

Leaching of Phthalate Esters from Microplastics into Seawater

Dissertations

zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen
- **Dr. rer. nat.** -

Fakultät der Christian-Albrechts-Universität
zu Kiel

Presented by
Jeyakumar Dhavamani

Supervised by
Prof. Eric P. Achterberg

Kiel, Germany - 2022

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Summary

The importance of understanding the leaching process of chemical additives from plastics in the marine environment has increased dramatically due to the increased loading of plastics to coastal and Open Oceans and the perceived risks of leachates because of their genotoxic effects. The relationship between the presence of plastic debris (PDs) and the concentration of additives could be used as a marker of exposure to plastic pollution in the ocean, but very few studies link these pollutants in seawater. In the current dissertation, the leaching process of six phthalic acid esters (PAEs) from three common consumer plastics was critically examined and properly addressed. The relationship between PAEs and plastic debris in the Red Sea was investigated. Major attempts to measure and control blank contamination problems were critically attempted and properly assigned.

One of the main problems of my study is that quantifying PAEs with good precision and reliability is a challenge due to blank contamination from the materials used in sampling and analysis. Great efforts have been made to measure and control the problems of blank contamination. The mass of PAEs in the blank by selected materials ranged from (3 ± 0.7 to 35 ± 6 ng) for liquid-liquid extraction (LLE) and from (5 ± 1.8 to 63 ± 15 ng) for solid-phase extraction (SPE). In the experiments, there are a few materials that have a high PAE contamination, such as sterile tips, PP syringes, and filtered and unfiltered artificial seawater (ASW) 96.3, 72.2, 110.0, and 57.0 ng, which were not used further. This information can be particularly useful in the analysis of PAEs, where it can be of great importance to obtain an accurate determination of plastic additives in a complex environment.

Average recoveries of PAEs in LLE (90-97%) were determined with successive aliquots of 2 mL, 1 mL, and 1 mL dichloromethane (DCM). In SPE, recoveries up to 86-90% were achieved with successive aliquots of 5, 3, and 2 mL of DCM and hexane (80:20) at a sample flow rate of 5 mL/min. The method quantification limits (MQLs) of PAEs obtained by the LLE and SPE methods were similar to the average reported MQLs (0.3-20 ng/L). In conclusion, this method is reliable in quantifying blank contamination and the MQL meets the requirements for the analysis of PAEs in seawater. Analysis times were reduced for LLE (1-2 hours) and SPE (6-7 hours), and peak resolution was good in most cases.

Summary

The current thesis reports a clear understanding of the leaching process of six PAEs from three common consumer plastics, low and high-density polyethylene (LDPE, HDPE) and recycled polyethylene (RP). The effects of salinity, temperature, and ultraviolet irradiation (UVR) on leaching were investigated. Temperature and UVR had a positive effect on the leaching rate while increasing salinity had a negative effect on the leaching rate. The approach used in this study to measure PAEs takes into account the re-adsorption loss of each target compound during the leaching process. Significant re-adsorption of PAEs was observed for all three polymers, which can reduce the amount of actual/total leachate in the dissolved phase by up to 30-80%. This is an important step in understanding the hazards and extent of exposure to additives from plastic pollution.

The second major focus of the work is to investigate the relationship between PAEs and PDs in the marine environment. We investigated the abundance of PDs and PAEs in the surface waters of Sharm Obhur Bay and the Red Sea. PAE concentrations in the study area ranged from 0.8 to 1224 ng/L, while the abundance of PDs ranged from (0.0301-0.0374 PDs/m³). A positive correlation was observed between the abundance of PDs and the concentration of PAEs, suggesting that a large portion of the dissolved PAE pool may be due to in situ leaching. The calculated ecological risk level (ERL) due to PAEs and PDs for Sharm Obhur and the Red Sea is currently at a low to moderate level.

Overall, the work presented in this thesis helps in improving the available knowledge of the leaching process of PAEs from plastics under different environmental conditions. Particularly, in proper assigning the re-adsorption loss in the measurement of total leachates in water. On the other hand, the latest information regarding the distribution of PAEs and plastic waste and their ecological risk in the surface waters of the Red Sea and Sharm Obhur Bay is reported. An improved method for the analysis of PAEs was developed, including the control of blank contamination, and the experimental conditions such as extraction time and temperature were optimized. This information may be useful in the analysis of PAEs where it can be of great importance to obtain a precise determination of plastic additives in the complex environment

Zusammenfassung

Die Bedeutung des Verständnisses des Auslaugungsprozesses chemischer Zusatzstoffe aus Kunststoffen in der Meeresumwelt hat aufgrund der zunehmenden Belastung der Küsten und der offenen Ozeane mit Kunststoffen und der wahrgenommenen Risiken von Auslaugungen aufgrund ihrer genotoxischen Wirkungen drastisch zugenommen. Die Beziehung zwischen dem Vorhandensein von Plastikmüll und der Konzentration von Additiven könnte als Marker für die Exposition gegenüber der Plastikverschmutzung im Meer dienen, aber nur sehr wenige Studien stellen eine Verbindung zwischen diesen Schadstoffen im Meerwasser her. In der vorliegenden Dissertation wurde der Auslaugungsprozess von sechs phthalsäureestern (PAEs) aus drei gängigen Verbraucherkunststoffen kritisch untersucht und richtig zugeordnet. Der Zusammenhang zwischen PAEs und Plastikmüll im Roten Meer wurde untersucht. Die wichtigsten Versuche zur Messung und Kontrolle der Verschmutzungsprobleme wurden kritisch eingeordnet.

Eines der Hauptprobleme meiner Studie ist, dass die Quantifizierung von PAEs mit guter Präzision und Zuverlässigkeit eine echte Herausforderung darstellt, da die bei der Probenahme und Analyse verwendeten Materialien eine Blindkontamination aufweisen. Es wurden große Anstrengungen unternommen, um die Probleme der Blindwertkontamination zu messen und zu kontrollieren. Die Masse der PAEs in der Blindprobe durch ausgewählte Materialien reichte von $(3 \pm 0,7$ bis 35 ± 6 ng) für die Flüssig-Flüssig-Extraktion (LLE) und von $(5 \pm 1,8$ bis 63 ± 15 ng) für die Festphasen-Extraktion (SPE). Bei den Experimenten gab es einige Materialien, die eine hohe PAE-Kontamination aufwiesen, wie sterile Spitzen, PP-Spritzen und gefiltertes und ungefiltertes künstliches Meerwasser (ASW) 96,3, 72,2, 110,0 und 57,0 ng, die nicht weiter verwendet wurden. Diese Informationen können insbesondere bei der Analyse von PAEs nützlich sein, wo es von großer Bedeutung sein kann, eine genaue Bestimmung von Kunststoffadditiven in einer komplexen Umgebung zu erhalten.

Durchschnittliche Wiederfindungen von PAEs in LLE (90-97 %) wurden mit aufeinanderfolgenden Aliquoten von 2 mL, 1 mL und 1 mL Dichlormethan (DCM) erzielt. Bei SPE wurden Wiederfindungen von bis zu 86-90 % mit aufeinanderfolgenden Aliquoten von 5, 3 und 2 mL DCM und Hexan (80:20) bei einer Probenflussrate von 5 mL/min erzielt. Die mit den LLE- und SPE-Methoden ermittelten Bestimmungsgrenzen (MQL) für PAEs entsprachen den durchschnittlichen berichteten MQLs (0,3-20 ng/L). Zusammenfassend lässt sich sagen, dass diese Methode zuverlässig bei der Quantifizierung von Blindkontaminationen ist und der MQL die Anforderungen für die Analyse von PAEs in Meerwasser erfüllt. Die Analysezeiten wurden für LLE (1-2 Stunden) und SPE (6-7 Stunden) reduziert, und die Peakauflösung war in den meisten Fällen gut.

Zusammenfassung

In der vorliegenden Arbeit wird ein klares Verständnis des Auslaugungsprozesses von sechs PAEs aus drei gängigen Verbraucherkunststoffen, Polyethylen niedriger und hoher Dichte (LDPE, HDPE) und recyceltem Polyethylen (RP), vermittelt. Die Auswirkungen von Salzgehalt, Temperatur und ultravioletter Bestrahlung (UVR) auf die Auslaugung wurden untersucht. Temperatur und UVR wirkten sich positiv auf die Auslaugungsrate aus, während ein steigender Salzgehalt eine negative Auswirkung auf die Auslaugungsrate hatte. Der in dieser Studie verwendete Ansatz zur Messung der PAEs berücksichtigt den Readsorptionsverlust der einzelnen Zielverbindungen während des Auslaugungsprozesses. Für alle drei Polymere wurde eine signifikante Readsorption von PAEs beobachtet, die die Menge des tatsächlichen/gesamten Sickerwassers in der gelösten Phase um bis zu 30-80 % reduzieren kann. Dies ist ein wichtiger Schritt zum Verständnis der Gefahren und des Ausmaßes der Belastung durch Zusatzstoffe aus der Kunststoffverschmutzung.

Der zweite Schwerpunkt der Arbeit ist die Untersuchung der Beziehung zwischen PAEs und PDs in der Meeresumwelt. Wir untersuchten die Häufigkeit von PDs und PAEs in den Oberflächengewässern der Bucht von Sharm Obhur und des Roten Meeres. Die PAE-Konzentrationen im Untersuchungsgebiet reichten von 0,8 bis 1224 ng/L, während die Häufigkeit von PDs zwischen 0,0301 und 0,0374 PDs/m³ schwankte. Es wurde eine positive Korrelation zwischen der Häufigkeit von PDs und der PAE-Konzentration festgestellt, was darauf hindeutet, dass ein großer Teil des gelösten PAE-Pools auf In-situ-Auswaschung zurückzuführen sein könnte. Das berechnete ökologische Risikoniveau (ERL) aufgrund von PAEs und PDs für Sharm Obhur und das Rote Meer liegt derzeit auf einem niedrigen bis mäßigen Niveau.

Insgesamt trägt die in dieser Arbeit vorgestellte Arbeit dazu bei, das vorhandene Wissen über den Auslaugungsprozess von PAEs aus Kunststoffen unter verschiedenen Umweltbedingungen zu verbessern. Insbesondere bei der korrekten Zuordnung des Readsorptionsverlustes bei der Messung der Gesamtauswaschung in Wasser. Andererseits wird über die neuesten Informationen über die Verteilung von PAEs und Kunststoffabfällen und deren ökologisches Risiko in den Oberflächengewässern des Roten Meeres und der Bucht von Sharm Obhur berichtet. Es wurde ein verbessertes Verfahren für die Analyse von PAEs entwickelt, das auch die Kontrolle der Leerwertkontamination umfasst, und die Versuchsbedingungen wie Extraktionszeit und -temperatur wurden optimiert. Diese Informationen können insbesondere bei der Analyse von PAEs nützlich sein, wo es von großer Bedeutung sein kann, eine genaue Bestimmung von Kunststoffzusätzen in einer komplexen Umgebung zu erha

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Contents

Summary	I
Zusammenfassung.....	III
Acknowledgments.....	V
Contents	VI
List of Figures.....	X
List of Tables	XII
List of abbreviations and symbols	XIII

Chapter 1. Introduction: Microplastic and chemical additives in the marine

Environment	1
1.1. Microplastics.....	2
1.2. Occurrence and sources of plastic pollution in the marine environment.....	4
1.3. Levels of pollution by microplastics in the marine environment	7
1.4. Physical and chemical impacts of microplastics in the marine environment	12
1.5. Parameters controlling the leaching of plastic additives into the marine environment.	17
1.6. Leaching protocols of phthalate esters from different polymers	19
1.7. Most common techniques used for phthalate esters analysis of microplastics and phthalate esters	23
1.8. Aim of the current study	25
1.9. Chapter overview	26

**Chapter 2. Improved method for GC-MS analysis in seawater of phthalates from
polyethylene following liquid-liquid and solid-phase extraction**

Abstract	31
2.1. Introduction.....	32
2.2. Materials and methods	33
2.2.1. Reagents and Materials	33
2.2.2. Instrumentation	34
2.2.3. Recommended procedures to avoid blank contamination	34
2.2.4. Liquid-liquid extraction	35
2.2.5. Solid-phase extraction.....	36

2.3.Results and Discussion	36
2.3.1. Programming of GC-MS analysis.....	36
2.3.2. Blank contamination levels.....	39
2.3.3. Optimization of liquid-liquid extraction	43
2.3.4. Optimization of the solid Phase extraction	45
2.3.5. Analytical performance and figures of merits	48
2.4.Real sample analysis	50
2.5.Conclusion and recommendations for future studies.....	51
Credit author contribution.....	52
Acknowledgements	52
Appendix supplementary information	53
 Chapter 3. The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater	 63
Abstract.....	65
3.1.Introduction.....	66
3.2.Materials and methods	68
3.2.1. Reagents and Materials\.....	68
3.2.2. Experimental setup.....	69
3.2.3. Extraction and analysis	70
3.2.4. Instrument calibration and method validation	71
3.2.5. Mass balance equations.....	71
3.3. Results.....	72
3.3.1. Dissolved concentration of leached PAEs (L_{dis}).....	72
3.3.2. Adsorption of spiked PAEs (S_{ads})	76
3.3.3. Total amount of PAEs leached ($A L_{total}$).....	78
3.3.4. Effect of salinity, temperature and UV radiation on the total amount of PAEs leached ($A L_{total}$)	79
3.3.5. Leaching rates	81
3.3.6. Diffusivity of PAEs.....	82
3.4.Discussion	85

3.5.Conclusions and Future Perspectives	90
Credit author contribution.....	91
Acknowledgements.....	91
Appendix supplementary information	92
Chapter 4. Partitioning of phthalate esters and plastic debris and their ecological risk level in surface waters of the Red Sea and the bay of Sharm Obhur	111
Abstract.....	113
4.1.Introduction.....	114
4.2.Materials and methods	115
4.2.1. Reagents and materials	115
4.2.2. Sampling	116
4.2.2.1.Net sampling	116
4.2.2.2.Surface water samples.....	117
4.2.3. Polymer identification of net-collected plastic particles.....	117
4.2.4. Extraction.....	118
4.2.5. GC-MS analysis.....	118
4.2.6. Method validation	119
4.2.7. Risk assessment related to PAEs and PDs.....	119
4.3.Results.....	121
4.3.1. Blank test experiment	121
4.3.2. Concentration of PAEs in surface seawater.....	121
4.3.3. Plastic debris abundance in surface seawater	123
4.3.4. Morphological and color characteristics of PDs in surface seawater	123
4.3.5. Correlation between PDs, PAEs and environmental parameters.....	125
4.3.6. Potential ecological risk of PAEs and PDs.....	127
4.4.Discussion.....	129
4.5.Conclusion and future perspectives	133
Credit author contribution.....	134
Acknowledgements.....	134
Appendix supplementary information	135

Chapter 5. Conclusions, future perspectives and limitations	141
5.1.General conclusions	142
5.2.Future perspectives and limitations	144
6. References.....	146
7. Statement of declaration.....	157

List of Figures

Figure 1.1: Global plastic production from 1950 -2016 (a), microplastic abundance in the seas worldwide (b) and the number of research publication on microplastic pollution (c).....	3
Figure 1.2: Microplastics, their transfer to other organisms, how they are distributed in the food chain, and how they affect human and animal health.....	5
Figure 1.3: Spatial distribution of the measured microplastic concentration (particles/m ³) as indicated by the color and size of the dots.....	11
Figure 2.1: GC-MS Chromatograms of PAE compounds at different retention times with conformational ions for the respective compounds	37
Figure 2.2: The effect of different parameters on the extraction efficiencies, desorption solvent (a) extraction cycle (b) half-life time in dark and light (c) and standard addition test (d)	45
Figure 2.3: Effect of different factors on the extraction efficiencies, elution solvent (a), elution cycle (b), half-life time at dark and light (c) and standard addition test (d)	47
Figure 3.1: Average concentrations of dissolved, adsorbed, and the ratio of adsorbed to dissolved spikes PAEs in experiment B for LDPE (a), RP (b) and HDPE (c) under the freshwater experiment conditions (S0.1 g/L), respectively	75
Figure 3.2: A comparison on the impact of the experimental conditions on the leaching of dissolved, adsorbed, and adsorbed-to-dissolved mass ratios of DBP and DEHP from LDPE (a & b), RP (c & d) and HDPE (e & f) polymers, respectively	77
Figure 3.3: The diffusion coefficient (D_e) for PEs versus the number of carbons (N_c) of PEs in fresh water (0.1 g/L), at high temperature (40°C), and UVR (354 nm) conditions for LDPE (a), RP (b) and HDPE (c). The D_e results of PEs with straight-chain (S_c) and branched chain carbons (B_c) were separated by dotted lines	84
Figure 4.1. Map showing the sampling area (Red Sea; Jeddah region in the insert) and enlarged (yellow arrow) locations of sampling stations in the bay of Sharm Obhur to the open waters of the Red Sea	116
Figure 4.2: The concentrations of PAEs (a) and plastic debris (b), distribution of \sum_6 PAEs (c) and plastic debris (d) in samples of surface water from Sharm Obhur (highlighted in yellow), Red Sea slope (highlighted in red) and the Red Sea shelf (highlighted in blue)	122

