzene	15000	22
a	Ethylbenzene	20	0,9	b	Total VOC/ Hydrocarbons	229000	203
a	m+p-Xylene	1800	12	c	MTBE	100	2,3
a	o-Xylene	60	2,7	c	ETBE	20	0,8
a	Styrene	*<10*	0,2	c	Benzene	80	0,4
a	1,3,5 Trimethylbenzene	2900	2,4	c	Toluene	10	1,9
a	1,2,4 Trimethylbenzene	5900	7,7	c	Ethylbenzene	2700	2,4
a	Total VOC/ Hydrocarbons	122000	65	c	m+p-Xylene	6000	12,3
b	MTBE	50	0,5	c	o-Xylene	50	6,2
b	ETBE	40	0,2	c	Styrene	*<10**	0,5
b	Benzene	50	0,5	c	1,3,5 Trimethylbenzene	6100	1,6
b	Toluene	1600	16	c	1,2,4 Trimethylbenzene	29000	6,1
b	Ethylbenzene	1800	10,5	c	Naphthalene	2500	0,4
b	m+p-Xylene	32000	36	c	Total VOC/Hydrocarbons	247000	134
b	o-Xylene	14000	26				

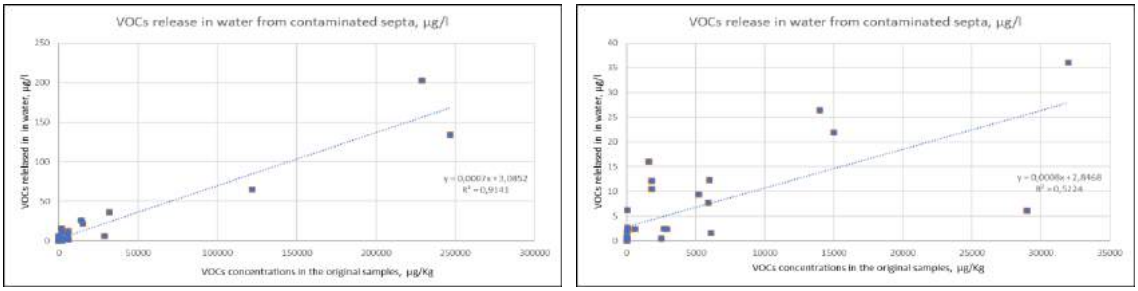

Figure 2a and 2b (scale expansion). Concentration of VOCs released in water versus original concentrations in soil.

Another similar experiment where some vial septa, afters being in contact with strong contaminated soils, has been washed with the laboratory cleaning procedures, evidenced nosignificative VOCs release in waters.

Conclusion

The tests showed the occurrence of cross- contamination phenomena as predictable in theabsence of washing procedure of the septa. In these conditions the tests provided some magnitude orders of the extent of such potential contaminations. Septa have been in contact with concentrations of the order of mg/Kg (or mg/l for liquid aqueous sample) and the observed release effects in these tests occur indicatively at levels ofμg/l. These considerations could be tentatively extrapolated to all container surfaces in contact with the samples, in a rough approximation.

References

1. C.B. Tuit, A.D. Wait; *Environmental Forensic*, 21(2020), pp 291-309.
2. P. Roose, J. Dewulf, U.A.TH. Brinkman, H. Van Langenhove, *Wat. Res.* 35 (2001), pp 1478–1488.
3. Unites States Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington DC 20460, *EPA Specification and Guidance for Contaminants-Free Sample Containers*, *Publication 9240.0 EPA 540/R-93/051PB93-963316*, Washington DC 1992, pp 15-16.
4. S.P. Levine, M.A. Puskar, P.P. Dimersky, B.J. Warner, C.S. Friedman, *Environ. Sci. Technol.* 17 (1983), pp. 125-127.
5. Bureau Veritas North America, *Methanol VialCross-Contamination Risk*, https://www.bvna.com/insight/methanol-vial- cross-contamination-risk (accessed 2023-06-07).
6. F. Welle, *Resources, Conservation and Recycling* 112 (2016), pp. 65–72.
7. J. Knapp, A. Allesch, W. Müller, A. Bockreis, *Waste Management* 69 (2017), pp. 88–100.
8. B. Tienpont, F. David, E. Dewulf, P. Sandra, *Chromatographia* 61 (2005), pp. 365-370.
9. A. Ulanowska, T. Ligor, A. Amann, B. Buszewski, *J. of Chromatographic Sci.* 50 (2012), pp. 10-14.

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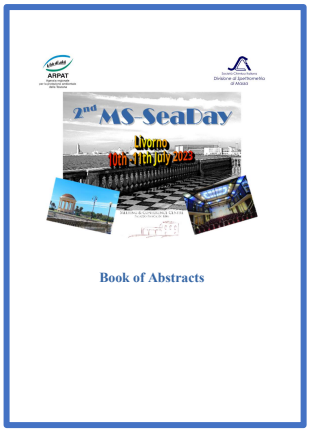

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