KLAIPĖDA UNIVERSITY

Elise Marie Clemence LORRE

PHTHALATE ESTERS IN BOREAL ESTUARINE SYSTEMS: SOURCES, DISTRIBUTION AND FATE

DOCTORAL DISSERTATION

Natural sciences, ecology and environmental sciences (N 012)

Klaipėda, 2025

This doctoral dissertation was prepared in the 2020–2025 period at Klaipeda University, based on the conferment of a doctorate right which was granted to Klaipeda University by order of Minister of Education, Science and Sport (Republic of Lithuania) No. V-160, signed on 22 February 2019.

Academic advisor

dr. Mindaugas ŽILIUS (Klaipėda University, Natural Sciences, Ecology and Environmental Sciences – N 012).

Research consultant

prof. dr. Federica BIANCHI (University of Parma, Italy, Natural Sciences, Ecology and Environmental Sciences – N 012).

This doctoral dissertation will be defended at a public meeting of the Board of Klaipėda University in Ecology and Environmental Sciences:

Chairman:

dr. Darius DAUNYS (Klaipėda University, Natural Sciences, Ecology and Environmental Sciences – N 012);

Members:

dr. Tatjana PAULAUSKIENĖ (Klaipėda University, Natural Sciences, Environmental Engineering – T 004);

dr. Marion KANWISCHER (Leibniz Institute for Baltic Sea Research, Germany, Natural sciences, Chemistry – N 003);

dr. Laure MALLERET (Aix-Marseille University, France, Natural Sciences, Chemistry – N 003);

prof. dr. Anna SOBEK (Stockholm University, Sweden, Natural Sciences, Ecology and Environmental sciences – N 012).

The dissertation will be defended in a public meeting of the Board in Ecology and Environmental Sciences, Klaipėda University, Marine Research Institute, Conference hall (201 a.) at 1 p. m. on 6th of June, 2025.

Address: Universiteto alley 17, LT-92294, Klaipėda, Lithuania.

This doctoral dissertation was sent out on May 6, and is available for review at the Library of Klaipeda University.

KLAIPĖDOS UNIVERSITETAS

Elise Marie Clemence LORRE

FTALATŲ ESTERIAI BOREALINĖSE ESTUARIJŲ SISTEMOSE: ŠALTINIAI, SKLAIDA IR LIKIMAS

DAKTARO DISERTACIJA

Gamtos mokslai, ekologija ir aplinkotyra (N 012)

Klaipėda, 2025

Disertacija rengta 2020–2025 metais Klaipėdos universitete pagal Lietuvos Respublikos švietimo, mokslo ir sporto ministro 2019 m. vasario 22 d. įsakymu Nr. V-160 Klaipėdos universitetui suteiktą Ekologijos ir aplinkotyros mokslo krypties doktorantūros teisę.

Vadovas

dr. Mindaugas ŽILIUS (Klaipėdos universitetas, gamtos mokslai, ekologija ir aplinkotyra – N 012).

Mokslinis konsultantas

prof. dr. Federica BIANCHI (Parmos universitetas, Italija, gamtos mokslai, ekologija ir aplinkotyra – N 012).

Daktaro disertacija ginama Klaipėdos universiteto Ekologijos ir aplinkotyros mokslo krypties taryboje:

Pirmininkas:

dr. Darius DAUNYS (Klaipėdos universitetas, gamtos mokslai, ekologija ir aplinkotyra – N 012);

Nariai:

dr. Tatjana PAULAUSKIENĖ (Klaipėdos universitetas, gamtos mokslai, aplinkos inžinerija – T 004);

dr. Marion KANWISCHER (Leibnizo Baltijos jūros tyrimų institutas, Vokietija, gamtos mokslai, chemija – N 003);

dr. Laure MALLERET (Marselio universitetas, Prancūzija, gamtos mokslai, chemija – N 003);

prof. dr. Anna SOBEK (Stokholmo universitetas, Švedija, gamtos mokslai, ekologija ir aplinkotyra – N 012).

Daktaro disertacija bus ginama viešame Ekologijos ir aplinkotyros mokslo krypties tarybos posėdyje 2025 m. birželio 6 d. 13 val. Klaipėdos universiteto Jūros tyrimų instituto konferencijų salėje (201 a.).

Adresas: Universiteto al. 17, LT-92294, Klaipėda, Lietuva

Daktaro disertacija išsiųsta 2025 m. gegužės 6 d. Disertaciją galima peržiūrėti Klaipėdos universiteto bibliotekoje.

Abstract

The present study provides a comprehensive evaluation of the occurrence, distribution, and fate of phthalate esters (PAEs) in boreal estuarine systems. Utilizing the Curonian Lagoon as a model ecosystem, it aimed to identify the key factors governing PAE dynamics in estuarine systems. The findings indicate that the estimated PAE inputs to the lagoon were generally lower than those of the outflow to the sea, suggesting that the lagoon serves as a convergence zone for PAEs before their release into the Baltic Sea. However, the role of the lagoon in regulating PAE throughputs varied seasonally and was modulated by meteorological factors, including ice cover and wind patterns. Among the estimated PAE sources, the main tributary emerged as the primary source to the Curonian Lagoon, although additional contributors were also present. While wastewater treatment plant discharges had a relatively minor impact, atmospheric deposition was suspected to be a significant yet understudied source. A key focus of the present study was to evaluate the role of suspended particulate matter (SPM) in the transport and distribution of PAEs. Often overlooked in conventional monitoring programs, SPM served as a crucial vector for distributing PAEs, particularly those with a higher molecular weight, throughout the ecosystem. The sedimentation of particles with attached PAEs contributed to sediment contamination, reinforcing the role of sediments as a major reservoir for PAEs in the ecosystem. Estimates indicated that several tonnes of PAEs had been retained in surface sediments for over 5 years, underscoring their long-term persistence in the aquatic environment. This accumulation raised concerns about potential ecological risks, as the study revealed significant threats to aquatic organisms in the lagoon. Comparative analysis with similar estuarine systems, such as the Szczecin and Vistula lagoons, demonstrated consistent long-term trends, further validating the Curonian Lagoon as a representative model for PAE distribution in boreal estuarine systems. In addition to assessing contamination patterns and ecological risks, this research introduces novel methodological advancements that enhance analytical precision in contaminant detection and provide unprecedented insights into PAE degradation processes, contributing to a broader understanding of the fate of pollutants in aquatic ecosystems.

Keywords:

Phthalate esters, Lagoon, Ecotoxicological Risk, Transcriptome analysis, Suspended Matter

Reziumė

Šiame darbe išsamiai įvertintas ftalatų esterių (PAE) paplitimas, pasiskirstymas ir likimas borealinėse estuarijų sistemose. Tyrimų tikslas buvo identifikuoti pagrindinius veiksnius, lemiančius PAE kaitą estuarijų aplinkoje, kaip modelinę ekosistemą naudojant Kuršių marias. Tyrimų rezultatai atskleidė, kad Kuršių marios didžiąją dalį laiko veikė kaip PAE šaltinis Baltijos jūrai. Vis dėlto marių kaip teršalų šaltinio arba "filtro" vaidmuo keitėsi priklausomai nuo sezoninių meteorologinių veiksnių – ledo dangos ar vėjo krypties. Pagrindiniu PAE šaltiniu Kuršių marioms buvo upės prietaka, tuo tarpu nuotekų valyklos darė santykinai nedidelį poveikį bendrajai taršai. Tikėtina, kad atmosferinė depozicija taip pat galėjo būti reikšmingas PAE taršos šaltinis, tačiau jis kol kas lieka mažai ištirtas.

Viena iš svarbių tyrimo krypčių buvo PAE identifikavimas skirtingose fazėse, siekiant įvertinti suspenduotų dalelių poveikį PAE pernašai ir pasiskirstymui Kuršių marių ekosistemoje. Nors šių dalelių vertinimas dažnai neįtraukiamas į standartines stebėsenos programas, paaiškėjo, kad jos yra svarbus vektorius, ypač pernešant didesnės molekulinės masės PAE junginius. Prie dalelių adsorbuoti PAE sedimentacijos metu pernešami ir į dugno nuosėdas, kurios yra pagrindinis jų rezervuaras ekosistemoje. Apskaičiuota, kad paviršinėse dugno nuosėdose yra sukaupta iki kelių tonų PAE, iš kurių dalis susikaupė prieš daugiau nei penkerius metus. Šis vertinimas parodo ilgalaikį PAE užsilaikymą Kuršių marių ekosistemoje. Atlikus rizikos vertinimą skirtingiems vandens organizmams paaiškėjo, kad PAE akumuliacija priekrantės ekosistemose gali kelti ekotoksiškumo pavojų.

Kuršių marių, Ščecino ir Vyslos lagūnų palyginimas atskleidė panašias ilgalaikes taršos tendencijas, patvirtinančias, kad Kuršių marios gali būti reprezentatyvus modelis PAE pasiskirstymui borealinėse estuarijų sistemose įvertinti. Be teršalų paplitimo ir ekotoksiškumo rizikos vertinimo, šiame tyrime panaudoti nauji metodologiniai sprendimai, kurie pagerino analitinį tyrimo tikslumą nustatant teršalus ir suteikė naujų įžvalgų apie PAE skaidymo procesus, taip prisidedant prie išsamesnio supratimo apie teršalų likimą vandens ekosistemose.

Reikšmingi žodžiai:

Ftalatų esteriai – Lagūna – Ekotoksiškumo rizika – Transkriptomo analizė – Suspenduota medžiaga

Table of Contents

LIST OF ORIGINAL PUBLICATIONS AND AUTHOR'S CONTRIBUTION	9
ABBREVIATIONS	11
1. INTRODUCTION	13
1.1. Phthalate esters: economical, societal and ecological importance	13
1.2. Sources of phthalate esters in the aquatic environment	15
1.3. The transport and partitioning of phthalate esters in the aquatic environment	16
1.4. The fate and transformation of phthalate esters in the aquatic environment	18
2. AIMS AND WORKING HYPOTHESES	21
2.1. Elements of novelty	22
2.2. The scientific and practical significance of the results	23
2.3. Scientific approval	24
3. MAIN METHODS	25
3.1. Study area	25
3.1.1. The Curonian Lagoon	27
3.1.2. Comparable lagoons (Vistula, Szczecin)	28
3.2. The extraction of phthalate esters	28
3.2.1. The dissolved phase	29
3.2.2. The particulate phase	30
3.2.3. The sediment phase	30
3.3. GC-MS analysis	31

4. RESULTS AND DISCUSSION	33
4.1. Challenges in the analytical detection of PAEs in environmental matrices	33
4.2. The role of the Curonian Lagoon in regulating the flux of PAEs	
into the Baltic Sea	36
4.2.1. Sources of PAEs in the lagoon: the investigation of compositional	
profiles	37
4.2.2. Seasonal variations in the transport and distribution of PAEs	39
4.3. The distribution of PAEs in the Curonian Lagoon	41
4.3.1. The role of suspended matter in the distribution of PAEs	42
4.3.2. Sediment as an efficient storage and a long-term representant	
of pollution	43
4.3.3. The fate of PAEs in the Curonian Lagoon	45
4.4. The ecological risk	50
4.5. The Curonian Lagoon as a model to study other Baltic lagoons	52
4.5.1. Dissimilarities	53
4.5.2. Similarities	54
5. RECOMMENDATIONS	57
6. CONCLUSIONS AND INSIGHTS	59
7. ACKNOWLEDGEMENTS	63
8. SUMMARY IN LITHUANIAN	65
9. REFERENCES	75
CURRICULUM VITAE	89
PUBLICATIONS	91

List of original publications and author's contribution

The material in this study has been presented in 4 original publications that were published in peer-reviewed scientific journals. They are referred to in the text by Roman numerals as follows:

- Lorre, E., Riboni, N., Bianchi, F., Orlandini, S., Furlanetto, S., Careri, M., Zilius, M., 2023. Quality by design in the optimization of the ultrasonic assisted solvent extraction for the GC-MS determination of plasticizers in sediments and shells. Talanta Open 100258. https://doi.org/10.1016/j.talo.2023.100258
- II. Lorre, E., Bianchi, F., Vybernaite-Lubiene, I., Mėžinė, J., Zilius, M., 2023. Phthalate esters delivery to the largest European lagoon: Sources, partitioning and seasonal variations. Environ. Res. 116667. https://doi.org/10.1016/j. envres.2023.116667
- III. Lorre, E., Bianchi, F., Mėžinė, J., Politi, T., Vybernaite-Lubiene, I., Zilius, M., 2024. The seasonal distribution of plasticizers in estuarine system: Controlling factors, storage and impact on the ecosystem. Environ. Pollut. 345, 123539. https://doi.org/10.1016/j.envpol.2024.123539
- IV. Lorre, E., Bianchi F., Broman E., Bonaglia S., Nascimento F.J.A, Samuiloviene A., Woźniczka A., Zilius M. 2024 Phthalate esters in baltic lagoons: Spatial distribution, ecological risks, and novel insights into their fate using transcriptomics. Sci. Total Environ. 957, 177526. https://doi.org/10.1016/j.scitotenv.2024.177526

For all original publications, Elise Lorre contributed to the study design, performed the sampling, data collection, pre-processing, post-processing, and data analysis, and wrote the draft manuscript.

Additional publications by the author of this thesis:

Jucyte-Cicine, A., Lorre, E., Petkuviene, J., Gasiunaite, Z.R., Durcova, E., Vybernaite-Lubiene, I., Zilius, M., 2025. Understanding the impact of summer festival on the dynamic of micropollutant delivery to coastal wastewater treatment plant. Emerg. Contam. 11, 100465. https://doi.org/10.1016/j.emcon.2024.100465

Jucyte-Cicine, A., **Lorre, E.**, Petkuviene, J., Gasiunaite, Z.R., Politi, T., Vybernaite-Lubiene, I., Zilius, M., 2024. Coastal wastewater treatment plants as a source of endocrine disrupting micropollutants: a case study of Lithuania in the Baltic Sea. Mar. Pollut. Bull. 200, 116084. https://doi.org/10.1016/j.marpolbul.2024.116084

Zilius, M., Barisevičiūtė, R., Bonaglia, S., Klawonn, I., Lorre, E., Politi, T., Vybernaite-Lubiene, I., Voss, M., Overlinge, D., Bukaveckas, P., 2024. The ef-

fects of variable riverine inputs and seasonal shifts in phytoplankton communities on nitrate cycling in a coastal lagoon. Front. Mar. Sci. 11, 1497246. https://doi. org/10.3389/fmars.2024.1497246

Cheung, H.L.S., Zilius, M., Politi, T., **Lorre, E**., Vybernaite-Lubiene, I., Santos, I.R., Bonaglia, S., 2025. Nitrate-Driven Eutrophication Supports High Nitrous Oxide Production and Emission in Coastal Lagoons. J. Geophys. Res. Biogeosciences 130, e2024JG008510. https://doi.org/10.1029/2024JG008510

Zilius, M., Cheung, H. L.S., Bukaveckas, P.A., Broman, E., Lorre, E., Politi, T., Vybernaite-Lubiene, I., Voss, M., Woźniczka, A., Nascimento, F.J.A., and Bonaglia, S., Eutrophication disrupts nitrogen cycling processes more than gene expression in lagoon waters and sediments. (Submitted).

Abbreviations

Abbreviation	Explanation				
ASE	Accelerated solvent extraction				
BBzP	Butyl benzyl phthalate				
CLP	Classification, labelling, and packaging				
DCM	Dichloromethane				
DEHA	Di(2-ethylhexyl) adipate				
DEHP	Di(2-ethylhexyl) phthalate				
DEP	Diethyl phthalate				
DF	Detection frequency				
DiBP	Diisobutyl phthalate				
DMP	Dimethyl phthalate				
DnBP	Dibutyl phthalate				
DOC	Dissolved organic carbon				
DOP	Di(n-octyl) phthalate				
GC-MS	Gas chromatograph-mass spectrometer				
HMW	High molecular weight				
Kow	Octanol-water partitioning coefficient				
LLE	Liquid-liquid extraction				
LMW	Low molecular weight				
MAE	Microwave-assisted extraction				
MDL	Method detection limit				
PA	Phthalic acid				
PAE	Phthalate esters/phthalic acid ester/phthalates				
PVC	Polyvinyl chloride				
QbD	Quality by design				
RQ	Risk quotient				
SPE	Solid-phase extraction				
SPM	Suspended particulate matter				
SPME	Solid-phase microextraction				
UAE	Ultrasonic-assisted extraction				
WWTP	Wastewater treatment plants				

1

Introduction

1.1. Phthalate esters: economical, societal and ecological importance

Phthalic acid esters (PAEs), commonly referred to as "phthalate esters" or "phthalates," are a group of organic compounds predominantly used as plasticizers. These additives play a key role in enhancing the flexibility of rigid polyvinyl chloride (PVC) materials, making them suitable for a wide range of applications. Plasticizers such as PAEs can constitute up to 30% of the total weight of PVC materials (Wypych, 2012). Beyond PVC, PAEs are used as viscosity control agents, solvents, adhesives, and components in personal care products (ECPI, 2018; Katsikantami et al., 2016; Wilkes et al., 2005).

Phthalates are the most widely used plasticizers globally, with an annual market demand of approximately 6 million tonnes. China and the Asia-Pacific region represent the largest area of PAE production, accounting for around 45% of the global market, while Europe contributes around 15% (Bu et al., 2020; ECPI, 2025). The widespread adoption of PAEs can be attributed to both their affordability and the versatile properties that they impart to plastic products, such as durability and temperature resistance. These qualities make PAEs essential for manufacturing products such as electrical wire sheathing and flooring (European Plasticisers, 2023; Wilkes et al., 2005).

Phthalates are a chemically diverse group of compounds that are not universally interchangeable in their applications. They are broadly categorized based on the car-

bon chain length of their side groups: low-molecular-weight (LMW) PAEs, with side chains of 1–6 carbons; and high-molecular-weight (HMW) PAEs, with side chains of 7–13 carbons (Katsikantami et al., 2016). Among HMW PAEs, Di(2-ethylhexyl) phthalate (DEHP) is the most commonly used plasticizer, enhancing the flexibility, elasticity, and durability of industrial products such as wires, cables, flooring, tar-paulins, wall coverings, synthetic leather, roofing membranes, and automotive materials (Katsikantami et al., 2016; Wilkes et al., 2005; Wypych, 2012). Conversely, LMW PAEs, such as dibutyl phthalate (DnBP) and diisobutyl phthalate (DiBP), are commonly utilized in medical devices, adhesives, paints, inks, and enteric coatings. The smallest LMW PAEs, including dimethyl phthalate (DMP) and diethyl phthalate (DEP), with hydrocarbon chains of only 1–2 carbons, are prevalent in domestic products, serving as solvents and fixatives in perfumes, cosmetics, and other household items (Katsikantami et al., 2016; Wilkes et al., 2005; Wypych, 2012).

The widespread use of PAEs has raised significant concerns regarding their potential impact on human health and the environment. The first official reports on the levels of PAEs in the environment appeared in the 1970s (Hites, 1973; Hites and Biemann, 1972). Since then, numerous studies have documented and monitored their presence across various environmental compartments, including the water column, sediments, and biota (Tran et al., 2022; Tuli et al., 2024), confirming their status as ubiquitous environmental contaminants. The evidence suggests that even at low concentrations, PAEs may disrupt biological processes in humans and wildlife, exhibiting teratogenic, mutagenic, and carcinogenic effects (Becker et al., 2004; Caldwell, 2012; Chang et al., 2021). Numerous studies have linked PAE exposure to endocrine system disruption, leading to reproductive toxicity and a variety of health disorders, including thyroid dysfunction, respiratory diseases, obesity, reproductive and kidney diseases, neurological conditions, cardiovascular ailments, and diabetes (Chang et al., 2021). In aquatic environments, PAEs pose significant ecological risks, affecting organisms at multiple levels. The exposure of embryos to PAEs can cause severe developmental abnormalities, including mortality, spinal deformities, necrosis, and cardiac edema, compromising survival and population stability (Y. Zhang et al., 2021). In adult organisms, PAEs have been linked to reproductive impairments, hormonal disruptions, and organ damage, with prolonged exposure leading to bioaccumulation and toxic effects up the food chain (Net et al., 2015b; Y. Zhang et al., 2021). Beyond direct toxicity, PAEs can disrupt ecosystem dynamics by altering community composition, potentially altering the nutrient cycle and destabilizing food webs (Wang et al., 2023; Zhu et al., 2019). Their impact on the growth of primary producers, such as algae (Gu et al., 2017; Wenchao et al., 2023), can also threaten nutrient cycling and ecosystem productivity. Given their potential impact on the ecosystem, further research is essential in order to understand their long-term ecological consequences and inform mitigation strategies.

Given their associated health and environmental risks, several PAEs, including DnBP, DiBP, DEHP, and benzyl butyl phthalate (BzBP), have been classified under the European Union's Classification, Labelling, and Packaging (CLP) regulation as reprotoxic substances, Category 1B (ECHA, 2025a). This classification indicates a presumed potential for reproductive toxicity in humans based on animal studies. Additionally, DnBP and BBP are categorized as Aquatic Acute 1, and BBP as Aquatic Chronic 1, reflecting their adverse impacts on aquatic ecosystems (ECHA, 2025a). The European Chemicals Agency (ECHA) further recognizes these four phthalates as endocrine disruptors for human health, with DEHP and DnBP also identified as endocrine disruptors in the environment (ECHA, 2025b).

As a result, the use of these PAEs is now heavily restricted under European regulations, requiring authorization for their application. These measures have led to a steady decline in the use of PAEs over time. For instance, PAEs constituted approximately 88% of the European plasticizer market in 2005, while by 2014, this share had declined to 70% (ECPI, 2018), with projections indicating a continued decrease in subsequent years. Despite this decline, PAEs continue to be widely used, posing risks to the well-being of living organisms. Numerous studies have confirmed the pervasive presence of PAE compounds in the environment (Gao and Wen, 2016; Puri et al., 2023; Y. Zhang et al., 2021).

1.2. Sources of phthalate esters in the aquatic environment

Coastal environments, particularly estuarine systems, receive substantial chemical inputs from agricultural, industrial, and domestic sources through both water and atmospheric pathways (Shen et al., 2019; Y. Zhang et al., 2021). In agricultural fields, surface runoff is a major contributor to PAE contamination in water bodies. The application of pesticides, biosolids, and fertilizers, which have been found to contain PAEs at concentrations ranging from 1.17 to 2795 $\mu g_{DW} kg^{-1}$ (Mo et al., 2008), leads to contamination as excess residues bind to soil particles and are later washed into rivers and estuaries (He et al., 2015). Additionally, plastic mulch and agricultural film, both widely used to improve crop yields, gradually degrade and release PAEs into the soil (Chen et al., 2013), where they can be transported by rainfall and irrigation runoff. Furthermore, plastic greenhouses and the volatilization of PAEs from contaminated sources contribute to atmospheric pollution (Wang et al., 2021), allowing these contaminants to be carried over long distances and subsequently deposited into water bodies (Woodrow et al., 2019).

Phthalates from both industrial and domestic sources enter the environment primarily through wastewater discharge. Industries such as plastic manufacturing, textiles, and chemical processing release PAEs into industrial wastewater, which, if inadequately treated, contaminates rivers, lakes, and coastal waters (Bergé et al., 2014;

Dargnat et al., 2009). Additionally, PAEs can evaporate from industrial materials and attach to airborne dust particles, which later settle onto land and water through atmospheric deposition (Gao et al., 2018; Wu et al., 2019). Similarly, domestic sources like personal care products, detergents, and plastic household items contribute significantly to wastewater pollution, as PAEs are washed down drains and often bypass complete removal in wastewater treatment plants (Bergé et al., 2014; Gao et al., 2014). Furthermore, indoor emissions from vinyl flooring, furniture, and synthetic textiles release PAEs into household air, where they bind to dust and can either be inhaled or transported outdoors through ventilation (Başaran et al., 2020; Lu and Zhu, 2021).

Wastewater treatment plants (WWTPs), which receive influents from domestic, agricultural, and industrial sources, are recognized as one of the most significant direct contributors to PAEs in aquatic systems (Gao et al., 2014). Conventional WWTPs, relying on standard treatment methods, have demonstrated highly variable PAE removal efficiency, ranging from as low as 14.2% to as high as 99.8% (Bai et al., 2022). However, recent advancements in wastewater treatment technology have improved the removal of organic pollutants including PAEs, offering promising solutions to mitigate their environmental impact (Wang et al., 2020).

While there are multiple potential contributors to environmental contamination, the complexity of environmental systems often poses challenges in identifying and quantifying individual sources of pollution. Semi-enclosed coastal estuarine systems, such as that of the Curonian Lagoon, offer unique opportunities to evaluate pollution dynamics through a "black-box" approach by estimating the mass balance of inputs and outputs. This lagoon receives 96% of its freshwater inflow from the Nemunas River, its major tributary (Jakimavičius and Kriaučiūnienė, 2013), along with several WWTPs which contribute localized inputs. It also features a single outflow, where it exchanges water with the Baltic Sea. These characteristics allow for the comprehensive monitoring of both inflow and outflow, facilitating the assessment of WWTP impacts and other pollutant sources. Consequently, the Curonian Lagoon provides an ideal case study for identifying and quantifying the sources of PAEs, enabling a deeper understanding of their behavior and distribution in semi-enclosed estuarine systems.

1.3. The transport and partitioning of phthalate esters in the aquatic environment

The ubiquitous use and continuous release of PAEs have rendered them pervasive environmental contaminants. Water serves as a major vector for both direct and indirect PAE exposure, given its central role in consumption, agriculture, and food production (Tuli et al., 2024). However, most studies have focused predominantly on the occurrence of PAEs in the water column, often neglecting their partitioning between dis-

solved and particulate phases (Bergé et al., 2013). Analytical workflows frequently lead to the removal of suspended particulate matter (SPM) during sample preparation, with only the dissolved phase being analyzed (He et al., 2013; Paluselli et al., 2018; Xu et al., 2022). Consequently, relatively few studies have provided a comprehensive assessment of PAE partitioning in aquatic environments (He et al., 2019; Zeng et al., 2009).

Understanding water-solid partitioning is crucial for elucidating the distribution and behavior of PAEs in the environment. Partitioning directly influences the bioavailability and accumulation patterns of these pollutants. It has been demonstrated that the sorption of chemicals onto sediments can reduce their bioavailability for uptake by organisms such as fish (Schrap and Opperhuizen, 1990). However, reduced bioavailability also limits biodegradation, allowing sorbed substances to persist and accumulate in the environment over extended periods. This persistence contributes to bioaccumulation and the potential for biomagnification, creating long-term environmental and health risks.

The sorption of PAEs onto particles (i.e., suspended matter or sediments) is largely governed by their hydrophobicity, typically expressed as the octanol-water partition coefficient (Log K_{ow} ; Gago et al., 1987). The Log K_{ow} for PAEs increases with the length of their alkyl chains (see Table 1), indicating that HMW PAEs are more likely to associate with particulate matter than LMW PAEs. Particle characteristics also play a critical role in the partitioning of PAEs. Hydrophobic compounds, such as PAEs, tend to be adsorbed preferentially onto the organic matter present in suspended particles or sediments (Staples et al., 1997b). Consequently, the degree of adsorption is typically correlated with the organic carbon content of these particles (Yang et al., 2013). Moreover, fine-grained particles have a larger surface area, providing more binding sites for adsorption and thus a greater capacity to retain organic matter, which can also enhance the sorption of PAEs (Lu et al., 2023).

Beyond hydrophobicity, the sorption of PAEs is also influenced by environmental factors, including temperature, pH, and salinity. Higher temperatures and pH levels generally reduce the sorption capacity of PAEs, increasing their solubility and promoting broader dispersion in the water column (Gao et al., 2016). In contrast, higher salinity enhances the sorption behavior of PAEs through the salting-out effect, leading to greater accumulation in sediments or adsorption onto particulate matter in saline environments (Yuwatini et al., 2013). This highlights the critical role of SPM in determining the distribution and fate of PAEs in aquatic environments. Investigating PAE partitioning is therefore essential for a more accurate understanding of PAE bioavailability, persistence, and potential ecological and health implications.

1.4. The fate and transformation of phthalate esters in the aquatic environment

Once introduced into aquatic environments, PAEs undergo various physical, chemical, and biological processes that govern their fate, transport, and transformation. Key mechanisms, such as adsorption, degradation, and partitioning, predetermine how PAEs are distributed across various compartments in water bodies (Staples et al., 1997b). The definitive removal of PAEs from the environment occurs through their degradation into smaller, linear compounds. This transformation can proceed via abiotic processes, such as hydrolysis and photolysis, or through microbial degradation, which has been recognized as the primary pathway for PAE breakdown in aquatic systems (Puri et al., 2023).

Abiotic degradation processes, while theoretically feasible, are generally too slow to significantly impact the persistence of PAEs in natural waters. For example, BBzP has an aqueous photolysis half-life exceeding 100 days, while DMP and DEHP have a half-life of approximately 3 and 2,000 years, respectively (Boll et al., 2020; Gledhill et al., 1980; Staples et al., 1997b). In contrast, microbial degradation can occur on a much faster timescale, ranging from days to years, depending on environmental conditions and the specific matrix involved (Boll et al., 2020).

Phthalate esters in the dissolved phase exhibit greater bioavailability to organisms and accessibility for microbial degradation than those adsorbed onto SPM or sediments (Staples et al., 1997b). Consequently, PAEs degrade, theoretically, more rapidly in the water column compared to sedimentary environments. Several environmental conditions, including oxygen availability, nutrient levels, temperature, and pH, influence PAE degradation rates. Aerobic conditions promote faster degradation than anaerobic conditions due to differences in microbial activity and metabolic pathways (Boll et al., 2020; Staples et al., 1997b). Optimal PAE degradation occurs at moderate to high temperatures, with peak efficiency observed at ~30 °C, a neutral pH (~7), and in nutrient-rich conditions that support microbial growth. In contrast, extreme pH values, low temperatures, and nutrient scarcity can significantly slow degradation, leading to prolonged PAE persistence in the environment (Chang et al., 2005; Kanaujiya et al., 2023; Li et al., 2007; Sharma et al., 2024).