List of figures

Figure 4.3: Distribution pattern of plastic debris of PDs (PDs/m ³) in different colors (a), shapes (b), polymer types (c) and sizes (d) at sampling sites. Inserts the average proportion (%) of Sharm Obhur and the Red Sea surface seawater	125
Figure 4.4: Correlations between abundance distribution of PDs and \sum_6 PAEs in Sharm Obhur (a), Red Sea, Shelf (b) and Red Sea, Slope (c)	127
Figure 4.5: Risk quotient and health index of PAEs (a), pollution load index (PLI) PDs and HI index of polyethylene polymer (b) for each sampling site and the average (insert Table) in Red Sea surface water samples	128

List of Tables

Table 1.1: Main sources of plastics and microplastics by usage sectors	6
Table 1.2: Levels of microplastic as determined by various methods	8
Table 1.3: Additives and their functions in plastics and their hazard classification	13
Table 1.4: Levels of phthalate esters in different marine water worldwide	15
Table 1.5: A summary of the most common leaching protocols to evaluate environmental plastics	21
Table 2.1: Physiochemical properties of phthalate esters	34
Table 2.2: GC–MS method parameters.....	37
Table 2.3: Calculated value from instrument, method validation calibration curves	38
Table 2.4: The total mass of DEPs (ng) in each sequence of blank testing for the corresponding materials added	41
Table 2.5: Blank contamination of PAEs in the liquid-liquid extraction method	42
Table 2.6: Blank contamination of PAEs in the solid-phase extraction method	42
Table 2.7: Figures of merits of the established and published methods for PAEs determination	49
Table 3.1: The dissolved leachate (ng/mL) at Q_m for LDPE, RP and HDPE polymer at different salinities (0.1, 20 and 40 g/L), temperature (10, 20 and 40°C), and UVR (354nm)	74
Table 3.2: Difference between the dissolved and calculated total leachate (ng/cm ²) at Q_m for LDPE, RP and HDPE polymer at freshwater condition (S 0.1 g/L)	79
Table 3.3: Leaching rate of PEs in fresh water (0.1 g/L), high temperature (40°C), and UVR (354 nm) treatments (i.e., fastest leaching conditions for each category) for LDPE, RP and HDPE polymer. In HDPE low temperature condition (10°C) were used to compare due its higher leaching then high temperature condition (40 °C)	83
Table 4.1: Average concentration (ng/L) pattern of PAEs in the surface seawater of Sharm Obhur and Red Sea. “ \sum PAEs” are the sum of each PAE from all 22 sampling sites. “Proportion” is the ratio of each \sum PAE to the total \sum PAE concentration.....	130

Abbreviations

ASW	Artificial Sea Water
ATR	Attenuated total reflectance
BBA	Bisphenol A
BBP	Benzyl butyl phthalate
BBzP	Butyl benzyl phthalate
BEP	Di(2-ethylhexyl) phthalate
BFRs	Rominate flame retardants
BFRs	Brominated flame retardants
BHT	Butylated hydroxytoluene
BPA	Bisphenol A
CMR	Carcinogenic, mutagenic and reprotoxic chemicals
CRM	Certified reference materials
DBP	Di-n-butyl phthalate
DBP	Di-n-butyl phthalate
DBT	Dibutyltin dichloride
DBTL	Dibutyltin dilaurate
DCM	Dichloromethane
DEHP	Di(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
DEP	Dimethyl phthalate
DHP	Diheptyl phthalate
DiBP	Diisobutyl phthalate
DINP	Diisononyl phthalate
DMEP	Bis(2-methoxyethyl) phthalate
DMP	Dimethyl phthalate

Abbreviations

DnBP	Di-n-butyl phthalate
DnNP	Dinonyl Phthalate
DnOP	Di-n-octyl phthalate
DnOP	Di-n-octyl phthalate
DOC	Dissolved organic carbon
EDC	Endocrine-disruptor
EDS	Energy-dispersive X-ray spectroscopy
EI	Electronic impact
ERL	Ecological risk level
FESEM	Field Emission Scanning Electron Microscope
FFEM	Fluorescence excitation-emission matrix
FID	Flame ionization detector
FTIR	Fourier-transform infrared spectroscopy
GC-MS	Gas Chromatography and Mass spectrometry
HBCD	Hexabromocyclododecane
HDPE	High-density polyethylene
HMW	High molecular weight
HPLC	High performance liquid chromatography
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
INB	Instrument blank
LCMS	Liquid chromatography and mass spectrum
LDPE	Low density polyethylene
LLE	Liquid-liquid extraction
LMW	low molecular weight
LOD	The instrumental detection
LOQ	Quantification limits
MEC	Measured Environmental Concentration

Abbreviations

MQLs	Method quantification limits
MRM	Multiple reaction monitoring
NPs	Nonylphenols
PAEs	Phthalic acid esters
PAHs	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PBT	Bioaccumulative and toxic
PC	Polycarbonate
PCB	Polychlorinated biphenyls
PCBs	Polychlorinated biphenyls
PDs	Plastic debris
PE	Polyethylene
PET	Polyethylene terephthalate
PLI	Pollution index
NEC	Predicted No Effect Concentration
POPs	Persistent organic pollutants
PP	Polypropylene
PRI	Polymer risk index
PS	Polystyrene
PVC	Polyvinyl chloride
RP	Recycled polyethylene
RQ	Risk quotient
RSD	Relative standard deviation
SIM	Single ion monitoring
SPC	Specific conductance
SPE	Solid-phase extraction
TBBPA	Tetrabromobisphenol A

Abbreviations

TDS	Total dissolved solids
TOC	Total organic carbon
UVR	Ultraviolet irradiation
°C	Degree Celsius
µg/L	Microgram/Liter
µg/mm ²	Microgram/square millimeter
µL	Microliter
g/kg	Gram/kilogram
g/L	Gram/Liter
g/mol	Gram-molecule
kg	Kilogram
m ⁻³	Cubic meter
mg L ⁻¹	Milligram/Liter
ml/min	Milliliter/minute
mm	Millimeter
mm ²	Square millimeter
ng	Nanogram
ng/cm ²	Nanogram/square centimeter
ng/L	Nanograms per liter
ng/mL	Nanogram/milliliter
PDs/m ³	Plastic Debris/cubic meter
pg/L	Picogram/Liter
pH	Potential of Hydrogen
µg/mL	Microgram/milliliter
lx	Lux

Chapter 1

Microplastics and chemical additives in the marine environment

Chapter 1. Microplastics and chemical additives in the marine environment

1.1. Microplastics

Plastics are ubiquitous in our world, either in the form of products or as pollutants. The versatile properties of plastics make them ideal materials for a wide range of household and industrial applications (Gewert et al. 2015, Xie et al. 2005). Over the last 60 years, average annual plastic production has increased from 1.5 to 311 million tons in 2014 and is expected to increase by about 1800 million tons by 2050 (UNEP 2016a)(Table 1.1a). Since the 1970s, plastics have been used in increasingly innovative ways and their release into the environment has increased dramatically. Originally, plastic pollution was thought to be an esthetic problem. Plastic waste enters the marine environment through runoff from land and sewage discharges or through maritime activities such as fishing and aquaculture (Law 2017). About 8 million tons of plastic waste enter the world's oceans every year, and the oceans may already contain over 150 million tons of plastic and this amount may increase to 250 million tons by 2050 (Gallo et al. 2018) (Table 1.1b).

Floating plastic debris in the ocean can be transported long distances by winds and currents and tends to accumulate in ocean eddies and gyres, and confined coastal waters (Sanchez-Avila et al. 2012). During the time that plastics remain in the ocean, the large plastic particles are slowly decomposed by weathering processes (Andrady 2011). As a result, most of the plastic debris in the oceans and coastal waters consists of small plastic fragments < 5 mm referred to as microplastics (MPs) (Eriksen et al. 2014). The presence of MPs in marine and freshwater environments has been documented by numerous studies (Shim and Thomposon 2015, Syversen 2015). Because of this concern for the environment, the number of research papers on microplastic pollution has increased (Figure 1.1c). (Source: Web of Science). In 2004, there were less than five publications on MPs, and eight years later there were 10. From 2014 to 2022, the number of publications per year is increased from 69 to 3065 The increase shows that MPs research has become an important topic.(Jiang 2021)

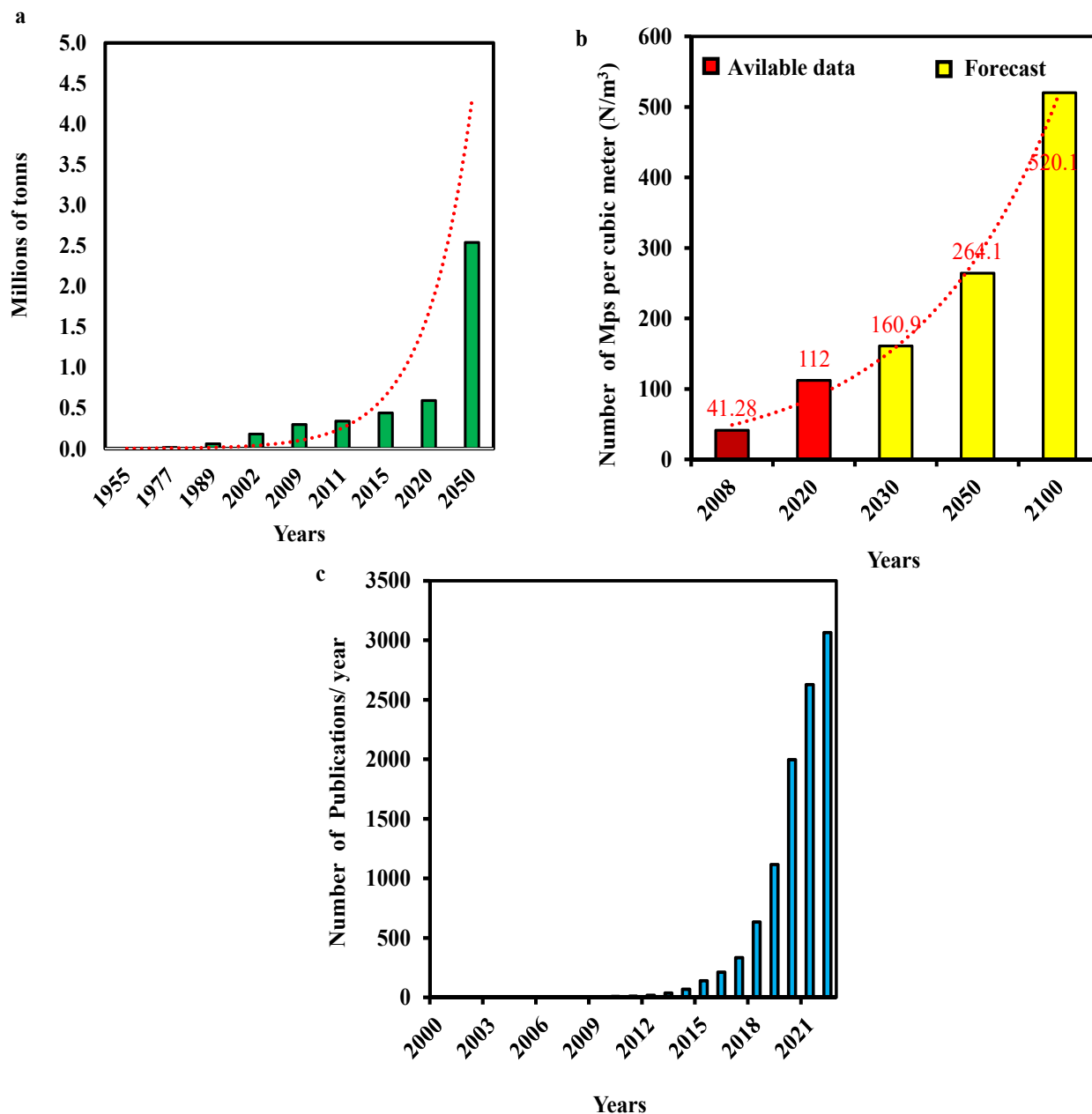


Figure 1.1 Global plastic production from 1950 to 2016 (a), micro plastic abundance in the worldwide sea (b) Adapted from plastic Europe 2017 and Plastics Europe Market Research Group (PEMRG) and Conversion Market & Strategy GmbH (D’ambrières 2019) and number research publication on microplastic pollution (c) (Bridson et al. 2021, GESAMP 2017) and updated (Source: Web of Science by using the search terms ‘plastic pellets’ and ‘MPs’).

1.2. Occurrence and sources of plastic pollution in the marine environment

Plastic debris is considered an important anthropogenic threat to marine systems (Anderson et al. 2016, Muniyasamy et al. 2008). Since the ocean has no boundaries, plastic trash moves anywhere. Currently, it is not easy to determine the sources and pathways of MPs into the ocean. There are likely ranges of sources, which differ between regions, with also temporal changes in source strengths. Most of the pollution we see today is historical in origin (Law 2017). A study by Lee et al. (2013) found that the majority of floating and beached plastic debris originated from coastal recreational activities and land-based sources in the northern South China Sea (Lee et al. 2013). Land sources include almost 80% of marine plastics (Andrady 2011). As 50% of the global population lives within 50 miles of the coast, terrestrial plastic waste reaches the coastal region through many waterways (Browne et al. 2010, Cole et al. 2011, Fatoki 2010). Researchers also found that large quantities of plastic debris derived from raw manufacturing materials were transported onto beaches following accidental spillage during handling and other processes (Tanhua et al. 2020). In the process of making plastic pellets and transporting them, plastic particles and chemicals are released into the environment E. Based on USEPA data; plastics emit 5g/kg during transportation and 0.4g/kg during the production of plastic pellets (D'ambrières 2019).

Ocean-based sources account for the remaining 20% of marine plastic debris, to which commercial fishing is the major contributing human activity. Currently, the amount of fishing gear lost to the environment has quadrupled: an estimated 640,000 tons of discarded fishing gear are added into the ocean every year, which amounts to approximately 10% of the total marine debris (Good et al., 2010). There is a significant relationship between the number of ocean-based plastic items found on beaches and the level of commercial fishing (Galgani et al. 2015). Inland plastics enter the ocean via surface runoff such as rivers and are estimated at 0.5 million tons/year (Galgani et al. 2015). Maritime activities release approximately 1.7 million tons/year of plastics into the ocean (Eunomia Research & Consulting Ltd. (2016).

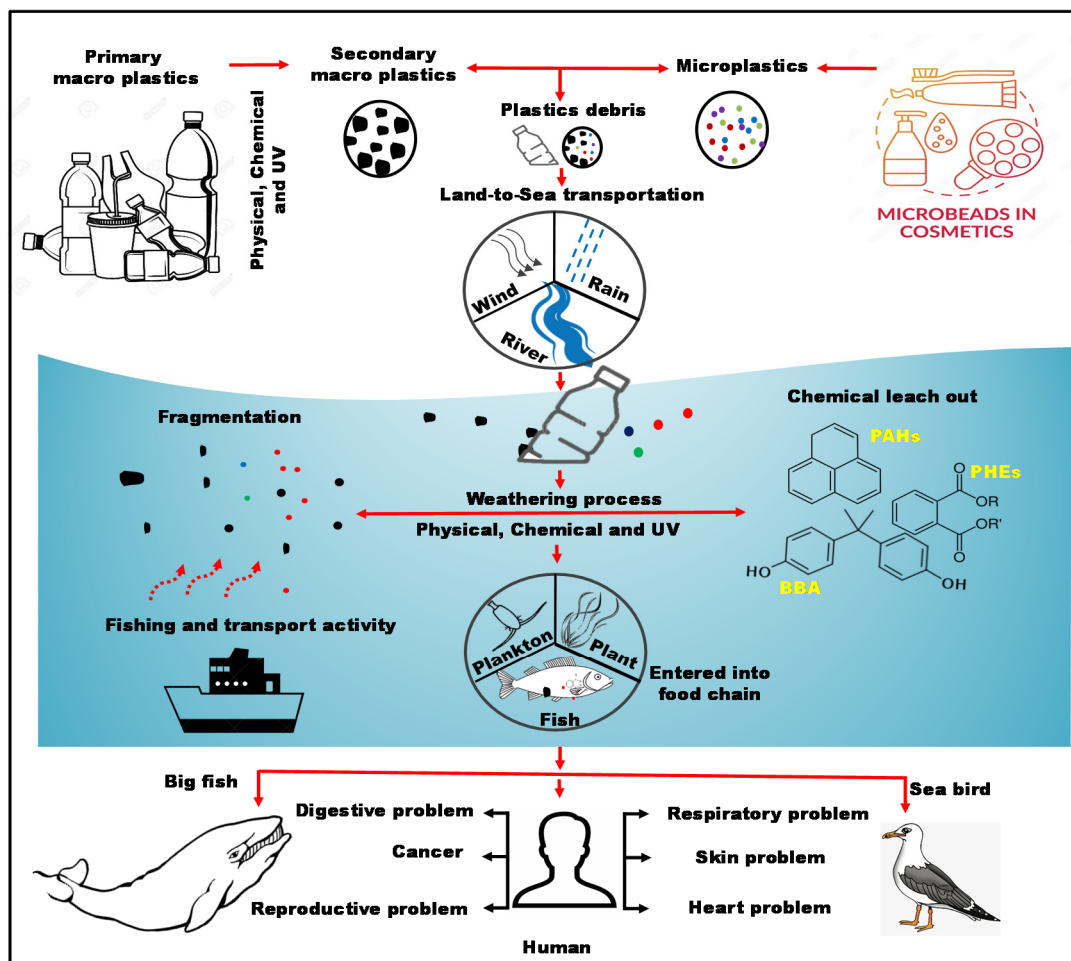


Figure 1.2 Microplastics, their transfer to other organisms, how they are distributed in the food chain, and how they affect human and animal health (Debroy et al. 2021, Liu et al. 2020a).

An average of 70 kg of plastic on each square kilometer of the seabed and worldwide, the estimated amount on beaches is five times greater and the overall average concentration is much higher at 2,000 kg/km²(Sherrington 2016)). The total amount of plastic entering the oceans from land worldwide by linking global data on improper management and population density has been estimated and this amount of plastic from land could increase by 2025 as reported (Sherrington 2016). In the schematic diagram (Figure 1.2), the pathway of microplastics, their transfer to other organisms, how they are distributed, and their effects on health to human and animals are shown. The main sources of marine plastics from various categories are listed in Table 1.1 (GESAMP 2017).

Table 1.1 Main sources of plastics and microplastics by usage sectors (Syversen 2015)

Category	Source sector	Description	Entry points
Producers/Converters	Plastic Producers, Fabricators & Recyclers	Pellets & fragments	Rivers, Coastline, Atmosphere
Sectoral, consumers	Agriculture	Greenhouse-sheets, pots, pipes, nutrient pills	Rivers, Coastline, Atmosphere
	Fisheries	Fishing gear, packaging	Rivers, Coastline (e.g. ports), Marine
	Aquaculture	Buoys, lines, nets, PVC pipes	Rivers, Coastline, Marine
	Construction	EPS, packaging	Rivers, Coastline, Atmosphere
	Terrestrial, Transportation	Pellets, tires, tire dust	Rivers, Coastline, Atmosphere
	Shipping/Offshore industry	Paints, pipes, clothes, miscellaneous, plastic-blasting, cargo	Rivers, Marine
	Tourism industry	Consumer goods, packaging, microbeads, textile fibers	Rivers, Coastline, Marine
	Textile industry	Fibers	Rivers, Coastline, Atmosphere
	Sport	Synthetic turf	Rivers, Coastline, Atmosphere
Individual consumers	Food & drink, single-use, packaging	Containers, plastic bags, bottles, caps, cups, plates, straws, spoons, etc.	Rivers, Coastline
	Cosmetics & personal care products	Microbeads, packaging, toothbrushes, etc.	Rivers, Coastline, Marine
	Textiles & clothing	Fibers	Rivers, Coastline, Atmosphere, Marine
Waste management	Solid waste,	Unmanaged or poorly managed waste disposal	Rivers, Coastline, Atmosphere
	Water & wastewater	Microbeads, fragments, fibers	Rivers, Coastline

1.3. Levels of pollution by microplastics in the marine environment

It is estimated that roughly 8 million tons of plastic litter enter the ocean on an annual basis (Jambeck et al. 2015, Tanhua et al. 2020). Currently, the fate of the majority of this plastic is unknown. Only a small fraction of the plastic input can be accounted for by scaling up observational estimates to a global scale (Law 2017). MPs have been found in the surface ocean and suspended sediments in the water column, but also sediments and the deep ocean. Plastic has also been found in freshwater, although there have been fewer studies than in the marine environment (Avio et al. 2017, Coyle et al. 2020). However, the distribution of marine plastics in the water column, or at the sea surface, is partially known from number of studies and reviews (Law 2017), although different sampling and measurement techniques make direct comparisons difficult (Woo et al. 2021). The concentration of MPs found is directly determined by the sampling method used, which can vary greatly from study to study. Concentrations of MPs obtained by a range of methods used for sampling, detection, and identification are listed in Table 1.

The use of different sampling and measurement methods makes direct comparisons between obtained results of MPs abundances difficult. Thus, several studies have been conducted worldwide, but few have used a single method to investigate the concentrations of MPs in the ocean (Tanhua et al. 2020). Some studies, which provide a global perspective on the distribution of MPs in the pelagic zone, take advantage of rapid measurement platforms and novel sampling platforms (Tanhua et al. 2020) (Figure 1.3).

Table 1.2. Levels of microplastic as determined by various methods*

Sampling Location	Sampling Method	Sample volume	Pre-Treatment	Instrumental Analysis Method	Amount of Particles	Ref
Scotland	Neuston net (335 µm)	from 16 to 557 m ³	Sieving	Micro-FTIR	4565 MPs /km ⁻²	Russell and Webster (2021)
Maowei Sea	Steel bucket	5 L each	Filtration (nylon membrane 5 µm pore size), chemical digestion (10% KOH)	Stereomicroscope, micro-FTIR	1.47–7.61 MPs/ L	Zhou et al. (2021))
Caspian Sea	Plankton net (300 µm)	141.37 m ³	Sieving (5 mm), filtration (S&S filter papers,	Stereomicroscope, polarized light microscope, FE-SEM with EDS, micro-Raman spectroscope	0.246 ± 0.020 MPs / m ³	Manbohi et al. (2021)
Nordic Seas	Water pump	100 L each	Filtration (stainless-steel mesh—5 mm, plankton net— 50 µm), chemical digestion (30% H ₂ O ₂), density separation (ZnCl ₂ —1.6 g/mL), sieving (stainless steel mesh— 2, 1, 0.5, 0.1, 0.05 mm)	Stereomicroscope, FTIR (only randomly selected MPs ground with potassium bromide, n = 200), scanning electron microscopy (SEM; randomly selected, n =	from 2.43 ± 0.84 to 1.19 ± 0.28 MPs /L	Jiang et al. (2019))

24), X-ray spectroscopy (EDS)						
Black Sea	Manta net (300 μm)	$84.5 \pm 6.3 \text{ m}^3$	Chemical digestion (3% H_2O_2 —only hand-sorted particles)	Stereomicroscope, optical microscope	$4.62 \times 10^4 \text{ MPs per km}^{-2}$, $0.62 \text{ MPs / m}^{-3}$	Berov and Klayn (2020))
South Yellow Sea	Water pump	100 L each	Filtration (stainless-steel mesh—5 mm, plankton net—50 μm), chemical digestion (30% H_2O_2), density separation (ZnCl_2 —1.6 g/mL), sieving (stainless steel mesh—2, 1, 0.5, 0.1, 0.05 mm)	Stereomicroscope, hot needle test, FTIR (only randomly selected items)	from 4.5 ± 1.8 to $6.5 \pm 2.1 \text{ MPs per L}$	(Jiang et al. 2019))
South China Sea	Water pump	2.041 L per second for ~10 min	Filtration (net—20 μm ; glass-fiber filters—0.4 μm)	Stereomicroscope, scanning electron microscope (SEM), micro-FTIR-ATR	1687 MPs per m^{-3} (estuary); 1900 MPs per m—C15:G203 (offshore)	Taha et al. (2021)
White Barents Kara Sea	Manta trawl (330 μm)	N/A	Sieving (5;1;0.3 mm mesh size)	FTIR, Nile Red staining	from 28,000 to 963,000 MPs per km	Torres-Agullo et al. (2021))

Chapter 1: Microplastics and chemical additives in the marine environment

Oman Sea	Water pump	10 L (each site; only 100 mL per sample was used)	Chemical digestion (30% H ₂ O ₂), filtration (glass microfiber filter; 1.2 mm)	Light microscope, micro-FTIR (only randomly selected particles; n = 150)	218 ± 17 MPs per L	Rodrigues et al. (2020)
Bohai Sea	Manta net (330 µm); Water pump	50 L (Water pump); N/A (Manta net)	Wet sieving (5 mm; 0.3 mm), chemical digestion (0.05 M Fe II; 30% H ₂ O ₂), density separation, filtration (glass-fiber filters; 0.7 µm)	Stereomicroscope, micro-FTIR	0.35 ± 0.13 MPs per m ³	Zhang et al. (2020))

* FTIR= Fourier-transform infrared spectroscopy, ATR= Attenuated total reflectance, FESEM= Field Emission Scanning Electron Microscope and EDS= energy-dispersive X-ray spectroscopy).

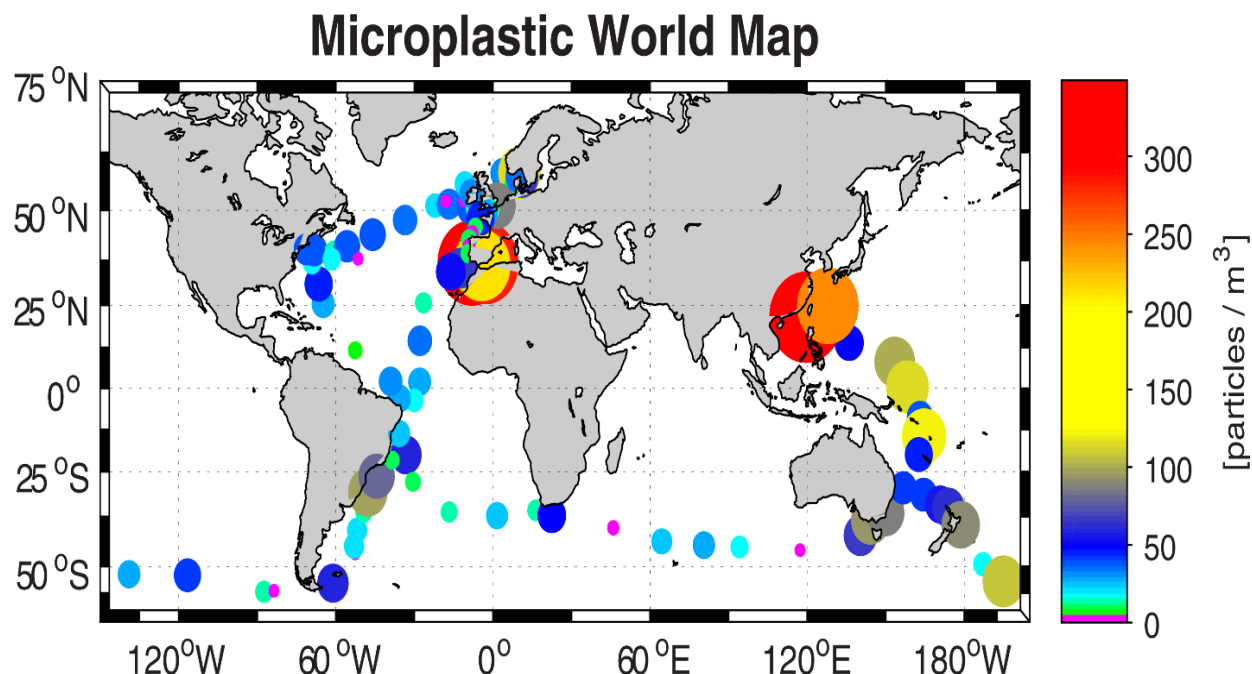


Figure1.3 Spatial distribution of the measured microplastic concentration (particles/m³) as indicated by the color and size of the dots (Tanhua et al., 2021).

MPs particles are abundant in this study, with an average abundance of 50 particles/m³, with concentrations ranging from 0.0 to 350 particles/m³. The highest levels were observed in the western tropical North Pacific, the South China Sea, and West Philippine Sea (243-349 particles/m³), the western Mediterranean Sea and Gulf of Cadiz (180 to 307 particles m⁻³), and the western tropical Pacific Ocean (86 particles m⁻³). The North Atlantic (25 particles m⁻³) and South Atlantic (29 particles m⁻³) had modest concentrations. Concentrations in the open ocean samples from the Southern Ocean were low (18 particles m⁻³). Due to higher particle concentrations near continents and in large ocean currents, the average concentration in the Southern Ocean increases to 34 particles/ m³ when samples are taken near South America, Australia, New Zealand, and Africa. Below is a map of global MP concentrations based on the results of the study (Tanhua et al. 2020).

1.4. Physical and chemical impacts of microplastics in the marine environment

The potential physical impacts of MPs and their accumulation in the environment have been documented as an emerging environmental concern (GESAMP 2017, UNEP 2016b). Benthic and invertebrate species, including corals (Hall et al. 2015), copepods (Lee et al. 2013), zooplankton and crustaceans (Cole et al. 2013, Watts et al. 2015), mollusks (Browne et al. 2008), sea cucumbers (Graham and Thompson 2009), barnacles (Thompson et al. 2004), lugworms, and polychaetes (Browne et al. 2013), have been reported to ingest plastic microparticles. The most common physical effects on organisms include digestive tract obstruction and respiratory tract irritation, potential impairment of critical biochemical processes, e.g., nutrient transfer from the gut or release to the deep sea (Avio et al. 2017, Gallo et al. 2018, Li et al. 2016). Further negative effects of microplastic particles on organisms include reduced fecundity (Lee et al. 2013), reduced feeding rate (Cole et al. 2013), reduced ability to excrete pathogenic bacteria (Browne et al. 2013), reduced energy reserves and balance (Watts et al. 2015), and reduced lysosome stability (von Moos et al. 2012).

One of the reasons for the success of plastics is the chemical additives because the additives give the plastics specific material properties such as color, flexibility, stability, and degradation resistance (Hahladakis et al. 2018a). Several thousand types of additives are used in various plastic products, which makes the chemical composition of plastic products extremely complicated (Rosato, 1998). Since most additives are not chemically or only weakly bound to the polymers (Hahladakis et al. 2018a), they can be leached out of the plastic product when in contact with media such as air, water, food, and so on. Thus, plastic pollution represents a source of chemical contaminants in the marine environment.

Table 1.3 Additives and their functions in plastic materials and their hazard classification (Hahladakis et al. 2018a)*

Functionality	Description	Level (%)	Chemical class	Example substances	Toxicity
Plasticizer	Improve fluidity of plastics during processing and flexibility at room temperature	10–70	Phthalates	DEHP	CMR, EDC, PBT
Antioxidant stabilizers	Prevent oxidation and deterioration caused by heat	0.1–3	Hindered phenolics	BHT	EDC
UV-absorbing stabilizers	Prevent the breakage of molecular bonds by UV light and radicals	0.1–3	Phenolic benzotriazoles	Bumetrizole, 2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol	PBT,
Thermal stabilizers	Inhibit thermal degradation of vinyl chloride resin during processing	0.5–3	Organotin compounds	DBT, DBTL	CMR
Flame retardants	Reduce the flammability of the material	1–25	Organo Bromides	HBCD, TBBPA	CMR, EDC, PBT,
Colorants	Enhance aesthetics and reduce light permeability	0.01–10	Inorganic pigments	Cadmium, chromium, and lead compounds	CMR

* BHT= butylated hydroxytoluene, DBT= Dibutyltin dichloride, DBTL=dibutyltin dilaurate, HBCD= Hexabromocyclododecane, TBBPA=Tetrabromobisphenol A, CMR= Carcinogenic, mutagenic and reprotoxic chemicals, EDC= Endocrine-disruptor and PBT= bioaccumulative and toxic).

Chapter 1: Microplastics and chemical additives in the marine environment

MPs represent the main source of various chemicals that are harmful to the aquatic environment, such as plasticizers, flame retardants, and antioxidants (Gao et al. 2011, Hahladakis et al. 2018b, UNEP 2016b). The most common additives observed in the marine environment are phthalate esters (PAEs), bisphenol A (BPA), nonylphenols (NPs), brominated flame retardants (BFRs), etc. (Alimi et al. 2018). Studies have confirmed that polymer additive contamination in marine waters ranges from pg/l to mg/l and that phthalates are the most commonly reported compounds (Keil et al. 2011, Net et al. 2015b, Pojana et al. 2007, Sanchez-Avila et al. 2012). PAEs account for about 92% of plasticizers produced and are therefore the most utilized plasticizers worldwide (He et al. 2013). The PAEs are considered as toxic chemicals that can have significant effects on reproduction in marine animals (Andrady 2011) and on the development of obesity and cancer in humans (Buckley et al. 2016, Ojeda et al. 2011, Wang et al. 2016). Given their potential environmental and health risks, six PAEs—dimethyl phthalates (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl benzyl phthalate (BBzP), di(2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP)—have been identified as priority pollutants by the US EPA and the European Commission (US EPA, 2014). The list of the most commonly used additives and their toxicity are summarized in Table 1.3.