The molecular structures of PAEs play a crucial role in their degradation kinetics. LMW PAEs degrade faster than HMW PAEs, for example. This difference is likely due to the steric effect of the side ester chains of PAEs, which prevent the binding of the hydrolytic enzymes necessary for their degradation (Liang et al., 2008). HMW PAEs are also more likely to be adsorbed onto SPM and sediments, reducing their bioavailability and degradation potential. Consequently, HMW PAEs are prone to long-term accumulation in the environment, while LMW PAEs are more readily bioremediated.

However, much of our current understanding of the degradation of PAEs comes from controlled laboratory studies, which may not accurately depict environmental conditions. Laboratory experiments frequently involve testing isolated chemicals as the sole carbon source for microbial growth, whereas natural environments contain a complex mixture of organic and inorganic compounds competing as substrates for microbial metabolism (Peterson and Staples, 2003). Moreover, laboratory experiments typically employ model microbial communities, often derived from sewage, that do not necessarily mimic the activity and composition of microbial communities in natural waters (Bouhajja et al., 2016; Zhu et al., 2020). While laboratory-controlled experiments remain an important tool for understanding pollutant degradation, their limitations underscore the need for complementary approaches to account for environmental complexity. Long-term field studies are therefore essential to validate laboratory findings, assess the real-world persistence of PAEs, and improve predictions of their environmental fate.

Integrating pollutant quantification with microbiome analyses in natural samples presents a promising approach. Advanced molecular tools, such as metagenomics and metatranscriptomics, offer powerful insights into microbial community composition and functional potential of PAE degradation in situ (Bouhajja et al., 2016). These methods allow for the identification of the genes and metabolic pathways associated with PAE biodegradation, providing a more holistic understanding of microbial processes in the ecosystem. Despite their potential, omics-based approaches are rarely employed to study the transformation of PAEs in natural environments (Wei et al., 2021; Zhu et al., 2020). Expanding the application of such tools could bridge knowledge gaps, enabling the more accurate assessment of the fate of PAEs and their transformation in complex aquatic systems.

2

Aims and working hypotheses

The general aim of this thesis is to elucidate the key factors controlling the dynamics and fate of PAEs within estuarine environments. To achieve this overreaching aim, the following specific objectives were delineated:

1. To develop a reliable analytical method for quantifying PAEs in sediments.

2. To quantify the PAE loads delivered to the Curonian Lagoon by different inputs (WWTPs, Nemunas River, Baltic Sea).

3. To assess the spatiotemporal variability in the distribution of PAEs within pelagic and benthic compartments of the Curonian Lagoon.

4. To evaluate the influence of suspended matter on the distribution behavior of PAEs in the water column.

5. To estimate the storage of PAEs in the Curonian Lagoon ecosystem.

6. To evaluate the distribution, potential risks, and fate of PAEs in selected coastal lagoons of the Baltic Sea, comparing them with the model Curonian Lagoon system.

In line with the specific objectives, the following hypotheses were formulated:

• Season-driven environmental fluctuations can significantly influence the release and distribution of PAEs within the Curonian Lagoon, potentially impacting their concentrations and transport mechanisms (objectives 2 and 3).

- Suspended matter plays a crucial yet often overlooked role in the distribution of PAEs. Despite its relevance, SPM has been insufficiently considered in previous investigations, and this study aims to address this gap (objectives 4 and 5).
- The Curonian Lagoon can serve as a valuable case study for elucidating the behavior of PAEs within estuarine ecosystems. This insight could facilitate the extrapolation of findings to similar coastal environments, particularly other lagoons in the Baltic Sea region (objective 6).

2.1. Elements of novelty

This doctoral study unveils novel insights into the mechanisms governing the distribution of pollutants in estuarine systems, with a focus on the previously underexplored Curonian, Vistula, and Szczecin lagoons, specifically concerning PAEs. It provides new data on pollution levels in these unique ecosystems, addressing a critical gap in the current understanding of phthalate contamination. Furthermore, the study employs mass balance estimations, also known as a "black-box" approach, to investigate anthropogenic pollutant sources within semi-enclosed lagoons. These systems possess the rare combination of geographical, morphological, and hydrodynamic characteristics necessary for such an analysis. This approach, which focuses on input and output estimations, has been successfully employed in the analysis of nutrient dynamics (Zilius et al., 2018); however, it is rarely utilized in studies of organic pollutant distribution due to its specific environmental constraints. This methodology significantly enhances our understanding of the transport, transformation, and fate of pollutants in estuarine environments, offering valuable insights into the complex interactions between natural processes and anthropogenic influences on ecosystems.

A key novel aspect of this study also lies in the comprehensive analysis of water column samples, systematically segregating them into dissolved and particulate phases. This approach facilitates the in-depth examination of the relative significance of each phase in the behavior and distribution of PAEs. The findings demonstrate that SPM, frequently overlooked in conventional monitoring studies due to practical constraints, plays a critical role in the partitioning of PAEs between dissolved and particulate forms. The partitioning observed between phases has significant implications for the fate and transport of these pollutants within estuarine environments.

Furthermore, the present study pioneers the application of advanced analytical tools. This includes the Quality by Design approach, which is used to optimize environmental analytical method, and shotgun transcriptomic analysis, which is performed on field samples from the Baltic lagoons. Transcriptomics analysis yields unprecedented insights into the microbial communities capable of degrading PAEs, thereby shedding light on the natural attenuation processes that mitigate pollutant impacts.

2.2. The scientific and practical significance of the results

The findings of this doctoral study hold substantial scientific and applied significance. From a scientific standpoint, the study significantly advances the current understanding of the distribution and fate of PAEs in estuarine systems, shedding light on the critical roles played by SPM and sediment in the transport, storage, and overall fate of PAEs within aquatic environments. This research not only underscores the importance of these matrices in influencing the behavior of PAEs, but also provides novel insights into their interactions and impact on pollutant dynamics in estuarine systems. Notably, it provides novel insights into the interaction between PAEs and microbial communities in sediment and the potential environmental risk posed by such pollutants.

While the focus of this research is specifically on PAEs, the innovative methodological approaches employed transcend this specific scope. The utilization of the "black-box" and Quality by Design approaches, transcriptomics analysis and analytical partitioning of PAE phases offer valuable tools and frameworks applicable beyond the scope of this research. These cutting-edge methodologies have the potential to be applied to a broader range of organic pollutants, thereby opening new avenues for future research. By refining these techniques, this research contributes to a better understanding of the role of estuarine systems in regulating the load of pollutants to adjacent coastal sites, and aims to establish a scientific basis for formulating recommendations for future environmental monitoring programs.

The findings of this study also hold important implications for environmental monitoring programs from an applied perspective. The results highlight the crucial importance of integrating SPM into monitoring frameworks to achieve a more comprehensive understanding of pollutant dynamics. The study demonstrates that SPM plays a pivotal role in the distribution, transport, and fate of PAEs, which can significantly affect the accuracy of environmental impact assessments if overlooked. By highlighting the substantial influence of SPM on pollutant behavior, this research advocates for the integration of SPM analysis into routine environmental monitoring programs. This integration would enhance the capability of monitoring programs to detect and evaluate the presence and movement of contaminants, thereby improving the reliability of the data used to assess the impacts of human activities, natural events, and policy interventions on aquatic environments. Furthermore, incorporating SPM into monitoring practices can aid in developing more effective strategies for pollution management and mitigation, ultimately leading to the better protection of water quality and ecosystem health. The insights gained from this study provide a foundation for revising existing monitoring practices and developing targeted recommendations for future environmental assessment efforts, ensuring that they are both scientifically robust and practically relevant.

2.3. Scientific approval

The results of this study have been presented at 3 international conferences:

ICES PICES Fourth Early Career Scientist Conference, St. John's, Newfoundland, Canada, July 2022 (Poster presentation) – "Distribution and behaviour of Phthalic Acid Ester in the water column of the largest European lagoon."

ASLO 2023; Association for Science of Limnology and Oceanography; Aquatic Sciences Meeting, Palma, Spain, June 2023. (Poster presentation) – "Impact of Suspended Matter on the transport and the degradation Kinetics of Phthalates Esters in the water column."

BSSC 2023; Baltic Sea Science Congress, Helsinki, Finland, August 2023. (Poster presentation) – "Comparative assessment of Phthalates Esters distribution in the coastal lagoons of the Baltic Sea: a compositional profile, spatial patterns and environmental control."

3

Main methods

3.1. Study area

Coastal environments, particularly estuarine systems, face increasing threats from anthropogenic pollution due to the large volumes of chemicals that they receive from domestic, agricultural, and industrial effluents (Shen et al., 2019; Y. Zhang et al., 2021). Estuaries, being situated at the interface between land and sea, function as "coastal filters" for pollutants. Within these systems, pollutants may be retained, transformed, or accumulated, thereby reducing the pollutant load on their pathway to the sea. However, the accumulation of pollutants has an adverse impact on the ecological health and water quality of estuarine and adjacent marine environments (Barletta et al., 2019; Roscher et al., 2021). Lagoons, representing a distinct sub-class of estuaries, are typically shallow water bodies separated from the open sea by natural barriers such as sandbanks, shingles, or rock formations (Johnson et al., 2007). Due to their unique morphology and hydrodynamics, lagoons are particularly relevant study areas for examining the behaviors and fate of pollutants within coastal ecosystems. Among other characteristics, boreal lagoons, in particular, are distinguished by periods of ice cover and ice-free conditions, dramatic temperature variations, and fluctuations in riverine inputs, which seasonally regulate nutrient and pollutant concentrations, as well as microorganism activity and composition (Idzelytė et al., 2020; Zilius et al., 2021, 2018).



Figure 1. The geographical locations of sampling stations in the lagoon areas (A – Curonian Lagoon; B – Vistula Lagoon; C – Szczecin Lagoon).

The present study primarily focused on the Curonian Lagoon (Fig. 1.A), located in the southeastern region of the Baltic Sea. The study aimed to comprehensively examine the sources and spatio-temporal distribution of PAEs within this lagoon system, as detailed in Papers II and III. The Curonian Lagoon represented a model system for this investigation. Subsequently, a comparative analysis was conducted with two other similar Baltic lagoons, the Vistula and Szczecin lagoons (Fig. 1.B and C), to

contextualize the findings and assess regional variations in PAE sources and distribution (Paper IV). The Szczecin and Vistula lagoons were selected for comparison due to their similar geomorphological features, comparable range of salinity despite varying riverine inputs (Szczecin > Curonian > Vistula), and different levels of productivity (Curonian > Szczecin > Vistula), resulting in varying compositions, levels of suspended matter, and activity of microorganisms. This allowed for the evaluation of whether comparable but distinct conditions could exert similar (or distinctive) control over PAE distribution, fate, and transformation in these ecosystems. The physicochemical conditions in these systems have been extensively described in previous studies (Bonaglia et al., 2025; Cheung et al., 2025).

3.1.1. The Curonian Lagoon

The Curonian Lagoon, with an area of \sim 1,600 km², is the largest coastal lagoon in Europe. This shallow (mean depth of 3.8 meters), microtidal estuarine system is situated along the southeastern coast of the Baltic Sea, spanning both Lithuania and the Russian Federation. The lagoon discharges into the sea through the narrow Klaipeda Strait and experiences occasional salinity increases in its northern section due to intermittent wind-driven events. They typically range from 1 to 2 PSU, with a maximum of 7 PSU (Zemlys et al., 2013).

The Curonian Lagoon receives direct discharges from several WWTPs situated along its perimeter, with the largest of these being the Klaipeda City WWTP, which serves approximately 150,000 inhabitants of the Klaipeda municipality and other small towns in the area. The WWTP has a design capacity of nearly 500,000 population equivalents (PE), with an actual loading of ~320,000 PE and a discharge of ~0.016 km³ per year (AB Klaipedos Vanduo pers. com.). Smaller WWTPs located on the Curonian Spit contribute additional inputs to the lagoon, particularly during the tourism season between June and September, when the resident population substantially increases. However, the predominant source of freshwater is the Nemunas River, with an annual average discharge of 16.4 km³, accounting for 96% of the total freshwater inflow (HELCOM, 2015; Jakimavičius and Kriaučiūnienė, 2013; Vybernaite-Lubiene et al., 2018). The river basin, covering an area of 97,864 km², drains urban, agricultural, and industrial regions across Lithuania, Belarus, and the Russian Federation, serving a population of over 5 million inhabitants.

The inflow from the Nemunas River results in distinct hydrodynamics and sedimentary conditions within the lagoon, dividing it into two main areas: the northern transitional area and the south-central confined area. The northern half is characterized by shallower depths (1.5–2 meters), shorter water renewal times (seasonal range of 50–100 days), and sandy sediments (Umgiesser et al., 2016; Zilius et al., 2014). In contrast, the central-southern half of the lagoon is deeper (mean depth of 3.5 me-

ters), characterized by longer water renewal times (seasonal range of 100–250 days), and dominated by organic-rich, silty sediments. Overall, the lagoon is vertically wellmixed due to its shallow depth and weak salinity gradients (Zilius et al., 2024, 2014).

The sampling strategy for each objective was meticulously designed to account for the diverse sources of input to the lagoon, including WWTPs, riverine inflows, and exchanges with the sea, as well as its distinct sedimentary environments – specifically the sandy transitional (hereinafter "sandy") and muddy confined (hereinafter "muddy") areas. This strategic approach ensured comprehensive coverage of the lagoon's varying hydrodynamic and sedimentary conditions, allowing for the more accurate assessment of spatial distribution and potential sources of pollutants.

3.1.2. Comparable lagoons (Vistula, Szczecin)

To evaluate the use of the Curonian Lagoon as a model system, a research framework was established in two similar systems: the Vistula and Szczecin lagoons, both located in the southern Baltic Sea. The Szczecin Lagoon, covering 687 km², is located between Poland and Germany and has an average depth of 3.8 meters. This lagoon is primarily influenced by the discharge of the River Odra (Oder), which contributes 94% of freshwater inputs. The Odra River, with a length of 854 kilometers and a basin area of 120,000 km², plays a crucial role in the hydrodynamics of the lagoon (Radziejewska and Schernewski, 2008). The Szczecin Lagoon is also significantly impacted by maritime transport, with a dredged channel 10.5 meters in depth crossing the lagoon, linking the mouth of the Odra River to the Szczecin harbor and the Baltic Sea.

The Vistula Lagoon, covering 838 km², is located between Poland and the Russian Federation. Unlike the Curonian and Szczecin lagoons, the Vistula Lagoon has a considerably smaller catchment area and tributaries, resulting in higher salinity levels and longer water residence times (Chubarenko and Margoński, 2008).

Similarly to the Curonian Lagoon, the Vistula and Szczecin lagoons are characterized by two dominant sedimentary environments: shallow sandy areas and deeper muddy areas. The shallow sandy areas, with a mean depth of 1.5 meters, are characterized by limited organic matter accumulation in sediments and high shear stress, resulting in frequent sediment resuspension. The deeper muddy areas, with a mean depth of 4 meters, are rich in organic deposits, indicating substantial organic input and prolonged water circulation.

3.2. The extraction of phthalate esters

The global industrial sector encompasses a substantial number of PAEs. However, the scope of this study focused on seven primary congeners: dimethyl phthal-

ate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), dibutyl phthalate (DnBP), butyl benzyl phthalate (BBzP), di(2-ethylhexyl) phthalate (DEHP), and di(notyl) phthalate (DOP). These specific PAEs were selected due to their widespread use, broadly recognized potential health risks, environmental persistence, and regulatory significance. Four of these phthalates, namely DiBP, DnBP, BBzP, and DEHP, are already included in several European Union watchlists, underscoring their importance as priority substances for environmental and public health monitoring (ECHA, 2025a). In contrast, although DMP, DEP, and DOP are not formally listed in major surveillance programs, they remain critical in environmental studies. These phthalates are frequently found in various environmental matrices, and their widespread presence in some ecosystems suggests that they may pose significant ecological risks (Miao et al., 2025).

In this study, PAEs were extracted from three distinct environmental matrices: the dissolved, particulate, and sediment phases. Due to the potential presence of PAEs within laboratories, extraction methods had to follow strict and clean protocols ensuring rigorous quality control measures. To ensure the reliability and accuracy of the results, all extraction techniques described below were validated in accordance with EURACHEM guidelines (Magnusson and Örnemark, 2018).

3.2.1. The dissolved phase

Phthalate esters can be extracted from the aqueous environment using various methods, such as solid-phase extraction (SPE), liquid-liquid extraction (LLE), and solid-phase microextraction (SPME) (Net et al., 2015a). Among these, SPE has emerged as the most widely utilized technique for the extraction of PAE from water samples due to its superior accuracy, reproducibility, and capacity to concentrate analytes from large water volumes (Tuli et al., 2024).

However, if the water samples contain excessive suspended particulate matter, the SPE system may become clogged, potentially compromising the efficiency of the SPE procedure. In this study, to mitigate the risk of compromised analysis and to gain more detailed insights into the distribution of PAEs, all water samples were filtered through pre-combusted (at 500 C for 6 hours) GF/F filters (0.7 μ m nominal pore size) to separate the dissolved and particulate phases. Subsequently, the SPE method was applied to extract the dissolved fraction of PAEs, following the EN ISO 18856:2005 international standard (European Committee for standardization, 2005).

The SPE is based on selective adsorption, where the target analytes – in this case, PAEs – are separated from the liquid matrix by passing the sample through a solid sorbent material. This study employed C18ec cartridges (Chromabond®, 6 mL/500 mg) as the sorbent. Subsequently, the retained analytes were eluted using a small volume

of solvent – specifically, 2 mL of ethyl acetate – to concentrate the analytes for later analytical detection. This procedure is detailed in Paper II.

3.2.2. The particulate phase

The analysis of PAEs bound to particulate matter requires the separation of SPM from the aqueous phase. Pre-combusted (at 500 °C for 6 hours) GF/F filters with a nominal pore size of 0.7 µm are commonly used for this purpose (Net et al., 2015a; Zeng et al., 2008). Filter pre-combustion is essential to minimize contamination during PAE analysis. After filtration, the filter containing the precipitated particulate matter must be treated as a solid sample. While various methods exist for extracting PAEs from solid samples, ultrasonic extraction is the most commonly used technique for SPM (Net et al., 2015a). In this study, an ultrasonic homogenizer (Bandelin Sonoplus HD 4200, equipped with a TS103 probe, BANDELIN electronic GmbH & Co. KG, Germany) was utilized for extraction. The filters were spiked with an internal standard and subjected to two rounds of ultrasonic extraction to ensure high recovery efficiency, using dichloromethane (DCM).

For particle-bound contaminants, concentrations are typically normalized by mass (e.g., $\mu g/g$) to clearly indicate the amount of contaminant associated with the particulate matter. In this study, the mass concentration was multiplied by the concentration of SPM in the water column to obtain the volumetric concentration of particulate-bound PAEs, expressed in $\mu g/L$. This volumetric concentration is crucial for calculating the total concentration of PAEs in the water column (as the sum of dissolved and particulate-phase concentrations) and for comparing the distribution of PAEs between the dissolved and particulate phases.

3.2.3. The sediment phase

The extraction of PAEs from sediment matrices has been carried out using a wide range of methods, including Soxhlet extraction, microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), and ultrasonic-assisted extraction (UAE). However, in contrast to liquid samples, no single method consistently outperforms the others when it comes to sediment extraction, as each technique presents its unique advantages and limitations. Techniques such as MAE and ASE require specialized equipment which may not be readily available in all laboratories, and pose the risk of cross-contamination between samples due to the cleaning procedures required between uses. Soxhlet extraction is effective, but it is time-intensive and requires large volumes of solvent (Net et al., 2015a).

In contrast, UAE offers a compelling advantage due to its simplicity and accessibility. It can be readily implemented in most analytical laboratories with basic equipment, such as a shaking table and an ultrasonic bath. Additionally, UAE allows for the si-

multaneous extraction of multiple samples, significantly reducing overall analysis time. However, optimizing parameters such as solvent type and volume, shaking time, and ultrasonic treatment duration can be challenging, as each factor can directly influence the extraction efficiency of organic pollutants (Ndwabu et al., 2023). Consequently, rigorous method optimization and validation are imperative to ensure optimal performance.

In this study, UAE was selected for the extraction of PAEs from sediment. The optimization and validation of the procedure are detailed in Paper I. The optimized parameters included shaking time, ultrasonic time, solvent type, and volume. The final extraction procedure involved transferring 2–4 g of dry sediment into a borosilicate glass bottle followed by the addition of the solvent (optimized condition: 17.5 mL of mixture of DCM and acetone in a 1:1 ratio). The bottles were closed, shaken (optimized condition: 15.0 min duration) on a horizontal shaking table and sonicated (optimized condition: 12.0 min duration) at room temperature in an ultrasonic bath. The supernatant was then separated from the sediment, evaporated under a gentle stream of nitrogen gas, and reconstituted in ethyl acetate for further analysis.

3.3. GC-MS analysis

Phthalates are commonly quantified using chromatographic techniques, with gas chromatography coupled to mass spectrometry (GC-MS) being the most widely used due to its capability for the simultaneous identification and quantification of target PAEs at low limits of detection (LOD), typically in the μ g/L or ng/g range.

The development of a GC-MS method requires the careful optimization of several parameters to ensure high sensitivity, selectivity, and resolution. Key GC parameters include column type and dimensions, carrier gas type and flow rate, oven temperature programming, and injection conditions. These factors influence analyte separation efficiency and analysis time. For MS, critical parameters such as ionization source, mass analyzer settings, detector voltage, and data acquisition mode directly impact detection sensitivity and specificity (Agilent, 2020).

In this study, a Shimadzu GC-2010 Plus gas chromatograph coupled with a GC-MS-TQ8040 mass spectrometer (Shimadzu Corporation, Japan) was employed. Helium (99.9999%) served as the carrier gas at a constant flow rate of 1 mL/min, with the GC injector operating in splitless mode at 250 °C. Chromatographic separation was achieved using an Rxi-5Sil MS capillary column (30 m × 0.25 mm i.d., 0.25 μ m film thickness; Restek®, USA) with the following temperature program: 60 °C for 2 minutes, 25 °C/min to 240 °C (held for 2 minutes), and 10 °C/min to 300 °C (held for 3 minutes). The transfer line and ion source temperatures were maintained at 280 °C and 230 °C, respectively. The mass spectrometer was operated in time-scheduled single-ion monitoring (SIM) mode, with the targeted and qualifier ions listed in Table 1.

Name	Acronym	Formula	CAS	Molecular weight (g mol ⁻¹)	Boiling point (°C)	Log K _{ow} ^a	Target ion (m/z)	Qualifier ions (m/z)
Dimethyl phthalate	DMP	$C_{10}H_{10}O_4$	131- 11-3	194.2	282	1.61	163	194–133
Diethyl phthalate	DEP	C ₁₂ H ₁₄ O ₄	84- 66-2	222.2	298	2.38	149	177–222
Diisobutyl phthalate	DiBP	C ₁₆ H ₂₂ O ₄	84- 69-5	278.3	327	4.11	149	223
Dibutyl phthalate	DnBP	C ₁₆ H ₂₂ O ₄	84- 74-2	278.3	340	4.45	149	223–278
Butyl Benzyl phthalate	BBzP	C ₁₉ H ₂₀ O ₄	85- 68-7	312.4	370	4.59	149	206–312
Di(2– ethylhexyl) phthalate	DEHP	C ₂₄ H ₃₈ O ₄	117- 81-7	390.6	386	7.5	149	167–279
Di(n-octyl) phthalate	DOP	C ₂₄ H ₃₈ O ₄	117- 84-0	390.6	380	8.06	149	279–261

Table 1. Physicochemical properties of targeted PAEs.

 K_{ow} : octanol–water partition coefficient; CAS: Chemical Abstract system. ^a Data from Staples et al. (1997).

4

Results and discussion

4.1. Challenges in the analytical detection of PAEs in environmental matrices

The present study aimed to provide novel insights into the distribution of PAEs in aquatic environments. Therefore, employing reliable analytical methods that adhere to validated protocols was crucial. Given the ubiquitous presence of PAEs in laboratory settings (e.g., in the air, materials, and chemicals), the risk of sample contamination was high, posing a challenge to the accurate quantification of PAEs in environmental samples (Net et al., 2015a). This was further exacerbated by the low concentrations of PAEs typically found in the natural environment and the complexity introduced by the diverse extraction methods available, each requiring time- and resource-intensive parameter optimization.

The goal of Paper I was to develop an optimized extraction method for PAEs in sediment matrices that strikes a balance between high analytical performance and operational efficiency. Specifically, the research sought to reduce the number of tests needed for optimization while concurrently upholding the robustness and reliability of the proposed methodology.

The study employed ultrasonic-assisted extraction (UAE) as the extraction method due to its simplicity and minimal equipment requirements. Notably, the Quality by Design (QbD) approach was applied for the first time to optimize UAE on environmental samples. QbD is a structured and systematic approach to method development that emphasizes understanding the relationships between critical process parameters and desired outcomes (Orlandini et al., 2013). By applying QbD, the research identified the optimal conditions for four key parameters in the extraction process: solvent type, solvent volume, shaking time, and ultrasonic extraction time. This approach minimized the number of tests required for optimization and substantially enhanced the robustness of the method, thereby improving extraction efficiency and reliability. When compared to alternative methodologies, the newly optimized approach employing standard instrumentation demonstrated a notable reduction in solvent consumption while simultaneously improving detection limits. The proposed method employed 17.5 mL of extraction solvent, representing a reduction in solvent volume compared to previously reported UAE-based methods for PAE extraction (Table 2). Additionally, the method demonstrated lower limits of detection (LODs) relative to other UAE-based approaches, achieving detection limits comparable to those obtained through miniaturized sample extraction techniques.

The implementation of QbD principles represents a significant advance in the field of environmental analysis. Using QbD allowed for the streamlining of the method development process, reducing the time and resources typically required for optimization. Furthermore, the use of QbD enhanced confidence in the final method, ensuring that it is both efficient and scientifically sound. This novel application sets a precedent for future studies in environmental analysis, highlighting the value of QbD as a tool for method optimization in complex analytical processes.

After optimizing the key parameters to ensure the best performance of the method, it was essential to conduct method validation to confirm both its accuracy and operational efficiency. Validation, as outlined in the EURACHEM guidelines (Magnusson and Örnemark, 2018), involves assessing several parameters, including selectivity, sensitivity, working range, limits of detection (LOD), limits of quantification (LOQ), accuracy in terms of trueness, and precision. Precision encompasses repeatability, intermediate precision, and reproducibility, while additional parameters such as ruggedness and uncertainty can also be considered. All of these parameters ensure that key challenges, such as the presence of interferences, are effectively controlled and monitored. Interference in PAE analysis can arise at various stages: during sampling if inappropriate materials (e.g., plastic) are used, during enrichment if the extraction materials or laboratory air are contaminated, or during GC-MS analysis if PAEs are present as contaminants in the instrument (European Committee for standardization, 2005).

Given that the method was newly developed and fully optimized for sediment matrices, comprehensive validation was performed to ensure its robustness (Paper I). Full validation may not be necessary for applications involving established methods, such as water sample analysis. In such scenarios, method verification can be conducted, allowing for the exclusion of certain parameters from the validation protocol. Consequently, in this study, the methods applied for PAE extraction in environmental matrices were either fully validated (for sediment) or verified (for water). All methods

34

exhibited recovery ranging from 73% to 111% across different matrices for all targeted analytes. The limits of detection were below 0.06 μ g L⁻¹ in water samples and below 0.7 ng g⁻¹ in sediment samples (Table 3). Furthermore, blank levels were carefully controlled, and no cross-contamination was observed throughout the analyses. These findings demonstrate that the analytical procedures utilized for PAE analysis adhered to strict high-quality protocols and that the laboratory and equipment were properly maintained, ensuring the accuracy of the reported results.

Reference	Extraction method	Compounds	Solvent	Solvent volume (ml)	LOD (ng g ⁻¹)
This study	UAE	7 PAEs + DEHA	DCM/AC (1:1)	17.5	0.1–0.7
Reid et al., 2009	ASE	4 PAEs	DCM/AC (1:1)	NS	15
Xu et al., 2008	UAE	DnBP, DEHP	DCM/AC (1:1)	NS	10
Huang et al., 2008	ASE	6 PAEs	Ethyl acetate	6	6–11
Ramirez et al., 2019	MAE	6 PAEs + DEHA	MeOH	15	15
Fernández- González et al., 2017	UAE + HS- SPME	6 PAEs	МеОН	5	1–79
Liu et al., 2014	Soxhlet	16 PAEs	DCM	300	1.12-8.59
Hassanzadeh et al., 2014	Soxhlet	DnBP, DEHP	DCM	300	1 (DnBP); 8 (DEHP)
Zhang et al., 2017	UAE	8 PAEs	DCM	50	0.12-1.60
Souaf et al., 2023	UAE	4 PAEs + DEHT	hexane/AC (1;1)	30	10-80
Cao et al., 2022	UAE	15 PAEs	AC/hexane (10:1)	20	0.27-5.85
Jiménez-Skrzypek et al., 2020	UAE + dSPE	10 PAEs + DEHA	Acetonitrile+DCM	22	LOQ: 0.020–4.0

Table 2. Comparison of LOD values achieved in this work (sediment samples) with previous studies on the determination of PAEs (from Paper I).