The concentrations of PAEs in seawater around the world are given in Table 1.4. PAEs are among the most commonly detected persistent organic pollutants (POPs) in the marine environment. DEHP and DINP are the most important plasticizers worldwide. Several studies have shown that phthalates can be found in the marine environment, with concentrations ranging from pg/L to µg/L, while the DEHP is the most concentrated phthalate found in marine waters (Table 1.4). As demand for PAEs and products that use them as raw materials increases, water pollution becomes more serious.

Table: 1.4 Levels of phthalate esters in different marine water worldwide*

Location	PAEs concentrations in seawater µg/L							Ref
	DMP	DEP	DnBP	DiBP	BBP	DEHP	DnOP	
Tees Bay, UK	0.001	0.5	0.55	1.1		2.2		Law et al. (1991)
Klang River estuary, Australia						3.1		(Tan 1995)
Eieti district, Italy	1		3		6			Cincinelli et al. (2017)
Taiwan	1		9			18		Yuan et al. (2003)
North Sea, Germany	0.002	0.067	0.0017		0.005	0.0022		Xie et al. (2005)
Surface waters, the Netherlands	0.004	2.3	3.1	1.8	0.9	0.0078		Vethaak et al. (2005b)
North sea, Germay	1		5		6			Xie et al. (2005)
Berlin, Germany	2				4			Xie et al. (2005)
Dutch coast, Netherlands	2	1			3			Vethaak et al. (2005a)
Bay of Biscay, Spain	7.5	0.03	0.83		0.008	0.064	0.0036	Prieto et al. (2007)
Arctic	0.00004	0.00014	0.00051	0.00022	0.00008	0.0004		Xie et al. (2007)
Barkley Sound, Canada			3			0.01		Keil et al. (2011)
Puget Sound, USA						0.06		Keil et al. (2011)
Caspian Sea, Iran	0.49	0.52						Hadjmohammadi et al. (2011)
Coastal seawater, Mediterranean	0.003	0.048			0.01	0.62	0.0036	Sanchez-Avila et al. (2012)

Chapter 1: Microplastics and chemical additives in the marine environment

Port sea, Mediterranean	0.004	0.0087			0.8	5.97	Sanchez-Avila et al. (2012)
River – sea interface, Mediterraneana						0.21	Sanchez-Avila et al. (2012)
Sea, Spain Liguarian Sea,						18.38	Fossi et al. (2012)
Mediterranean Sea, Italy						23.42	Fossi et al. (2012)
Chinna-Average			12		60		Net et al. (2015b)
NW Mediterranean Sea,	0.002	0.0253	0.1032	0.089	0.0003	0.331	Paluselli et al. (2018a)
NW Pacific marginal Sea, China			0.063 to 0.169 *				Paluselli and Kim (2020)
Northern South China Sea			0.06 to 1.5 *				Cao et al. (2022)

* **Not available for each PAEs**

It is critical to understand the aquatic ecological risk assessment of PAEs, which is done by accurately determining the concentration of these compounds in the aquatic environment. Phthalates (DMP, DEP, DiBP, DnBP, BBP, DEHP, DnOP, DnNP) have been found in a wide range of organisms, including 18 different species, from primary producers (plankton and macroalgae) to the captive spiny dogfish (*Squalus acanthias*). No biomagnification of phthalates in the food web has been observed (Mackintosh et al., 2004). Workers also detected phthalates (DMP, DEP, DPRP, DiBP, DnBP, DHP, BBP, DEHP, DOHP, DnOP, DNP, and DiDP) in fish at concentrations ranging from 0.2 to 1.23 µg/g (Cheng et al., 2013).

1.5. Parameters controlling the leaching of plastic additives into the marine environment

Plastics in the ocean have been found at various depths, from the surface to sediments and beaches. Concentrations vary greatly from place to place due to hydrophobic, geomorphological, and anthropogenic factors (Galgani et al. 2015). Due to their low rate of degradation, they accumulate in the marine environment for decades. Plastics are subject to weathering process. During weathering, chemical additives are leached from the plastics, organic pollutants are adsorbed, and chemical intermediates such as oligomers and chemical fragments are accumulated, which can lead to bioaccumulation/toxicity.

Plastics can leach additives depending on their properties, chemical characteristics of the additives, and environmental factors. A polymer can have a crystalline structure with molecules arranged in a regular pattern, or an amorphous internal structure with the molecules randomly arranged (Satkowski 1990). The state of a polymer chain is however typically neither fully crystallized nor amorphous. Highly crystalline structures are glassy polymers and highly amorphous structures are called rubbery polymers. The density of the polymer increases with the percentage of crystalline structure. Glass transition temperature, density, crystallinity, degree of crosslinking, and branching are factors that determine the leaching rate of additives. The reported diffusivity of additives in plastics is greater for rubbery polymers (HDPE $D = 10 \times 10^{-10} \text{ cm}^2 \text{ S}^{-1}$ versus PVC $D = 10 \times 10^{-14} \text{ cm}^2 \text{ S}^{-1}$) than for glassy polymers (Prasad et al. 1994).

Additives are dispersed in the porous structure of the polymer. Each polymer has a unique diameter with various pore sizes; the additives pass through the voids and other gaps between the polymer molecules. The leaching rate depends on the chemical characteristics, size, and shape of the additives (Hahladakis et al. 2018a). The correlation between the pore size of the polymer and the size of the additives is directly related to the leaching rate; the larger the pores, the lower the leaching (Zaharescu and Lungulescu 2016). The average molecular weight of substances used as additives in plastics is estimated to be in the range of 200 - 2000 g/mol (Davis 2013). A higher molecular weight means a large molecule and a slow migration rate, and vice versa. The rule of thumb is used to develop additives with low migration rates by designing them with high molecular weight structures. On the other hand, the high molecular weight compounds are typically more expensive and so far, many companies use low molecular weight additives, which are more easily leached. The leaching process is facilitated for low molecular weight compounds but also by higher molecular weight compounds, which do not penetrate the pores easily.

Chapter 1: Microplastics and chemical additives in the marine environment

The water solubility of additives plays a crucial role in the leaching process where compounds of a high water solubility are leached first. The compound DMP is a common plasticizer that is relatively easily released from polymers due to its high water solubility (~5.0 g/L) (Bradlee 2003). In contrast, higher molecular weight phthalates such as DEHP are more resistant to leaching, due to their hydrophobicity and low water solubility (2.49×10^{-6} g/L) (Bradlee 2003). The low boiling point of low molecular weight additives such as formaldehyde, vinyl chloride, ethylene, and butadiene gases leach relatively easily and have a high tendency to migrate quickly even at ambient temperatures (100°C) (Davis 2013, Hansen et al. 2013). Leaching of additives that are chemically bound (reactive) to polymers e.g. Tetrabromobisphenol A (TBBPA) requires the cleavage of covalent bonds; leaching of non-reactive compounds appears thus more important than leaching of reactive compounds. These chemicals are reactively bonded to the polymer matrix and require cleavage of covalent bonds before migration can take place (Debenest et al. 2010). Functional additives like phthalates (DEHP) are covalently linked to the host polymer and can leach into the environment (Hahladakis et al. 2018a, Hansen et al. 2013). Chemical and physical factors in the environment such as dissolved organic carbon (DOC) concentration, pH, temperature, salinity, wave action, and UV radiation have a major influence on the leaching of chemicals from plastics into sea water (Suhrhoff and Scholz-Böttcher 2016). Dissolved organic carbon (DOC) in the environment also facilitates leaching due to hydrophobic interactions (Bauer and Herrmann 1998)(Bauer & Herrmann 1998). The leaching rate of phthalates is higher in vegetable oil than in water due to their lipophilicity (Romera-Castillo et al. 2018). The hydrophilicity of compounds is generally represented by the octanol/water coefficient ($\log K_{ow}$) (Bradlee 2003).

The leaching potential of additives from plastic polymers to natural water decreases in acid solutions of low pH and high ionic strength (Suhrhoff and Scholz-Böttcher 2016). The effect of salinity (ionic strength) depends on the inherent chemical properties of individual additives and cannot be generalized for the group of compounds (Suhrhoff and Scholz-Böttcher 2016). The leaching behavior of PAEs in seawater and distilled water has been reported (Suhrhoff and Scholz-Böttcher 2016). The rate of leaching increases with higher temperatures where 4 mg/kg/h of DEHP leached from PVC into Diprivan infused (lipid solution) at 32°C and up to 24.9 mg/kg/h at 37°C. Higher exposures would occur for Propoven and Intralipid, or if the infusate were at 37°C (Rose et al. 2012).

The influence of UV radiation (300 nm) on the leaching behavior of additives is reportedly small (Suhrhoff and Scholz-Böttcher 2016). The reasons could be the surface changes of the plastics, such as swelling, which creates a leaching barrier, or photodegradation of the leachate in the aqueous phase since many additives are UV sensitive. For example, acetyltri-n-butyl citrate (ATBC; 0.06 mg/g) of plastics was detected in the aqueous phase of polyethylene (PE) under UV radiation (300 15 w lamp). In the sample under UV irradiation, tributyl citrate (TBC; 0.62 mg/g) and tributyl aconite (TBA; 0.0009 mg/kg) of plastics were detected. These compounds may be formed via photodegradation of ATBC. However, the sum of TBC and TBA is not equal to ATBC, which may indicate the loss of decay products by UV irradiation. Direct analysis of the additives remaining in the polymer after UV treatment compared to the starting material could lead to a more reliable leaching rate (Suhrhoff and Scholz-Böttcher 2016)

Turbulence in the water column may have a strong influence on the leaching rate of additives. Concentration gradients on the surface of MPs are reduced by turbulence (Shareef et al., 2006). Researchers have found a positive relationship between turbulence and leaching (Suhrhoff and Scholz-Böttcher 2016). Turbulence significantly increases the diffusion rate of low molecular weight compounds. According to Suhrhoff et al. 2016, the release of BPA from PVC increases 28 to 79 times under turbulent conditions. Due to their hydrophobicity, phthalates have a lower leaching rate in water (0.020 - 121 mg/l) (Vitali et al. 1997) (DeFoe 1990). Since steady-state equilibrium cannot be established at the polymer-water interface, the relatively low molecular weight compounds diffuse into solution. Because of the turbulent flow, oxidation products form in the aqueous phase. (Suhrhoff and Scholz-Böttcher 2016).

1.6. Leaching protocols of phthalate esters from different polymers

A number of studies have been examined via leaching of additives (incl. PAEs) from plastics to precisely determine the extent and rate of release, bioavailability, and ecotoxicity. A summary of the most common protocols for analysis of additives including PAEs from plastics is summarized in Table 1.5. The leaching behavior is often evaluated by analyzing the mass loss in the main sample or the dissolved concentration in the surrounding media (Bach et al. 2014, Bridson et al. 2021, Rani et al. 2015, Suhrhoff and Scholz-Böttcher 2016, Tüzüm Demir and Ulutan 2013). In the batch mode of separation, phthalates leaching into aqueous environments is challenging due to

their low solubility, and high hydrophobicity and there are no standard methods to study leaching processes. Re-adsorption of PAEs on plastics after leaching can significantly affect transport and dissolved concentrations in the marine environment. This type of measurement may overestimate or underestimate the compounds due to their adsorption behavior, which makes it difficult to explain genotoxic effects (Wei et al. 2019). Therefore, it is necessary to improve the knowledge of the leaching process of additives in the marine environment (Bridson et al. 2021, Rodrigues et al. 2019). Whenever there is a possibility of adsorption by the polymer and the total leaching concentration is in the ultra-trace (ng- μ g/L) range, the adsorption loss ratio should be determined to evaluate the total amount of additive leached (Zhang et al. 2017b).

There are some dynamic or semi-dynamic approaches in which samples are subjected to continuous flow, such as column percolation (Bandow et al. 2017, Fikarová et al. 2019). Dynamic approaches, while somewhat more complex to implement and to achieve dynamic conditions is to use infinite solid-phase sinks that have a high adsorption capacity for additives, and columns and other materials can introduce contamination with additives from the sink itself (Henkel et al. 2019, Sun et al. 2019, Ye et al. 2020). The need to recover the infinite sink from the leaching medium and extract the analyzes from the solid phase adds additional steps that further complicate this approach (Bridson et al. 2021). Nonetheless, studies have investigated the direct adsorption and desorption kinetics of micropollutants with plastics (POPs, PAHs, PAEs, Pesticides, etc.), and these studies have only measured the adsorption and desorption of the spiked compounds without considering leaching of similar compounds from the polymer itself (Razanajatovo et al. 2018).

Table 1.5. A summary of the most common leaching protocols to evaluate environmental plastics*

Plastic properties (type; origin; size)	Leaching parameters (method; media; solid to liquid ratio; time; temperature; mixing)	Analytical technique	Analytes	Ref
PVC, PS; new product, micro	Static; fresh water; 0.1–5.0 g/L; 1–24, (Coffin et al., 2019b)	TOC, FEEM	Phthalates, BPA	Liu et al. (2020c)
PE, PVC; new product; meso	Static; seawater; 0.4–1.5 g/600 mL; 1–12 weeks (time series); 22 °C; periodic agitation	GC-MS	Phthalates	Paluselli et al. (2019)
PE, PS, PET, PVC; new product; micro	Semi-dynamic; water, salt water; 5 g/500 mL; 1–78 d (time series); RT; 150 rpm	GC-MS	Phthalates, organophosphites, oligomers	Suhrhoffer and Scholze-Böttcher (2016)
PE, PVC; new product; micro	Dynamic, seawater; 50 mg/mL (dynamic leaching repeated up to 40 cycles) at 0.25 mL/min (time series); ND; ND	HPLC	Phthalates, BPA	Fikarov et al. (2019)
PE, PVC, PS; recycled product; micro	Dynamic (DIN 19528); water; 0.3–10 L/kg at flow rate of 0.2–0.32 mL/min; total contact time 5 h (time series); ND; ND	Conductivity, pH, turbidity, TOC, cations, ICP-AES, GC-MS	Phthalates, NIAS, Cl, Ca, Cu, Ti, Zn, Cd, Pb	Bandow et al. (2017)

Chapter 1: Microplastics and chemical additives in the marine environment

PVC; new product; micro	Static (with infinite sink), water; 1 g/100 mL; 4–95 d (time series); 4–45 °C; no mixing	HPLC	Dibutyl phthalate	Ye et al. (2020)
PVC; new product; micro	Static (with infinite sink), KCl solution; 85 mg/40 mL; 0.5–50 d (time series); RT; 125 rpm	GC-MS	Phthalates	Henkel et al. (2019)
PS, plastic mixture; marine debris (beached); micro	Static; Fulmarus glacialis stomach oil; 7.5–25 g/L; 8 h-90 d; 40 °C; 120 rpm	GC-MS	Phthalates, phenolic antioxidants, benzotriazoles, organophosphates	Kühn et al. (2020)
PE, PP, PS, nylon, acetal, polyester, latex, isoprene; new products; macro-micro	Static; simulated seabird and fish gastric fluid; 0.01 g/mL; 16 h (single time point); 24 °C, 38 °C; 100 rpm	LC-MS	Phthalates, BPA derivatives, organophosphates, alkylphenols	Coffin et al. (2019)
PS; new product; macro-micro	Static; simulated invertebrate & vertebrate gastric fluid, salt water; 1 g/100 mL; 24 h (single time point); 18 °C, 24 °C; 100 rpm	LC-MS, bioassay	Phthalates, BPA derivatives	Coffin et al. (2019)

* Size = mega (> 1 m), macro (25–1000 mm), meso (5–25 mm), micro (< 5 mm), ND= not detected, PS= Polystyrene, PVC= Polyvinyl chloride, PET= polyethylene terephthalate, BBA= bisphenol A, TOC= Total organic carbon, FFEM= fluorescence excitation-emission matrix, ICP-AES= Inductively Coupled Plasma-Atomic Emission Spectroscopy, GC MS= Gas

chromatography and Mass spectrometry, HPLC= High performance liquid chromatography, LCMS= liquid chromatography, and mass spectrum).

Nowadays, (Fikarová et al. 2019) have reported a series of dynamic methods to evaluate MPs leaching using a flow-based system with SPE columns for additive pre-concentration. The use of infinite solid-phase sinks with high adsorption capacity for additives has been used to achieve dynamic conditions. In this way, additives are continuously adsorbed to the sink and removed from the aqueous phase, ensuring that leaching is not limited by solubility (Henkel et al. 2019, Sun et al. 2019, Ye et al. 2020). Many pollution studies use polymeric resins such as Amberlite® XAD® (cross-linked polystyrene copolymer), Tenax® (poly (2,6-diphenyl-p-phenylene oxide), silicone, and polyethylene. However, the sinks themselves can introduce additional contaminants into the environmental study, however, this effect can be minimized by pre-cleaning of such materials prior to use (Sun et al. 2019).

Additional steps are required to extract the analytes from the solid phase and recover the infinite sink from the leaching medium. Alternatively, determine release kinetics by removing the solid phase sink and replacing it with fresh material. This process typically involves rapid release in the first few hours or days, followed by gradual release over weeks or months. As indicated by Chen et al., 2019a; Fikarov et al., 2019, the kinetics of release follow either first-order or pseudo first-order (Chen et al. 2019, Fikarová et al. 2019).

1.7. Most common techniques used for phthalate esters analysis of microplastics and phthalate esters

Using a single analytical method to fully and reliably identify MPs in complex environmental matrices is challenging (Woo et al. 2021). Therefore, it is common to combine more than two analytical methods. In general, MPs analysis is performed in two steps: first, a physical analysis (microscopy), followed by chemical analysis (spectroscopy) to determine the plastic composition (Jung et al. 2018, Wang et al. 2018). Each method and various combinations have advantages and limitations. For example, microscopy is simple and easy to use, while chemical confirmation is not. Microscopy has been complemented by FTIR spectroscopy, which can be used for both plastic morphology and chemical confirmation. One of the limitations of this method is the possibility of overlooking small and transparent plastic parts.

To reduce false-negative data, Raman spectroscopy is now also be used alongside FTIR (Dowarah and Devipriya 2019). Features of the Raman technique: - Non-destructive analysis - Detection of plastics less than 10 μ m in diameter - Automatic mapping (FPA reflectance). However, there are still no optimal methods for the detection of MPs (Ma et al. 2013, Razanajatovo et al. 2018).

In general, identification and quantification of PAEs are performed by chromatographic techniques such as liquid chromatography (LC) or gas chromatography (GC) (Ma et al. 2013), GC equipped with mass spectrometry (MS) is the most popular technique employed for PAE determination with low LOD (ng/L or ng/g) (Net et al. 2015a). The separation of PAEs using GC–MS is commonly performed on a 30 m \times 0.25 mm I.D, the non-polar capillary column with 0.25 μ m film of 5% phenyl–95% methyl polysiloxane such as DB-5MS and HP-5MS ((Net et al. 2015a). The PAEs can be detected with electron capture (ECD), photoionization (PID), or MS detectors. ECD is relatively sensitive to PAEs (Jaworek and Czaplicka 2013), while with an MS detector each PAE can be ionized by electronic impact (EI) or more rarely by chemical ionization (CI) (Bester et al. 2001), and be detected in full scan, single ion monitoring (SIM), selected ion storage (SIS), tandem MS (MS/MS) or multiple reaction monitoring (MRM) mode. Splitless injection functions enable to improvement of the instrument limit of detection (LODs) of PAEs. The PAEs in environmental matrices can also be characterized with high sensitivity by ion mobility spectrometry (IMS), an analytical technique that emerged in 1970 (Li et al., 2002). The PAEs can also be quantified using the LC. However, compared to GC–MS, a lower sensitivity is obtained with LC-MS. Indeed, LC is more appropriate for analyzing MPEs and degradation products of PAEs than PAEs themselves PAE separation with LC is commonly achieved on a polar C₁₈ ODS (octadecylsilyl groups column) analytical column, using a mobile phase containing an organic solvent such as methanol or ACN and Milli-Q water, both generally buffered (e.g., with 10 mM ammonium formate, ammonium acetate) or acidified (0.05–0.1% AcOH or TFA). FTIR is a simple technique allowing to obtain an infrared spectrum of e.g. absorption, and emission of a matrix (Zhang and Chen 2014). This technique uses a unique fingerprint of materials and is very useful in qualitative analysis. It is based on the principle that a compound that has a covalent bond and a dipole moment can absorb frequencies of electromagnetic radiation (Higgins, 2013; Thermo Scientific, 2013).

1.8. Aim of the current study

Nowadays, there is growing worried over microplastics because of their occurrence in the marine environment, increasing concentrations over time, detection in a wide range of marine biota and potential for trophic transfer and biomagnification in food chains (Hong et al, 2017). A key concern of microplastic pollution also is whether they pose a risk to marine ecosystems and human health. However, there is a lot of uncertainty associated with this task. All this information suggests that PAEs from plastic debris are a concern, and confirms that assessing exposure, and associated risks is essential, especially in the marine environment. So, to evaluate the risk of microplastics to marine environments, data on the exposure and effect levels of microplastics are required. Thus, the availability of a simple and low-cost system for preconcentration and subsequent determination of this class of chemicals in marine and fresh water represents an important task in recent years. Thus, the overall objectives of the current thesis are focused on:

- i. Reviewing the essential background information's on the sampling, occurrence, mode of action, various sources, toxicity, their impact to marine biota and analytical methodology for the PAEs and plastic pollution and their determination. On the other hand, a brief overview of the chemistry of PAEs and the most common protocols for their determination in complex matrices.
- ii. Improving the analytical protocols for the cleanup and measuring the phthalic acid esters in seawater. In the current study attempts to develop a reliable strategy for the sample prep and analysis of PAEs will be fully studied.
- iii. Studying the impact of blank contamination control, extraction time, temperature salinity, and ultraviolet irradiation (UVR) on the leaching of PAEs from polyethylene.
- iv. Establishing a reliable and highly efficient strategy involving the use of solvent extraction for liquid-liquid extraction (LLE) for minimizing blank contamination during PAEs measurements.
- v. Understanding the leaching of the phthalic acid esters (PAEs) from common consumer plastics from different plastics under seawater conditions and their relationship with plastics in marine environments. To the best of our knowledge, no study has been performed in the Red Sea water. Thus, proper assigning of the relationship between the PAEs and abundance of plastic waste in the Red Sea is of great importance

1.9. Chapter overview

Nowadays, great interest has been oriented towards the leaching process of phthalate esters from plastics in seawater under laboratory conditions microplastics and to determine the relationship between phthalate esters and plastics in the seawater environment. Thus, the current work in this dissertation can be outlined as follows:

Chapter 1: This chapter includes a brief introductory on the necessary theoretical background of plastic production, pollution, and the pathway to the marine environment. This is followed by the life cycle of plastics in the marine environment. The effects of the weathering process, temperature, wave action, and UV radiation on the leaching behavior of plastics. The chemical composition during leaching and the toxicological effects of leaching, especially phthalate esters. In addition, this chapter reviews current methods for studying leaching with their advantages and disadvantages and highlights key research findings.

Chapter 2: This chapter is deeply focused on analyzing PAEs with good precision and reliability is a challenge due to contamination from the materials used in sampling, processing, and analysis. The work in this chapter describes a reliable method to measure and control the blank contamination of PAEs and optimize the experimental conditions, such as extraction time and temperature, for liquid-liquid extraction (LLE) and solid-phase extraction (SPE). The analytical instrument (GC-MS) parameters, such as injector temperature, flow rate, and temperature gradients, were optimized for more intensive elution of chromatographic peaks. A manuscript based on this chapter is ready for submission for peer-reviewed publication:

Jeyakumar dhavamani, Aaron J. Beck, Martha Gledhill, Mohammad S. El-Shahawi, Mohammad I. Orif, Iqbal M.I. Ismail, Eric P. Achterberg. **Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction.** All authors contributed significantly to this study. Jeyakumar Dhavamani: performed all analyzes and wrote the original draft, Aaron J. Beck: co-supervision: data quality maintenance, Mohammad El-Shahawi Soror: review and editing, Martha Gledhill: co-supervision, Eric P. Achterberg; supervision. Mohammad I. Oriff and Iqbal MI Ismail: Resources, Fundraising.

Chapter overview

Chapter 3: This chapter covers the leaching of six phthalic acid esters (PAEs) named dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DNOP) from three common consumer plastics was investigated: Low and High-Density Polyethylene (LDPE, HDPE) and Recycled Polyethylene (RP). The effects of salinity, temperature, and ultraviolet irradiation (UVR) on leaching were investigated. One of the main objectives of this chapter is to measure the effects of high hydrophobicity of PAEs on the leaching process. The work in this chapter was previously published as:

Jeyakumar Dhavamani, Aaron J. Beck, Martha Gledhill, Mohammad S. El-Shahawi, Mohammad W. Kadi, Iqbal M.I. Ismail, Eric P. Achterberg. **The effects of salinity, temperature, and UV irradiation on the leaching and adsorption of phthalate esters from polyethylene in seawater.** Science of the Total Environment. DOI: 10.1016/j.scitotenv.2022.155461

Author contributions: All authors contributed substantially to this study. Jeyakumar Dhavamani: Performed all analyzes and wrote the original draft, Aaron J. Beck: co-supervision: data curation, Mohammad El-Shahawi Soror: review and editing, Martha Gledhill: co-supervision, Eric P. Achterberg; supervision. Mohammad W. Kadi and Iqbal MI Ismail: Resources, Fundraising.

Chapter 4: This chapter is deeply oriented towards proper understanding and assigning the relationship between phthalate esters and plastic pollution in seawater and the abundance partitioning of PDs and PAEs in the surface water of Sharm Obhur Bay (a semi-enclosed bay on the east coast of the Red Sea, near Jeddah, Saudi Arabia.) and the Red Sea water. The correlation between PAEs, PDs, and environmental parameters is reported, and the ecological risk level (ERL) of PDs and PAEs was calculated. The work in this chapter was submitted for publication and it is under review as:

Chapter overview

Jeyakumar Dhavamani, Aaron J. Beck , Martha Gledhill, Mohammad S. El-Shahawi, Mohammad I. Orif, Iqbal M.I. Ismail, Eric P. Achterberg. **Partitioning of phthalate esters and plastic waste and their ecological risk levels in the surface waters of the Red Sea and Sharm Obhur Bay, Environmental pollution, under review.**

Authors' contributions: All authors contributed substantially to this study. Jeyakumar Dhavamani: Performed all analyzes and wrote the original draft, Aaron J. Beck: Co-supervision: data curation, Mohammad El-Shahawi Soror: review and editing, Martha Gledhill: Co-supervision, Eric P. Achterberg; Supervision. Mohammad I. Oriff and Iqbal MI Ismail: Resources, Fundraising.

Chapter 5: This chapter includes the conclusion, concluding remarks for the oceanographic community, including suggestions, future perspectives to address gaps in current knowledge and limitations.

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Chapter 2

Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

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2. Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

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Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Abstract

Phthalates or phthalic acid esters (PAEs) are ubiquitous in the laboratory environment, including ambient air, reagents, sampling devices, and various analytical instruments, thereby making it difficult to reliably analyze natural water samples with low PAE concentrations. In the current study, a reliable method for the analysis of PAEs was developed, including control of blank contamination, and the experimental conditions such as extraction time and temperature were optimized. The mass of PAEs in blank tests of selected materials ranged from 3 ± 0.7 to 35 ± 6 ng for liquid-liquid extraction (LLE) and from 5 ± 1.8 to 63 ± 15 ng for solid-phase extraction (SPE). For both extraction methods, higher blank values were measured for dibutyl phthalate (DBP) (35 ± 6 ng, 12 ± 3 ng), and DEHP (63 ± 12 ng, 23 ± 5 ng) in LLE and SPE, respectively. Average recoveries of PAEs in LLE were 90-97% and obtained with successive aliquots of 2 mL, 1 mL, and 1 mL dichloromethane (DCM). For SPE, recoveries up to 86-90% were achieved with successive aliquots of 5, 3, and 2 mL DCM at a sample flow rate of 5 ml/min. Under the optimized conditions, the method quantification limits (MQL) for PAEs was 10-20 ng/L for LLE and 10-35 ng/L for SPE. Moreover, the dissolved concentrations of PAEs from LDPE measured by the LLE method ranged < 1.5 to 5.83 ng/cm², and those measured by SPE ranged from 1.0 to 256 ng/L, in seawater samples of Sharm Obhur. The method has considerably lower MQL values for LLE and SPE than average reported values of 10-100 ng/L and 30-100 ng/L, respectively. The method offers a reliable approach for quantifying blank contamination and the MQL values meets the requirements for analysis of PAEs in seawater. The overall results suggest that LLE or SPE combined with GC-MC could be an easy and efficient method to quantify PAEs residue levels in seawater.

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

2.1. Introduction

Phthalic acid esters (PAEs) are the main group additives and are used as plasticizers to improve the flexibility and pliability of plastics. Phthalic acid esters account for about 92% of plasticizers produced and the most produced and consumed plasticizers worldwide (Bradlee 2003, Stenmarck and et al. 2013). Some PAEs are considered endocrine-disrupting chemicals that can have significant effects on reproduction in marine animals and on the development of obesity and cancer in humans (Heudorf et al. 2007, Meeker et al. 2009, Zhang et al. 2021). Given their potential environmental and health risks, six PAEs (dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), di(2-Ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP)) have been identified as priority pollutants by *U.S.* Environmental Protection Agency (US-EPA) and the European Commission (2005/84/EC) 2005, U.S 2014).

The pre-treatment for PAEs analysis in natural water samples is typically conducted by liquid-liquid extraction (LLE), solid-phase extraction (SPE), soxhlet extraction, and ultrasonication. The detection techniques for PAEs mainly concern gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) (Bridson et al. 2021, Sánchez-Avila et al. 2011, Zhang et al. 2017a). Generally, analysis with small sample volumes of 10-20 ml mainly used LLE to determine PAEs, with a relatively short extraction time (2-5 h). In environmental samples including freshwaters and seawaters, where sample volumes of 2-10 L are used, SPE has been employed for PAE analysis. The extraction efficiency for SPE ranges between 65 to 95%, with extraction times between 5 and 8 h for a volume of 1-2 L (Bridson et al. 2021).

Contamination by reagents and materials used as part of the analytical protocols of sampling, processing, and analysis of PAEs, forms an important challenge for PAE quantification with good precision and reliability. Most materials used in the field during sampling and in laboratories are sources of plastic contamination including samplers, lab coats, and other apparel worn by laboratory personnel, analytical instruments, water used to clean equipment before use, sponges or brushes used to clean equipment before use, synthetic polymer gloves and plastic sample containers. Extreme care must be taken to minimize contamination when collecting and analyzing water samples for microplastics. Contamination can significantly affect sample concentrations

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

leading to overestimation. It is often not possible to confidently eliminate all contamination from samples during laboratory processing. A literature review showed that over the last 20 years, data on blank contamination appeared in 242 out of 552 articles (44%). In many cases, authors omit information regarding blank contamination. (Bogdanowicz et al. 2021). This may mean that no cross-contamination occurred in a given study, or it was sufficiently low to be considered negligible. Nonetheless, such information should be included in publications, because it may prove useful from the point of view of discussions regarding cross-contamination. Since the blank contamination is one of the major issues in PAE quantification, a blank measurement of each material and device was conducted individually to determine background contamination. The overall goal of the current study is to develop a reliable method for PAE analysis. The work includes the evaluation of PAEs contamination in all materials involved in the experiments and analysis, as well as optimization of extraction conditions such as liquid-liquid extraction and solid-phase extraction.

2.2. Materials and methods

2.2.1. Reagents and materials

A standard mixture of PAEs, including three low molecular weight (LMW) compounds, DMP, DEP, DBP, and three high molecular weight (HMW) compounds, BBP, di(2-ethylhexyl) phthalate (DEHP), and DnOP at concentrations of 20,000 mg/L in methanol (purity 99.8%) was purchased from Sigma Aldrich (Germany). The most common properties of the PAEs are listed in Table 2.1 (Hermabessiere et al. 2017). Dichloromethane (DCM), MeOH, and ethyl acetate were purchased from Sigma Aldrich (HPLC grade). Working solutions of PAEs were prepared in isooctane and stored in the dark at 4°C for a maximum of two weeks. All experiments were performed in artificial seawater (ASW) prepared according to Sunda et al (2008) (3.0 mg L⁻¹ NaF, 20 mg/L SrCl₂·6H₂O, 30 mg/L H₃BO₃, 100 mg/L KBr, 700 mg/L KCl, 1 mg/L CaCl₂·2H₂O, 4000 mg/L Na₂SO₄, 10.780 mg/L MgCl₂·6H₂O, 23,500 mg/L NaCl, 20 mg/L Na₂SiO₃·9H₂O, and 200 mg/L Na HCO₃) in ultrapure water (Milli-Q, resistivity 18.2 MΩ cm). Phthalate impurities in the ASW were removed by SPE with a polypropylene column (Chromabond Easy, 6 ml, 200 mg, Macherey-Nagel, Germany) at a flow rate of 5 ml/min; the ASW was sterilized in an autoclave (JASC-80JSR, Korea). All glass materials were baked at 400°C for 4 h.

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table 2.1. Physiochemical properties of phthalate esters (PAEs) *

Compounds	Molar mass	Alkyl chain length	Molecular Formula	Log K _{ow} (at 25°C)	Log K _{Aw} (at 25°C)
Dimethyl Phthalate (DMP)	194.2	1	C ₁₀ H ₁₀ O ₄	1.61	-5.40
Diethyl Phthalate (DEP)	222.4	2	C ₁₂ H ₁₄ O ₄	2.54	-5.01
Di-N-Butyl Phthalate (DBP)	278.4	4	C ₁₆ H ₂₂ O ₄	4.27	-4.27
Benzyl Butyl Phthalate (BBP)	312.6	8	C ₁₉ H ₂₀ O ₄	4.70	-4.08
Di(2-Ethylhexyl)Phthalate (DEHP)	390.6	6	C ₂₄ H ₃₈ O ₄	7.73	-2.80
Di-N-Octyl Phthalate (DnOP)	390.6	8	C ₂₄ H ₃₈ O ₄	8.60	-2.80

* log_{ow}=Octanol-Water partition coefficient, log_{aw}=Air-water partition coefficient).