AC: Acetone; ASE: accelerated solvent extraction; DCM: Dichloromethane; DEHA: Di(2ethylhexyl) adipate; DEHT: Dioctyl Terephthalate; dSPE: dispersive solid phase extraction; HS-SPME: headspace solid-phase extraction; MAE. Microwave assisted extraction; MeOH: Methanol; NS: Not specified; UAE: Ultrasonic assisted extraction

PAE	Dissolved		Particulate		Sediment	
congeners	MDL	Recovery	MDL	Recovery	MDL	Recovery
	(µg L ⁻¹)	(%)	(µg L ⁻¹)	(%)	$(ng g^{-1})$	(%)
DMP	0.06	105	0.02	70	0.2	73
DEP	0.06	97	0.02	74	0.1	80
DiBP	0.05	99	0.01	91	0.3	92
DnBP	0.03	109	0.01	96	0.2	83
BBzP	0.03	106	0.04	82	0.7	90
DEHP	0.04	111	0.01	103	0.2	100
DOP	0.04	109	0.03	96	0.2	87
D4-DBP	-	92	-	90	-	89
D4-DEHP	-	96	-	87	-	84
D4-DOP	-	93	-	85	-	78

Table 3. Control quality data for each PAE and each of the matrices targeted in the study (from supplementary data of paper IV).

Method Detection limit (MDL) = 3*Ss (Ss – sample standard deviation of n replicates spiked sample analyses; n = 10). Recovery rate (%) based on n replicates spiked sample analyses at 1 µg L⁻¹ for the water phase (dissolved and particulate; n = 10) and 1 ng g⁻¹ for the sediment phase (n = 10).

4.2. The role of the Curonian Lagoon in regulating the flux of PAEs into the Baltic Sea

The Curonian Lagoon exhibits unique hydrological and morphological characteristics, rendering it an ideal system for investigating the retention and release of pollutants during their transit from land to sea. This study employed a "black-box" approach to quantify the mass balance of PAE inputs from various sources, with a primary focus on the Nemunas River, contributions from WWTPs along the lagoon, and outflow to the Baltic Sea (see Paper II). The difference between these inputs and outputs was used to evaluate whether the lagoon functions as a sink for PAEs or as an intermediary system that accumulates PAEs from various sources before later exporting them to the Baltic Sea.

The findings reveal that from June 2021 to May 2022, the Nemunas River was the primary source of PAEs to the lagoon, delivering approximately 2.74 tons. In contrast, WWTPs discharged negligible quantities (<0.02 tons) during this period. Concurrently, 5.19 tonnes of PAEs were exported from the lagoon to the Baltic Sea (see Paper II). These findings suggest that during the study period the lagoon exported more PAEs to the Baltic Sea than it received from the sources examined, implying the existence of other contributing factors beyond those considered in this study. However, the lagoon to the study is the study of the study.
goon's role in regulating PAE flux is dynamic and subject to temporal and seasonal variability. For example, during winter, the opposite pattern emerged, with inflows surpassing outflows by 159%, suggesting the substantial retention of PAEs within the Curonian Lagoon (Fig. 2). This implies that the lagoon can temporarily shift to a PAE sink. These findings raise a critical question: If PAEs within the lagoon are not predominantly sourced from the river, what other sources may contribute to their presence?



Figure 2. Monthly PAE loads from June 2021 to May 2022 at the inflow (Nemunas River) and outflow (Klaipeda Strait) of the Curonian Lagoon, and the proportion of dissolved or particulate-bound PAEs (from Paper II).

4.2.1. Sources of PAEs in the lagoon: the investigation of compositional profiles

The PAEs in aquatic environments can originate from various sources, including industrial, domestic, and agricultural activities. Although PAEs are primarily used as plasticizers, each congener has a distinct function and application. Analyzing the profiles of specific PAEs within the lagoon system can provide valuable insights into their sources.

4. Results and discussion

Figure 3 illustrates the compositional profiles of PAEs obtained during winter and summer at the different stations, including potential source points within the lagoon and at its outflow. During the winter period (December–February), the compositional profiles of the lagoon and outflow stations were strikingly similar to those of the river, with DEHP being the dominant congener. DEHP is commonly used as a plasticizer in PVC, making it the most widely used PAE globally, with a high potential for leaching into the environment during PVC manufacturing, use, and/or disposal (Wilkes et al., 2005). Its extensive application across various industries suggests a significant impact of industrial activities on the Nemunas River. The similar compositional profiles observed in the water column along the river, lagoon, and outflow gradient reinforce the point that the river serves as the primary source of PAEs to the lagoon during winter.

In contrast, during the summer (June–August), PAE sources appear to be more diversified. Due to lower precipitation in summer, the flow of the river decreased substantially, reducing PAE inputs to less than 0.1 tonnes in July and August, compared to over 1.6 tonnes in January 2021 (Fig. 2). Despite the diminished flow, compositional similarities between the river and lagoon stations, particularly at the transitional "sandy" station, suggest that the river continues to have a notable influence on PAE distribution within the lagoon even during periods of low flow. During the summer, both the river and lagoon appear to be more strongly impacted by domestic sources, as evidenced by the increased presence of lower-molecular-weight PAEs (Fig. 3), which are typically found in products such as insect repellents and personal care items (Al-Saleh and Elkhatib, 2016; Karunamoorthi and Sabesan, 2010). The heightened recreational use of the river and lagoon during the warmer months likely contributes to the release of these domestic pollutants.

While smaller PAEs were present in higher proportions at the river and lagoon stations, DEHP remained the dominant congener at the lagoon's outflow during the summer (Fig. 3). It is noteworthy that this study did not measure the contribution of the atmospheric deposition of PAEs to the lagoon. Zeng et al. (2010) suggest that atmospheric deposition could significantly influence PAE concentrations in the waters adjacent to industrial zones, which may be particularly relevant to the lagoon outflow station located near the industrial area of Klaipėda. As semi-volatile organic pollutants, PAEs can be emitted into the atmosphere from various anthropogenic sources, including industrial activities, plastic production, waste incineration, the use of household products, and agricultural practices (Gao et al., 2018; Lu et al., 2020; Wu et al., 2019; Zhang et al., 2020). Once released, PAEs exist either in the gas phase, primarily LMW PAEs, or as particle-bound pollutants, predominantly HMW PAEs (Darbre, 2018). Their transfer to aquatic environments occurs through multiple atmospheric processes, including wet deposition (via precipitation, such as rain and snow), dry deposition (the settling of aerosol-bound PAEs), and gas-water exchange (direct absorption from the atmosphere into surface waters) (Mi et al., 2023). These mechanisms

4. Results and discussion



Figure. 3. Compositional profiles of PAEs obtained during the winter and summer seasons at different stations, including potential source points (WN: WWTP Nida; WK: WWTP Klaipeda; Inflow: Nemunas River) and measurements from within the lagoon (sandy and muddy sites) and at its outflow (data from Papers II and III). *Data collected in July 2021.

have already been demonstrated as pathways for PAE transport into aquatic systems (Mi et al., 2023; Xie et al., 2007, 2005). Consequently, the continuous atmospheric deposition of PAEs throughout the year may represent a significant source of contamination for both the lagoon and the Baltic Sea.

4.2.2. Seasonal variations in the transport and distribution of PAEs

Seasonality can significantly influence the transport of pollutants within a lagoon system through various environmental fluctuations. Temporal changes in temperature, precipitation, and wind patterns may influence pollutant distribution. Water temperature, for example, can indirectly impact the distribution of PAEs by affecting microbial activity, water density and phase transitions. During the warmer summer months, the microbial community is expected to be more active than in colder periods, facilitating the more efficient degradation of PAEs in the environment (Kumawat et al., 2022; Xu et al., 2022). Additionally, temperature can induce stratification in the water column, preventing the vertical mixing and deposition of pollutants (Elçi, 2008). In temperate and boreal regions, lagoons frequently become ice-covered during low-temperature periods. The ice sheet can act as a temporary reservoir, trapping PAEs from the water and atmosphere (Stocker et al., 2007).

Wind and precipitation patterns exert a direct impact on the hydrodynamics of the lagoon, influencing both the dispersion of pollutants and water residence time, which can shape the accumulation patterns of PAEs. Increased precipitation, peaking during the autumn and winter seasons in Lithuania, increases surface runoff, which can transport PAEs from the catchment area into the lagoon. Furthermore, intense precipitation events can also lead to overflows from wastewater systems, further increasing the input of pollutants to aquatic ecosystems (Gajewska et al., 2024). On the other hand, precipitation can also induce a dilution effect, reducing the concentration of PAEs in the water (Dong et al., 2022). Consequently, quantifying such factors remains extremely challenging in ambient environmental conditions, as they can either offset or exacerbate one another at certain points.

The primary objective of this study was to identify key factors that may supersede others in influencing PAE dynamics (see Papers II and III). Although no definitive seasonal patterns were observed, several findings merit further investigation. First, ice cover formation in the lagoon during the coldest month (typically January) appears to have a significant effect on the retention of PAEs in the water column. The concentrations of PAEs in the inflow of the Nemunas River to the lagoon fluctuated between 0.01 μ g/L (July 2021) and 0.25 μ g/L (May 2022) (Fig. 2A, Paper II). However, in January 2022, with the presence of frozen snow that had accumulated in the river, PAE concentrations spiked to 0.74 μ g/L, tripling the typical levels in the inflow. Previous studies have demonstrated that a surface of snow can effectively adsorb organic pollutants from the atmosphere, particularly at temperatures below 0 °C (Lei and Wania, 2004), creating a temporary reservoir for these contaminants. During snowmelts, these pollutants are released into surrounding water bodies (Meyer and Wania, 2008), thus explaining the high concentration found during snowmelt events.

The findings of the present study also demonstrate that water circulation within the lagoon regulates the concentration and distribution of PAEs. The highest PAE levels in the water column were observed during contrasting seasons: winter, characterized by cold temperatures and high river discharge; and summer, characterized by warmer temperatures and lower river discharge. During the winter months, when riverine inputs were elevated, the sandy station experienced direct flushing from the river, resulting in the highest observed PAE concentrations. Conversely, in summer, when river discharge was at its lowest, the confined muddy site exhibited prolonged water residence time, leading to higher PAE concentrations due to the accumulation of pollutants (Fig. 4). This suggests that, over the course of the seasons, pollutants tend to spread in different areas within the lagoon depending on the hydrodynamics. Furthermore, it indicates that during certain seasons (most likely winter), PAEs originating from the river are more likely to be flushed out of the lagoon due to its short residence time, while in other seasons (most likely summer) they are more prone to remaining and accumulating within the lagoon.



Figure 4. Seasonal variations in the total PAE concentration in the water column (dissolved and particulate-bound phases). Labelling by letters indicates the outcome of post-hoc pairwise comparisons – i.e., bars sharing the same letter are not statistically significantly different (p > 0.05) (modified from Paper III).

4.3. The distribution of PAEs in the Curonian Lagoon

The spatial distribution of PAEs is influenced not only by the fluctuation of environmental conditions across seasons, but also by their physico-chemical properties. Due to their strong hydrophobicity, PAEs (especially those of higher molecular weight) will tend to adsorb to suspended matter and later accumulate in the sediment (Mohammadian et al., 2016; Staples et al., 1997b). Consequently, PAEs rarely remain in the dissolved phase of the water column. However, due to analytical constraints and time-consuming processes, many studies have focused solely on the dissolved phase and thus failed to investigate suspended matter (Bergé et al., 2013). In this study, all samples from the water column were partitioned between the dissolved and particulate phases to investigate the role of SPM in the distribution of PAEs in the aquatic environment.

4.3.1. The role of suspended matter in the distribution of PAEs

The analysis of all samples in this study consistently revealed that LMW PAEs (i.e., carbon chain < 4C) were predominantly found in the dissolved phase. In contrast, HMW PAEs (i.e., carbon chain > 4C) were primarily associated with particulate matter (Fig. 5). This finding aligns with the physicochemical properties of PAEs, as LMW PAEs exhibit lower log K_{ow} , indicating a smaller tendency to be attached to the particulate phase (Staples et al., 1997b). Therefore, the importance of SPM in the distribution of PAEs in the environment depends on the relative abundance of LMW and HMW PAEs. If HMW congeners are more prevalent in the environment, suspended matter will be the dominant phase for their presence; conversely, if LMW PAEs are more common, they will primarily remain dissolved.



Figure 5. The relationship between the proportion of HMW PAEs and the proportion of total PAEs associated with particles in water column samples. The data represents the combined results from water samples collected in this study (from Papers II, III, and IV).

The present study observed a wide range of SPM concentrations at the study sites, ranging from 3 to 113 mg/L. However, SPM concentration was a poor predictor of PAE concentration or partitioning in the Curonian Lagoon. This likely results from the complex nature of the adsorption and desorption processes of PAEs onto SPM. While SPM concentration can indeed play a role, numerous other factors, such as the organic content of SPM, salinity, dissolved organic carbon (DOC), and other environmental variables, also play a significant role in determining the extent of PAE adsorption and desorption (Al-Omran and Preston, 1987; Yang et al., 2013; Zhou and Liu, 2000).

The study detailed in Paper III revealed that higher concentrations of dissolved and particulate-bound PAEs were associated with elevated DOC levels and increased δ^{13} C values in SPM. An increase in DOC concentration can facilitate the desorption of PAEs from SPM into the aqueous phase (Mitsunobu and Takahashi, 2006), thereby increasing the proportion of dissolved PAEs. Conversely, higher δ^{13} C values, typically observed during spring and summer phytoplankton blooms in the Curonian Lagoon (Lesutiene et al., 2014; Remeikaitė-Nikiene et al., 2016), were correlated with increased levels of particulate-bound PAEs, suggesting that phytoplankton may provide a suitable substrate for PAE adsorption.

All of this demonstrates that environmental conditions exert a profound effect on the distribution of PAEs, rendering the prediction of their behaviour a complex challenge. Nonetheless, this study also confirms that SPM plays a crucial role in the sorption of hydrophobic contaminants in aquatic systems. Accordingly, further research and monitoring programs should include SPM as an important compartment.

4.3.2. Sediment as an efficient storage and a long-term representant of pollution

The PAEs accumulate within the sediment by settling with SPM onto surface sediment (Staples et al., 1997b). The sedimentary environment of the Curonian Lagoon comprises two distinct macroareas: a shallow, transitional area comprising sandy, low-organic sediments (712.8 km²); and a deeper, confined area containing muddy, organic-rich sediments (871.2 km²; Zilius et al., 2018, 2014). Therefore, sediment type strongly influences the distribution of PAEs within each lagoon compartment. Notably, muddy sediments in the Curonian Lagoon exhibited higher PAE concentrations (90–254 ng g⁻¹) than sandy sediments (5–28 ng g⁻¹) (Fig. 6). This pattern can be attributed to the hydrophobic nature of organic compounds like PAEs, which preferentially accumulate in the organic matter fractions of sediment particles (Xu and Li, 2009; Zhou and Rowland, 1997), thereby resulting in elevated concentrations within organic-rich sediments.



Figure 6. Seasonal variations in total plasticizers concentrations in sediment (0–2 cm and 2–5 cm layers). Letter labels indicate the outcome of post-hoc pairwise comparisons – i.e., bars sharing the same letters are not statistically significantly different (p > 0.05). Note that different sediment layers were not compared between sites due to negligible differences (p > 0.05) (modified from Paper III).

The present study reveals that approximately 7.5 tonnes of plasticizers have been accumulated within the upper 5 cm of sediment in the Curonian Lagoon (Paper III). An additional estimate suggests that 4.1 tonnes of plasticizers have been deposited over the past 5 years. These findings indicate that around 3 tonnes of plasticizers identified within the upper 5 cm of sediment have likely persisted for more than 5 years, highlighting the long-term persistence of such compounds in estuarine sediments. Notably, the vast majority (~90%) of these plasticizers are accumulated in the organic-rich areas of the lagoon.

The estimated stock of plasticizers in the Curonian Lagoon is approximately 2.6 kg km², significantly higher than those reported for open coastal areas such as the Bohai Sea and Yellow Sea (Table 4), where the estimated stocks are 0.3 and 0.2 kg km⁻², respectively (Mi et al., 2019). However, this remains considerably lower than the amount of plasticizers observed in Taihu Lake (170 kg km⁻²; W. Zhang et al., 2021) and the Hangzhou, Taizhou, and Wenzhou Bays (230, 82, and 52 kg km⁻², respec-

tively; Hu et al., 2020). These estimates rely on assumptions such as uniform sedimentation rates and consistent plasticizer concentrations across the study area. Nevertheless, such comparisons offer valuable insights into the relative scale of plasticizer pollution in various aquatic environments globally.

Location	Inventory 2(tons)	System surface area (km ²)	Content (kg km ⁻²)	Reference
Curonian Lagoon	4.1	1584	2.6	This Study
Bohai Sea	21	78000	0.27	Mi et al., 2019
Yellow Sea	66	380000	0.17	Mi et al., 2019
Taihu Lake	390	2338	170	W. Zhang et al., 2021
Hangzhou bay	82	350	230	Hu et al., 2020
Taizhou bay	28	342	82	Hu et al., 2020
Wenzhou bay	26	500	52	Hu et al., 2020

Table 4. Normalized content of plasticizers in the surface sediment of various marine and freshwater systems (from Paper III).

Moreover, the presence of mollusk shells can facilitate the accumulation of organic pollutants in the benthic compartment, particularly in areas with low organic matter content. In Paper I of this study, conducted at a station with fine sandy sediments, PAE concentrations were substantially higher in live zebra mussel shells, followed by shell debris, and lowest in surrounding sandy sediments. This pattern may be attributed to the higher organic content of the shells and the filtration capacity of live mussels (De Solla et al., 2016; Topić Popović et al., 2023). Additionally, the size of the shells appeared to influence the extent of PAE accumulation. The study further reveals that larger unionid shell debris contained significantly (p < 0.05) higher amounts of PAEs than smaller debris from zebra mussels and gastropods. Thus, the surface area of shells represents an important factor in determining the accumulation of organic pollutants within coastal ecosystems.

4.3.3. The fate of PAEs in the Curonian Lagoon

The fate of PAEs in the Curonian Lagoon is influenced by their transport to the sea, accumulation in sediments, and biodegradation processes (Papers II–IV). As previously discussed, the export of PAEs via lagoon outflow to the sea acts as an effective removal pathway. While sedimentation also transfers PAEs from the water column to the sediment, their long-term accumulation in the sediment creates a significant reservoir for these compounds. However, once PAEs settle in the sediment, their subsequent behaviour remains a critical question.

4. Results and discussion

Hydrodynamic processes, including sediment resuspension and flushing, can transport PAEs out of the Curonian Lagoon, but they do not eliminate these compounds from the environment. Biodegradation is the primary mechanism for the ultimate breakdown and removal of PAEs within aquatic systems (Boll et al., 2020; Staples et al., 1997b). This involves the transformation of PAEs into smaller linear compounds through complex pathways (Fig. 8), making the investigation of these processes extremely challenging. Conventional laboratory experiments have been widely used to study these processes, but they often utilize controlled conditions with single isolated bacterial strains, failing to capture the complexity of natural environments (Hu et al., 2021). A more holistic approach can rely on the analysis of pollutants alongside microbiome data from environmental samples. In this study (Paper IV), genomic data was compared with the KEGG PATHWAY database to identify actively expressed genes associated with the aerobic degradation of phthalic acid (PA) to protocatechuate (Fig. 7). The results indicate that PA biodegradation is more likely to occur within sediments rather than in the water column, as critical active genes for this process were consistently absent in the water column (see Table 5). This finding contrasts with controlled laboratory results, which typically show that PAEs degrade more rapidly in the water column within a few days, while in sediments, degradation can take weeks to months (Yuwatini et al., 2006). However, in natural environments, degradation rates can be influenced by external factors, and the water column often lacks the specific bacterial strains required for efficient degradation (Balovi et al., 2021). As a result, in situ PAE degradation can result in a significantly longer half-life, ranging from several years to even hundreds of years (Y. Zhang et al., 2021).

The analysis indicates that Gram-positive bacteria played a minimal role in PA degradation, as only one of the six transcripts associated with the Gram-positive degradation pathway was detected. Conversely, Gram-negative bacteria were primarily responsible for the degradation process (Table 5). Furthermore, the taxonomic annotation revealed that the genes involved in the aerobic degradation pathway were mainly attributed to unclassified bacteria, unclassified Proteobacteria, Alpha- and Betaproteobacteria, Chloroflexi, and Actinobacteria (see Fig. S4 and S5 in Paper IV). These are the common classes of bacteria capable of degrading PAEs (Hu et al., 2021; Liang et al., 2008).

4. Results and discussion



Figure 7. Aerobic degradation pathways of phthalic acid (phthalate, PA) into protocatechuate, along with the corresponding genes involved in the pathway. K18251: phthalate
3,4-dioxygenase subunit alpha; K18252: phthalate 3,4-dioxygenase subunit beta; K18253: phthalate 3,4-dioxygenase ferredoxin component; K18254: phthalate 3,4-dioxygenase ferredoxin reductase component; K18255: phthalate 3,4-cis-dihydrodiol dehydrogenase; K18256: 3,4-dihydroxyphthalate decarboxylase; K18068: phthalate 4,5-dioxygenase; K18069: phthalate 4,5-dioxygenase reductase component; K18067: phthalate 4,5-cis-dihydrodiol dehydrogenase; K04102: 4,5-dihydroxyphthalate decarboxylase (from Paper IV).

In the Curonian Lagoon, the biodegradation of PA appeared to be slightly more active in sandy sediments compared to muddy sediments. This difference is likely attributable to the quality of organic carbon in these environments, which influences microbial activity and substrate preference (Bartoli et al., 2021). During microbial degradation, PAEs can serve as a carbon source for microorganisms, but breaking down the PAE molecule requires a significant investment of energy due to its complex chemical structure (Durante-Rodríguez et al., 2024). In organic-rich environments such as muddy sediments, microorganisms have access to more readily degradable carbon sources, such as sugars, amino acids, and other simple organic compounds. These substrates are metabolically easier to process, leading microorganisms to preferentially utilize them over energy-intensive PAEs (Hu and Wan, 2006). Conversely, in sandy sediments, where organic carbon content is low, microorganisms face limited options for carbon sources. In such nutrient-poor conditions, they are more likely to utilize PAEs as carbon sources despite the higher energy demand for their degrada-

lable 5. RNA transcripts for multiple genes associated with Gram-negative and Gram-positive bacteria that participate in the microbial degradation of phthalates (aerobic degradation pathway) in the water column and sediment of the Curonian (CL), Vistula (VL), and Szczecin lagoons (SL). The table shows average normalized sequence counts (counts per million mapped reads, CPM) and standard deviation based on three replicates (n = 3) (from Paper IV).

	Lagoon	Station	SUM transcripts	Gram neg	gative				Gram pos	itive
			for all genes	K18068	K18069	K18067	K04102	Total gram	K18251	Total
								negative		gram posi- tive*
Water	CL	Sandy	6 ± 10	0	0	1 ± 3	4 ± 8	6 ± 10	0	0
column		Muddy	10 ± 8	4 ± 7	1 ± 3	0	5 ± 3	10 ± 8	0	0
	VL	Sandy	11 ± 4	7 ± 6	0	0	4 ± 4	11 ± 4	0	0
		Muddy	1 ± 2	0	0	0	1 ± 2	1 ± 2	0	0
	SL	Sandy	13 ± 13	6 ± 10	1 ± 2	0	7 ± 8	13 ± 13	0	0
		Muddy	20 ± 22	8 ± 9	1 ± 2	1 ± 3	10 ± 9	20 ± 22	0	0
Sediment	CL	Sandy	154 ± 105	68 ± 29	0	13 ± 25	68 ± 74	149 ± 110	6 ± 9	6 ± 9
		Muddy	12 ± 21	5 ± 13	0	2 ± 4	5 ± 9	12 ± 21	0	0
	VL	Sandy	92 ± 69	34 ± 26	0	8 ± 12	48 ± 44	89 ± 69	2 ± 6	2 ± 6
		Muddy	40 ± 28	15 ± 14	0	7 ± 10	16 ± 22	38 ± 27	2 ± 5	2 ± 5
	SL	Sandy	101 ± 65	68 ± 34	0	4 ± 6	29 ± 28	101 ± 65	0	0
		Muddy	73 ± 82	27 ± 51	0	6 ± 10	39 ± 25	73 ± 82	0	0
* 1 +1 0		and of the						1210051	F-J:*F: -	

In the Gram-positive degradation pathway, transcripts associated solety with the genes denoted as K18221 were identified, while transcripts pertaining to genes K18252, K18253, K18254, K18255, and K18256 were not detected.

4. Results and discussion

tion. This selective use of PAEs in sandy sediments highlights the adaptation of microbial communities in response to environmental constraints and resource availability. It also underscores the importance of sediment composition and organic content in determining the fate of PAEs in aquatic ecosystems.

The fate of PAEs in the Curonian Lagoon is governed by two distinct macro-areas that influence their behaviour differently. In the transitional sandy area, river flushing likely facilitates the transport of PAEs out of the lagoon. The low organic content in sediments in this area also limits the potential for PAE adsorption, leading to their removal from the lagoon ecosystem. In contrast, the confined muddy area fosters the accumulation of PAEs within the ecosystem. The longer water residence time due to reduced river influence in this zone allows PAEs to settle into organic-rich sediments where microbial degradation may occur, although activity remains limited.



Figure 8. The diverse biodegradation pathways of PAEs in the environment.

This study demonstrates the great potential of genomic tools in understanding the fate of pollutants in the environment. These advanced tools enable the identification and characterization of the microbial communities involved in pollutant degradation, providing valuable insights into the metabolic pathways and genetic mechanisms underlying these processes (Bouhajja et al., 2016; Mishra et al., 2021). However, their widespread application is hindered by several limitations. Due to their technical complexity and high analytical costs, metagenome analyses are not easily accessible to all researchers, resulting in a significant gap in the available data (Bouhajja et al., 2016). Furthermore, the lack of comprehensive databases restricts our ability to fully

interpret transcriptomic information related to pollutant degradation. In the present study, the main focus was on a single degradation pathway transforming PA into protocatechuate. However, multiple pathways exist for the complete breakdown of PAEs (Fig. 8). One of these pathways involves the initial degradation of PAEs into PA (i.e., the "upstream degradation pathway"), which is often considered a limiting step in the overall degradation process (Wei et al., 2021). Characterizing these additional pathways is crucial in the creation of a more comprehensive assessment of the fate of PAEs in the environment. Therefore, in future research, efforts should be made to enrich genomic databases to gain a more holistic understanding of organic pollutant degradation. This knowledge will ultimately contribute to more effective environmental management and pollution mitigation strategies.

4.4. The ecological risk

The widespread distribution of PAEs in the Curonian Lagoon raises concerns about potential risks to the organisms inhabiting this aquatic ecosystem. Prolonged exposure to organic pollutants, such as PAEs, can have a significant impact on aquatic organisms (Staples et al., 1997a; Y. Zhang et al., 2021). To evaluate the potential environmental risk of PAEs in the Curonian Lagoon, the risk quotient (RQ) was calculated by comparing measured concentrations with the predicted no-effect concentrations (PNEC) of PAEs (European Commission, 2003). PNEC values for algae, crustaceans, and fish in water and sediment were obtained from Li et al. (2017) and Zhang et al. (2018). The ecological risk in the water phase was categorized into three levels based on the RQ value: low risk (RQ < 0.01), medium risk ($0.01 \le RQ < 1$), and high risk (RQ > 1). For the sediment phase, high risk was defined as RQ > 1 for PAE congeners with a log K_{ow} between 3 and 5 (e.g., DMP, DEP, DiBP, DnBP), and RQ > 10 for those with a log K_{ow} > 5 (e.g., DEHP) (European Commission, 2023; Li et al., 2017). Due to insufficient data, BBzP in both phases and DOP in sediment were excluded from the risk assessment.

The ecological risk assessment for the pelagic compartment of the Curonian Lagoon, based on the average concentrations of PAEs over four seasons, reveals comparable trends in both the sandy and muddy sites (Fig. 9). DMP, DEP, and DnBP posed a low risk to all organisms in these areas. Algae appeared to be the most vulnerable group – mainly to DEHP, which represented a high risk at both sites, and to DOP, which posed a high risk at the muddy site and a medium risk at the sandy site. Crustaceans were exposed to a medium level of risk from DEHP and DOP across both sites, while fish faced a medium level of risk from DiBP and DEHP at both sites, as well as from DOP at the transitional sandy site in particular. These results indicate that the spatial distribution of PAEs alone does not drastically alter ecological risk trends, as both sites exhibited comparable patterns of contamination.



Figure 9. A risk assessment of six PAEs in the water phase of the muddy and sandy sites in the Curonian Lagoon. Risk Quotient (RQ) values were calculated based on the mean concentrations of PAEs. RQ values represent the potential effect on algae (square), crustaceans (triangle), and fish (cross) (modified from Paper III).

The ecological risk assessment was also extended to a single season across three different lagoons, facilitating comparative analysis (Fig. 10). The assessment revealed overall moderate ecological risks in all systems. However, the specific PAEs posing the highest risks to algae, crustaceans, and fish varied between lagoons. While DEHP, DOP, and DiBP posed risks to most organisms in the Curonian and Vistula lagoons, only DMP presented a medium risk to all targeted organisms in the Szczecin Lagoon. Although the high risk thresholds were not reached, the persistent presence of PAEs remains a considerable threat to lagoon ecosystem stability. Furthermore, the temporal variation of PAEs in the pelagic compartment plays a crucial role in risk levels. For example, when assessing all seasons, the Curonian Lagoon presented a potentially high risk for some organisms (Fig. 9), whereas a single-season assessment suggested only a medium risk (Fig. 10). Given the transient nature of PAEs in the pelagic environment, relying solely on single-season assessments may either understate or overstate long-term ecological threats. Therefore, a comprehensive risk assessment should prioritize data collection across multiple time points throughout the year to ensure a more accurate evaluation.



Figure 10. A risk assessment of six PAEs in the water phase of the three studied lagoons (Curonian Lagoon – CL; Vistula Lagoon – VL; Szczecin Lagoon – SL). Risk Quotient (RQ) values were calculated based on the mean concentrations of PAEs. RQ values represent the potential effect on algae (cross), crustaceans (triangle), and fish (square) (from Paper IV).

In the sediment assessment, none of the compounds exceeded the ecological risk thresholds. However, these findings should be interpreted cautiously, as sediment risk assessment relies on several assumptions. These include the equal sensitivity of pelagic and benthic organisms to PAEs, the assumption of the thermodynamic equilibrium of PAE concentrations in different phases, and the use of a generic partition method to estimate sediment-water partitioning (European Commission, 2003). Therefore, determining the toxicity of PAEs in the sediment of the Curonian Lagoon remains challenging. Overall, the situation suggests that the current PAE concentrations in the Curonian Lagoon could have a significant impact on the structure and function of micro- and macro-organisms, as well as their biological activities (Arfaeinia et al., 2019; Liu et al., 2016). Consequently, careful monitoring and management of the lagoons are essential in order to prevent further adverse effects on their ecosystems.

4.5. The Curonian Lagoon as a model to study other Baltic lagoons

This study hypothesized that the Curonian Lagoon could serve as a representative model for investigating the spatial variability and accumulation patterns of PAEs within Baltic lagoon ecosystems. To validate this, a comparative analysis was carried out in the Curonian Lagoon and two similar lagoon systems – the Vistula and Szczecin lagoons. This inter-system comparison aimed to determine whether the spatial distribution and accumulation of PAEs observed in the Curonian Lagoon are consistent with patterns found in other similar coastal environments.

4.5.1. Dissimilarities

The primary distinctions between the studied lagoons lay in the sources of PAEs affecting each system. In the Szczecin and Curonian lagoons, with large tributaries, riverine input played a significant role in determining the PAE levels within these systems. Conversely, the Vistula Lagoon, with small tributaries, was not as strongly influenced by river inputs. This becomes evident when examining PAE concentrations: in the Szczecin Lagoon, PAE levels in river inflows were notably higher compared to the concentrations at sampling stations within the lagoon, whereas the opposite pattern was observed in the Vistula Lagoon (Fig. 11). Additionally, the compositional profile of PAEs at the inflow to the Szczecin Lagoon was dominated by congeners with lower molecular weights, particularly DMP. This pattern was also observed at the sandy station and lagoon outflow (Fig. 12). In contrast, the inflow to the Vistula Lagoon exhibited a more balanced distribution of PAE congeners, potentially even dominated by species with higher molecular weights. However, within the Vistula Lagoon itself, lower-molecular-weight congeners become predominant, accounting for over 95% of the PAEs detected. This high proportion of lower-molecular-weight congeners within the lagoon suggests potential leakage from domestic wastewater sources occurring shortly before the sampling events.



Figure 11. Mean total PAE concentrations in the water column (dissolved and particulatebound phases) in the three Baltic lagoons under study (Curonian, Vistula, and Szczecin) (from Paper IV). The error bars refer to the standard deviation (n = 3). NA – not analyzed.

4. Results and discussion

These results underscore the need for tailored monitoring strategies for each lagoon to address its unique pollution sources and environmental conditions. A strategic, sitespecific approach would enable the more precise identification and assessment of local pollution sources, facilitating effective management and mitigation efforts.



Figure 12. The relative contributions (%) of individual plasticizers in the water column at each sampling station within the Curonian (CL), Vistula (VL), and Szczecin (SL) lagoons (from Paper IV).

4.5.2. Similarities

While the differences between the studied systems were most pronounced in the water column, notable similarities emerged in the sediment. Given the propensity of PAEs to accumulate in sediment, their concentration in this compartment accurately reflects long-term pollution trends. A significant similarity across all systems was the consistently higher concentration levels of PAEs in muddy sediments (Fig. 13).

The elevated concentrations of PAEs detected at the muddy sites can be attributed to the accumulation of organic pollutants in fine-grained deposits and high organic content in sediments (Lu et al., 2023; Yang et al., 2013). These characteristics enhance the settling and retention of PAEs in surface sediments for extended periods. Consequently, a comprehensive understanding of the hydrodynamics and sedimentary conditions in each area is crucial when assessing the pollution levels of organic contaminants.



Figure 13. The mean total PAE concentrations in the sediments of the three Baltic lagoons under study (Curonian, Vistula, and Szczecin) (from paper IV). The error bars refer to the standard deviation (n = 3).



Figure 14. The relative contribution (%) of individual plasticizers in the water column and sediment within the Curonian (CL), Vistula (VL), and Szczecin (SL) lagoons (from Paper IV).

Another notable similarity observed across the studied lagoons was the compositional profile of PAEs in the sediment, specifically the predominance of DEHP, followed by DnBP (Fig. 14). Although these PAEs were not consistently the most abundant in the water column, they exhibited high persistence in the sediments across all studied systems. This persistence highlights a crucial aspect of the long-term ecological risks posed by PAEs. Furthermore, while DEHP is regulated under the European framework for environmental pollutants, DnBP is not (European Commission, 2008). This regulatory gap has led to the exclusion of DnBP from many monitoring programs, potentially allowing its accumulation to continue unmonitored.

Developing sampling strategies that consider the sources of PAEs in the water column is crucial, as short-term pollution dynamics directly impact the ecosystem. It is equally vital to comprehend the hydrodynamic and sedimentary characteristics of the studied area in order to accurately evaluate long-term pollution trends in sediments. Moreover, it is advisable to expand monitoring programs to encompass a broader range of pollutants, including those not currently regulated, so as to adopt a more comprehensive approach to environmental management. 5

Recommendations

Based on the findings of this study, several recommendations are proposed to enhance the monitoring of PAEs within state programs. These recommendations aim to support the more comprehensive evaluation of PAE occurrence, transport dynamics, and potential environmental risks in transitional and coastal waters.

Current state monitoring programs follow European Union guidelines, limiting the analysis of PAEs to DnBP and DEHP in WWTP outflows and restricting DEHP monitoring to aquatic environments (rivers, lagoons, and the sea). However, given the broader distribution of PAEs in the environment and their potential ecological and human health impacts, the expansion of the list of target analytes is strongly recommended. The results of this study indicate that additional PAEs are present in Lithuanian waters, suggesting the need for a more comprehensive monitoring approach. Importantly, all PAEs analyzed in this study can be extracted and quantified simultaneously, making the inclusion of additional compounds a cost-effective improvement to the current monitoring framework.

The analysis of PAEs in water monitoring is subject to ambiguity regarding the specific matrices analyzed, which may lead to inconsistencies in data interpretation. The method detailed in the EN ISO 18856 standard commonly used for PAE analysis relies on solid-phase extraction (SPE), which is particularly sensitive to SPM. During periods of high SPM levels, such as algal blooms or strong sediment resuspension

events, pre-filtration is often necessary to prevent column clogging, whereas at other times, samples may be analyzed without filtration. This variability can significantly influence the measured concentrations of PAEs. Therefore, to improve comparability and ensure consistent data collection, it is essential to establish a clear and standard-ized protocol that defines the analyzed matrix (filtered vs. unfiltered samples) and maintain consistency across different monitoring periods.

Finally, DEHP, the only PAE currently monitored in the aquatic environment in Lithuania, is an HMW compound with a strong tendency to bind to SPM rather than remain dissolved in the water. In scenarios where economic or logistical constraints necessitate the selection of a single matrix for monitoring, prioritizing SPM over the dissolved phase would provide a more representative assessment of DEHP contamination and its environmental fate.

6

Conclusions and insights

- A robust, ready-to-use analytical method for the detection of PAEs in sediment was optimized and validated, ensuring its applicability for routine environmental assessment. This method offers lower quantification limits compared to other methods found in the literature and requires a reduced volume of solvents, making it more environmentally friendly. Furthermore, this study introduces a novel approach for method optimization in environmental chemistry, contributing to advancements in analytical methodologies for contaminant detection and enhancing the precision of environmental monitoring practices.
- 2. Mass balance calculations assessing the input and output loads of PAEs indicate that the Nemunas River served as the primary source of PAEs to the Curonian Lagoon ecosystem, while the contribution of WWTPs discharging directly into the lagoon was comparatively minor. On an annual basis, the Curonian Lagoon exports more PAEs to the Baltic Sea than it receives from the river, suggesting the presence of additional contributing sources beyond those analyzed within the scope of this study. Atmospheric deposition is suspected to play a significant role in the delivery of PAEs to this system, highlighting the necessity of further investigations into atmospheric pathways and their potential influence on contaminant levels in the lagoon.

- 3. The Curonian Lagoon plays a dynamic role in regulating PAE throughput to the sea, influenced by seasonal meteorological events and environmental factors. During winter, the ice cover in the lagoon can trap pollutants, which are subsequently released into the water. Additionally, wind patterns and hydrodynamics can shape the distribution of PAEs within the lagoon. This underscores the intricate interplay between the lagoon's regulatory function and the prevailing environmental conditions.
- 4. The distribution of PAEs within the Curonian Lagoon ecosystem is governed by their molecular weight. Lower-molecular-weight phthalate esters were predominantly found in the dissolved phase, whereas higher-molecular-weight PAEs were primarily associated with SPM, accounting for around 50% of the total PAEs detected. SPM acted as a vector that facilitated the accumulation of PAEs in sediments, where higher-molecular-weight compounds were most prevalent. These findings highlight the importance of including SPM in future investigations of hydrophobic organic pollutants to ensure accurate assessments of their environmental presence and potential impacts.
- 5. Sediment serves as an effective reservoir for PAEs, particularly in confined, organic-rich environments. This study estimates that over 7 tonnes of PAEs were present in the surface sediment of the Curonian Lagoon, with more than 3 tonnes suspected to have been stored for over 5 years. Notably, these estimates do not account for the presence of shell material in the sediment, which, as demonstrated in this study, also contributes to the retention of PAEs. This underscores the widespread and persistent presence of such contaminants in the estuarine environment.
- 6. The Curonian Lagoon serves as a suitable model for understanding the distribution of PAEs within coastal lagoon ecosystems, particularly concerning long-term trends. DEHP and DiBP emerged as the predominant PAEs in the sediments across all studied systems, with their concentrations being consistently higher in areas with organic-rich sediments. The primary distinction among the studied lagoons was attributed to varying sources of pollution. Riverine and industrial discharges were identified as the main contributors to PAE contamination in the Curonian and Szczecin lagoons. In contrast, the Vistula Lagoon was primarily affected by domestic runoff from surrounding areas, which was the principal source of PAEs. These findings underscore the importance of considering both environmental conditions and pollution sources when assessing the ecological impacts of PAEs in lagoon ecosystems.
- 7. The investigation of three lagoon systems revealed a comparable number of actively transcribed genes responsible for converting phthalic acid to protocatechuate. This finding suggests that the conversion of phthalic acid to protocatechuate is not a rate-limiting step in the PAE degradation process in these

lagoons. While the transcriptomic approach employed in this study provided valuable information regarding the occurrence of degradation, it has limitations, particularly in terms of the limited understanding of other metabolic pathways involved in PAE degradation that it engenders. Therefore, future research should address these knowledge gaps to enhance our understanding of complete PAE degradation mechanisms in environmental contexts.

8. The levels of PAEs found in the Baltic lagoons are comparable to or lower than those observed globally, posing a medium risk to living organisms, including algae, crustaceans, and fish. Furthermore, specific PAEs present in the Curonian Lagoon pose a potentially high risk to organisms. These findings highlight the importance of the continuous monitoring and risk assessment of PAEs in these ecosystems, as even low concentrations can have adverse effects on aquatic life and disrupt the ecological balance. Further research is needed to better understand the long-term implications of PAE exposure for the health of aquatic organisms, especially those inhabiting sediments.

7

Acknowledgements

I would like to express my deepest gratitude to my supervisor, Dr. Mindaugas Zilius. I will always be thankful for the opportunity, freedom, trust, and unwavering support you have given me throughout this PhD. Our collaboration began during my master's studies, and I sincerely hope it will continue long after my PhD.

I am also very grateful to my advisor, Prof. Federica Bianchi, for her invaluable insights into my work and for warmly welcoming me to Parma University during my visit.

This research would not have been possible without the support of all of my colleagues. To everyone who, at some point, took the time to help me: an immense thank you!

A special thanks to Tobia Politi for your many hours spent for sampling and helping me. To Jovita Mėžinė for always doing the best maps; to Irma Vybernaitė-Lubienė and Jolita Petkuvienė whose help was always appreciated; to Dr. Stefano Bonaglia, for offering me new opportunities to explore the Scandinavian fjords; and to Greta Kilmonaitė, for her friendship and our daily lunches together.

I would also like to extend my sincere thanks to the Marine Research Institute of Klaipeda University and its PhD committee, particularly Dr. Jurate Lesutiene and Prof. Habil. Dr. Sergej Olenin, for their guidance throughout this journey. My appreciation also goes to Egle Navickiene and Egle Karimžanova for their administrative assistance, as well as to my fellow PhD students at the institute for the shared laughter and conversations, which provided invaluable emotional support.

Finally, this journey would not have been possible without the love and encouragement of my wonderful family and amazing friends. Despite the distance, you were always present, and for that, I am eternally grateful. MERCI!

This research was supported by the Doctoral Study Program in Ecology and Environmental Sciences at Klaipeda University. Additional funding was provided by the Coastal Environment and Biogeochemistry Laboratory at the Marine Research Institute, Klaipeda University, and by the Research Council of Lithuania (Grant No. S-MIP-22-47).

8

Summary in Lithuanian

ĮVADAS

Ftalatų esteriai (PAE), dar žinomi kaip ftalatai, yra organinių junginių grupė, dažniausiai naudojama kaip plastifikatoriai siekiant padidinti polivinilchloridą (PVC) turinčių medžiagų lankstumą. Be PVC gaminių, ftalatų esteriai taip pat naudojami ir kituose produktuose, tokiuose kaip klijai, tirpikliai ar asmens higienos priemonės (ECPI, 2018; Katsikantami ir kt., 2016; Wilkes ir kt., 2005). Ftalatų esteriai yra plačiausiai pasaulyje naudojami plastifikatoriai, o jų metinis poreikis siekia apie 6 milijonus tonų. Tai chemiškai įvairi junginių grupė, kurių neįmanoma taip lengvai pakeisti kitais, siekiant gaminiui suteikti panašias savybes. Įprastai pagal anglies grandinės ilgį PAE yra klasifikuojami į mažos molekulinės masės (LMW) ir didelės molekulinės masės (HMW) ftalatus (Katsikantami ir kt., 2016).

Didelės molekulinės masės PAE, tokie kaip bis(2-etilheksilo) ftalatas (DEHP), dažniausiai naudojami pramonėje: kabelių, grindų dangų ir automobilių detalių gamyboje. Mažos molekulinės masės PAE, tokie kaip dibutilftalatas (DnBP), diizobutilftalatas (DiBP) ir dietilftalatas (DEP), dažniau naudojami gaminat medicinos prietaisus, kosmetiką ir buitinės paskirties produktus (Katsikantami ir kt., 2016; Wilkes ir kt., 2005; Wypych, 2012). Nepaisant PAE svarbos pramonėje, jų naudojimas kelia riziką aplinkai, gyvų organizmų ir žmogaus sveikatai. Tyrimai rodo, kad tam tikras PAE kiekis gali sutrikdyti endokrininę sistemą, sukelti reprodukcijos sutrikimus, skydliaukės funkcijos pažeidimus, kvėpavimo ligas, nutukimą ir neurologinius sutrikimus (Becker ir kt., 2004; Caldwell, 2012).

Aplinkoje ftalatų esteriai yra patvarūs, todėl kelia ilgalaikę grėsmę organizmams ir visos ekosistemos funkcionavimui. Europos Sąjungoje keli iš PAE – DBP, DiBP, DEHP ir benzilbutilftalatas (BzBP) – yra klasifikuoti kaip toksiški, veikiantys reprodukciją, todėl yra griežtai reguliuojami (ECHA, 2025a). Nors šių PAE naudojimas per pastaruosius metus gerokai sumažėjo, jie vis dar dažnai aptinkami aplinkoje, todėl yra būtina nuolatinė stebėsena ir prevencija.

Į estuarijas ir kitas priekrantės sistemas teršalai patenka iš upės baseine esančių buitinių, žemės ūkio ir pramonės šaltinių (Shen ir kt., 2019; Zhang ir kt., 2021). Pagrindiniai PAE patekimo į vandens ekosistemas keliai yra išleidžiamos nevalytos ar tik iš dalies išvalytos nuotekos, emisijos į atmosferą arba paviršinės lietaus nuotekos. Nuotekų valyklos laikomos vienomis iš pagrindinių PAE šaltinių paviršiniams vandenims (Gao ir kt., 2014). Tačiau atskirti konkretaus šaltinio indėlį į taršą yra gana sudėtinga dėl jų įvairovės. Vertinant pasklidusios PAE taršos sulaikymą priekrantės sistemose, Kuršių marios yra idealus modelis, dėl savo išskirtinių geomorfologinių savybių leidžiantis sudaryti srautų masių balansą pagal vadinamąjį "juodosios dėžės" principą.

Dėl plataus PAE naudojimo šie junginiai nuolat aptinkami vandenyje, dugno nuosėdose ir gyvuosiuose organizmuose. Vanduo yra pagrindinis tiesioginis ir netiesioginis PAE pernašos vektorius, nes jis yra naudojamas žemės ūkyje, maisto gamyboje ir kasdieninėje žmogaus veikloje (Tuli ir kt., 2024). Analizuojant PAE junginius vandenyje, dėl efektyvesnės ekstrakcijos suspenduotos dalelės (SPM) yra pašalinamos iš mėginio, todėl nustatomos tik ištirpusios PAE frakcijos (He ir kt., 2013; Paluselli ir kt., 2018; Xu ir kt., 2022). Tuo tarpu PAE pasiskirstymas tarp skirtingų fazių yra svarbus veiksnys, lemiantis jų biologinį prieinamumą, galimą bioakumuliaciją, degradaciją ir užsilaikymą aplinkoje. PAE sorbcija prie SPM lemia jų pernašą į dugno nuosėdas, dėl to gali sumažėti šių junginių momentinis poveikis organizmams, gyvenantiems, vandens storymėje, tačiau taip prailgėja jų buvimas ekosistemoje. Sorbcijos intensyvumas priklauso nuo PAE molekulių hidrofobiškumo, SPM savybių ir aplinkos sąlygų (Gago ir kt., 1987; Lu ir kt., 2023; Yang ir kt., 2013). Todėl tiriant PAE sklaidą yra būtina atsižvelgti į atitinkamus aplinkos veiksnius, lemiančius PAE ekologinį poveikį ir ilgalaikę elgseną ekosistemoje.

Į vandenį patekusius ftalatų esterius veikia įvairūs fizikiniai, cheminiai ir biologiniai procesai, lemiantys jų pernašą, transformaciją ir likimą. Mikrobiologinė degradacija yra pagrindinis PAE skaidymo kelias vandens ekosistemoje, kurį reguliuoja deguonies bei maistinių medžiagų prieinamumas, pačių PAE molekulinė struktūra (LMW vs. HMW) ir jų pasiskirstymas tarp fazių (Boll ir kt., 2020; Puri ir kt., 2023; Staples ir kt., 1997b). Vis dėlto didžioji dalis turimų žinių apie PAE skaidymą yra paremta laboratoriniais eksperimentais, kurie ne visuomet atspindi realias aplinkos sąlygas. PAE degradacijos tyrimai *in situ* sąlygomis, taikant pažangius molekulinius įrankius – metagenomo ir metatranskriptomo analizę – gali suteikti naujų įžvalgų apie šių teršalų skaidymo mechanizmus. Šie molekuliniai metodai leidžia identifikuoti mikrobiomą, mikroorganizmus ir genus, dalyvaujančius PAE degradacijoje, taip pagilindami supratimą apie šių junginių natūralią transformaciją (Bouhajja ir kt., 2016). Didesnis šių molekulinių metodų taikymas galėtų praturtinti žinias apie PAE biodegradacijos dinamiką ir užsilaikymą aplinkoje.

Tikslai ir darbo hipotezės

Pagrindinis šio darbo tikslas – atskleisti svarbiausius veiksnius, lemiančius ftalatų esterių (PAE) kaitą ir likimą estuarijų aplinkoje. Siekiant šio tikslo, buvo iškelti uždaviniai:

- 1. sukurti patikimą analitinį metodą PAE kiekiams nuosėdose nustatyti;
- įvertinti skirtingų šaltinių (nuotekų valymo įrenginių, Nemuno upės, Baltijos jūros) svarbą PAE srautams į Kuršių marias;
- 3. įvertinti PAE pasiskirstymo kaitą laike ir erdvėje, skirtinguose Kuršių marių ekosistemos komponentuose;
- 4. ištirti suspenduotų dalelių įtaką PAE pasiskirstymui vandens storymėje;
- 5. apskaičiuoti sukauptus PAE kiekius Kuršių marių ekosistemoje;
- 6. įvertinti PAE pasiskirstymą, galimą riziką ir likimą pasirinktose Baltijos jūros pakrantės lagūnose, palyginant jas su modeline Kuršių marių sistema.

Remiantis tyrimo uždaviniais buvo suformuluotos šios hipotezės:

- Sezoninių aplinkos veiksnių svyravimai gali reikšmingai pakeisti PAE išsiskyrimą ir pasiskirstymą Kuršių mariose, veikdami jų koncentraciją ir pernašos mechanizmą (2 ir 3 uždavinys).
- Suspenduotos dalelės vandenyje atlieka svarbų, tačiau dažnai neįvertinamą vaidmenį PAE pasiskirstyme. Nepaisant SPM reikšmės, anksčiau atliktuose PAE tyrimuose SPM buvo nepakankamai tirta, todėl šis darbas atskleidžia SPM poveikį PAE pasiskirstymui Kuršių mariose (4 ir 5 uždavinys).
- Kuršių marios gali būti laikomos tinkamu modeliu, padedančiu suprasti PAE elgseną estuarinėse sistemose. Tokie duomenys gali būti pritaikomi kitų panašių priekrančių lagūnų tyrimams, ypač Baltijos jūros regione (6 uždavinys).

Naujumo elementas ir rezultatų reikšmė

Šioje studijoje pateikiamos naujos įžvalgos apie teršalų pasiskirstymo mechanizmus estuarijų sistemose, ypatingą dėmesį skiriant PAE taršai Kuršių mariose, Vyslos ir Ščecino lagūnose – ekosistemose, kurios šiuo aspektu iki šiol buvo menkai ištirtos. Taikant "juodosios dėžės" (angl. *black-box*) principą, buvo identifikuojami antropogeniniai taršos šaltiniai pusiau uždarose vandens sistemose ir sudaromas srautų masių balansas, pasinaudojant išskirtinėmis šių ekosistemų geografinėmis, morfologinėmis ir hidrodinaminėmis savybėmis.

Vienas iš svarbiausių metodologinių tyrimo aspektų – sisteminga PAE analizė ištirpusioje ir su dalelėmis susietoje fazėje, vandens mėginiuose. Toks PAE fazių atskyrimas atskleidžia fundamentinį, tačiau dažnai ignoruojamą SPM poveikį PAE pernašai ir pasiskirstymui vandens ekosistemose. Tradicinėse stebėsenos programose įprastai PAE neanalizuojamas skirtingose fazėse, todėl gali būti netinkamai įvertinamas šių teršalų kiekis ar neteisingai interpretuojama jų elgsena aplinkoje.

Šiame darbe taip pat pristatoma pažangi analitinė tyrimų metodika, apimanti "*Ko-kybės valdymo pagal dizainą*" (angl. *Quality by Design*, QbD) koncepcijos taikymą, optimizuojant analitinius metodus aplinkos cheminiuose tyrimuose ir transkriptomo analizę, naudojamą nustatant PAE skaidyme dalyvaujantį mikrobiomą (genus ir mi-krobiotą). Transkriptomo analizė leidžia pirmą kartą išsamiai įvertinti mikrobiomą, dalyvaujantį PAE transformacijose estuarijų ekosistemose.

Šioje studijoje taikyti metodai ir principai – masių spektrometrija, masių balanso sudarymas, skirtingų PAE fazių identifikavimas, transkriptomo analizė – yra universalūs ir gali būti pritaikyti platesniam organinių teršalų spektrui tirti, taip atveriant naujas kryptis ateities aplinkos tyrimuose.

Rezultatai ir diskusija

Iššūkiai tiriant PAE aplinkos komponentuose

Ftalatų esteriai yra plačiai paplitę tiek gamtoje, tiek ir laboratorijų aplinkoje, todėl jų analitinis aptikimas aplinkos komponentuose kelia nemažai iššūkių. Maža šių junginių koncentracija aplinkoje ir didelė ekstrakcijos metodų įvairovė apsunkina optimalios analizės strategijos pasirinkimą (Net ir kt., 2015a). Daugelis esamų metodų yra reiklūs laikui arba dideliam tirpiklių kiekiui ekstrakcijos metu. Siekiant įveikti šiuos iššūkius, tyrimo metu buvo sukurtas ir optimizuotas PAE ekstrakcijos iš nuosėdų metodas, užtikrinantis aukštą analitinį preciziškumą bei veiklos efektyvumą (I publikacija).

Tyrimo metu buvo atrinkta ultragarsu atliekama PAE ekstrakcija (UAE), pasižyminti paprastumu bei minimaliu įrangos poreikiu, o metodui optimizuoti analizuojant aplinkos mėginius pirmą kartą buvo pritaikytas "*Kokybės valdymo pagal dizainą*" principas. Sistemiškai įvertinus pagrindinius optimizacijos proceso parametrus, tirpiklio tipą, jo tūrį, mėginio kratymo laiką bei ultragarso poveikio trukmę, pavyko nustatyti optimalias ekstrakcijos sąlygas. Lyginant su alternatyviais metodais, optimizuotas metodas leido sumažinti tirpiklio sunaudojimą bei pagerinti nustatymo ribas, taip užtikrinant patikimą rezultatą. Šio tyrimo metu taip pat buvo atlikta metodo verifikacija ir validacija, kuri parodė aukštą preciziškumą atkūrimo sąlygomis skirtingose matricose, žemas nustatymo ribas ir kryžminės taršos nebuvimą, o tai patvirtina, kad analitinės procedūros atitiko griežtus kokybės kontrolės standartus ir užtikrino duomenų patikimumą.

Kuršių marių vaidmuo reguliuojant ftalatų esterių (PAE) srautą į Baltijos jūrą

Tyrimo rezultatai parodė, kad 2021 m. birželio – 2022 m. gegužės laikotarpiu pagrindinis PAE šaltinis Kuršių marioms buvo Nemuno upė, atnešusi apie 2,74 tonos PAE. Tuo tarpu nuotekų valymo įrenginiai neturėjo reikšmingos įtakos PAE srautams į marias (<0,02 tonos). Per tą patį laikotarpį į Baltijos jūrą iš Kuršių marių su ištekėjimu buvo išnešta apie 5,19 tonos PAE (žr. II publikaciją), tai rodo, kad be šiame tyrime identifikuotų PAE šaltinių egzistuoja ir kiti, neištirti taršos šaltiniai. Šie rezultatai leidžia daryti prielaidą, kad metų kontekste Kuršių marios daugiausia funkcionuoja kaip tarpinė sistema, kaupianti PAE iš įvairių šaltinių ir vėliau juos eksportuojanti į Baltijos jūrą. Vis dėlto, marių vaidmuo reguliuojant PAE srautus yra kintantis ir priklauso nuo sezoninių veiksnių.

Žiemos metu Nemuno upė buvo ypač užteršta DEHP junginiu, kuris, tikėtina, pateko su nuotekomis iš pramonės. Todėl šiuo periodu Kuršių marių vandenyje vyravo DEHP. Didelis Nemuno upės debitas taip pat sąlygojo ir trumpą vandens užsilaikymą pačiose mariose, dėl to DEHP dominavo ir ištekėjime iš marių į Baltijos jūrą. Vasarą, priešingai, upė į marias atnešė didesnę dalį mažos molekulinės masės PAE (LMW PAE), dažnai siejamus su buitine tarša (Al-Saleh ir Elkhatib, 2016; Karunamoorthi ir Sabesan, 2010). Taip pat vasaros metu upės įtaka marių vandens srautui į jūrą buvo mažesnė, nors DEHP liko dominuojančiu junginiu ištekančiame marių vandenyje, greičiausiai dėl atmosferinės taršos iš netoliese esančios Klaipėdos pramoninės zonos.

Sezoniniai veiksniai, tokie kaip ledo danga ir pokyčiai vandens masių judėjime, taip pat reikšmingai veikė PAE pasiskirstymą aplinkoje. Pavyzdžiui, žiemą, kai upės paviršius buvo padengtas ledu ir sniegu, buvo išmatuota iki trijų kartų už vidutinę metinę didesnė PAE koncentracija, o tai leidžia manyti, kad ledas gali veikti kaip laikinas teršalų kaupiklis. Tuo tarpu vasarą, sumažėjus upės debitui, pailgėjo vandens užsilaikymas pačiose Kuršių mariose, o tai lėmė intensyvų PAE kaupimąsi, ypač už-darose marių zonose.

PAE pasiskirstymas Kuršių mariose

Ftalatų esterių pasiskirstymą Kuršių mariose lemia ne tik sezoniškumo veikiamos aplinkos sąlygos, bet ir pačių PAE specifinės fizikinės ir cheminės savybės. Dėl didelės PAE hidrofobijos, jie įprastai jungiasi prie vandenyje suspenduotų dalelių, o vėliau, vykstant jų sedimentacijai, PAE pernešami į dugno nuosėdas (Mohammadian ir kt., 2016; Staples ir kt., 1997b). Visuose analizuotuose mėginiuose buvo pastebėta, kad mažos molekulinės masės ftalatai (LMW PAE) dažniausiai aptinkami ištirpusioje fazėje, tuo tarpu didesnės molekulinės masės junginiai (HMW PAE) labiau susiję su vandenyje suspenduotomis dalelėmis. Todėl SPM vaidmuo reguliuojant PAE pasiskirstymą mariose didžiąja dalimi priklauso nuo santykinio LMW ir HMW kiekio.