2.2.2. Instrumentation

The concentrations of PAEs in the extracts were analyzed by GC-MS (QPplus-2010, Shimadzu, Japan) using electron ionization (EI) conditions. The initial temperature of the column oven was 250°C. A HP 5MS, 30 m capillary column was used (30 m × 0.25 mm i.d. × 0.25 m, 5% phenylmethyl siloxane, Agilent HP -5MS) with a temperature program of 60°C (2 min hold), ramp 5°C min⁻¹ to 310, and 5 min hold. Helium was used as the carrier gas (2 ml/min). The ion source temperature was 220°C and the interface temperature was 250°C. The injection was performed in "splitless" mode, with a spitting time of 0.98 min and a purge flow of 30 ml/min. Target compounds were positively identified by comparing their retention times and target ions to specific reference ions. Instrument performance was calibrated using an eleven-point calibration curve. There were lower range calibration standards of 1, 2, 4, 8, 16, and 20 ng/L and higher range calibration standards of 20, 40, 80, 160, 200, and 400 ng/L.

2.2.3. Recommended procedures to avoid blank contamination

To avoid background contamination in experiments and standard solutions, as well as in the extraction procedures, all materials used were plastic-free except in cases where the material was

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

not replaceable. To determine the method blank value, the contamination of materials and instruments was measured individually. The detailed information on the materials and testing procedures are given in the Supplementary information (Table S2. 1). As a first step, instrument blank values analyses (without any injection) were performed and the signal intensity at characteristic mass to charge ratio (m/z) values for quantization ions (Q) was obtained for each PAE. Then, the organic solvents DCM, MeOH, and isooctane were directly injected into GC-MS and the difference between their signal intensity and instrumental blank was measured to calculate the contamination of each solvent. Consumable materials including micropipette tips (autoclaved), GC-MS vials, syringes, syringe filters, solid-phase extraction cartridges, test tubes, test tube caps, sodium sulfate, and laboratory sample preparation instruments including nitrogen units, and vacuum units were treated separately with 5 mL of DCM and MeOH and the leached contaminants were GC-MS analyzed. The extracts were evaporated to near dryness at 25°C with N₂ gas using a TurboVap LV (Model N- EVAP 111, USA). The extracts were then reconstituted with isooctane (1.0 mL). The extracted samples were stored at -20 °C until further analysis. All the samples obtained were stored at -20 °C until analyzed by GC-MS. Based on the difference in m/z values at each step; the mass of PAEs from each material was calculated. The workstations were cleaned before using DCM: MeOH (80:20) at each step and a laboratory coat (cotton clothing) was worn at all times during the study.

2.2.4. Liquid-liquid extraction

The extraction efficiency of the target analyte (PAEs) from spiked ASW (10 mL) was performed by liquid-liquid extraction with successive aliquots of solvents (DCM, ethyl acetate, n-hexane). The combined extracted volume was pre-concentrated to 0.2 mL and stored as described above (Section 2.3). The extracted samples were stored at 4°C until analysis. We employed the standard addition method (SAM) as an efficient approach to correct for matrix effect and obtain an overall evaluation of the extraction efficiency at different concentrations. The method quantification limits were determined using the standard addition method (Frenna, Mazzola, et al. 2012). Known concentrations of standard PAEs (5, 10, 20, 40, 80, 160, and 200 ng/L) were spiked into 10 mL ASW and extracted by the same procedure.

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

2.2.5. Solid-phase extraction

Concentrations of dissolved 6PAEs in ASW (1L) were determined by SPE (Teflon column, Chromabond Easy, 6 ml, 200 mg, Macherey-Nagel, Germany). The samples were spiked with a mix of 6PAEs at 200 ng/L. A vacuum system (J.T. Baker, The Netherlands) was used for pre-concentration. The SPE cartridges were conditioned with 5 mL of methanol, followed by 5 mL of DCM and 5 mL of ultrapure water, all at a flow rate of 5 ml/min. Samples were aspirated through the cartridges at a flow rate of 2-10 ml/min. The cartridge was finally rinsed with 5 mL \times 3 ultrapure water and dried under vacuum for 30 min. Elution was performed with 10 mL of DCM with successive aliquots of 5, 3, and 2 mL. The combined volume of the extract was pre-concentrated to 0.2 mL as and stored as described above (section 2.3). The quantification limits of methods were also performed using a range of concentrations of 5, 10 20, 40, 80, 166, and 200 ng/L that were dosed to 2 L of ASW and extracted by the described procedure.

2.3. Results and Discussion

2.3.1. Programming of GC-MS analysis

In the method development for gas chromatography-mass spectrometry, the infusion is the first step. Thus, the preparation process in MS involves the isolation of a single precursor ion and subsequent dissociation of the precursor ion into characteristic product ions. First, the individual standards were injected into the GC-MS in full scan mode (50-1500 m/z mass range). Ion with the highest abundances for each analyte was selected as the base peak. As shown in Table 2.2, a total of 6 different certified reference materials (CRM) were monitored by GC-MS to cover all target contaminants and the surrogate standards. To confirm the positive finding of the CRM, one or two molecular ions and their ratio shall be measured (CommissionDecision2002/657/EC 2002). The transition with the highest intensity was selected as the quantitative transition (Q). The GC-MS spectra for PAEs were obtained by selecting the base peak at m/z 163 $[M-OCH_3]^+$ for dimethyl phthalate and m/z 149 $[C_9H_9O_2]^+$ for all other PAEs as the characteristic precursor ion. The main ion m/z 149 resulted from the fragmentation with the loss of the alkyl ester groups and a furan ring formation (Bradlee 2003). A similar fragmentation pattern is found for the other phthalates, except

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

for DMP. In the mass spectrum of DMP, the molecular ion is detected at m/z 194. The most abundant ion is at m/z 163, corresponding to the loss of a methoxy group ($M-31$) (Bradlee 2003).

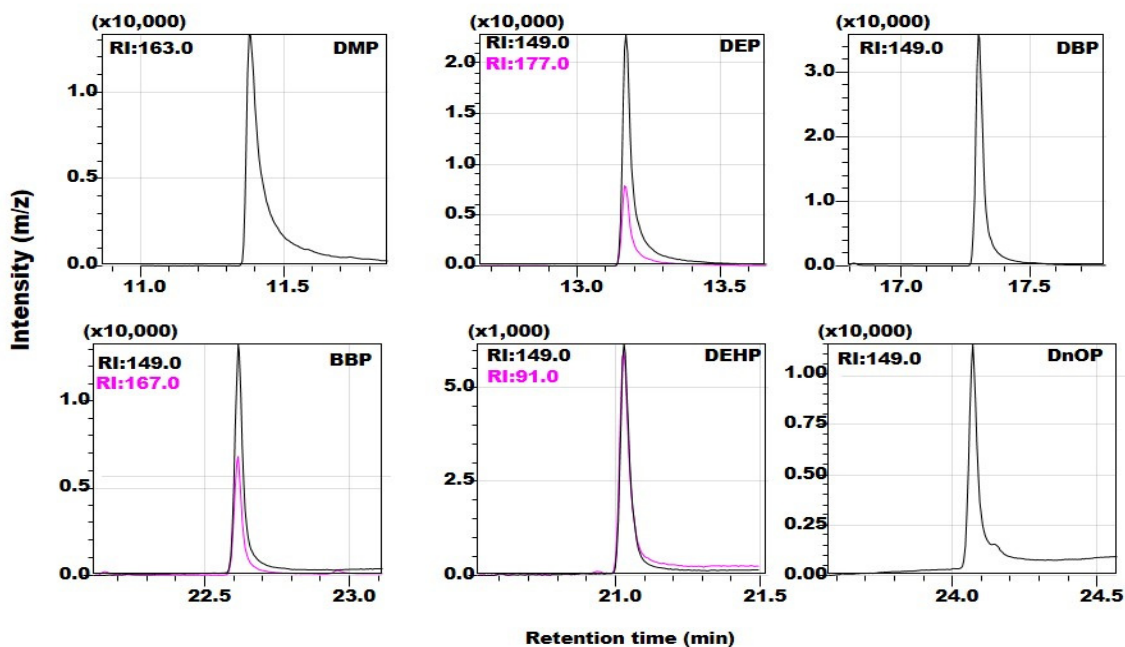


Figure 2.1 GC-MS chromatograms for PAE compounds at different retention times with reference ions for the respective compounds* (m/z =mass to charge ratio, RI=Reference ions)

Table 2.2. GC–MS method parameters*

Compounds	RT (min)	Mol weight (g.mol)	Reference ion (m/z)	Signal intensity	Ratio
Dimethyl phthalate	11.38	194.18	169	100	97
Di-n-butyl phthalate	13.16	278.34	149, 77	100, 83	87
Diethyl phthalate	17.29	222.24	149	100	58
Butyl benzyl phthalate	21.03	390.56	149, 167	100,37	45
Di(2-ethylhexyl) phthalate	22.6	312.4	149, 91	100, 74	52
Di-n-octyl phthalate	24.06	390.6	149	100	63

* RT; retention time, Mol weight; molecular weight).Parameters of GC, such as injector temperature (250°C), flow rate (2 ml/min), and temperature gradients were optimized for higher

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

intensity elution of chromatographic peaks. The optimized GC parameters are shown in section 2.2. The intensity observed in this study ranged from 52-97 (Table 2.2). These conditions are typical for the analysis of phthalates by GC-MS and result in sufficient resolution of the most important phthalates. As previously reported, these conditions have a good compromise between resolution and speed of analysis (Bradlee 2003, Sánchez-Avila et al. 2011).

Table 2.3. Calculated value from instrument, method validation calibration curves*

Compounds	LOD	LOQ	R ²	R%	RSD	LLE	SPE
	(ng/L)	(ng/L)		(200 ng/L)	%	MQL (ng/L)	MQL (ng/L)
Dimethyl phthalate	1.5	5	0.979	102.3	12	10	10
Di-n-butyl phthalate	3.0	10	0.952	102.6	11.3	20	35
Diethyl phthalate	1.5	5	0.983	106.8	13.2	10	15
Butyl benzyl phthalate	1.5	5	0.953	98.5	9.26	10	10
Di(2-ethylhexyl) phthalate	3.0	10	0.905	99.1	8.6	20	20
Di-n-octyl phthalate	1.5	5	0.936	94.3	5.6	10	10

* The instrumental detection (LOD) and quantification limits (LOQ) by instrument; R= recovery percentage; method quantification limit (MQL) by liquid-liquid (LLE) and solid-phase extractions (SPE) are listed.

Good linearity responses were obtained for all target contaminants over the entire concentration range tested, and an eleven-point calibration was analyzed in triplicate at 1, 2, 4, 8, 16, 20 ng/L (R^2 ; 0.998) and 20, 40, 80, 160, 200, 400 ng/L (R^2 ; 0.996) for all target contaminants. Quantification was performed using the standard external method in the selected ion monitoring

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

mode (SIM). The regression coefficient of linearity was greater than 0.99 with a relative standard deviation (RSD) of 18%. The instrumental detection limit (LOD) was calculated using the signal-to-noise ratio (S/N) of the lowest concentration used. The optimized parameters for GC-MS calibration and instrument detection limits are listed in Table 2.3. Performance of this instruments was calibrated based on the European regulation, article number; 10/2011/EU (Commission 2011).

2.3.2. Blank contamination levels

Plastic parts are a significant component of materials needed during this study. Such as rubber seals on GC-MS, nylon lining on N₂ and vacuum units, polypropylene housing on the SPE, and most salts are stored in polyethylene containers. In Table, S2.1 details are provided about all the materials and suspected plastic parts of each material. Each material was analyzed for contamination with PAEs by GC-MS. Table (S2.2-S2.7) shows the increasing GC-MS background contamination by each material introduced into the method. The various materials used in the method have different concentrations of PAEs. For example, the mass of DEP increased starting from the instrument blank sample by adding all the required materials sequentially as presented in Table 2.4. The mass value of DBPs in the instrument blank (GC-MS) alone was 8 ± 2 ng and increased to 96.3, 72.2, 110.0, and 57.0 ng using autoclaved micropipette tips (0.2 mL, polypropylene, CAAP, Denmark), syringes (5 mL, disposable, polyethylene, KSA), PTFE syringe filters (0.2 μ m, polypropylene, Whatman, UK), and artificial seawater (ASW). After considering the pre-concentration, the given mass values were found equivalent to 0.008, 0.1, 0.8, 0.11, and 0.05 ng/L, respectively. Because of the sterilization process, such autoclaves affect the chemical stability of tips and this may lead to chemical leaching. The PP housing and black rubbers in the syringe filter are also affected by DCM, leading to contamination. The list of suspected plastic parts in the materials is given in Table S2.1. Due to high blank contamination, the use of sterile tips, syringes, syringe filters, and unfiltered ASW was avoided in the LLE and SPE extraction methods. Other selected materials listed in Tables 2.5 and 6 with DEP concentration below 40 ng (0.008 ng/L), were further used for extraction analysis in this study.

Overall, the mass of PAEs in the blanks ranged from 3 ± 0.2 to 35 ± 6 ng for LLE and 6 ± 2 to 65 ± 17 ng for SPE, as shown in Tables 2.5, and 2.6. The highest mass was found for DEP (35.0 ng)

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

followed by DEHP (18 ng) (Table 2.5). Indeed, DBP and DEHP are the most commonly used plasticizers and account for half of the total PE production in Western Europe (PlasticsEurope 2019). The mass of DMP, DBP, BBP, and DnOP was estimated to be 10, 12.7, 9.4, and 3.2 ng, respectively for LLE blank (Table 2.5). Due to the higher number of materials involved in SPE, the mass of DEP, DBP, and DEHP was measured as 63.1, 23.0, and 24.5 ng, respectively (Table 2.6). The mass of PAEs in the LLE blanks and SPE blanks was subtracted from the pre-concentrated sample.

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table 2.4. The total mass of DEPs (ng) in each sequence of blank testing for the corresponding materials added

DEP	GC-MS INB	DCM	Micro pipette tips	Micro pipette tips (Sterile)	GC vial	Syringe	Syringe filter	Na2 SO4	N ₂ Unit	Iso octane	MeOH	Vacuum unit	SPE column	Line Tubes	ASW	ASW filter
GC-MS Instrument																
blank	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Dichloromethane																
(DCM)		7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Micropipette tips			8.0			8.0	8.0	8.0	8.0	8.0		8.0	8.0	8.0	8.0	8.0
Micropipette tips (Sterile)				69.3												
GC vial																
Syringe						37.0	37.0									
Syringe filter							38.0									
Sodium sulphate			6.0	6.0	6.0	6.0	6.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Nitrogen Unit			6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Iso-octane																
MeOH											8.0		8.0			
Vacuum unit											5.0	5.0	5.0			
SPE cartridge													10.0			
Tubes (silicon)														3.0		
ASW																
ASW filtered															25.0	2.0
Total	8.0	15.0	35.0	96.3	27.0	72.0	110.0	32.0	32.0	32.0	37.0	37.0	55.0	35.0	57.0	34.0

*DEP=dimethyl phthalate, INB=instrument blank, MeOH= methanol, SPE=soil phase extraction column and ASW is the artificial seawater

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table 2.5. Blank contamination of PAEs in the liquid-liquid extraction method

Materials	PAEs (ng)					
	DMP	DEP	DBP	DEHP	BBP	DnOP
GC-MS Instrument blank	3.1	8	3.36	3.2	0.96	0.8
DCM	1.89	7	2.94	2.52	0.77	0.7
Micropipette tips	2.32	8	2.8	2.96	0.88	0.72
GC vial	0	0	0	6	6	0
Sodium sulphate	0.93	3	1.26	1.11	0.3	0.3
Nitrogen Unit	1.62	6	2.34	2.4	0.54	0.72
Iso octane	0	3.1	0	0	0	0
Total	9.86	35.1	12.7	18.19	9.45	3.24

Table 2.6. Blank contamination of PAEs in the solid-phase extraction method

Materials	PAEs (ng)					
	DMP	DEP	DBP	DEHP	BBP	DnOP
GC-MS Instrument blank	3.1	8	3.36	3.2	0.96	0.8
DCM	1.89	7	2.94	2.66	0.77	0.7
MeOH	2.56	10	3.36	2.96	0.88	0.8
Vacuum unit	1.45	5	2.1	2.05	0.55	0.55
SPE cartridge	2.5	10	3.8	0	1.1	1.1
Tubes	0.87	3	1.05	1.11	0.3	0.27
ASW filter	0	0	0	0	0	0
Micropipette tips	2.32	8	2.8	2.96	0.88	0.72
GC vial	0	0	0	6	6	0
Sodium sulphate	0.93	3	1.26	1.11	0.3	0.3
Nitrogen Unit	1.62	6	2.34	2.4	0.54	0.72
Iso octane	0	3.1	0	0	0	0
Total	17.24	63.1	23.01	24.45	12.28	5.96

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

2.3.3. Optimization of liquid-liquid extraction

As PAEs are weak polar compounds, weak and nonpolar solvents were better than medium and strong polar solvents for PAEs extraction. Following prior studies (Bridson et al. 2021, Zhang et al. 2017b), dichloromethane (DCM), ethyl acetate, and n-hexane were used as extraction solvents, and we evaluated their extraction efficiency. Good efficiency was achieved with DCM and hexane as nonpolar solvents, related to the fact that the dielectric, as well as the auto pyrolytic constants of both solvents, are very low (Liu et al., 2013; Gao et al., 2014; Net et al., 2014). The highest yield was obtained with DCM (80-90% yield), followed by n-hexane (75-89% yield), the mixture of DCM: ethyl acetate (80:20) (60-72% yield), and ethyl acetate (65-75% yield) (Figure 2.2a), which might result from its larger polarity (Christian 2004). However, the recovery of DMP was low (65-80%), which might result from its lower partitioning coefficient of octanol and water ($\log K_{ow} = 1.6$), and its high solubility in water (4000 mg L⁻¹). In contrast, the $\log K_{ow}$ of DnOP, DBP, and DEHP were 4.5, 7.6, and 8.4, and their solubility in water was 11.2, 0.27, and 0.09 mg L⁻¹ (Christian, 2020).