Prie SPM prisijungę ftalatų esteriai lengvai nusėda į dugno nuosėdas, kurios tampa jų ilgalaikiu rezervuaru (Staples ir kt., 1997b). Kuršių mariose PAE daugiausia kaupiasi akumuliacinėje marių zonoje, kurioje vyrauja dumblo nuosėdos. Šiose zonoje sulaikoma apie 90 % bendro PAE kiekio. Tikėtina, kad tokį PAE pasiskirstymo dėsningumą lemia jų hidrofobiškumas, dėl kurio jie linkę kauptis didelį organinės medžiagos kiekį turinčioje smulkiojoje nuosėdų frakcijoje. Šio tyrimo duomenimis, viršutiniame 5 cm Kuršių marių nuosėdų sluoksnyje yra susikaupę apie 7,5 tonos PAE, iš kurių apie 3 tonos susikaupė prieš daugiau nei penkerius metus. Šie rezultatai atskleidžia ilgalaikį PAE užsilaikymą estuarijų nuosėdose.

Pagrindinis ftalatu esteriu skaidymo mechanizmas gamtoje vra biodegradacija procesas, kurio metu šios medžiagos yra transformuojamos į mažesnius, mažiau kenksmingus junginius (Boll ir kt., 2020; Staples ir kt., 1997b). Visgi natūralūs PAE skaidymo keliai yra sudėtingi ir dažnai sunkiai atkuriami laboratorinėmis salygomis (Hu ir kt., 2021). Jie galėtų būti geriau suprasti paraleliai atliekant teršalų analizę ir aplinkos mikrobiomo (genų ir mikrobiotos) tyrimus. Ypač vertingos įžvalgos gaunamos pasitelkus genomo tyrimo įrankius (pvz.: transkriptomo analizę), kurie leidžia identifikuoti mikroorganizmų bendrijas ir jų metabolizmo kelius. Šio tyrimo metu gauti genomo duomenys rodo, kad PAE biodegradacija koduojančių genų ekspresija intensyviausiai vyksta dugno nuosėdose, tuo tarpu šių genų ekspresija nevyko vandens storymėje. Šie rezultatai kontrastuoja su prieš tai vykdytais laboratoriniais eksperimentais, kurie parodė didesnius skaidymo greičius vandenyje. Šio tyrimo rezultatai rodo, kad estuarijose aktyviausiai PAE skaidė gram neigiamos bakterijos, tuo tarpu gam teigiamų bakterijų poveikis skaidymui buvo nedidelis. Idomu ir tai, kad PAE biodegradacija, vertinant pagal genų ekspresiją, intensyviau vyko smėlyje, negu dumblo nuosėdose, tikėtina, dėl skirtingo organinės anglies prieinamumo bakterijoms. Šie rezultatai pabrėžia genomo tyrimo metodų svarbą analizuojant mikrobiologinius procesus ir teršalų skaidymo mechanizmus. Vis dėlto tokių metodų taikymas turi ir iššūkių: didelė tyrimo kaina, reikalinga specifinė įranga, techniniai sunkumai ir ribota informacija apie mikrobiomą duomenų bazėje. Todėl genomo duomenų bazės plėtra ir tobulinimas yra būtini siekiant išsamesnio aplinkos teršalų degradacijos procesų supratimo.

Ekotoksiškumo rizika

Estuarijų aplinkoje plačiai paplitę PAE kelia susirūpinimą dėl galimos rizikos šiose ekosistemose gyvenantiems organizmams (Staples ir kt., 1997a; Zhang ir kt., 2021). Atliekant ekotoksiškumo rizikos vertinimą, visų tirtų lagūnų vandenyje nustatyta, kad bendras rizikos lygis žuvims, dumbliams ir vėžiagyviams buvo vidutinio

lygio. Tiek seklaus smėlio, tiek ir gilesnėse dumblo kaupimosi zonose buvo nustatyta panaši tendencija, rodanti, kad vien geografinis PAE pasiskirstymas neturi esminės įtakos bendram ekotoksiškumo rizikos pobūdžiui. Tačiau laikinas PAE koncentracijos kitimas vandens storymėje gali turėti didelę reikšmę rizikos lygio vertinimui. Pavyzdžiui, įvertinus visus sezonus, Kuršių mariose kai kuriems organizmams nustatyta galimai didelė rizika dėl PAE taršos lygio. Kai buvo analizuojami tik vieno sezono duomenys nustatyta jau tik vidutinė rizika. Atsižvelgiant į PAE koncentracijos kaitą vandens storymėje, rizikos vertinimas remiantis vieno sezono duomenimis gali lemti ilgalaikės ekologinės grėsmės pervertinimą arba nepakankamą įvertinimą.

Vertinant ekotoksiškumo riziką dugno nuosėdose nenustatyta, kad analizuojamų PAE junginių koncentracija viršytų leistinas rizikos vertes, sukeliančias poveikį bentosui. Tačiau šių rezultatų negalima interpretuoti vienareikšmiškai dėl naudojamo vertinimo metodo neapibrėžtumo. Esama situacija rodo, kad dabartinė PAE koncentracija tirtose lagūnose gali turėti neigiamą poveikį mikro- ir makroorganizmų bendrijos sudėčiai, funkcijoms ir jos biologiniam aktyvumui. Todėl, siekiant išvengti tolesnio neigiamo poveikio ekosistemai, būtinas nuoseklus Kuršių marių stebėjimas ir atsakinga taršos prevencija.

Kuršių marios kaip modelis kitų Baltijos lagūnų tyrimams

Kuršių marios šioje studijoje buvo naudojamos kaip modelis PAE pasiskirstymui ir užsilaikymui Baltijos lagūnose vertinti. Todėl tyrimų metu gauti rezultatai iš Kuršių marių buvo palyginti su rezultatais iš Vyslos ir Ščecino lagūnų. Pagrindinis skirtumas tarp šių lagūnų buvo PAE šaltinis: į Kuršių marias ir Ščecino lagūną PAE pateko su intensyvia upių prietaka iš žemyninės dalies dėl nuotekų išleidimo iš pramonės. Tuo tarpu Vyslos lagūną PAE srautas su upės prietaka buvo nedidelis. Analizuojant PAE sudėtį upės prietakoje į Vyslos lagūną nustatyta, kad didžioji dalis PAE yra iš aplink lagūną išsidėsčiusių buitinių taršos šaltinių. Skirtumai pagal PAE sudėtį pačiose lagūnose daugiausiai atsispindėjo vandens storymėje, tuo tarpu dugno nuosėdose PAE sudėtis buvo panaši tarp lagūnų ir jo kaupimosi tendencijos buvo vienodos. Didžiausia PAE koncentracija buvo išmatuota dumblo nuosėdose su dideliu organinės medžiagos kiekiu, tai rodo ilgalaikį šių junginių sulaikymą tirtose sistemose. Atsižvelgiant į tai, kad PAE sudėtis dugno nuosėdose visose trijose lagūnose buvo panaši, galima teigti, kad Kuršių marios yra tinkamas modelis ilgalaikiam PAE pasiskirstymui ir kaupimuisi estuarijų sistemose vertinti.

Išvados

 Tyrimo metu buvo optimizuotas ir validuotas analitinis metodas, tinkamas naudoti ftalatų esterių (PAE) kokybinei ir kiekybinei analizei nuosėdose, vykdant rutininę aplinkos būklės stebėseną. Šis metodas, palyginus su literatūroje aprašytais metodais, turi žemesnes kiekybines nustatymo ribas ir reikalauja mažesnio tirpiklių kiekio, todėl yra draugiškesnis aplinkai. Be to, tyrime pateikiamas naujas požiūris į metodų optimizavimą aplinkos chemijoje, prisidedantis prie teršalų aptikimo analitinių metodikų pažangos ir didinantis aplinkos stebėsenos tikslumą.

- 2. Masės balanso skaičiavimai, vertinant PAE įnešimo ir išnešimo srautus, parodė, kad pagrindinis PAE šaltinis Kuršių marių ekosistemoje buvo Nemuno upė, o nuotekų valyklų indėlis, tiesiogiai išleidžiant nuotekas į marias, buvo palyginti nedidelis. Per metus iš Kuršių marių į Baltijos jūrą pateko daugiau PAE, nei jų buvo atnešama su upe, kas rodo, jog egzistuoja papildomi šaltiniai, kurie nebuvo identifikuoti šiame tyrime. Tikėtina, kad atmosferinė depozicija gali būti reikšmingas PAE patekimo kelias į marių ekosistemą. Todėl ateityje būtina įvertinti galimą atmosferinių procesų įtaką taršos mastui mariose.
- 3. Kuršių marios vaidmuo reguliuojant PAE srautus į jūrą priklauso nuo sezoninių meteorologinių reiškinių ir aplinkos veiksnių. Pavyzdžiui, žiemą ledas gali sugerti teršalus, kurie vėliau tirpsmo metu atpalaiduojami į vandens storymę. Taip pat vėjo kryptis ir hidrodinaminiai procesai daro įtaką PAE pasiskirstymui mariose. Tai parodo sudėtingą sąveiką tarp marių reguliacinės funkcijos ir vyraujančių aplinkos sąlygų.
- 4. Ftalatų esterių pasiskirstymas Kuršių marių ekosistemoje priklauso ir nuo jų molekulinės masės. Mažos molekulinės masės PAE daugiausia buvo aptikti ištirpusioje fazėje, o didesnės molekulinės masės junginiai buvo adsorbuoti suspenduotų dalelių (SPM), sudarydami apie 50 % visų nustatytų PAE. SPM veikė kaip vektorius, sedimentacinių procesų metu pernešantis PAE iš vandens storymės į dugno nuosėdas. Todėl dugno nuosėdose buvo daugiausiai susikaupę didesnės molekulinės masės PAE junginiai. Šie rezultatai rodo, kad, siekiant tiksliai įvertinti hidrofobiškų organinių teršalų paplitimą ir poveikį estuarijų aplinkai, yra būtina analizuoti SPM.
- 5. Nuosėdos, ypač akumuliacinėje Kuršių marių zonoje, kurioje kaupiasi organinė medžiaga, veikia kaip efektyvus PAE rezervuaras. Įvertinta, kad paviršiniame Kuršių marių nuosėdų sluoksnyje yra susikaupę daugiau nei 7 tonos PAE, iš kurių virš 3 tonų galimai susikaupė prieš daugiau nei 5 metus. Svarbu pažymėti, kad nebuvo įvertintos moliuskų kriauklių duženos, kurios, kaip parodė šis tyrimas, taip pat adsorbuoja PAE. Tai patvirtina, kad PAE yra plačiai paplitę teršalai, ilgam pasiliekantys lagūnų aplinkoje.
- 6. Kuršių marios yra tinkamas modelis siekiant suprasti PAE pasiskirstymo dėsningumus pakrančių lagūnų ekosistemose, ypač ilgalaikėje perspektyvoje. DEHP ir DiBP buvo pagrindiniai ftalatų esteriai, aptikti visų tirtų lagūnų nuosėdose. Šių junginių koncentracija buvo didesnė daugiau organinės medžiagos turinčiose dumblo nuosėdose. PAE junginių šaltiniai tirtose lagūnose skyrėsi: Kuršių mariose ir Ščecino lagūnoje dominavo upių ir pramoninių nuotekų prietaka, o Vyslos lagūnoje – tarša iš buitinių šaltinių. Todėl vertinant PAE
poveikį lagūnų ekosistemoms būtina atsižvelgti tiek į aplinkos sąlygas, tiek į taršos šaltinių pobūdį.

- 7. Trijose tirtose lagūnose buvo aptikta panaši ftalio rūgšties virsmą į protokatechuatą kuoduojančių genų ekspresija. Tai leidžia daryti prielaidą, kad šis virsmas nėra ribojantis etapas PAE skaidymo procese. Nors šiame tyrime taikyta transkriptomo analizė suteikė daug vertingos informacijos apie PAE skaidymo proceso eigą, ji turi ir tam tikrų apribojimų, ypač dėl ribotos duomenų bazės apie kitus PAE virsmus. Todėl ateities tyrimai turėtų būti orientuoti į šias žinių spragas.
- 8. Taršos lygis ftalatų esteriais Baltijos lagūnose buvo panašus arba mažesnis nei vidutinis pasaulinis lygis, keliantis vidutinį pavojų gyviems organizmams: dumbliams, vėžiagyviams ir žuvims. Visgi kai kurie specifiniai Kuršių mariose aptikti PAE junginiai gali kelti didelę grėsmę organizmams. Šie rezultatai pabrėžia nuolatinio PAE monitoringo ir rizikos vertinimo svarbą, kadangi net ir nedidelės koncentracijos gali turėti neigiamą poveikį vandens organizmams ir sutrikdyti ekologinę pusiausvyrą. Siekiant geriau suprasti ilgalaikes PAE poveikio pasekmes vandens organizmų sveikatai, ypač gyvenantiems dugno nuosėdose, būtini išsamesni tyrimai.

9

References

- Agilent, 2020. Mass Spectrometer Optimization. https://www.agilent.com/cs/library/eseminars/public/mass-spectrometer-optimization-agilent-webinar-janyary232020.pdf.
- Al-Omran, L.A., Preston, M.R., 1987. The interactions of phthalate esters with suspended particulate material in fresh and marine waters. Environ. Pollut. 46, 177– 186. https://doi.org/10.1016/0269-7491(87)90075-3
- Al-Saleh, I., Elkhatib, R., 2016. Screening of phthalate esters in 47 branded perfumes. Environ. Sci. Pollut. Res. 23, 455–468. https://doi.org/10.1007/s11356-015-5267-z
- Arfaeinia, H., Fazlzadeh, M., Taghizadeh, F., Saeedi, R., Spitz, J., Dobaradaran, S., 2019. Phthalate acid esters (PAEs) accumulation in coastal sediments from regions with different land use configuration along the Persian Gulf. Ecotoxicol. Environ. Saf. 169, 496–506. https://doi.org/10.1016/j.ecoenv.2018.11.033
- Bai, L., Dong, X., Wang, F., Ding, X., Diao, Z., Chen, D., 2022. A review on the removal of phthalate acid esters in wastewater treatment plants: from the conventional wastewater treatment to combined processes. Environ. Sci. Pollut. Res. 29, 51339–51353. https://doi.org/10.1007/s11356-022-20977-6
- Baloyi, N.D., Tekere, M., Maphangwa, K.W., Masindi, V., 2021. Insights Into the Prevalence and Impacts of Phthalate Esters in Aquatic Ecosystems. Front. Environ. Sci. 9. https://doi.org/10.3389/fenvs.2021.684190

- Barletta, M., Lima, A.R.A., Costa, M.F., 2019. Distribution, sources and consequences of nutrients, persistent organic pollutants, metals and microplastics in South American estuaries. Sci. Total Environ. 651, 1199–1218. https://doi.org/10.1016/j. scitotenv.2018.09.276
- Bartoli, M., Nizzoli, D., Zilius, M., Bresciani, M., Pusceddu, A., Bianchelli, S., Sundbäck, K., Razinkovas-Baziukas, A., Viaroli, P., 2021. Denitrification, Nitrogen Uptake, and Organic Matter Quality Undergo Different Seasonality in Sandy and Muddy Sediments of a Turbid Estuary. Front. Microbiol. 11, 3524.
- Başaran, B., Soylu, G.N., Yılmaz Civan, M., 2020. Concentration of phthalate esters in indoor and outdoor dust in Kocaeli, Turkey: implications for human exposure and risk. Environ. Sci. Pollut. Res. 27, 1808–1824. https://doi.org/10.1007/ s11356-019-06815-2
- Becker, K., Seiwert, M., Angerer, J., Heger, W., Koch, H.M., Nagorka, R., Roßkamp, E., Schlüter, C., Seifert, B., Ullrich, D., 2004. DEHP metabolites in urine of children and DEHP in house dust. Int. J. Hyg. Environ. Health 207, 409–417. https:// doi.org/10.1078/1438-4639-00309
- Bergé, A., Cladière, M., Gasperi, J., Coursimault, A., Tassin, B., Moilleron, R., 2013. Meta-analysis of environmental contamination by phthalates. Environ. Sci. Pollut. Res. 20, 8057–8076. https://doi.org/10.1007/s11356-013-1982-5
- Bergé, A., Gasperi, J., Rocher, V., Gras, L., Coursimault, A., Moilleron, R., 2014. Phthalates and alkylphenols in industrial and domestic effluents: Case of Paris conurbation (France). Sci. Total Environ. 488–489, 26–35. https://doi.org/10.1016/j. scitotenv.2014.04.081
- Boll, M., Geiger, R., Junghare, M., Schink, B., 2020. Microbial degradation of phthalates: biochemistry and environmental implications. Environ. Microbiol. Rep. 12, 3–15. https://doi.org/10.1111/1758-2229.12787
- Bonaglia, S., Cheung, H.L.S., Politi, T., Vybernaite-Lubiene, I., McKenzie, T., Santos, I.R., Zilius, M., 2025. Eutrophication and urbanization enhance methane emissions from coastal lagoons. Limnol. Oceanogr. Lett. 10, 140–150. https://doi. org/10.1002/lol2.10430
- Bouhajja, E., Agathos, S.N., George, I.F., 2016. Metagenomics: Probing pollutant fate in natural and engineered ecosystems. Biotechnol. Adv. 34, 1413–1426. https:// doi.org/10.1016/j.biotechadv.2016.10.006
- Bu, S., Wang, Y., Wang, H., Wang, F., Tan, Y., 2020. Analysis of global commonly-used phthalates and non-dietary exposure assessment in indoor environment. Build. Environ. 177, 106853. https://doi.org/10.1016/j.buildenv.2020.106853
- Caldwell, J.C., 2012. DEHP: Genotoxicity and potential carcinogenic mechanisms— A review. Mutat. Res. Mutat. Res. 751, 82–157. https://doi.org/10.1016/j.mrrev.2012.03.001

- Cao, Y., Li, J., Wu, R., Lin, H., Lao, J.-Y., Ruan, Y., Zhang, K., Wu, J., Leung, K.M.Y., Lam, P.K.S., 2022. Phthalate esters in seawater and sediment of the northern South China Sea: Occurrence, distribution, and ecological risks. Sci. Total Environ. 811, 151412. https://doi.org/10.1016/j.scitotenv.2021.151412
- Chang, B.V., Liao, C.S., Yuan, S.Y., 2005. Anaerobic degradation of diethyl phthalate, di-n-butyl phthalate, and di-(2-ethylhexyl) phthalate from river sediment in Taiwan. Chemosphere 58, 1601–1607. https://doi.org/10.1016/j.chemosphere.2004.11.031
- Chang, W.-H., Herianto, S., Lee, C.-C., Hung, H., Chen, H.-L., 2021. The effects of phthalate ester exposure on human health: A review. Sci. Total Environ. 786, 147371. https://doi.org/10.1016/j.scitotenv.2021.147371
- Chen, Y., Wu, C., Zhang, H., Lin, Q., Hong, Y., Luo, Y., 2013. Empirical estimation of pollution load and contamination levels of phthalate esters in agricultural soils from plastic film mulching in China. Environ. Earth Sci. 70, 239–247. https://doi. org/10.1007/s12665-012-2119-8
- Cheung, H.L.S., Zilius, M., Politi, T., Lorre, E., Vybernaite-Lubiene, I., Santos, I.R., Bonaglia, S., 2025. Nitrate-Driven Eutrophication Supports High Nitrous Oxide Production and Emission in Coastal Lagoons. J. Geophys. Res. Biogeosciences 130, e2024JG008510. https://doi.org/10.1029/2024JG008510
- Chubarenko, B., Margoński, P., 2008. The Vistula Lagoon, in: Schiewer, U. (Ed.), Ecology of Baltic Coastal Waters. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 167–195. https://doi.org/10.1007/978-3-540-73524-3 8
- Darbre, P.D., 2018. Overview of air pollution and endocrine disorders. Int. J. Gen. Med. 11, 191–207. https://doi.org/10.2147/IJGM.S102230
- Dargnat, C., Teil, M.-J., Chevreuil, M., Blanchard, M., 2009. Phthalate removal throughout wastewater treatment plant: Case study of Marne Aval station (France). Sci. Total Environ. 407, 1235–1244. https://doi.org/10.1016/j.scitotenv.2008.10.027
- De Solla, S.R., Gilroy, È.A.M., Klinck, J.S., King, L.E., McInnis, R., Struger, J., Backus, S.M., Gillis, P.L., 2016. Bioaccumulation of pharmaceuticals and personal care products in the unionid mussel Lasmigona costata in a river receiving wastewater effluent. Chemosphere 146, 486–496. https://doi.org/10.1016/j.chemosphere.2015.12.022
- Dong, L., Lin, L., Pan, X., Zhang, S., Lv, Z., Mi, C., 2022. Distribution Dynamics of Phthalate Esters in Surface Water and Sediment of the Middle-Lower Hanjiang River, China. Int. J. Environ. Res. Public. Health 19. https://doi.org/10.3390/ ijerph19052702
- Durante-Rodríguez, G., de Francisco-Polanco, S., Fernández-Arévalo, U., Díaz, E., 2024. Engineering bacterial biocatalysts for the degradation of phthalic acid esters. Microb. Biotechnol. 17, e70024. https://doi.org/10.1111/1751-7915.70024

- ECHA (European Chemicals Agency), 2025a. Table of harmonised entries in Annex VI to CLP, https://echa.europa.eu/information-on-chemicals/annex-vi-to-clp [Accessed: Jan. 20, 2025].
- ECHA (European Chemicals Agency), 2025b. Candidate List of Substances of Very High Concern for Authorisation. https://echa.europa.eu/candidate-list-table [Accessed: Jan. 20, 2025].
- ECPI, European Council for Plasticisers and Intermediates, 2025. Plasticisers, https:// www.plasticisers.org/ [Accessed: Jan. 22, 2025].
- ECPI, European Council for Plasticisers and Intermediates, 2018. Plasticisers and Flexible PVC (2018). https://www.plasticisers.org/wp-content/uploads/2018/10/ ECPI_proposal_V19b_15022016.pdf.
- Elçi, Ş., 2008. Effects of thermal stratification and mixing on reservoir water quality. Limnology 9, 135–142. https://doi.org/10.1007/s10201-008-0240-x
- European Commission, 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality 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. https://eurlex.europa.eu/eli/dir/2008/105/2013-09-13.
- European Commission, 2003. European Commission Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substance, Part II. Brussels, Belgium. EU/2005/84/EC, 2005.
- European Committee for standardization, 2005. EN ISO 18856. Water Quality Determination of Selected Phthalates Using Gas Chromatography/mass Spectrometry. ISO copyright office, Geneva, pp. 1–32. ISO 18856:2004).
- European Plasticisers, 2023. Applications. https://www.plasticisers.org/plasticisers/ applications/.
- Fernández-González, V., Moscoso-Pérez, C., Muniategui-Lorenzo, S., López-Mahía, P., Prada-Rodríguez, D., 2017. Reliable, rapid and simple method for the analysis of phthalates in sediments by ultrasonic solvent extraction followed by head spacesolid phase microextraction gas chromatography mass spectrometry determination. Talanta 162, 648–653. https://doi.org/10.1016/j.talanta.2016.10.068
- Gago, Federico., Alvarez-Builla, Julio., Elguero, Jose., Diez-Masa, J.C., 1987. Correlation of octanol/water partition coefficients with hydrophobicity measurements obtained by micellar chromatography. Anal. Chem. 59, 921–923. https://doi.org/10.1021/ac00133a029

- Gajewska, M., Fitobór, K., Artichowicz, W., Ulańczyk, R., Kida, M., Kołecka, K., 2024. Occurrence of specific pollutants in a mixture of sewage and rainwater from an urbanized area. Sci. Rep. 14, 18119. https://doi.org/10.1038/s41598-024-69099-8
- Gao, D., Li, Z., Wang, H., Liang, H., 2018. An overview of phthalate acid ester pollution in China over the last decade: Environmental occurrence and human exposure. Sci. Total Environ. 645, 1400–1409. https://doi.org/10.1016/j.scitotenv.2018.07.093
- Gao, D., Li, Z., Wen, Z., Ren, N., 2014. Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China. Chemosphere 95, 24–32. https://doi. org/10.1016/j.chemosphere.2013.08.009
- Gao, D.-W., Wen, Z.-D., 2016. Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. Sci. Total Environ. 541, 986–1001. https://doi.org/10.1016/j.scito-tenv.2015.09.148
- Gao, M., Gong, X., Lv, M., Song, W., Ma, X., Qi, Y., Wang, L., 2016. Effect of Temperature and pH on the Sorption of Dibutyl Phthalate on Humic Acid. Water. Air. Soil Pollut. 227, 55. https://doi.org/10.1007/s11270-016-2759-5
- Gledhill, W.E., Kaley, R.G., Adams, W.J., Hicks, Orville., Michael, P.R., Saeger, V.W., LeBlanc, G.A., 1980. An environmental safety assessment of butyl benzyl phthalate. Environ. Sci. Technol. 14, 301–305. https://doi.org/10.1021/es60163a001
- Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F., 2017. Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. Aquat. Toxicol. 191, 122–130. https://doi.org/10.1016/j.aquatox.2017.08.007
- Hassanzadeh, N., Sari, A.E., Khodabandeh, S., Bahramifar, N., 2014. Occurrence and distribution of two phthalate esters in the sediments of the Anzali wetlands on the coast of the Caspian Sea (Iran). Mar. Pollut. Bull. 89, 128–135. https://doi. org/10.1016/j.marpolbul.2014.10.017
- He, L., Gielen, G., Bolan, N.S., Zhang, X., Qin, H., Huang, H., Wang, H., 2015. Contamination and remediation of phthalic acid esters in agricultural soils in China: a review. Agron. Sustain. Dev. 35, 519–534. https://doi.org/10.1007/s13593-014-0270-1
- He, W., Qin, N., Kong, X., Liu, W., He, Q., Ouyang, H., Yang, C., Jiang, Y., Wang, Q., Yang, B., Xu, F., 2013. Spatio-temporal distributions and the ecological and health risks of phthalate esters (PAEs) in the surface water of a large, shallow Chinese lake. Sci. Total Environ. 461–462, 672–680. https://doi.org/10.1016/j.scitotenv.2013.05.049

- He, Y., Wang, Q., He, W., Xu, F., 2019. The occurrence, composition and partitioning of phthalate esters (PAEs) in the water-suspended particulate matter (SPM) system of Lake Chaohu, China. Sci. Total Environ. 661, 285–293. https://doi. org/10.1016/j.scitotenv.2019.01.161
- HELCOM, 2015. Updated Fifth Baltic Sea pollution load compilation (PLC-5.5). Baltic Sea Environment Proceedings No. 145.
- Hites, R.A., 1973. Analysis of Trace Organic Compounds in New England Rivers. J. Chromatogr. Sci. 11, 570–574. https://doi.org/10.1093/chromsci/11.11.570
- Hites, R.A., Biemann, K., 1972. Water Pollution: Organic Compounds in the Charles River, Boston. Science 178, 158–160. https://doi.org/10.1126/science.178.4057.158
- Hu, H., Fang, S., Zhao, M., Jin, H., 2020. Occurrence of phthalic acid esters in sediment samples from East China Sea. Sci. Total Environ. 722, 137997. https://doi. org/10.1016/j.scitotenv.2020.137997
- Hu, R., Zhao, H., Xu, X., Wang, Z., Yu, K., Shu, L., Yan, Q., Wu, B., Mo, C., He, Z., Wang, C., 2021. Bacteria-driven phthalic acid ester biodegradation: Current status and emerging opportunities. Environ. Int. 154, 106560. https://doi.org/10.1016/j. envint.2021.106560
- Hu, X., Wan, J., 2006. Study of biodegradation properties of phthalate esters in aqueous culture conditions. J. Synth. Lubr. 23, 71–80. https://doi.org/10.1002/jsl.12
- Huang, P.-C., Tien, C.-J., Sun, Y.-M., Hsieh, C.-Y., Lee, C.-C., 2008. Occurrence of phthalates in sediment and biota: Relationship to aquatic factors and the biota-sediment accumulation factor. Chemosphere 73, 539–544. https://doi.org/10.1016/j. chemosphere.2008.06.019
- Idzelytė, R., Mėžinė, J., Zemlys, P., Umgiesser, G., 2020. Study of ice cover impact on hydrodynamic processes in the Curonian Lagoon through numerical modeling. Oceanologia 62, 428–442. https://doi.org/10.1016/j.oceano.2020.04.006
- Jakimavičius, D., Kriaučiūnienė, J., 2013. The climate change impact on the water balance of the Curonian Lagoon. Water Resour. 40, 120–132. https://doi.org/10.1134/ S0097807813020097
- Jiménez-Skrzypek, G., González-Sálamo, J., Varela-Martínez, D.A., González-Curbelo, M.Á., Hernández-Borges, J., 2020. Analysis of phthalic acid esters in sea water and sea sand using polymer-coated magnetic nanoparticles as extraction sorbent. J. Chromatogr. A 1611, 460620. https://doi.org/10.1016/j.chroma.2019.460620
- Johnson, D.E., Bartlett, J., Nash, L.A., 2007. Coastal lagoon habitat re-creation potential in Hampshire, England. Mar. Policy 31, 599–606. https://doi.org/10.1016/j. marpol.2007.03.004
- Kanaujiya, D.K., More, A., Chhantyal, A.K., Karn, R., and, K.P., 2023. Biodegradation of low, medium and high molecular weight phthalate by Gordonia sp. in a

batch system: Kinetics and phytotoxicity analyses. Bioengineered 14, 195–211. https://doi.org/10.1080/21655979.2023.2229094