The percentage yield increased with increasing the molecular weight of PAEs. Generally, the water solubility of the alkyl phthalate ester varies inversely with the length of the alkyl side chain (Bradlee 2003). To determine the recovery rate, the mean peak area of each analyte was determined for an ASW spiked with the analyte (n = 6). To improve the extraction efficiency with DCM, the recovery experiments were performed with a number of extraction cycles of 1-5. The reasonable extraction yield was obtained at 2-3 cycles, as shown in Figure 2.2b. As the number of extraction cycles increased from 2 to 3, the recovery rate increased to 90-97%. Multiple extractions by DCM enhanced the mass transfer of the PAEs from the aqueous to the organic phase (Christian, 2020). Maximum PAEs extraction was achieved after 3 cycles and remained constant which is most likely attributed to their equilibrium concentrations. Thus, in the subsequent experiments, three extraction cycles were adopted.

Photolysis experiments were performed with compact fluorescent tubes (Philips CFL, 15W,) for 120 hours. The irrigation cycle was 12 h and the distance between the test tube and the lamp was about 10 cm. The light intensity was measured with a light intensity meter and was about 165 lx. The dark experiment was performed without light and also by covering the tubes with aluminum foil. The PAE in the tubes placed in the dark were affected only by hydrolysis, while PAEs in light

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

was affected by both hydrolysis and photolysis. The LMW-PAEs were degraded by 10-25% under light conditions, in contrast, HMW-PAEs the degradation rate ranged from 8-10% (Figure 2.2c). After 10 days, more than 80% of the original concentration was still present in tubes placed in sunlight. This behavior is attributed to the hydrolysis of the ester group by cleavage of the C-O bond as reported (Balabanovich and Schnabel, 1998; Lau et al., 2005). The half-life ($t_{1/2}$) of DEHP varied between 0.2 -2 days whereas for DMP it was 9.3 days) (Cousins I 2002). The degradation of HMW-PAEs (BBP, DEHP, and DnOP) in the dark was negligible and in good agreement with the data reported (Wang et al. 2019). To estimate the stability and the limit of quantification of the LLE method, 10 mL of ASW was prepared with different concentrations (5, 10, 20, 40, 80, 160, 200 ng/L) of PAEs. The estimated value was compared with the mean value obtained with the same range (5-200 ng/L) of PAEs in 0.2 mL isooctane. The estimated recoveries were linear with a range from 10-200 and ng/L (Figure 2.2d). The minimum quantification limit of LLE obtained was 10-20 ng/L (Table 2.3). These results demonstrate the feasibility and reliability of the established method for PAEs determination in water samples (AFNOR 2005, Marine 2002)

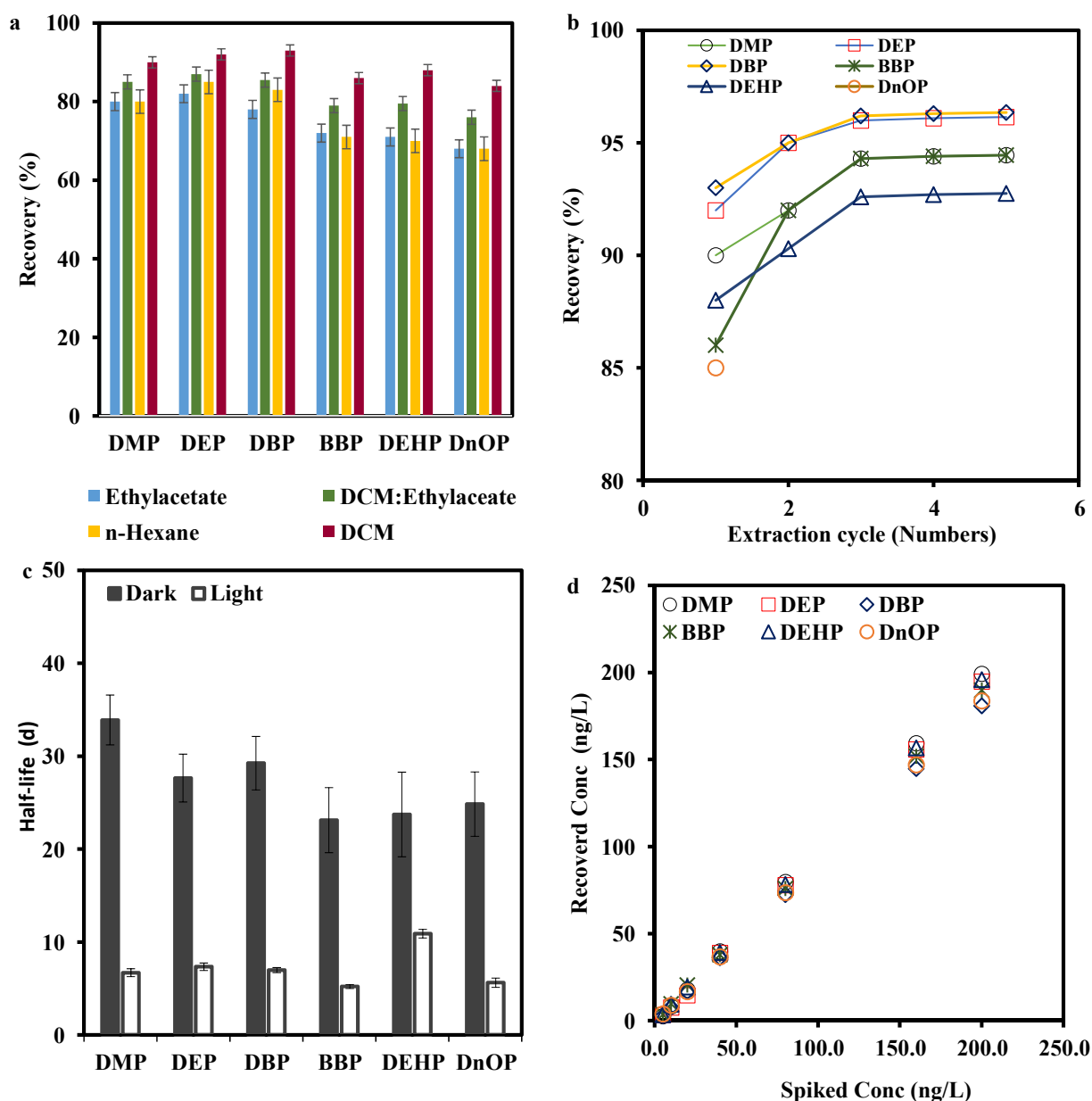


Figure 2.2. The effect of different factors on extraction efficiencies: Optimization of desorption solvent (a), investigation of the extraction cycle (b), investigation of the half-life time in dark and light (c), standard addition test (d).

2.3.4. Optimization of the solid-phase extraction

To optimize the eluting agent, different solvents were used to evaluate their effects on extraction efficiency, including 100% dichloromethane (DCM), MeOH, and the mixture of DCM: MeOH (50:50) and (80:20). The samples were initially spiked with PAEs to give a final PAEs

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

concentration of 200 ng/L. At a low flow rate (1 ml/min), the sample volumes were then percolated over the SPE column and the concentration of each PAEs in the effluent was then measured. The results showed that the best extraction results were obtained with DCM: MeOH (80:20) as desorption solvent when the volume of desorption solvent exceeded 5 mL. In solid-phase extraction, maximum recovery (80-90%) of PAEs was achieved with DCM: MeOH (80:20), followed by DCM (75-89%), MeOH (60-72%), and DCM: MeOH (50:50) (65-75%) (Figure 2.3a). With DCM, the recovery percentage of PAEs also increased by raising the molecular weight of the PAEs. The solubility of HMW-PAEs in water is low thus it extracts easily in a non-polar solvent like DCM (5 mL) (Liu et al., 2013; Gao et al., 2014; Net et al., 2014). The color of the extract of the DCM; Methanol was deeper than dichloromethane, which is attributed to the increased polarity of the mixed solvent (DCM: Methanol) which dissolves the polar impurities (Li et al. 2004). So far, only DCM was adopted in the next study as a proper eluting solvent.

To determine the recovery rate, the mean peak area of each analyte was determined for an ASW spiked with the analyte ($n = 6$). To improve the extraction efficiency with DCM, the recovery experiments were performed over a number of 1 to 5 elution times 1-5. As mentioned before (section 3.3) a good extraction yield (90-92%) was obtained at between 2 to 3 cycles, as shown in Figure 2.3b. No further increase was measured at 3 to 5 extraction cycles. To reduce the extraction time, and the adsorption efficiency of the column the experiments were performed under different sample flow rates. PAEs were first spiked into the samples to give the final PAEs concentration of 200 ng/L. The sample solutions were percolated through the SPE-packed columns at various flow rates ranging from 2 to 20 ml/min. The adsorption efficiency of the column decreased on increasing sample flow rate, and a reasonable efficiency was observed between 5-10 ml/min flow rate. Generally at a low flow rate, good equilibration of the species with the solid phase packed column is high (Christian, 2020).

It is worth mentioning that, the adsorption efficiency was reduced by 8-12% at 15 ml/min and by 15-22% at 20 ml/min (Figure 2.3c). To estimate the limit of quantification of the SPE approach, the samples were initially spiked with PAEs to give various known final concentrations of 1, 5, 10, 20, 40, 80, 160, and 200 ng/L of ASW. The estimated value was compared with the mean value obtained with the same range (5-200 ng/L) of PAEs in 0.2 mL isooctane. The estimated average recoveries were linear with a wide range of concentrations (10 -200 ng/L) as illustrated in Figure

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

2.3d. Minimum quantification limits of SPE were achieved in the range of 10 to 35 ng/L (Table 3).

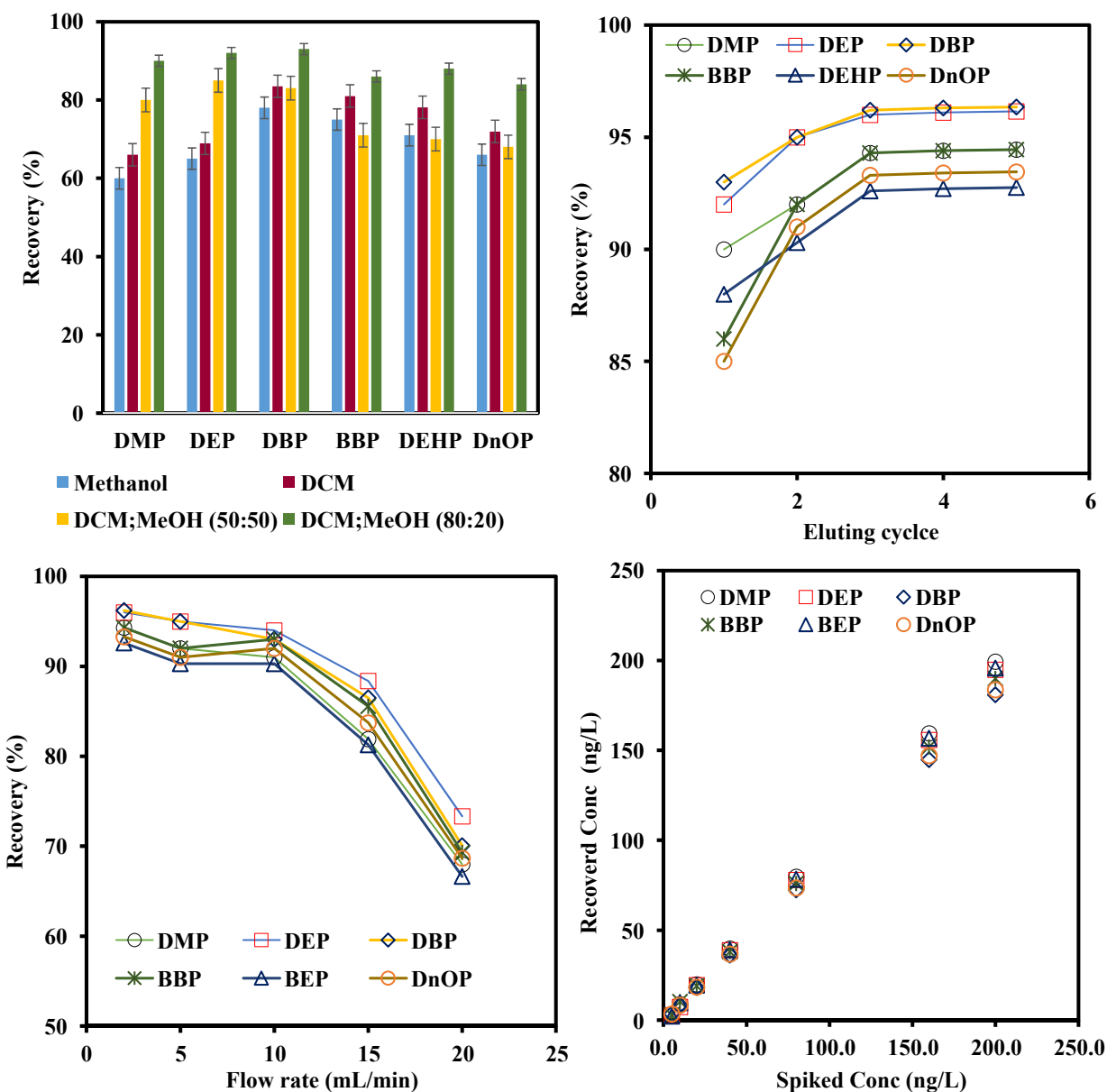


Figure 2.3. The effect of different influencing factors on extraction efficiencies: Optimization of elution solvent (a), investigation of elution cycle (b), investigation of the half-life time at dark and light (c), standard addition test (d)

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

2.3.5. Analytical performance and figures of merits

Most studies reported blank or background contamination without reporting target PAE (Sibali et al. 2013). Studies reported blank contamination of dimethyl phthalate (DMP), nonylphenol (NP), and nonylphenol monoethoxylate (NMP) of 0.07-0.8 ng/L (Paluselli et al. 2018b, Sánchez-Avila et al. 2011). Although DEP, DBP, and occasionally DnBP have been detected in blank samples, their values were below 0.07 ± 0.02 ng/L, 0.8 ± 0.3 ng/L, and 0.7 ± 0.2 ng/L, respectively (Paluselli et al. 2018b). These values are higher than the blank contaminants determined in the current study. The highest measured mass of DBP and DEHP in the LLE blank (35 ± 6 and 18.2 ng, respectively; Table 2.5) corresponded to 0.035 and 0.018 ng/L, respectively, after accounting for the pre-concentration. Similarly, the mass of DBP (63.0 ng) and DEHP (25 ng) measured in the SPE blanks corresponds to 0.06 and 0.02 ng/L, respectively. Blank contamination control and evaluation are particularly important for samples with low environmental concentrations.