- Karunamoorthi, K., Sabesan, S., 2010. Laboratory evaluation of dimethyl phthalate treated wristbands against three predominant mosquito (Diptera: Culicidae) vectors of disease. Eur Rev Med Pharmacol Sci 14, 443–448.
- Katsikantami, I., Sifakis, S., Tzatzarakis, M.N., Vakonaki, E., Kalantzi, O.-I., Tsatsakis, A.M., Rizos, A.K., 2016. A global assessment of phthalates burden and related links to health effects. Environ. Int. 97, 212–236. https://doi.org/10.1016/j. envint.2016.09.013
- Kumawat, M., Sharma, P., Pal, N., James, M.M., Verma, V., Tiwari, R.R., Shubham, S., Sarma, D.K., Kumar, M., 2022. Occurrence and seasonal disparity of emerging endocrine disrupting chemicals in a drinking water supply system and associated health risk. Sci. Rep. 12, 9252. https://doi.org/10.1038/s41598-022-13489-3
- Lei, Y.D., Wania, F., 2004. Is rain or snow a more efficient scavenger of organic chemicals? Atmos. Environ. 38, 3557–3571. https://doi.org/10.1016/j.atmosenv.2004.03.039
- Lesutienė, J., Bukaveckas, P.A., Gasiūnaitė, Z.R., Pilkaitytė, R., Razinkovas-Baziukas, A., 2014. Tracing the isotopic signal of a cyanobacteria bloom through the food web of a Baltic Sea coastal lagoon. Estuar. Coast. Shelf Sci. 138, 47–56. https://doi.org/10.1016/j.ecss.2013.12.017
- Li, B., Chi, J., Wu, W., Wang, Z., 2007. Effect of Nutrients and Light on Biodegradation of Dibutyl Phthalate and Di-2-ethylexyl Phthalate in Haihe Estuary. Bull. Environ. Contam. Toxicol. 79, 80.
- Liang, D.-W., Zhang, T., Fang, H.H.P., He, J., 2008. Phthalates biodegradation in the environment. Appl. Microbiol. Biotechnol. 80, 183. https://doi.org/10.1007/ s00253-008-1548-5
- Liu, H., Cui, K., Zeng, F., Chen, L., Cheng, Y., Li, H., Li, S., Zhou, X., Zhu, F., Ouyang, G., Luan, T., Zeng, Z., 2014. Occurrence and distribution of phthalate esters in riverine sediments from the Pearl River Delta region, South China. Mar. Pollut. Bull. 83, 358–365. https://doi.org/10.1016/j.marpolbul.2014.03.038
- Liu, N., Wang, Y., Yang, Q., Lv, Y., Jin, X., Giesy, J.P., Johnson, A.C., 2016. Probabilistic assessment of risks of diethylhexyl phthalate (DEHP) in surface waters of China on reproduction of fish. Environ. Pollut. 213, 482–488. https://doi.org/10.1016/j.envpol.2016.03.005
- Lu, H., Zhu, Z., 2021. Pollution characteristics, sources, and health risk of atmospheric phthalate esters in a multi-function area of Hangzhou, China. Environ. Sci. Pollut. Res. 28, 8615–8625. https://doi.org/10.1007/s11356-020-11135-x
- Lu, M., Jones, S., McKinney, M., Kandow, A., Donahoe, R., Faulk, B.C., Chen, S., Lu, Y., 2023. Assessment of phthalic acid esters plasticizers in sediments of coastal

Alabama, USA: Occurrence, source, and ecological risk. Sci. Total Environ. 897, 165345. https://doi.org/10.1016/j.scitotenv.2023.165345

- Lu, S., Yang, D., Ge, X., Li, L., Zhao, Y., Li, C., Ma, S., Yu, Y., 2020. The internal exposure of phthalate metabolites and bisphenols in waste incineration plant workers and the associated health risks. Environ. Int. 145, 106101. https://doi. org/10.1016/j.envint.2020.106101
- Magnusson, B., Örnemark, U., 2018. Eurachem Guide: The Fitness for Purpose of Analytical Methods–A Laboratory Guide to Method Validation and Related Topics, 2nd edn, 2014. www.eurachem.org.
- Meyer, T., Wania, F., 2008. Organic contaminant amplification during snowmelt. Water Res. 42, 1847–1865. https://doi.org/10.1016/j.watres.2007.12.016
- Mi, L., Xie, Z., Xu, W., Waniek, J.J., Pohlmann, T., Mi, W., 2023. Air–Sea Exchange and Atmospheric Deposition of Phthalate Esters in the South China Sea. Environ. Sci. Technol. 57, 11195–11205. https://doi.org/10.1021/acs.est.2c09426
- Mi, L., Xie, Z., Zhao, Z., Zhong, M., Mi, W., Ebinghaus, R., Tang, J., 2019. Occurrence and spatial distribution of phthalate esters in sediments of the Bohai and Yellow seas. Sci. Total Environ. 653, 792–800. https://doi.org/10.1016/j.scitotenv.2018.10.438
- Miao, Q., Ji, W., Dong, H., Zhang, Y., 2025. Occurrence of phthalate esters in the yellow and Yangtze rivers of china: Risk assessment and source apportionment. J. Environ. Sci. 149, 628–637. https://doi.org/10.1016/j.jes.2024.03.006
- Mishra, S., Lin, Z., Pang, S., Zhang, W., Bhatt, P., Chen, S., 2021. Recent Advanced Technologies for the Characterization of Xenobiotic-Degrading Microorganisms and Microbial Communities. Front. Bioeng. Biotechnol. 9. https://doi.org/10.3389/ fbioe.2021.632059
- Mitsunobu, S., Takahashi, Y., 2006. Study of the Water Solubility and Sorption on Particulate Matters of Phthalate in the Presence of Humic Acid Using 14C Labelled Di-(2-Ethylhexyl)Phthalate. Water. Air. Soil Pollut. 175, 99–115. https://doi.org/10.1007/s11270-006-9115-0
- Mo, C.-H., Cai, Q.-Y., Li, Y.-H., Zeng, Q.-Y., 2008. Occurrence of priority organic pollutants in the fertilizers, China. J. Hazard. Mater. 152, 1208–1213. https://doi. org/10.1016/j.jhazmat.2007.07.105
- Mohammadian, S., Ghanemi, K., Nikpour, Y., 2016. Competitive adsorption of phthalate esters on marine surface sediments: kinetic, thermodynamic, and environmental considerations. Environ. Sci. Pollut. Res. 23, 24991–25002. https://doi. org/10.1007/s11356-016-7723-9
- Ndwabu, S., Malungana, M., Mahlambi, P., 2023. Comparison of Ultra-Sonication and Microwave Extraction Followed by Filtration or Filtration and Solid-Phase Extraction Clean-Up for PAH Determination from Sediment and Sludge: Human

Health and Ecological Risk Assessment. Appl. Sci. 13. https://doi.org/10.3390/app13095619

- Net, S., Delmont, A., Sempéré, R., Paluselli, A., Ouddane, B., 2015a. Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): A review. Sci. Total Environ. 515–516, 162–180. https://doi.org/10.1016/j. scitotenv.2015.02.013
- Net, S., Sempéré, R., Delmont, A., Paluselli, A., Ouddane, B., 2015b. Occurrence, Fate, Behavior and Ecotoxicological State of Phthalates in Different Environmental Matrices. Environ. Sci. Technol. 49, 4019–4035. https://doi.org/10.1021/ es505233b
- Orlandini, S., Pinzauti, S., Furlanetto, S., 2013. Application of quality by design to the development of analytical separation methods. Anal. Bioanal. Chem. 405, 443– 450. https://doi.org/10.1007/s00216-012-6302-2
- Paluselli, A., Fauvelle, V., Schmidt, N., Galgani, F., Net, S., Sempéré, R., 2018. Distribution of phthalates in Marseille Bay (NW Mediterranean Sea). Sci. Total Environ. 621, 578–587. https://doi.org/10.1016/j.scitotenv.2017.11.306
- Peterson, D.R., Staples, C.A., 2003. Degradation of Phthalate Esters in the Environment, in: Staples, C.A. (Ed.), Series Anthropogenic Compounds: Phtalate Esters. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 85–124. https://doi.org/10.1007/b11464
- Puri, M., Gandhi, K., Kumar, M.S., 2023. The occurrence, fate, toxicity, and biodegradation of phthalate esters: An overview. Water Environ. Res. 95, e10832. https:// doi.org/10.1002/wer.10832
- Radziejewska, T., Schernewski, G., 2008. The Szczecin (Oder-) Lagoon, in: Schiewer, U. (Ed.), Ecology of Baltic Coastal Waters. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 115–129. https://doi.org/10.1007/978-3-540-73524-3_5
- Ramirez, M.M.B., Caamal, R.D., Osten, J.R. von, 2019. Occurrence and seasonal distribution of microplastics and phthalates in sediments from the urban channel of the Ria and coast of Campeche, Mexico. Sci. Total Environ. 672, 97–105. https:// doi.org/10.1016/j.scitotenv.2019.03.472
- Reid, A.M., Brougham, C.A., Fogarty, A.M., Roche, J.J., 2009. Accelerated solventbased extraction and enrichment of selected plasticisers and 4-nonylphenol, and extraction of tin from organotin sources in sediments, sludges and leachate soils. Anal. Chim. Acta 634, 197–204. https://doi.org/10.1016/j.aca.2008.12.032
- Remeikaitė-Nikienė, N., Lujanienė, G., Malejevas, V., Barisevičiūtė, R., Žilius, M., Garnaga-Budrė, G., Stankevičius, A., 2016. Distribution and sources of organic matter in sediments of the south-eastern Baltic Sea. J. Mar. Syst. 157, 75–81. https://doi.org/10.1016/j.jmarsys.2015.12.011

- Roscher, L., Fehres, A., Reisel, L., Halbach, M., Scholz-Böttcher, B., Gerriets, M., Badewien, T.H., Shiravani, G., Wurpts, A., Primpke, S., Gerdts, G., 2021. Microplastic pollution in the Weser estuary and the German North Sea. Environ. Pollut. 288, 117681. https://doi.org/10.1016/j.envpol.2021.117681
- Schrap, S.M., Opperhuizen, A., 1990. Relationship between bioavailability and hydrophobicity: Reduction of the uptake of organic chemicals by fish due to the sorption on particles. Environ. Toxicol. Chem. 9, 715–724. https://doi.org/10.1002/ etc.5620090604
- Sharma, K., Nayarisseri, A., Singh, S.K., 2024. Biodegradation of plasticizers by novel strains of bacteria isolated from plastic waste near Juhu Beach, Mumbai, India. Sci. Rep. 14, 30824. https://doi.org/10.1038/s41598-024-81239-8
- Shen, C., Wang, Y., Shen, Q., Wang, L., Lu, Y., Li, X., Wei, J., 2019. Di-(2-ethylhexyl) phthalate induced the growth inhibition and oxidative damage in the microalga Chlorella vulgaris. E&ES 227, 052054.
- Souaf, B., Methneni, N., Beltifa, A., Turco, V.L., Danioux, A., Litrenta, F., Sedrati, M., Mansour, H.B., Di Bella, G., 2023. Occurrence and seasonal variation of plasticizers in sediments and biota from the coast of Mahdia, Tunisia. Environ. Sci. Pollut. Res. 30, 48532–48545. https://doi.org/10.1007/s11356-023-25687-1
- Staples, C.A., Adams, W.J., Parkerton, T.F., Gorsuch, J.W., Biddinger, G.R., Reinert, K.H., 1997a. Aquatic toxicity of eighteen phthalate esters. Environ. Toxicol. Chem. 16, 875–891. https://doi.org/10.1002/etc.5620160507
- Staples, C.A., Peterson, D.R., Parkerton, T.F., Adams, W.J., 1997b. The environmental fate of phthalate esters: A literature review. Chemosphere 35, 667–749. https:// doi.org/10.1016/S0045-6535(97)00195-1
- Stocker, J., Scheringer, M., Wegmann, F., Hungerbühler, K., 2007. Modeling the Effect of Snow and Ice on the Global Environmental Fate and Long-Range Transport Potential of Semivolatile Organic Compounds. Environ. Sci. Technol. 41, 6192– 6198. https://doi.org/10.1021/es062873k
- Topić Popović, N., Lorencin, V., Strunjak-Perović, I., Čož-Rakovac, R., 2023. Shell
 Waste Management and Utilization: Mitigating Organic Pollution and Enhancing
 Sustainability. Appl. Sci. 13. https://doi.org/10.3390/app13010623
- Tran, H.T., Lin, C., Bui, X.-T., Nguyen, M.K., Cao, N.D.T., Mukhtar, H., Hoang, H.G., Varjani, S., Ngo, H.H., Nghiem, L.D., 2022. Phthalates in the environment: characteristics, fate and transport, and advanced wastewater treatment technologies. Bioresour. Technol. 344, 126249. https://doi.org/10.1016/j.biortech.2021.126249
- Tuli, A., Suresh, G., Halder, N., Velpandian, T., 2024. Analysis and remediation of phthalates in aquatic matrices: current perspectives. Environ. Sci. Pollut. Res. 31, 23408–23434. https://doi.org/10.1007/s11356-024-32670-x

- Umgiesser, G., Zemlys, P., Erturk, A., Razinkova-Baziukas, A., Mėžinė, J., Ferrarin, C., 2016. Seasonal renewal time variability in the Curonian Lagoon \hack\newline caused by atmospheric and hydrographical forcing. Ocean Sci. 12, 391–402. https://doi.org/10.5194/os-12-391-2016
- Vybernaite-Lubiene, I., Zilius, M., Saltyte-Vaisiauske, L., Bartoli, M., 2018. Recent Trends (2012–2016) of N, Si, and P Export from the Nemunas River Watershed: Loads, Unbalanced Stoichiometry, and Threats for Downstream Aquatic Ecosystems. Water 10. https://doi.org/10.3390/w10091178
- Wang, J., Tian, Y., Wei, J., Lyu, C., Yu, H., Song, Y., 2023. Impacts of dibutyl phthalate on bacterial community composition and carbon and nitrogen metabolic pathways in a municipal wastewater treatment system. Environ. Res. 223, 115378. https://doi.org/10.1016/j.envres.2023.115378
- Wang, R., Ji, M., Zhai, H., Liu, Y., 2020. Occurrence of phthalate esters and microplastics in urban secondary effluents, receiving water bodies and reclaimed water treatment processes. Sci. Total Environ. 140219. https://doi.org/10.1016/j.scitotenv.2020.140219
- Wang, X., Zhang, Y., Huang, B., Chen, Z., Zhong, M., Wang, W., Liu, X., Fan, Y. nan, Hu, W., 2021. Atmospheric phthalate pollution in plastic agricultural greenhouses in Shaanxi Province, China. Environ. Pollut. 269, 116096. https://doi. org/10.1016/j.envpol.2020.116096
- Wei, S.T.-S., Chen, Y.-L., Wu, Y.-W., Wu, T.-Y., Lai, Y.-L., Wang, P.-H., Ismail, W., Lee, T.-H., Chiang, Y.-R., 2021. Integrated Multi-omics Investigations Reveal the Key Role of Synergistic Microbial Networks in Removing Plasticizer Di-(2-Ethylhexyl) Phthalate from Estuarine Sediments. mSystems 6, 10.1128/msystems.00358-21. https://doi.org/10.1128/msystems.00358-21
- Wenchao, W., Zhang, D., Sophocleous, M., Qu, Y., Jing, W., Chalermwisutkul, S., Russel, M., 2023. Measuring the effects of diethyl phthalate microplastics on marine algae growth using dielectric spectroscopy. Sci. Total Environ. 865, 161221. https://doi.org/10.1016/j.scitotenv.2022.161221
- Wilkes, C., Summers, J., Daniels, C., 2005. PVC handbook. Hanser Munich.
- Woodrow, J.E., Gibson, K.A., Seiber, J.N., 2019. Pesticides and Related Toxicants in the Atmosphere, in: de Voogt, P. (Ed.), Reviews of Environmental Contamination and Toxicology Volume 247. Springer International Publishing, Cham, pp. 147–196. https://doi.org/10.1007/398_2018_19
- Wu, Y., Sun, J., Zheng, C., Zhang, X., Zhang, A., Qi, H., 2019. Phthalate pollution driven by the industrial plastics market: a case study of the plastic market in Yuyao City, China. Environ. Sci. Pollut. Res. 26, 11224–11233. https://doi.org/10.1007/ s11356-019-04571-x

- Wypych, G. (Ed.), 2012. 13 PLASTICIZERS IN VARIOUS INDUSTRIAL PROD-UCTS, in: Handbook of Plasticizers (Second Edition). William Andrew Publishing, Boston, pp. 431–520. https://doi.org/10.1016/B978-1-895198-50-8.50015-1
- Xie, Z., Ebinghaus, R., Temme, C., Caba, A., Ruck, W., 2005. Atmospheric concentrations and air–sea exchanges of phthalates in the North Sea (German Bight). Atmos. Environ. 39, 3209–3219. https://doi.org/10.1016/j.atmosenv.2005.02.021
- Xie, Z., Ebinghaus, R., Temme, C., Lohmann, R., Caba, A., Ruck, W., 2007. Occurrence and Air–Sea Exchange of Phthalates in the Arctic. Environ. Sci. Technol. 41, 4555–4560. https://doi.org/10.1021/es0630240
- Xu, G., Li, F., Wang, Q., 2008. Occurrence and degradation characteristics of dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in typical agricultural soils of China. Sci. Total Environ. 393, 333–340. https://doi.org/10.1016/j.scitotenv.2008.01.001
- Xu, H., Liu, Y., Xu, X., Lan, H., Qi, W., Wang, D., Liu, H., Qu, J., 2022. Spatiotemporal variation and risk assessment of phthalate acid esters (PAEs) in surface water of the Yangtze River Basin, China. Sci. Total Environ. 836, 155677. https://doi. org/10.1016/j.scitotenv.2022.155677
- Xu, X.-R., Li, X.-Y., 2009. Sorption behaviour of benzyl butyl phthalate on marine sediments: Equilibrium assessments, effects of organic carbon content, temperature and salinity. Mar. Chem. 115, 66–71. https://doi.org/10.1016/j.marchem.2009.06.006
- Yang, F., Wang, M., Wang, Z., 2013. Sorption behavior of 17 phthalic acid esters on three soils: Effects of pH and dissolved organic matter, sorption coefficient measurement and QSPR study. Chemosphere 93, 82–89. https://doi.org/10.1016/j. chemosphere.2013.04.081
- Yuwatini, E., Hata, N., Kuramitz, H., Taguchi, S., 2013. Effect of salting-out on distribution behavior of di(2-ethylhexyl) phthalate and its analogues between water and sediment. SpringerPlus 2, 422. https://doi.org/10.1186/2193-1801-2-422
- Yuwatini, E., Hata, N., Taguchi, S., 2006. Behavior of di (2-ethylhexyl) phthalate discharged from domestic waste water into aquatic environment. J. Environ. Monit. 8, 191–196.
- Zemlys, P., Ferrarin, C., Umgiesser, G., Gulbinskas, S., Bellafiore, D., 2013. Investigation of saline water intrusions into the Curonian Lagoon (Lithuania) and twolayer flow in the Klaipėda Strait using finite element hydrodynamic model. Ocean Sci. 9, 573–584. https://doi.org/10.5194/os-9-573-2013
- Zeng, F., Cui, K., Xie, Z., Liu, M., Li, Y., Lin, Y., Zeng, Z., Li, F., 2008. Occurrence of phthalate esters in water and sediment of urban lakes in a subtropical city, Guangzhou, South China. Environ. Int. 34, 372–380. https://doi.org/10.1016/j. envint.2007.09.002

- Zeng, F., Lin, Y., Cui, K., Wen, J., Ma, Y., Chen, H., Zhu, F., Ma, Z., Zeng, Z., 2010. Atmospheric deposition of phthalate esters in a subtropical city. Atmos. Environ. 44, 834–840. https://doi.org/10.1016/j.atmosenv.2009.11.029
- Zeng, F., Wen, J., Cui, K., Wu, L., Liu, M., Li, Y., Lin, Y., Zhu, F., Ma, Z., Zeng, Z., 2009. Seasonal distribution of phthalate esters in surface water of the urban lakes in the subtropical city, Guangzhou, China. J. Hazard. Mater. 169, 719–725. https:// doi.org/10.1016/j.jhazmat.2009.04.006
- Zhang, Qinghao, Sun, Y., Zhang, Qingnan, Hou, J., Wang, P., Kong, X., Sundell, J., 2020. Phthalate exposure in Chinese homes and its association with household consumer products. Sci. Total Environ. 719, 136965. https://doi.org/10.1016/j.scitotenv.2020.136965
- Zhang, W., Li, X., Guo, C., Xu, J., 2021. Spatial distribution, historical trend, and ecological risk assessment of phthalate esters in sediment from Taihu Lake, China. Environ. Sci. Pollut. Res. 28, 25207–25217. https://doi.org/10.1007/s11356-021-12421-y
- Zhang, Y., Jiao, Y., Li, Z., Tao, Y., Yang, Y., 2021. Hazards of phthalates (PAEs) exposure: A review of aquatic animal toxicology studies. Sci. Total Environ. 771, 145418. https://doi.org/10.1016/j.scitotenv.2021.145418
- Zhang, Z.-M., Zhang, H.-H., Li, J.-L., Yang, G.-P., 2017. Determination of Phthalic Acid Esters in Seawater and Sediment by Solid-phase Microextraction and Gas Chromatography-Mass Spectrometry. Chin. J. Anal. Chem. 45, 348–356. https:// doi.org/10.1016/S1872-2040(17)60999-X
- Zhou, J.L., Liu, Y.P., 2000. Kinetics and equilibria of the interactions between diethylhexyl phthalate and sediment particles in simulated estuarine systems. Mar. Chem. 71, 165–176. https://doi.org/10.1016/S0304-4203(00)00047-5
- Zhou, J.L., Rowland, S.J., 1997. Evaluation of the interactions between hydrophobic organic pollutants and suspended particles in estuarine waters. Water Res. 31, 1708–1718. https://doi.org/10.1016/S0043-1354(96)00323-5
- Zhu, F., Doyle, E., Zhu, C., Zhou, D., Gu, C., Gao, J., 2020. Metagenomic analysis exploring microbial assemblages and functional genes potentially involved in di (2-ethylhexyl) phthalate degradation in soil. Sci. Total Environ. 715, 137037. https://doi.org/10.1016/j.scitotenv.2020.137037
- Zhu, F., Zhu, C., Zhou, D., Gao, J., 2019. Fate of di (2-ethylhexyl) phthalate and its impact on soil bacterial community under aerobic and anaerobic conditions. Chemosphere 216, 84–93. https://doi.org/10.1016/j.chemosphere.2018.10.078
- Zilius, M., Barisevičiūtė, R., Bonaglia, S., Klawonn, I., Lorre, E., Politi, T., Vybernaite-Lubiene, I., Voss, M., Overlinge, D., Bukaveckas, P., 2024. The effects of variable riverine inputs and seasonal shifts in phytoplankton communities on nitrate cycling in a coastal lagoon. Front. Mar. Sci. 11, 1497246.

- Zilius, M., Bartoli, M., Bresciani, M., Katarzyte, M., Ruginis, T., Petkuviene, J., Lubiene, I., Giardino, C., Bukaveckas, P.A., de Wit, R., Razinkovas-Baziukas, A., 2014. Feedback Mechanisms Between Cyanobacterial Blooms, Transient Hypoxia, and Benthic Phosphorus Regeneration in Shallow Coastal Environments. Estuaries Coasts 37, 680–694. https://doi.org/10.1007/s12237-013-9717-x
- Zilius, M., Vybernaite-Lubiene, I., Vaiciute, D., Overlingė, D., Grinienė, E., Zaiko, A., Bonaglia, S., Liskow, I., Voss, M., Andersson, A., Brugel, S., Politi, T., Bukaveckas, P.A., 2021. Spatiotemporal patterns of N₂ fixation in coastal waters derived from rate measurements and remote sensing. Biogeosciences 18, 1857–1871. https://doi.org/10.5194/bg-18-1857-2021
- Zilius, M., Vybernaite-Lubiene, I., Vaiciute, D., Petkuviene, J., Zemlys, P., Liskow, I., Voss, M., Bartoli, M., Bukaveckas, P.A., 2018. The influence of cyanobacteria blooms on the attenuation of nitrogen throughputs in a Baltic coastal lagoon. Biogeochemistry 141, 143–165. https://doi.org/10.1007/s10533-018-0508-0

CURRICULUM VITAE

Elise was born on May 30, 1996, in Saint-Brieuc, France. She began her academic journey in the field of chemistry, earning her bachelor's degree from Rennes 1 University in 2018. Throughout her undergraduate studies, she developed a growing interest in environmental issues, particularly the impact of chemical substances on marine ecosystems. This passion led her to pursue a master's degree in Marine Chemistry at the University of Bretagne Occidentale in Brest, France (2018–2020), with courses and research conducted at the prestigious European Institute for Marine Studies (IUEM).

During the first year of her master's program, Elise was involved in research focused on the detection and analysis of heavy metals in seagrass beds from diverse geographic locations. This early exposure to environmental work and analytical techniques deepened her appreciation for the complexity of marine pollution. However, it was during her second-year internship that her academic interests took a more defined direction. Working on the analysis of organic pollutants in environmental samples, Elise discovered a strong interest in persistent organic contaminants and their longterm effects on marine ecosystems.

This pivotal internship held at Klaipeda University in Lithuania, gave her practical experience in advanced analytical chemistry and helped her build strong academic connections. Motivated by this experience and eager to delve further into the subject, Elise decided to continue her academic path by enrolling in Klaipeda University's PhD program in 2020.

Alongside her PhD, she has been actively working as an analytical chemist at the Coastal Environment and Biogeochemistry Laboratory, part of the Marine Research Institute at Klaipeda University. Her role focuses on developing and validating analytical methods to detect and quantify organic pollutants in a variety of environmental matrices, including water, wastewater and sediments. Her work contributes to a broader understanding of pollution dynamics in coastal environments and supports efforts in environmental monitoring and protection.

Elise's academic path demonstrates a strong commitment to environmental science, a solid foundation in analytical chemistry, and a passion for contributing to marine pollution research at both local and global levels.

10

Publications

PAPER I

Talanta Open 8 (2023) 100258



Quality by design in the optimization of the ultrasonic assisted solvent extraction for the GC-MS determination of plasticizers in sediments and shells

Elise Lorre ^a, Nicolò Riboni ^b, Federica Bianchi ^{b, c, *}, Serena Orlandini ^d, Sandra Furlanetto ^d, Maria Careri ^b, Mindaugas Zilius ^{a, b}

^a Marine Research Institute, Klaipeda University, Klaipeda 92295, Lithuania

^b Department of Chemistry, Life Science and Environmental Sustainability, University of Parma, Parco Area delle Scienze 17/A, Parma 43124, Italy

^c Interdepartmental Center for Energy and Environment (CIDEA), University of Parma, Parco Area delle Scienze, Parma 43124, Italy

^d Department of Chemistry "U. Schiff", University of Florence, Via U. Schiff 6, Sesto Fiorentino, Florence 50019, Italy

ARTICLE INFO

ABSTRACT

Keywords: Phthalate esters Adipate Quality by design Ultrasonic-assisted solvent extraction Sediment Mollusc shells Phthalate esters (PAEs) are the most widely used plasticizers worldwide and are considered as ubiquitous environmental contaminants. Due to both their ubiquity and potential health and environmental risks, their determination is a matter of worldwide concern. In the present study, an efficient method based on ultrasonicassisted solvent extraction followed by gas chromatography-mass spectrometry is proposed for the simultaneous determination of PAEs and di(2-ethylhexyl) adipate in estuarine sediments and shells. Method optimization was successfully carried out according to Quality by Design principles. Shaking time, ultrasonic time, extractant volume, and solvent type were selected as Critical Method Parameters. Validation proved method reliability for the determination of the investigated analytes, achieving detection limits in the 0.1–0.7 ng gr⁻¹ and 0.1–0.5 ng gr⁻¹ range for sediments and shells, respectively. A good precision was obtained with RSD < 20% and trueness (recovery) in the 73(\pm 7)–120(\pm 10)% and 70(\pm 10)–111(\pm 3)% range, for sediments and shells, respectively. Finally, the method was applied to analyze sediment samples and molluse shells, collected from the Curonian Lagoon (Southeast Baltic Sea). Di(2-ethylhexyl) phthalate was present in all the analyzed samples, thus pointing out its ubiquity in estuarine environment. PAEs were also found in shell debris or living mussels, highlighting them as a hotspot of organic contaminants, especially in transitional environments, where accumulation of organic rich deposits is limited.

1. Introduction

Phthalate esters (PAEs) are the most widely used plasticizers worldwide. Their presence as additives in plastics, cosmetics, wall covering, floors, and packaging materials make them ubiquitous compounds in daily life [1,2]. Being classified as endocrine disruptors, these compounds could exert adverse health effects on both humans and wildlife in all environments [3].

In aquatic environments, owing to their relative high hydrophobicity, PAEs are adsorbed onto suspended particles and settled down to surface sediment [4]. Consequently, sediments can act as a temporal storage for PAEs. In Europe, the concentration of PAEs in aquatic systems has decreased over the last decades due to the introduction of strict regulations on their use in industry [5,6]. Nevertheless, the level of PAEs observed in some areas is still high: for example in the Baltic region, concentrations over 3900 and 800 ng g⁻¹ were determined in settling deposits and sediments, respectively [6,7]. To overcome the restrictions on the use of PAEs, many manufacturers have replaced them with phthalate-free alternatives such as adipates, among which di (2-ethylhexyl) adipate (DEHA), thus contributing to increase their presence in the plasticiser market and, ultimately, in the environment [6].

The quantification of PAEs in sediments can be a real challenge since it requires the use of reliable analytical methods to avoid overestimation

https://doi.org/10.1016/j.talo.2023.100258

Received 21 August 2023; Received in revised form 10 October 2023; Accepted 18 October 2023

Available online 20 October 2023 2666-8319/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

^{*} Corresponding author at: Department of Chemistry, Life Science and Environmental Sustainability, University of Parma, Parco Area delle Scienze 17/A, Parma 43124, Italy.

E-mail address: federica.bianchi@unipr.it (F. Bianchi).

E. Lorre et al.

[8]. The extraction of PAEs from solid matrices such as sediments has been reported using a large variety of methods, like Soxhlet, microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), ultrasonic-assisted extraction (UAE) or solid-phase microextraction (SPME) [8–15]. The major advantage of the UAE approach relies on its ease of implementation in every analytical lab, requiring simple devices.

In recent years, Quality by Design (QbD) principles have been introduced by US Food and Drug Administration [16] for assuring quality in pharmaceutical products and processes. This quality paradigm has been outlined also in International Council for harmonisation (ICH) Pharmaceutical Development guideline Q8 [17] and its use has been implemented in Analytical Method Development ICH Q14 guideline [18]. Analytical Quality by Design (AQbD) consists of a systematic framework in analytical method development, and represents an important ally of the analytical researcher, in particular for the set-up of separation procedures [19,20]. Even if the most important field of AQbD application is still pharmaceutical analysis [21-23], recently few applications have been reported in food [24,25] and environmental analysis [26,27], confirming the great potential of this sound-science based approach. QbD principles are founded on Design of Experiments (DoE) and Risk Analysis, and their application makes it possible to efficiently drive method development, both clarifying the effects of multiple parameters on the analytical output and identifying the method operable design region (MODR). The latter is defined as the multivariate zone where it is assured that the analytical target is achieved with a selected level of probability [21].

In this study, for the first time in the literature, QbD was applied to the development of an extraction procedure in environmental analysis. Despite DoE optimization of UAE extraction has been already proposed in the literature [9,25,28–30], the application of QbD to extraction techniques is still limited to one example in food analysis, associated with the extraction of polyphenols from a vegetable matrix [31].

In this study, QbD was applied to optimize the UAE conditions for the GC-MS determination of several PAEs and DEHA in estuarine sediments. Validation was carried out to assess method reliability for the determination of the analytes at trace levels in samples collected from the Curonian Lagoon (Southeast Baltic Sea). The obtained results will provide a better understanding of the pollution levels in one of the most important lagoons of the Baltic region, since the compositional profiles of PAEs in the sediment of the area are still understudied and rarely considered in the state monitoring programs.

2. Material and methods

2.1. Chemicals and materials

Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Dibutyl phthalate (DBP), ButylBenzyl phthalate (BBzP), Di(2-ethylhexyl) phthalate (DEHP), Di-n-octyl phthalate (DOP), Diisobutyl phthalate (DiBP), and Di(2-ethylhexyl) adipate (DEHA) standards (all purity grade > 98%) were purchased from HPC Standard GmbH (Cunnersdorf, Germany).

Internal standards D4-ring deuterated dibutyl phthalate (D4-DBP, 98%) and D4-ring deuterated di-n-octyl phthalate (D4-DDP, 98%) were purchased from LGC Standard Sp. Z O.O. (Lomianki, Poland). Capillary grade ethyl acetate (EA), supratrace grade dichloromethane (DCM), acetone (>99.8% purity) (AC) and supergradient grade methanol (MeOH) were purchased from VWR International GmbH (Vienna, Austria). Both the stock (1000 mg L⁻¹) and the working (10 mg L⁻¹) solutions were prepared in EA and stored in the dark at -20 °C until analysis. To avoid background pollution, the laboratory equipment used for sampling and analysis was exclusively made of glass or stainless steel. Prior to use, all glass apparatus was soaked in 10% HCl for 12 h, rinsed with distilled water, dried, burned at 500 °C for 6 h, and finally pre-rinsed with MeOH. Talanta Open 8 (2023) 100258

2.2. Optimization of the ultrasonic assisted extraction

The Critical Method Attributes (CMAs) [19,22] corresponding to the responses to be investigated and maximized, were chosen as the chromatographic areas of DiBP, DBP, BBzP, DEHP. The Critical Method Parameters (CMPs) of the extraction [19,22], namely the factors that could potentially impact the CMAs, were represented by three quantitative factors, i.e. shaking time (SHA time), ultrasonic time (US time) and extraction volume (VOL), and one qualitative factor, namely solvent type (SOLV type). The knowledge space, that is the experimental domain of the CMPs explored by DoE, was the following: SHA time, 10.0-20.0 min: US time, 10.0-20.0 min: VOL, 10.0-20.0 mL (Table A1), The investigated solvents were DCM and the solvent mixtures DCM/AC and DCM/MeOH, both in 1:1 ratio. The final optimized conditions for the ultrasonic assisted extraction parameters, together with the MODR interval in brackets, are the following: SHA time, 15.0 min, US time, 12.0 min (10.5-15.0 min), SOLV type, DCM/AC; VOL, 17.5 mL (15.1-20.0 mL).

The extraction procedure was as follows: dry sediment (2.5 g) was weighed and transferred to a 100 mL borosilicate glass bottle, then the extraction solvent (optimized condition: 17.5 mL of DCM/AC) was added. The bottles were closed, shaken (optimized condition: 15.0 min) on a horizontal shaking table (KS 4000 ic control, IKA, Germany) and sonicated (optimized condition: 12.0 min) at room temperature in an ultrasonic bath (setting power: 100%; Sonorex Digiplus DL 512 H, Bandelin, Germany). Supernatant was separated from sediment, evaporated under a gentle stream of nitrogen gas, and dissolved in 1.5 mL of EA. Prior to GC-MS analysis, the extracts were filtered through 0.22 μ m polytetrafluoroethylene filters (Frisenette, Denmark).

NemrodW software (NemrodW, LPAI sarl, Marseille, France) was used for planning the symmetric screening matrix and for related data treatment. MODDE 13 software (Sartorius Data Analytics AB, Göttingen, Germany) was used to generate the Central Composite Design (CCD) in Response Surface Methodology (RSM), to perform related data treatment and to identify the MODR through Monte-Carlo simulations [32]. When performing risk analysis, the target for defect per million opportunities (DPMO) [33] was set at 100,000, matching with a risk of error of 10%.

2.3. GC-MS analysis

A Shimadzu GC-2010 Plus gas chromatograph equipped with a GCMS-TQ8040 mass spectrometer (Shimadzu Corporation, Japan) was used for GC-MS analyzes. Helium (99.9999%) was used as the carrier gas at a constant flow rate of 1 mL/min; the GC injector was operated in splitless mode at 250 °C, whereas chromatographic separation was performed on a Rxi-5Sil MS capillary column ($30 \text{ m} \times 0.25 \text{ mm}$ i.d., 0.25 µm film thickness; Restek & Bellefonte, USA) using the following temperature program: 60 °C, held for 2 min, to 240 °C at 25 °C/min, held for 2 min, to 300 °C at 10 °C/min, held for 3 min. Transfer line and ion source were maintained at 280 °C and 230 °C, respectively. The mass spectrometer was operated in time scheduled single-ion monitoring mode (SIM) by recording the current of the ions reported in Table A2. Signal acquisition and data handling were performed using the LabSo-lutions (Shimadzu Corporation) software.

2.4. Method validation

A blank analysis was performed prior to validation to establish the absence of contamination in the matrices used as reference (shells and sediments). Then method validation was performed according to EUR-ACHEM guidelines [34] under the optimized conditions using the blank matrices. Detection (yD) and quantitation limits (yQ) expressed in terms of signals were obtained by the analysis of blank sediments spiked with each analyte at 0.2 mg s⁻¹. Both the mean (y_b) and the standard deviation (s_b) of 10 replicated measurements were calculated. Finally, detection

E. Lorre et al.

(LOD) and quantitation (LOQ) limits were obtained by projection of the corresponding signals vD and vO through a calibration plot v = f(x) onto the concentration axis. Linearity was assessed using a matrix-matching calibration (sediment and shells) on 6 concentration levels: LOQ, 2, 10, 50, 100, 150 ng g^{-1} for all the analytes, with the only exception of BBzP (LOQ, 4, 10, 50, 100, 150 ng g⁻¹), by performing 3 replicated measurements per level. D4-DBP and D4-DOP were used as internal standard at the concentration of 10 ng g⁻¹. Homoscedasticity was verified by applying the Hartley test. Lack of fit and Mandel's fitting tests [35] were also performed to assess the goodness of fit and linearity ($\alpha =$ 0.01). The significance of the intercept (α =0.05) was evaluated by performing a Student's t-test. Repeatability and intermediate precision were evaluated as relative standard deviations (RSD%) on 3 concentration levels: LOQ, 25 and 150 ng g-1, performing four replicated measurements per level. Intermediate precision was estimated over three days, verifying homoscedasticity of data and performing the analysis of variance (ANOVA) at the 95% confidence level. Trueness was calculated for each analyte in terms of recovery rate (RR%) by performing 8 replicated measurements at LOQ, 25 and 150 ng g $^{-1}.~\rm RR\%$ was calculated as follows: RR%=(c_{exp}/c_{spk})•100 where c_{exp} is the observed concentration and c_{spk} is the concentration of the fortified matrix. Finally, the matrix effect (ME) was calculated at LOQ, 25 and 150 ng gusing the following formula:

 $ME(\%) = A1 / A2 \times 100$

where A1 is average peak area of each analyte/internal standard in matrix extract and A2 is that in DCM/AC (1:1, v/v) at the same concentration.

2.5. Real sample analysis

Sediment samples from the oligobaline Curonian Lagoon (salinity < 0.5 [36]), which is located along the southeast coast of the Baltic Sea, were collected in August 2021 (Site A and B) and in February 2022 (Site C) from two principal sedimentary environments: deeper confined (3.5 m depth) and shallow transitional (~1.5 m depth). Confined area (Site A, 55°17'14.3" N/ 21°01'17.4"E) has a longer water renewal time and organic-rich deposits (organic carbon content (Corg) = 12.0%, median grain size = 0.042 mm). Shallower transitional area (Site B, 55°26'40.1" N/ 21°10'57.8"E; Site C 55°20'25.9"N/21°11'24.4"E) is characterized by shorter water renewal time, and sandy sediments with low organic matter content ($C_{org} = 0.1-0.3\%$, median grain size = 0.209-0.223 mm). Sediments were collected using a hand corer with stainless-steel tubes (i. d. 8 cm, length 30 cm) at each of the three sites. The upper 0-5 cm layer was subsampled, transferred to glass jars and kept at -20 °C. Prior to analysis, sediments were freeze-dried for 48 h, grinded using agate mortar and pestle, and homogenized by sieving through a stainless-steel sieve (0.5 mm, Retsch GmbH, Germany). At stations with fine sand deposits (Site B and C), large debris of shells (> 0.5 mm) were also separated and mashed prior to analysis. In total, four types of shells (only Site C): i) a mixture of 95% gastropods + mussels together and 5% of unionid, ii) unionid, iii) gastropods, and iv) mussels were submitted to analysis.

3. Results and discussion

Phthalate esters can be categorized into two main groups: low molecular weight (LMW; 3–7 carbon atoms in their chemical backbone) and high molecular weight compounds (HMW; 7–13 carbon atoms in their chemical backbone). These two groups have different applications, toxicological properties, and legal requirements. In the present study, our focus was on LMW PAEs, as HMW PAEs are not associated with adverse health effects and do not cause endocrine disruption [2,37]. Among the LMW PAEs, only four compounds, namely DBP, BB2P, DEHP and DIBP are classified as very dangerous substances by REACH [2], Talanta Open 8 (2023) 100258

suggesting their use as model compounds for the optimization of the extraction conditions.

The choice of the extraction solvent is always a critical step in the development of a novel extraction procedure. A wide variety of organic solvents have been proposed to extract PAEs from sediment, including hexane/AC mixtures (1:1, v:v) [9,12], pure DCM or mixture of solvents, such as DCM/MeOH (1:1, v:v) or DCM/AC (1:1, v:v) [13,38,39]. Unfortunately, most organic solvents are potentially dangerous, and extra care must be taken during sample handling, recycling or disposal [40].

Preliminary results (data not shown) showed that the use of hexane, both pure and in mixture with AC was able to produce lower GC-MS responses compared to DCM, so only the performance of DCM (pure or in mixture with MeOH) or AC was evaluated. DCM has been used to extract PAEs from different matrices including water, sediment, plastic toys, food, and other biological matrices [41,42]. However, the addition of water-miscible solvents, such as AC or MeOH, could increase the extraction canability of the mixture due to both increased hydrophilicity and water/organic solvent partitioning. As previously reported, residual water present in the sample can reduce the extraction efficiency [43] by reducing the wettability of the surface, thus limiting the extraction of the most apolar compounds. By contrast, the use of a water-miscible organic modifier facilitates PAEs extraction by allowing a better penetration of the solvents into the sample [44]]. In this context, the extraction of PAEs relies on the establishment of weak London dispersion forces, dipole-based interactions, and hydrogen bonding with carboxylic groups of the analytes.

In this study, following the Quality by Design framework, the optimization of the UAE extraction conditions was carried out in subsequent steps, consisting of a screening phase for obtaining preliminary information on the effects of the investigated CMPs on the CMAs, and a subsequent RSM to obtain a map of the predicted CMAs values throughout the experimental domain. The final step was represented by the definition of the MODR.

3.1. Screening phase

Preliminary experiments were carried out to identify the CMPs, i.e. the factors potentially affecting the effectiveness of UAE, and to select their experimental domain for the screening phase (Table A1). To reduce environmental impact, cost and time, the range of factors, such as extraction time (shaking and ultrasonic) and solvent volume, was selected based on the minimum value reported in the literature (10/15 min extraction time [10,33] and 5/10 ml of solvent [10,39]).

A Free-Wilson model was postulated to investigate the relationship between the CMPs and the CMAs [45]. This includes one constant term A_0 and a number of coefficients for each factor equal to the number of considered levels minus one. Each factor was investigated at three levels, so the model comprised two coefficients for each factor:

 $y = A_0 + A_1A + A_2A + B_1B + B_2B + C_1C + C_2C + D_1D + D_2D$

In this model, y is the response (CMA), namely the area of each of the considered compounds, A is *SHA time*, B is *US time*, C is *SOLV* type and D is *VOL* (see Table A1). A $3^4//9$ symmetric screening matrix was selected to estimate the coefficients, thus highlighting the effect of the considered factors for each CMA. This type of matrix is very useful to achieve preliminary information on both the significant factors to be considered in the study and on the experimental domain to be studied in subsequent RSM [45,46]. The experimental domain investigated and the $3^4//9$ symmetric screening matrix used in this study are reported in Table A1.

Graphical analysis of effects was performed, and the results obtained from the different models are plotted in Fig. 1 referring to DiBP (Fig. 1a, b), DBP (Fig. 1c,d), BB2P (Fig. 1e,f) and DEHP (Fig. 1g,h). In the plots on the left (Fig. 1a,c,e,g) the bar length is related to the effect of the change of level of each CMP on the CMA; each bar is related to a specific pair of levels. Hence, the bar named as b1/2-1 shows the entity of the effect





Fig. 1. Screening graphic analysis of effects for DiBP (a, b), DBP (c, d), BB2P (e, f), DEHP (g, h). In (a, c, e, g) plots the length of the bar is related to the entity of the effect produced by a change of level of the factors; the statistically significant effects are coloured in orange. In (b, d, f, h) plots the bar length is related to the effect of the single level on the response.

when changing level between the medium one (2) and the low one (1) of the first considered CMP, i.e. shaking time. In the same way, b1/3-1refers to the effect observed when moving from high level (3) to low level (1) of the same CMP. In the plots on the right (Fig. 1b,d,f,h) the length of the bar is related to the effect of each single level of the CMPs (blue bar, low level; green bar, medium level; red bar, high level) on the CMA.

The analysis of the results reported in Fig. 1 allowed for the identification of the CMPs levels leading to a maximization of the CMAs. As for SHA time, the best level was the medium one for all the analytes, apart from DEHP, for which no significant effect was observed. Thus, this factor was set at 15 min (medium level) for RSM. As concerns US time, the graphs for all the analytes evidenced that the maximization of all the four CMAs was in general obtained at low-medium levels. consequently the new experimental domain to be studied in the RSM phase was moved towards low levels, corresponding to 8-15 min. As for SOLV type, it was observed that for DiBP and DBP the best results were obtained by using DCM/AC, whereas for DEHP the best solvents were either DCM or DCM/ AC. Finally, the highest extraction for BBzP was obtained by using the DCM/MeOH mixture. On the basis of these findings, considering that DCM/AC was able to effectively extract the majority of the analytes, the DCM/AC mixture was selected as the best compromise for the simultaneous extraction of the investigated PAEs. As for VOL, increased peak areas were obtained at medium and high level for both DiBP and DBP. whereas low levels were required for BBzP extraction. The effect of this factor was not significant on DEHP. Since no common trend was observed for this CMP, the same experimental domain as in the screening was studied in the RSM phase. The experimental conditions to be investigated through RSM are summarized in Table A1.

3.2. Response surface methodology

To obtain detailed information on the effects of both US time and VOL, RSM was carried out. RSM makes use of optimization designs to generate tridimensional response surfaces or bidimensional contour plots which allow the dataset to be described and previsions of the response values to be made with the purpose of selecting the optimal conditions [33,47].

A second-order polynomial model was postulated linking the CMPs to the CMAs and investigating the presence of curvature. The following equation was hypothesized relating the response *y* (CMA) to the independent factors *x_i* (CMPs), where linear (β_i), interaction (β_{ij}) and quadratic (β_{ij}) coefficients are included, β_0 is the intercept and ε is the experimental error.

$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon$

A CCD was used to estimate the coefficients of the model. This design is composed by 2^k Full Factorial Design points, 2k star points and nexperiments at the center of the design. In this case, the factorial and the star points lied equidistant from the center, obtaining a circumscribed CCD [45,46]. The factors were studied at five levels (- α , -1, 0, +1, + α), where α =1.41. Due to the low number of factors involved, the design was replicated for obtaining a reliable estimate of the experimental variance, for a total of eighteen experiments including two experiments at the center of the experimental domain. No mathematical transformation of the responses was required. Model refining led to negligible improvements in the quality parameters of the model, thus all the coefficients were retained, regardless of their statistical significance. All the four models resulted valid and significant by ANOVA (p < 0.05), apart from DiPB, whose model was not valid due the high reproducibility observed (DiPB reproducibility = 0.935). However, for DiPB, as for all the other responses, the quality parameters of the model (Table A3) were good [33]. The values of coefficient of determination R^2 ranged from 0.716 to 0.914, whereas the values of coefficient of predicted variation Q^2 were in the 0.39-0.814 range. Therefore, all the models could be used for drawing and investigating the contour plots.

The coefficient plots, which provide a graphical representation of both the weight and the significance of the model terms, are represented in Fig. A1. *US time* exerted a significant positive effect on DiBP and DBP, whereas *VOL* exerted a significant positive effect on all the responses, with a higher weight (longer bar) with respect to *US time*. Negative quadratic effects were found for DiBP (both for time and volume) and for DBP (volume). There was a significant *US time***VOL* positive interaction only for DEHP.

The contour plots obtained plotting VOL vs. US time are reported in Fig. 2. The best results for each CMA corresponded to the red-coloured

4



Fig. 2. RSM contour plots of the CMAs obtained plotting volume of extractant vs. ultrasonic time.

zones, where the maximization of the responses was achieved. For all the CMAs high values of VOL were preferred, whereas for US time different behaviors were observed, even if a medium value of this CMP could represent a good compromise among the different CMAs.

This aspect was confirmed by observing the sweet spot plot shown in Fig. A2. This plot is built by overlaying all contour plots for individual CMAs to define the area where all the required response criteria are met. The color scale, reported in the legend, makes it possible to distinguish the zone where all the CMAs requirements are satisfied, highlighted in bright green and corresponding to the sweet spot, as well as the zones where three, two, one, or no CMA requirements are satisfied, coloured in green, teal, blue and white, respectively. The following minimum and target values were set for the CMAs: DiBP, 7800–8000; DBP, 7300–7500; BBzP, 1900–2000; DEHP, 6800–7000. These values were fixed by considering the responses measured when running the CCD, in particular, the target values were approximately between the median and the 75% quartile of the measured results, to obtain a good compromise for all the CMAs.

The MODR was established considering not only the predicted average values of the CMAs but also the probability of fulfilling the CMA requirements, combining the information of calculated models and Monte-Carlo simulations [32]. The desired level of probability that the requirements for the CMAs are met was set to 90%, corresponding to 10% risk of failure, thus meaning a target value for DPMO equal to 100, 000 [32]. The model error was included in the predictions of response distributions and the risk of failure was plotted in the probability map shown in Fig. 3. The MODR was calculated from a robust set-point which corresponded to 13.1 min and 18.0 min for US time and VOL, respectively, and was represented by the green zone, included in the 10% isoprobability line. The MODR interval, reported in Table A1, was calculated as: US time, 10.5-15.0 min; VOL, 15.1-20.0 min. The MODR was validated by testing four verification points spanning the interval and verifying the satisfaction of the CMA targets. The working point was selected to reduce both the time required for the extraction and the amount of solvent used, limiting costs and environmental pollution. The following conditions were set: US time, 12.0 min; VOL, 17.5 mL.

Talanta Open 8 (2023) 100258

Talanta Open 8 (2023) 100258



Fig. 3. Probability map by plotting ultrasonic time vs. volume of extractant. The risk of failure is represented; the MODR is coloured in green and is included in the 10% isoprobability line.

3.3. Method validation

Table 1

DEHP

DOP

0.2

0.2

E. Lorre et al.

The method was validated by operating under the optimized conditions. Validation was carried out considering some of the most used and harmful PAEs as target analytes: as previously stated, all the selected compounds are present in lists of substances of very high concern and widely found in the environment [48,49]. In addition to their inclusion in the US EPA priority pollutant list [50], DEHP, DBP, DIBP and BBzP have been recognized as compounds having endocrine disrupting effects to human health according to Regulation (EC) No 2021/2045 [51]. DEHP has been also identified as having endocrine disrupting properties for which there is scientific evidence of probable serious effects to the environment. Among the target analytes, DEHP, DBP, DOP, and BBzP were prohibited in childcare products; in addition, the use of BBzP, DBP, DEHP in the electrical and electronic equipment was banned since 2019 [52]. To extend the potential applications of the optimized method, DMP and DEP were also included into the validation study. Although these PAEs are not classified as carcinogenic, mutagenic, endocrine disruptors, or reproductive toxicant substances by REACH, they are present in the US EPA priority pollutant list [50]. Finally, due to the increasing use of phthalate-free alternatives like adipate in the plasticizer market and environment [6], DEHA was also investigated for potential environmental discharge due to its widespread use as a low-toxic phthalate-free alternative [53].

LOD and LOQ values in the 0.1–0.7 and 0.4–2 ng $g^{\rm -1}$ range for

sediments, and in the 0.1-0.5 and 0.4-2 range for shells, were achieved (Table 1 and Table 2)

Linearity was assessed in the LOQ-150 ng g⁻¹ range for each analyte (Tables 1 and 2). As for precision, good results were obtained in terms of intermediate precision, with RSDs always lower than 20%. No significant difference among the mean values obtained over 3 days was observed by applying ANOVA (p > 0.05). Since no certificated reference material was available for PAEs in sediment, the trueness of the method was evaluated by spiking sediment and shells samples. Recovery rates -RR% in the 73(±7)-120(±10)% range and 70(±10)-111(±3)% range in sediment and shells respectively, were calculated at LOO, 25 and 150 ng g^{-1} , thus assessing the efficiency of the developed method. A matrix effect in the 12-25% range was observed, thus confirming that matrixmatched calibrations have to be performed. Finally, a preconcentration factor of 12 was calculated. An example of a GC-MS chromatogram of a sediment sample spiked with the investigated analytes is shown in Fig. A3.

Overall, it can be stated that the LOD values achieved by the proposed method were either better or comparable with those reported in previous studies (Table 3). It has to be highlighted that the reported LODs and LOQs are lower compared to other UAE-based methods, obtaining limits similar to those achieved by using miniaturized sample extraction techniques (Table 3). Despite the preconcentration factor may not be remarkable, the proposed method offers several additional advantages including low costs, speed, and the availability of the instrumentation in all analytical labs. With respect to the traditional Soxhlet extraction, which is time- and solvent-consuming, UAE, MAE, and ASE can be considered as greener approaches, i.e. more time- and solvent-efficient techniques. As reported in Table 3, the proposed method is based on the use of 17.5 ml of extraction solvent, which is lower than those used in previous UAE-based methods for PAEs extraction [9,12,41,54]. Although MAE and ASE require lower solvent consumption with respect to UAE, concerns related to the cleaning of sample cells need to be considered, since potential cross contamination can occur, thus affecting the achievement of reliable results especially when trace analyzes are performed [8,11]. UAE can be also considered an efficient technology in terms of energy consumption, since it does not require high temperature and pressure conditions. Another key point of this technique relies on its high versatility, allowing for the optimization of many extraction variables depending on the matrix and the targeted analytes.

3.4. Analysis of real sample

20

10

9

3

To demonstrate the feasibility and applicability of the proposed method, solid matrices (lithogenic particles + shells debris or live mollusc individuals, n = 27) from 9 different sedimentary environments of the Curonian Lagoon were analyzed. Samples and the obtained results are given in Tables 4 and A4.

Plasticizers were detected in all samples, indicating that PAEs are

 110 ± 20

 100 ± 10

 100 ± 10

87±3

89±2

88±4

,	., .0						1						
Analyte	LODs (ng g ⁻¹)	s LOQs g ⁻¹) (ng g ⁻¹)	Regression coefficient* b(±s _b) Sediment	Repeatability (RSD%) Level (ng g ⁻¹)			Intermediate precision (RSD%) Level (ng g ⁻¹)			Trueness (RR%%±SI Level (ng g	Trueness (RR%%±SD) Level (ng g ⁻¹)		
				LOQ	25	150	LOQ	25	150	LOQ	25	150	
DMP	0.2	0.8	1.72 (±0.02)	7	4	2	20	10	10	90±20	73±8	80±10	
DEP	0.1	0.4	1.55 (±0.02)	8	2	2	20	20	10	100 ± 20	80±10	78±9	
DiBP	0.3	0.8	0.76 (±0.03)	3	3	2	9	9	5	120 ± 10	92±8	85±4	
DBP	0.2	0.7	0.76 (±0.02)	6	2	0.8	10	6	2	120 ± 10	83±6	82±2	
BBzP	0.7	2	1.90 (±0.04)	9	5	3	9	7	2	100 ± 10	90±7	84±2	
DFHA	0.1	0.5	1 96 (+0.04)	7	5	2	20	10	10	100 ± 20	90+10	86+9	

2

LODs LOOs regression coefficients precision (RSDs%) and trueness (RR%) for sediment samples

0.71 (±0.01)

0.71 (±0.01)

Regression equation: y = b x. Intercept not significant for all the analytes.

0.6

0.7

E. Lorre et al.

Table 2

LODs, LOQs, regression coefficients, precision (RSDs%) and trueness (RR%) for shell samples.

Analyte	LODs (ng g ⁻¹)	LOQs (ng g ⁻¹)	Regression coefficient* b(±s _b) Shell	Repeatability (RSD%)			Interme (RSD%)	Intermediate precision (RSD%)			Trueness (RR%%±SD)		
				LOQ	25	150	LOQ	25	150	LOQ	25	150	
DMP	0.2	0.5	1.45 (±0.01)	10	6	1	20	10	2	70±10	84±5	78.0 ± 0.7	
DEP	0.1	0.4	1.328 (±0.007)	20	9	2	10	7	2	90±20	90±20	87±2	
DiBP	0.2	0.6	0.62 (±0.01)	10	4	4	10	3	2	77±8	90±4	96±4	
DBP	0.1	0.4	0.667 (±0.003)	10	2	2	9	2	2	90±10	97±2	98±2	
BBzP	0.5	2	1.82 (±0.01)	20	1	2	10	2	3	90±10	108 ± 1	109±2	
DEHA	0.4	1	1.77 (±0.01)	10	5	2	10	3	1	90±10	104±5	111 ± 3	
DEHP	0.1	0.4	0.693 (±0.006)	10	10	7	10	8	4	96±9	100 ± 10	102±7	
DOP	0.2	0.7	0.695 (±0.004)	3	2	0.3	3	2	1	89±2	98±2	100.9 ± 0.3	

* Regression equation: *y* = *b x*. Intercept not significant for all the analytes.

Table 3

Comparison of LOD values achieved in this work (sediment samples) with previous studies for determination of PAEs.

Refs.	Extraction method	Compounds	Solvent	Solvent volume (ml)*	LOD (ng g ⁻¹)
This study	UAE	7 PAEs + DEHA	DCM/AC (1:1)	17.5	0.1-0.7
[11] Reid et al., 2009	ASE	4 PAEs	DCM/AC (1:1)	NS	15
[13] Xu et al., 2008	UAE	DBP, DEHP	DCM/AC (1:1)	NS	10
[55] Huang et al., 2008	ASE	6 PAEs	Ethyl acetate	6	6-11
[56] Ramirez et al., 2019	MAE	6 PAEs + DEHA	MeOH	15	15
[10] Fernández-González et al., 2017	UAE + HS-SPME	6 PAEs	MeOH	5	1-79
[57] Liu et al., 2014	Soxhlet	16 PAEs	DCM	300	1.12-8.59
[58] Hassanzadeh et al., 2014	Soxhlet	DBP, DEHP	DCM	300	1 (DBP); 8 (DEHP)
[41] Zhang et al., 2017	UAE	8 PAEs	DCM	50	0.12-1.60
[12] Souaf et al. 2023	UAE	4 PAEs + DEHT	hexane/AC (1;1)	30	10-80
[9] Cao et al. 2022	UAE	15 PAEs	AC/hexane (10:1)	20	0.27-5.85
[54] Jiménez-Skrzypek et al., 2020	UAE + dSPE*	10 PAEs + DEHA	AC+DCM	22	LOQ:
					0.020-4.0

* m-dSPE: micro-dispersive solid-phase extraction;

MAE. Microwave assisted extraction

ASE: accelerated solvent extraction

HS-SPME: headspace solid phase extraction

dSPE: dispersive solid phase extraction

NS: Not specified.

ubiquitous pollutants in the Curonian Lagoon. Regarding the compositional profiles of sediments, only 4 plasticizers were detected in the samples collected from the Curonian Lagoon. DEHP was the most predominant PAE and was observed in all samples, accounting for 66–93% of the total plasticizer amount. DiBP and DBP were the other predominant PAEs and were detected in 94% and 77% of the samples, respectively. However, their relative contribution to the total concentration of plasticizers ranged only from 0 to 26% and 0 to 8%, respectively, which is in line with the values observed in other contaminated areas [59]. These findings can be explained considering that DEHP is characterized by a high octanol-water partitioning coefficient (K_{ow}) compared to other PAEs (Table A2), thus indicating its tendency to be bound with particles [4]. In previous years, PAEs, particularly DEHP, accounted for the vast majority of plasticizers used. However, due to health concerns and legal restrictions, numerous phthalate plasticizer manufacturers have converted to phthalate-free alternative compounds such as adipates or citrates, which now account for a significant part of the EU plasticizer market [6,60]. DEHA was detected only at Station A (Table 4) at a concentration level ($6.5 \pm 1.6 \text{ ng}$ g⁻¹) lower than those of other PAEs identified at this station. However, its detection in the Curonian Lagoon sediments encourages the development of new monitoring programs including the evaluation of alternatives free-phthalate plasticizers.

Hydrophobic organic compounds such as PAEs are mainly accumulated in the organic matter fractions of particles [61], explaining the

Table 4

Average concentrations (ng g⁻¹, n = 3) of PAEs in sediment samples collected in the Curonian Lagoon (SE Baltic Sea).

Station	Sediment Type C_{org} (%) Concentration \pm SD (ng g^{-1})										
			DMP	DEP	DiBP	DBP	BBzP	DEHA	DEHP	DOP	Total
А	Sediment	$15.10 {\pm} 0.09$	<lod< td=""><td><lod< td=""><td>12±3</td><td>< LOD</td><td><lod< td=""><td>6 ± 2</td><td>60±20</td><td><lod< td=""><td>80±20</td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td>12±3</td><td>< LOD</td><td><lod< td=""><td>6 ± 2</td><td>60±20</td><td><lod< td=""><td>80±20</td></lod<></td></lod<></td></lod<>	12±3	< LOD	<lod< td=""><td>6 ± 2</td><td>60±20</td><td><lod< td=""><td>80±20</td></lod<></td></lod<>	6 ± 2	60±20	<lod< td=""><td>80±20</td></lod<>	80±20
В	Fine sediment	$0.41 {\pm} 0.01$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	6 ± 2	< LOD	6 ± 2
В	Shells debris mix ^a	-	< LOD	< LOD	2.3 ± 0.5	0.7 ± 0.3	< LOD	< LOD	6 ± 1	< LOD	9 ± 2
С	Fine sediment	$0.44 {\pm} 0.01$	< LOD	< LOD	2.5 ± 0.1	1.5 ± 0.3	< LOD	< LOD	36±1	< LOD	40±2
С	Shells debris mix ^b	1.76 ± 0.02	< LOD	< LOD	3.1 ± 0.4	1.9 ± 0.3	< LOD	< LOD	27.0 ± 0.7	< LOD	32 ± 1
С	Gastropod shells debris	2.0 ± 0.1	< LOD	< LOD	2.3 ± 0.6	2.0 ± 0.8	< LOD	< LOD	20 ± 1	< LOD	25±3
С	Unionid shells debris	4.61 ± 0.03	< LOD	< LOD	2.6 ± 0.3	1.6 ± 0.4	< LOD	< LOD	40±6	< LOD	44±7
С	Zebra mussel shells debris	2.34 ± 0.06	< LOD	< LOD	2.1 ± 0.3	1.5 ± 0.2	< LOD	< LOD	22.6 ± 0.5	< LOD	26.3 ± 0.9
С	Mussel shells ^c	$9.16{\pm}0.03$	< LOD	< LOD	3.1 ± 0.4	2.7 ± 0.5	< LOD	< LOD	73±9	< LOD	$80{\pm}10$

^a Mix of gastropod and mussel shells debris.

^b Mix of gastropods (~50%), mussels (~45%), and unionid (~5%).

^c Alive individuals; Corg – organic carbon content.

E. Lorre et al.

highest concentrations of PAEs in most organic loaded sediments (Station A, C_{org} = 15.10±0.09%; PAEs: 80±20 ng g⁻¹). However, other factors such as the presence of shells of mollusc can facilitate the accumulation of organic pollutants in the benthic compartment. At Station C, a significant difference (p < 0.001) in concentration levels among the type of sediment and shells was observed. The shells of live zebra mussels had a significantly (p < 0.001) higher concentration of PAEs than the rest of the samples (sediments and shell debris), which may be explained by considering both the higher organic content of the shells, and the particle filtration of live mussels. Mussels, and in general benthic filter-feeders, are largely used for monitoring water quality as they can bioaccumulate the contaminants dissolved in water, sequestered in surficial sediment, or bound to particulates in the water column [62,63]. Therefore, the surface of shells can be an important factor for determining the accumulation of organic pollutants. As shown in Table 4, results showed that larger unionid shells had a significantly (p < 0.05) higher amount of PAEs (44 \pm 7 ng g $^{-1}$) compared to smaller zebra mussels and gastropods (26.3 \pm 0.9 ng g⁻¹ and 25 \pm 3 ng g⁻¹, respectively). As for the shell debris mix, it has to be noticed that unionid shells represented a small portion of the mixture (~5% of the total weight), thus explaining the similar concentration between the shell debris mixture $(32\pm1 \text{ ng g}^{-1})$ and gastropod and mussel shells, which represented the 95% of remaining debris (Table A4). These findings suggest that the presence of shells debris or living molluscs in organic poor sediments canincrease the potential retention of organic pollutants in environment depending on the type of shells.

In general, the total concentration of plasticizers in the analyzed samples ranged from 6 ± 2 ng g⁻¹ to 80 ± 20 ng g⁻¹. This range of concentration is relatively lower compared to that found in other regions of the world, especially in China, where concentrations in sediment often exceeds 500 ng g⁻¹ and can reach over 2400 ng g⁻¹ [10,64]. Although collected sediment samples are not necessarily representative of pollution level in the Baltic area, the ubiquity of PAEs in all samples encourage the development of further monitoring studies in estuarine systems such as the Curonian Lagoon.

4. Conclusions

The results obtained in this study demonstrate that a novel reliable UAE-GC-MS method is applicable for the simultaneous determination of different PAEs in sediment samples, with good precision, recovery and detection limits. The optimization of the UAE parameters was efficiently carried out by Quality by Design principles, making it possible to identify the MODR. For the first time in the literature QbD is applied for developing an extraction procedure in environmental analysis. The developed method allowed the simultaneous determination of 8 plasticizers that are rarely considered all together in monitoring programs. Among the investigated analytes, DEHP was present in all the samples collected from estuarine system. PAEs were also found attached to the shell debris or live mussels, highlighting them as a hotspot in organic poor sandy sediments. An important future perspective is the application of the developed methodology for the determination of PAEs in complex environmental matrices, including sediments with a different level of organic matter or shell debris, to improve results deriving from environmental monitoring strategies.

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.

Data availability

Data will be made available on request.

Acknowledgments

This research was supported by the Doctorate Study Programme in Ecology and Environmental Sciences, Klaipéda University. This work has benefited also from the equipment and framework of the COMP-R Initiative, funded by the 'Departments of Excellence' program of the Italian Ministry for Education, University and Research (MIUR, 2023–2027) allocated to both the Department of Chemistry, Life Sciences and Environmental Sustainability of the University of Parma and the Department of Chemistry "Ugo Schiff", University of Florence.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.talo.2023.100258.

References

- M. Rahman, C.S. Brazel, The plasticizer market: an assessment of traditional plasticizers and research trends to meet new challenges, Prog. Polym. Sci. 29 (2004) 1223–1248, https://doi.org/10.1016/j.progpolymsci.2004.10.001.
- [3] R. Hauser, A.M. Calafat, Phthalates and human health, Occup. Environ. Med. 62 (2005) 806–818, https://doi.org/10.1136/oem.2004.017590.
- [4] C.A. Staples, D.R. Peterson, T.F. Parkerton, W.J. Adams, The environmental fate of phthalate esters: a literature review, Chemosphere 35 (1997) 667–749, https://doi. org/10.1016/s0045-6535(97)00195-1.
- (B) 10.1019 S0073635247 (0175917) (B) EC European Commission, Directive 2008/105/EC of the European parliament and the council of 16 December 2008 of environmental quality standards in the field of water policy, amending and subsequently repealing council directives 82(176/ EEC, 83/513/EEC, 84/156/EEC, 86/280/EEC and amending directive 2000/60. EC of the European parliament and of the council, OJ L 348/84, (2008). (6] R. Nagorka, J. Soschoreck, Trends for plasticizers in German freshwater
- [6] R. Nagorka, J. Koschorreck, Trends for plasticizers in German freshwater environments – evidence for the substitution of DEHP with emerging phthalate and non-phthalate alternatives, Environ. Pollut. 262 (2020), 114237, https://doi.org/ 10.1016/j.envpol.2020.114237.
- [7] S. Josefsson, Contaminants in Swedish offshore sediments 2003–2021. Results from the national environmental monitoring programme, Geological Survey of Sweden (SGU), Uppsala, 2022. http://um.kb.se/resolve?um=um:nbn:se:naturvardsverket: diva-10289 (accessed June 9, 2022).
- B S. Net, A. Delmont, R. Sempéré, A. Paluselli, B. Ouddane, Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): a review, Sci. Total Environ. 515–516 (2015) 162–180, https://doi.org/10.1016/j. scitotenv.2015.02.013.
- [9] Y. Cao, J. Li, R. Wu, H. Lin, J.Y. Lao, Y. Ruan, K. Zhang, J. Wu, K.M.Y. Leung, P.K. S. Lam, Phthalate esters in seawater and sediment of the northern South China Sea: occurrence, distribution, and ecological risks, Sci. Total Environ. 811 (2022), 151412, https://doi.org/10.1016/j.scitotenv.2021.151412.
- [10] V. Fernández-González, C. Moscoso-Pérez, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, Reliable, rapid and simple method for the analysis of phthalates in sediments by ultrasonic solvent extraction followed by head spacesolid phase microextraction gas chromatography mass spectrometry determination, Talanta 162 (2017) 648–653, https://doi.org/10.1016/j. talanta.2016.10.068.
- [11] A.M. Reid, C.A. Brougham, A.M. Fogarty, J.J. Roche, Accelerated solvent-based extraction and enrichment of selected plasticisers and 4-nonylphenol, and extraction of in from organoin sources in sediments, Judges and leachate soils, Anal. Chim. Acta 634 (2009) 197–204, https://doi.org/10.1016/j. aca.2008.12.032.
- [12] B. Souaf, N. Methneni, A. Beltifa, V.L. Turco, A. Danioux, F. Litrenta, M. Sedrati, H. B. Mansour, G. Di Bella, Occurrence and seasonal variation of plasticizers in sediments and biota from the coast of Mahdia, Tunisia, Environ. Sci. Pollut. Res. 30 (2023) 48532–48545, https://doi.org/10.1007/s11356-023-25687-1.
- [13] G. Xu, F. Li, Q. Wang, Occurrence and degradation characteristics of dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in typical agricultural soils of China, Sci. Total Environ. 393 (2008) 333–340, https://doi.org/10.1016/j. scitotenv.2008.01.001.
- [14] F. Zeng, K. Cui, Z. Xie, M. Liu, Y. Li, Y. Lin, Z. Zeng, F. Li, Occurrence of phthalate esters in water and sediment of urban lakes in a subtropical city, Guangzhou, South China, Environ. Int. 34 (2008) 372–380, https://doi.org/10.1016/j. envint.2007.09.002.
- [15] X. Zheng, B.T. Zhang, Y. Teng, Distribution of phthalate acid esters in lakes of Beijing and its relationship with anthropogenic activities, Sci. Total Environ. 476-477 (2014) 107-113, https://doi.org/10.1016/j.scitotenv.2013.12.111.
- [16] FDA, US Food and Drug Administration, Guidance for Industry. PAT- a framework for innovative pharmaceutical development, Manufacturing, and Quality Assurance. https://www.fda.gov/media/71012/download, 2004, (2004). (accessed March 2, 2023).

E. Lorre et al.

- [17] ICH Harmonised Tripartite Guideline, Pharmaceutical development Q8(R2) international council for harmonisation of technical requirements for pharmaceuticals for human use. https://database.ich.org/sites/default/files/Q8% 2882962942041idleine off (2009)
- [18] ICH Harmonised Tripartite Guideline, Analytical procedure development Q14 international council for harmonisation of technical requirements for pharmaceuticals for human use. https://database.ich.org/sites/default/files/ICH_ Q14_Document_Step2_Guideline_2022_0324.pdf, (2022).
- [19] S. Orlandini, S. Pinzauti, S. Furlanetto, Application of quality by design to the development of analytical separation methods, Anal. Bioanal. Chem. 405 (2013) 443–450, https://doi.org/10.1007/s00216-012-6302-2.
- [20] E. Rozet, P. Lebrun, P. Hubert, B. Debrus, B. Boulanger, Design Spaces for analytical methods, TrAC Trends Anal. Chem. 42 (2013) 157–167, https://doi.org. 10.1016/j.trac.2012.09.007.
- [21] R. Deidda, S. Orlandini, P. Hubert, C. Hubert, Risk-based approach for method development in pharmaceutical quality control context: a critical review, J. Pharm. Biomed. Anal. 161 (2018) 110-121, https://doi.org/10.1016/j.jpba.2018.07.050.
- [22] S. Orlandini, G. Hancu, Z.I. Szabó, A. Modroiu, L.A. Papp, R. Gotti, S. Furlanetto, New trends in the quality control of enantiomeric drugs: quality by designcompliant development of chiral capillary electrophoresis methods, Molecules 27 (2022), https://doi.org/10.3390/molecules27207058.
- [20] C. Ordandini, B. Pasquini, M. Stocchero, S. Pinzauti, S. Furlanetto, An integrated quality by design and mixture-process variable approach in the development of a capillary electrophoresis method for the analysis of almostriptan and its impurities, J. Chromatogr. A 1339 (2014) 200-209, https://doi.org/10.1016/j. chroma.2014.07.088
- [24] C. Ancillotti, S. Orlandini, L. Ciofi, B. Pasquini, C. Caprini, C. Droandi, S. Furlanetto, M.D. Bubba, Quality by design compliant strategy for the development of a liquid chromatographyl-rander mass spectrometry method for the determination of selected polyphenols in Diospyros kaki, J. Chromatogr. A 1569 (2018) 79–90, https://doi.org/10.1016/j.chroma.2018.07.046.
- [25] F. Bianchi, F. Fornari, N. Riboni, C. Spadini, C.S. Cabassi, M. Iannarelli, C. Carraro, P.P. Mazzeo, A. Bacchi, S. Orlandini, S. Furlanetto, M. Careri, Development of novel corrystal-based active food packaging by a quality by design approach, Food Chem. 347 (2021), 129051, https://doi.org/10.1016/j.foodchem.2021.129051.
- [26] F. Bettazzi, S. Orlandini, L. Zhang, S. Laschi, M.M. Nilsen, A. Krolicka, T. Baussant, I. Palchetti, A simple and selective electrochemical magneto-assay for sea lice eDNA detection developed with a quality by design approach, Sci. Total Environ. 791 (2021), 148111, https://doi.org/10.1016/j.scitotenv.2021.148111.
- [27] L. Renai, C.V.A. Scordo, A.E. Ghadraoui, S. Santana-Viera, J.J.S. Rodriguez, S. Orlandini, S. Furlanetto, D. Fibbi, D. Lambropoulou, M.D. Bubba, Quality by design optimization of a liquid chromatographic-tandem mass spectrometric method for the simultaneous analysis of structurally heterogeneous pharmaceutical compounds and its application to the rapid screening in wastewater and surface water samples by large volume direct injection, J. Chromatogr. A 1649 (2021), 462225, https://doi.org/10.1016/j.chroma.2021.462225.
- [28] H. Alasalwar, Z. Yildim, Ultrasound-assisted extraction of antioxidant phenolic compounds from Lavandula angustifolia flowers using natural deep eutectic solvents: an experimental design approach, Sustain. Chem. Pharm. 22 (2021), 100492, https://doi.org/10.1016/j.scp.2021.100492.
- [29] I. Ganesh Moorthy, J. Prakash Maran, S. Muneeswari Surya, S. Naganyashree, C. S. Shivamathi, Response surface optimization of ultrasound assisted extraction of pectin from pomegranate peel, Int. J. Biol. Macromol. 72 (2015) 1323–1328, https://doi.org/10.1016/jijbiomac.2014.10.037.
- [30] P. Katsampa, E. Valasmedou, S. Grigorakis, D.P. Makris, A green ultrasoundassisted extraction process for the recovery of antioxidant polyphenols and pigments from onion solid wastes using Box-Behnken experimental design and kinetics, Ind. Crops Prod. 77 (2015) 535–543, https://doi.org/10.1016/j. indcrop.2015.09.039.
- [31] L. Marzullo, O. Ochkur, S. Orlandini, L. Renai, R. Gotti, O. Koshovyi, S. Furlanetto, M.D. Bubba, Quality by Design in optimizing the extraction of (poly)phenolic compounds from Vaccinium myrtillus berries, J. Chromatogr. A 1677 (2022), 463329, https://doi.org/10.1016/j.chroma.2022.463329.
- [32] M.Á. Herrador, A.G. Asuero, A.G. González, Estimation of the uncertainty of indirect measurements from the propagation of distributions by using the Monte-Carlo method: an overview, Chemom. Intell. Lab. Syst. 79 (2005) 115-122, https://doi.org/10.1016/j.chemolab.2005.04.010.
- [33] L. Eriksson, E. Johansson, N. Kettaneh-Wold, C. Wikström, Design of Experiments, Principles and Applications, MKS Umetrics AB, Umeå, Sweden, 2000.
 [34] B. Magnusson, U. Örnemark, Eurachem Guide: The Fitness For Purpose of
- [34] B. Magnusson, U. Ornemark, Eurachem Guide: The Fitness For Purpose of Analytical Methods-A Laboratory Guide to Method Validation and Related Topics, 2nd ed., 2014. www.eurachem.org (2018).
 [35] J. Mandel, The Statistical Analysis of Experimental Data, Wiley, New York, 1964.
- [35] J. Mandel, The Statistical Analysis of Experimental Data, Wiley, New York, 1964.
 [36] P. Zemlys, C. Ferrarin, G. Umgieszer, S. Gulbinskas, D. Bellaffore, Investigation of saline water intrusions into the Curonian Lagoon (Lithuania) and two-layer flow in the Klaipėda Strait using finite element hydrodynamic model, Ocean Sci. 9 (2013)
- Kapeda stratu using imite element nyurodynamic model, ocean sci. 9 (2013) 573–584, https://doi.org/10.5194/os-9-573-2013.
 [37] EP, European Plasticisers, Ortho-phthalates, https://www.plasticisers.org/plastic iser/ortho-phthalates/, (2018).

- [38] H. Chen, W. Mao, Y. Shen, W. Feng, G. Mao, T. Zhao, L. Yang, L. Yang, C. Meng, Y. Li, X. Wu, Distribution, source, and environmental risk assessment of phihalate esters (PAEs) in water, suspended particulate matter, and sediment of a typical Yangtze River Delta City, China, Environ. Sci. Pollut. Res. 26 (2019) 24609–24619, https://doi.org/10.1007/s11356-019-05259-9.
- [39] R. Li, J. Liang, H. Duan, Z. Gong, Spatial distribution and seasonal variation of phthalate esters in the Juluong River estuary, Southeast China, Mar. Pollut. Bull. 122 (2017) 38-46, https://doi.org/10.1016/j.marpolbull.2017.05.062.
- [40] United States consumer product safety commission, prohibition of children's toys and child care articles containing specified phthalates. Final rule, Federal register, (2017).
- [41] Z.M. Zhang, H.H. Zhang, J.L. Li, G.P. Yang, Determination of phthalic acid esters in seawater and sediment by solid-phase microextraction and gas chromatographymass spectrometry, Chin. J. Anal. Chem. 45 (2017) 348–356, https://doi.org/ 10.1016/S1872-2040(17)60999-X.
- [42] Y. Guo, K. Kannan, Challenges encountered in the analysis of phthalate esters in foodstuffs and other biological matrices, Anal. Bioanal. Chem. 404 (2012) 2539–2554, https://doi.org/10.1007/s00216-012-5999-2.
- [43] O.P. Heemken, N. Theobald, B.W. Wenclawiak, Comparison of ASE and SFE with soxhlet, sonication, and methanolic sapoilfication extractions for the determination of organic micropollutants in marine particulate matter, Anal. Chem. 69 (1997) 2171–2180, https://doi.org/10.1021/ac9606951.
- [44] F. Fornari, F. Bianchi, N. Riboni, F. Casoli, A. Bacchi, P.P. Mazzeo, P. Pelagatti, M. Careri, Metal-organic framework-based magnetic dispersive micro-solid-phase extraction for the gas chromatography-mass spectrometry determination of polycyclic aromatic compounds in water samples, J. Chromatogr. A 1671 (2022), 463010, https://doi.org/10.1016/j.chroma.2022.463010.
- [45] G.A. Lewis, D. Mathieu, R. Phan-Tan-Luu, Pharmaceutical Experimental Design, CRC Press, 1998.
- [46] J.L. Groupy, Methods for Experimental Design: Principles and Applications for Physicists and Chemists, Elsevier, 1993.
- [47] M.A. Bezerra, R.E. Santelli, E.P. Oliviera, L.S. Villar, L.A. Escaleira, Response surface methodology (RSM) as a tool for optimization in analytical chemistry, Talanta 76 (2008) 965–977, https://doi.org/10.1016/j.talanta.2008.05.019.
- [48] ECHA (European Chemicals Agency), Candidate list of substances of very high concern for authorisation. https://echa.europa.eu/substances-restricted-und er-reach/ (2023).
 [49] Y. Liu, Y. Tang, Y. He, H. Liu, S. Tao, W. Liu, Riverine inputs, spatiotemporal
- [49] Y. Liu, Y. Tang, Y. He, H. Liu, S. Tao, W. Liu, Riverine inputs, spatiotemporal variations, and potential sources of phthalate esters transported into the Bohai Sea from an urban river in northern China, Sci. Total Environ. 878 (2023), 163253, https://doi.org/10.1016/j.scitotenv.2023.163253.
- [50] EPA, Priority Pollutant List, https://www.epa.gov/sites/default/files/2015-09/do cuments/priority-pollutant-list-epa.pdf, (2014).
- [51] EC European Commission, Commission regulation (EU) 2021/2045 of 23 november 2021 amending annex XIV to regulation (EC) No 1907/2006 of the European parliament and of the council concerning the registration, Evaluation, authorisation and restriction of chemicals (REACH) (Text with EEA relevance) (OJ L 418 24.11.2021, p. 6, ELI: http://data.europa.eu/eli/reg/2021/2045/oj), (2021).
- [52] ECHA (European Chemicals Agency), Hot topics, phthalates https://echa.europa. eu/hot-topics/phthalates, (2023).
 [53] G. Malarvannan, M. Onghena, S. Verstraete, E. van Puffelen, A. Jacobs,
- [53] G. Malarvannan, M. Onghena, S. Verstratet, E. van Puffelen, A. Jacobs, I. Vanhorebeek, S.C.A.T. Verbruggen, K.F.M. Joosten, G.V. den Berghe, P.G. Jorens, A. Covaci, Phthalate and alternative plasticizers in indwelling medical devices in pediatric intensive care units, J. Hazard. Mater. 363 (2019) 64–72, https://doi. org/10.1016/j.hlpzmat.2018.09.087.
- [54] G. Jiménez-Siczypek, J. González-Sálamo, D.A. Varela-Martínez, M.Á. González-Curbelo, J. Hernández-Borges, Analysis of phthalic acid esters in sea water and sea sand using polymer-coated magnetic nanoparticles as extraction sorbent, J. Chromatogr. A 1611 (2020), 460620, https://doi.org/10.1016/j. chroma.2019.460620.
- [55] P.C. Huang, C.J. Tien, Y.M. Sun, C.Y. Hsieh, C.C. Lee, Occurrence of phthalates in sediment and biota: relationship to aquatic factors and the biota-sediment accumulation factor, Chemosphere 73 (2008) 539–544, https://doi.org/10.1016/j. chemosphere.2008.06.019.
- [56] M.M.B. Ramirez, R.D. Caamal, J.R. von Osten, Occurrence and seasonal distribution of microplastics and phthalates in sediments from the urban channel of the Ria and coast of Campeche, Mexico, Sci. Total Environ. 672 (2019) 97–105, https://doi.org/10.1016/j.scitotenv.2019.03.472.
- [57] H. Liu, K. Cui, F. Zeng, L. Chen, Y. Cheng, H. Li, S. Li, X. Zhou, F. Zhu, G. Ouyang, T. Luan, Z. Zeng, Occurrence and distribution of phthalate esters in riverine sediments from the Pearl River Delta region, South China, Mar. Pollut. Bull. 83 (2014) 358–365, https://doi.org/10.1016/j.marpoblul.2014.03.038.
- [58] V. Hassanzadeh, A.E. Sari, S. Khodabandeh, N. Bahramifar, Occurrence and distribution of two phthalate esters in the sediments of the Anzali wetlands on the coast of the Caspian Sea (Iran), Mar. Pollut. Bull. 89 (2014) 128–135, https://doi. org/10.1016/j.marpolbul.2014.10.017.
- org/10.1016/j.marpolbul.2014.10.017.
 Y.S. Lee, J.E. Lim, S. Lee, H.B. Moon, Phthalates and non-phthalate plasticizers in sediment from Korean coastal waters: occurrence, spatial distribution, and

Talanta Open 8 (2023) 100258

٥

E. Lorre et al.

ecological risks, Mar. Pollut. Bull. 154 (2020), 111119, https://doi.org/10.1016/j. arnolbul 2020 111119

- [60] ECPI, European council for plasticisers and intermediates, plasticisers and flexible [60] ECPI, European council for plasticisers and intermediates, plasticisers and flexible PVC (2018). https://www.plasticisers.org/wp-content/uploads/2018/10/EC PI proposal V190 15022016.pdf (2018).
 [61] A.A. Koelmans, F. Gillissen, W. Makatita, M.V.D. Berg, Organic carbon normalisation of PCB, PAH and pesticide concentrations in suspended solids, Water Res. 31 (1997) 461–470, https://doi.org/10.1016/S0043-1354(096)00280-1.
 [62] S.R. De Solla, E.A.M. Gillory, J.S. Klinck, L.E. King, R. McInnis, J. Struger, S. M. Backus, P.L. Gillis, Bioaccumulation of pharmaceuticals and personal care

Talanta Open 8 (2023) 100258

- products in the unionid mussel Lasmigona costata in a river receiving wastewater effluent, Chemosphere 146 (2016) 486–496, https://doi.org/10.1016/j.
 (63) N. Topić Popović, V. Lorencin, I. Strunjak-Perović, R. Čož-Rakovac, Shell waste management and utilization: mitigating organic pollution and enhancing sustainability, ApJ. Sci. 13 (2023), https://doi.org/10.390/app13010623.
 (64) S. Net, R. Sempéré, A. Delmont, A. Paluselli, Ouddane, occurrence, fate, behavior and ectoxicological state of phthalates in different environmental matrices, Environ. Sci. Technol. 49 (2015) 4019–4035, https://doi.org/10.1021/es505233b.

10

PAPER II

Lorre, E., Bianchi, F., Vybernaite-Lubiene, I., Mėžinė, J., Zilius, M., 2023. Phthalate esters delivery to the largest European lagoon: Sources, partitioning and seasonal variations. Environ. Res. 116667. https://doi.org/10.1016/j.envres.2023.116667

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version
Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

PAPER III

Lorre, E., Bianchi, F., Mėžinė, J., Politi, T., Vybernaite-Lubiene, I., Zilius, M., 2024. The seasonal distribution of plasticizers in estuarine system: Controlling factors, storage and impact on the ecosystem. Environ. Pollut. 345, 123539. https://doi.org/10.1016/j.envpol.2024.123539

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

PAPER IV

Lorre, E., Bianchi F., Broman E., Bonaglia S., Nascimento F.J.A, Samuiloviene A., Woźniczka A., Zilius M. 2024 Phthalate esters in baltic lagoons: Spatial distribution, ecological risks, and novel insights into their fate using transcriptomics. Sci. Total Environ. 957, 177526. https://doi.org/10.1016/j.scitotenv.2024.177526

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Puslapis pasiekiamas tik spausdintinėje versijoje / Page available only in a print version

Klaipėdos universiteto leidykla

Elise Marie Clemence Lorre PHTHALATE ESTERS IN BOREAL ESTUARINE SYSTEMS: SOURCES, DISTRIBUTION AND FATE Doctoral dissertation

FTALATŲ ESTERIAI BOREALINĖSE ESTUARIJŲ SISTEMOSE: ŠALTINIAI, SKLAIDA IR LIKIMAS Daktaro disertacija

Klaipėda, 2025

SL 1335. 2025 04 23. Apimtis 10,76 sąl. sp. l. Tiražas 20 egz. Klaipėdos universiteto leidykla, Herkaus Manto g. 84, 92294 Klaipėda Tel. (8 46) 398 891, el. paštas: leidykla@ku.lt, interneto adresas: http://www.ku.lt/leidykla/ Spausdino UAB "Vitae Litera", Savanorių pr. 137, 44146 Kaunas