A variety of methods can be used for the extraction and quantification of PAEs from seawater. Limits of quantification vary depending on the extraction method (LLE, SPE, Soxhlet, ultrasound, etc.) and quantification technique (GC-MS, GC-MS/MS, HPLC, UPLC, etc.). The quantification limits of PAEs in different techniques for the LLE and SPE extraction methods for published works are listed in Table 2.7. The average limits of quantification are in the range of (10-100 ng/L) for the LLE method and (10.0-180 ng/L) for the SPE method using GC-MS. In some studies, the limits of quantification obtained with GC-MS/MS were in a higher range (0.2.-0.53 ng/L), and UPLC with MS / MS detection was in the range of (3-8 ng/L). The MQL obtained by LLE and SPE were comparatively 5-10 higher than the MQL obtained by similar techniques (GC-MS), which may be due to the careful selection of experimental materials. In conclusion, our analytical method provides reliable quantification of blank contamination and increases the MQL. It is clear that some of these published methods revealed high MQL with significant interference issues, whereas the MQL achieved by the developed protocol was much lower than the maximum permissible limit of PAEs ($> 1.3 \mu\text{g L}^{-1}$) in fresh and marine water as per the EU guideline (JOUE 2013, Net et al. 2015a). Our approach provided better MQL, and will contribute to assessments of blank contamination from different materials during LLE and

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table 2.7. Figures of merits of the established and published methods for PAEs determination *

S.N o	Compounds	Method	Techniques	MQL (ng/L)	References
1	6 PAEs	LLE	LC-MS/MS	20-60	Liu et al. (2013)
2	15 PAEs	LLE	GC-MS	19.8-99	Adewuyi (2012)
3	6 PAEs	LLE	GC-MS	10	Net et al. (2015b)
4	6 PAEs	SPE	GC-MS	1.5-3	He et al. (2013)
5	DMP, DEP, DnBP, DEHP	SPE	GC-FID/MS	3-60 n	Fatoki (2010)
6	6 PAEs	LLE	GC-MS	150-430	Kim (2007)
7	7 PAEs	SPE	GC-MS	60-90	Farajzadeh et al. (2008)
8	15 PAEs	SPE	GC-MS	80-2547	Zhang and Chen (2014)
9	11 PAEs	SPE	GC-MS	33-99	He et al. (2013)
10	8 PAEs	SPE	UPLC-MS/MS	3	Liou et al. (2014)
11	DMP, DAIP, DnBP, BBzP	SPE	GC-MS	6.6-26.4	Farajzadeh and Jonsson (2007)
12	6 PAEs	SPE	GC-MS	3.3-20	Sánchez-Avila et al. (2011)
13	6 PAEs	SPE	GC-MS/MS	0.5-3	Aragón et al. (2013)
14	DEP and DEHP	SPE	GC-MS	90	Alhaddad et al. (2021)
15	7PAEs	SPE	GC-FID	20-63	Paluselli and Kim (2020)
16	7 PAEs	SPE	GC-MS	40-130	Paluselli et al. (2018a)
17	16 PAEs	SPE	GC-MS	180	Zhang et al. (2017a)
18	6 PAEs	LLE	GC-MS	10-20	Current work
19	6 PAEs	SPE	GC-MS	10-35	Current work

*The list of PAEs used in each study is given in the supplementary information Table S2.8; LC-MS = liquid chromatography and mass spectrum, FID= flame ionization detector, UPLC = ultra performance liquid chromatography.

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

SPE extraction and analysis, which help improve the method detection limits. Thus, it can be concluded that the established protocol can substitute other expensive analytical methods and be practiced in environmental laboratories for the direct determination of PAEs in water samples. Thus, this method meets the requirements for the analysis of PAEs in water samples.

2.4. Real sample analysis

The analytical utility of the established LLE and SPE methods to detect the leached PAEs from LDPE polymer, and PAEs in surface waters of the Red Sea, were critically tested. To detect the leached PAEs concentrations from LDPE, the polymer surface was rinsed with ultrapure water and cut into squares of $1.0 \times 1.0 \text{ cm}^2$. For each experiment, $\sim 100 - 150 \text{ mg}$ of the polymer (17 cm^2) was added to 30 mL screw-cap tubes containing ASW (10 mL). The tubes were tightly sealed with polytetrafluoroethylene (PTFE) septa, covered with aluminum foil, and shaken continuously for 6 h at 85 rpm. The dissolved concentrations of all PAEs from LDPE ranged from < 1.5 to 5.83 ng cm^{-2} . The highest amounts of PAEs released were DBP ($5.83 \pm 1.4 \text{ ng/cm}^2$), DEHP ($4.2 \pm 0.8 \text{ ng/cm}^2$), and DBP ($4.8 \pm 1.3 \text{ ng/cm}^2$). Indeed, DBP (LMW) and DEHP (HMW) are the most commonly used plasticizers and account for half of the total PE production in Western Europe (PlasticsEurope 2019). The absence of other target compounds (DMP, DEP, and BBP) is due to their low concentrations ($< 4 \text{ ng/mL}$) in the polymer, low leaching rates, high affinity to the polymer, or losses during the production process (Satkowski 1990). This is consistent with the fact that plasticizer leaching is concentration-dependent, with the rate decreasing with decreasing concentration (Wei et al. 2019). Similarly, the reported DBP levels from PE ranged from (1.2 ng/cm^2 over 1-78 days; Suhrhoff and Scholz-Boettcher 2016) to (8 ng/cm^2 over 1-12 weeks) (Paluselli, Fauvelle, et al. 2018).

The concentrations of PAEs in real seawater were measured following SPE extraction. The water samples were collected from 11 sampling sites along the Bay of Sharm Obhuur (Figure 4.1). The individual PAEs concentrations ranged from 1.0 - 256 ng/L , while the arithmetic average for individual PAEs concentrations ranged from 188 ng/L . High levels of DEP, DBP, and BEP were detected in all samples and ranging from 143 - 276 , 8.9 - 238 , and 138 - 282 ng/L , respectively. In contrast, DMP (3.4 - 16.6 ng/L), BBP (1.6 - 4.7 ng/L), and DnOP (2.2 - 49.1 ng/L) were generally

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

lower in most samples, The compounds DEP, DBP, and BEP accounted for more than 87% of the sum of concentrations of Σ_6 PAEs, with DEP (34%), DBP (22%) and BEP (33%). The concentration of the other three compounds represented between 4 - 6% of the total concentration, with the proportion for DMP (3%), BBP (6%), and DnOP (3%). This concentration range is consistent with data reported for other regions e.g. Σ_6 PAEs have been reported in the Mediterranean Sea: 17.4- 8442 ng/L (Sanchez-Avila et al. 2012), 130-1330 ng/L (Paluselli et al. 2018b) and 168-689 ng/L (Paluselli and Kim 2020) and northern Europe: 76-1440 ng/L (Turner and Rawling 2000).

2.5. Conclusion and recommendations for future studies

This study demonstrates the analytical utility and sensitivity of the established protocol for quantifying PAEs. Possible sources of blank contamination are highlighted with their impact on quantification limits in LLE and SPE extraction. A low level of blank contamination seems to be inevitable during experiments, despite many strict guidelines which do not necessarily guarantee the complete elimination of secondary contamination. Therefore, a detailed protocol is important to evaluate the control of blank contamination. The blank contamination with PAEs determined in the current study was 0.003- 0.04 and 0.005 – 0.06 ng/L for the materials used in the LLE and SPE methods, respectively. Failure to consider this could lead to an overestimation of the results obtained in the study. The MQL of PAEs obtained with the LLE and SPE methods are similar to the average reported MQL (0.3-20 ng/L). The current study provided lower MQL, and contributes to the assessment of blank contamination of different materials during LLE and SPE extraction and analysis, which helps to improve the MQL. In conclusion, this method is reliable in quantifying blank contamination and the MQL meets the requirements for the analysis of PAEs in seawater. We consider that it is important to standardize the methodology of sampling, separation, and identification of blank values with respect to PAE studies. This information is of prime importance in the analysis of PAEs, where it is of great value to achieve reliable determination of plastic additives in complex environmental samples. The eminence control of separable data is of great importance to assess the current environmental pressures associated with microplastic pollution.

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

CRedit authorship contribution statement

All authors made significant contributions to this study. Jeyakumar dhavamani: Performed all analyses and writing- original draft preparation, Aaron J. Beck: Co-supervision: Data curation, , Mohammad El-Shahawi Soror: Reviewing and editing, Martha Gledhill: Co-supervision, Eric P Achterberg; Supervision. Mohammad I. Oriff and Iqbal MI Ismail: Resources, funding acquisition.

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Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Appendix. Supplementary information

Table S2.1: The materials and testing procedures used in performing the blank interference test

Materials	Brand name	Suspected plastic parts	Testing procedure
GC-MS Instrument blank	Shimadzu-QP2010 Plus	Green Septa	A blank run without injection under the optimized conditions described in section 2.2
Dichloromethane (DCM)	Shimadzu	Polyethylene cap or dispenser	1 mL of solvent directly injected to GC-MS
GC vial	SUPELCO	Vial cap with Silicon septa	1ml of solvent directly transferred to GC vial injected directly
Nitrogen Unit	TurboVap LV Model N- EVAP 111	Unknown plastic lining housing and tips	Directly transferred 5 ml of solvent into a glass vial and pre-concentrated into 1 mL and injected into GC-MS
Micropipette tips	Caap, Denmark	0.2ml, Polypropylene tips	By using a micro-pipette, 5ml of solvent is transferred to a glass vial and 1 mL of it is pre-concentrated and injected into the GC-MS

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Micropipette tips (Sterile)	Caap, Denmark	0.2ml, Polypropylene tips	By using a sterile micro-pipette, 5ml of solvent is transferred to a glass vial and 1 mL of it is pre-concentrated and injected into the GC-MS
Syringe	ACTEST	Polyethylene housing and injection rubber	By using a syringe , 5ml of solvent is transferred to a glass vial and 1 mL of it is pre-concentrated to 1ml and injected into the GC-MS
Syringe filter	Whatman (0.2µm)	Polypropylene housing	By using a syringe with filter , 5ml of solvent is transferred to a glass vial and 1.0 mL of it is pre-concentrated and injected into the GC-MS
Sodium sulfate	Pan Reac- Applichem	Polyethylene bottle	Micropipette tips were used to transfer 5ml of solvent to a glass vial. The solvent was filtered through 5g of sodium sulfate and pre-concentrated into 1ml and injected into GC-MS.
Iso-octane	Shimadzu	Polyethylene cap or dispenser	0.2 µl of solvent directly injected to GC-MS
Methanol	Shimadzu	Polyethylene cap or dispenser	Directly transferred 5 ml of solvent into a glass vial and pre-concentrated into 1 ml and injected into GC-MS
Vacuum unit	J.T. Baker	Plastic luer fitting, delivery tips	A 5 ml solution passed through a vacuum unit and was collected in a glass vial. The solution was pre-concentrated into 1 ml of isooctane and injected into the GC-MS.

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

SPE cartridge	Chromabond Easy Macherey-Nagel, Germany	Polyethylene column housing	Solvent is passed through a SPE catheter connected to a vacuum unit and collected in a glass vial. The solvent is then pre-concentrated into 1 ml of isooctane and injected into the GC-MS.
ASW	Manually prepared (see section 2.2)	MiliQ unit, plastic salt containers	The cartridges were directly added with 1 L ASW flowing at 1 mL per minute. The sample was eluted with 5 mL of DCM and pre-concentrated into 1 ml of isooctane and then injected into a GC-MS.
ASW filtered	Manually prepared (see section 2.2)		ASW filtered from the above experiment step was again aspirated into SPE cartridges at 1 mL per minute. The sample was eluted with 5 mL of DCM and pre-concentrated into 1ml of isooctane and then injected into a GC-MS.
Tubes (silicon)	SHPI	Polyurethane tube	By using a silicon tubes, the cartridges were added with 1 L filtered ASW flowing at 1 mL per minute. The sample was eluted with 5 mL of DCM and pre-concentrated into 1 ml of isooctane and then injected into a GC-MS.

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table S2.2: The total mass of DMPs (ng) in each sequence of blank testing for the corresponding materials added

DMP	INB	DCM	Micro pipette tips	Micro pipette tips (Auto cleaved)	GC vial	Syringe	Syringe filter	Na ₂ SO ₄	N ₂ Unit	Iso octane	MeOH	Vacuum unit	SPE column	Line Tube	ASW	ASW filter
INB	2.48		2.48	2.48	2.48	2.48		2.48	2.48	2.48	2.48	2.48		2.48	2.48	
DCM	0	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89	1.89
Micropipette tips	0		2.32			2.32	2.32	2.32	2.32	2.32		2.32	2.32	2.32	2.32	2.32
Micropipette tips (Auto cleaved)				19.404												
GC vial																
Syringe						9.25	9.25									
Syringe filter							9.88									
Na ₂ SO ₄	0		1.86	1.86	1.86	1.86	1.86	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Nitrogen Unit			1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62
Iso-octane									0	0						
Methanol											2.56		2.56			
Vacuum unit											1.45	1.45	1.45			
SPE cartridge													2.5			
Tube (silicon)														0.87		
ASW															7	
ASW filtered																0.54
Total	2.48	1.89	10.17	27.254	7.85	19.42	26.82	9.24	9.24	9.24	10.93	10.69	13.27	10.1	16.24	7.3

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table S2.3: The total mass of DMPs (ng) in each sequence of blank testing for the corresponding materials added

DEP	INB	DCM	Micro pipette tips	Micro pipette tips (Auto clave)	GC vial	Syringe	Syring e filter	Na ₂ SO ₄	N2 Unit	Iso octane	MeOH	Vacuum unit	SPE column	Line Tube	ASW	ASW filter
INB	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
DCM		7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Micropipette tips			8.0			8.0	8.0	8.0	8.0	8.0		8.0	8.0	8.0	8.0	8.0
Micropipette tips (Auto cleaved)				69.3												
GC vial					0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Syringe					0.0	37.0	37.0									
Syringe filter							38.0									
Na ₂ SO ₄			6.0	6.0	6.0	6.0	6.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Nitrogen Unit			6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Iso-octane										0.0				0.0		
Methanol											8.0		8.0			
Vacuum unit											5.0	5.0	5.0			
SPE cartridge												0.0	10.0			
Tube (silicon)												0.0		3.0		
ASW												0.0			25.0	
ASW filtered												0.0				2.0
Total	8.0	15.0	35.0	96.3	27.0	72.0	110.0	32.0	32.0	32.0	37.0	37.0	55.0	35.0	57.0	34.0

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table S2.4: The total mass of DBP s (ng) in each sequence of blank testing for the corresponding materials added

DBP	INB	DCM	Micro pipette tips	Micro pipette tips (Auto clave)	GC vial	Syringe	Syringe filter	Na ₂ SO ₄	N ₂ Unit	Iso octane	MeOH	Vacuum unit	SPE column	Line Tube	ASW	ASW filter
INB	3.36	3.36	3.36	3.36	3.36	3.36	3.36	3.36	3.36	3.36	3.36	3.36	3.36	3.36	3.36	3.36
DCM		2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94
Micropipette tips			2.8			2.8	2.8	2.8	2.8	2.8		2.8	2.8	2.8	2.8	2.8
Micropipette tips (Auto cleaved)				24.948												
GC vial																
Syringe						12.95	12.95									
Syringe filter							15.58									
Na ₂ SO ₄			2.52	2.52	2.52	2.52	2.52	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26
Nitrogen Unit			2.34	2.34	2.34	2.34	2.34	2.34	2.34	2.34	2.34	2.34	2.34	2.34	2.34	2.34
Iso-octane																
Methanol											3.36		3.36			
Vacuum unit											2.1	2.1	2.1			
SPE cartridge													3.8			
Tube (silicon)														1.5		
ASW															1.25	
ASW filtered																0.78
Total	3.36	6.3	13.9	36.1	11.1	26.91	42.49	12.7	12.7	12.7	15.36	14.8	21.96	13.75	22.95	13.48

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table S2.5: The total mass of BBP s (ng) in each sequence of blank testing for the corresponding materials added

BBP	INB	DCM	Micro pipette tips	Micro pipette tips (Auto clave)	GC vial	Syringe	Syringe filter	Na ₂ SO ₄	N2 Unit	Iso octane	MeOH	Vacuum unit	SPE column	Line Tube	ASW	ASW filter
INB	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
DCM	0	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77
Micropipette tips	0	0	0.88	0	0	0.88	0.88	0.88	0.88	0.88	0	0.88	0.88	0.88	0.88	0.88
Micropipette tips (Auto cleaved)				6.23												
GC vial																
Syringe						4.44	4.44									
Syringe filter							4.94									
Na ₂ SO ₄	0	0	0.6	0.6	0.6	0.6	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Nitrogen Unit	0	0	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Iso-octane																
Methanol											0.88	0	0.88			
Vacuum unit											0.55	0.55	0.55			
SPE cartridge													1.1			
Tube (silicon)														0.3		
ASW															2.75	
ASW filtered																0.22
Total	0.96	1.73	3.75	9.107	2.87	8.19	13.13	3.45	3.45	3.45	4	4	5.98	3.75	6.2	3.67

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table S2.6: The total mass of DEHPs (ng) in each sequence of blank testing for the corresponding materials added

DEHP	INB	DCM	Micro pipette tips	Micro pipette tips (Autoclave)	GC vial	Syringe	Syringe filter	Na ₂ SO ₄	N ₂ Unit	Iso octane	MeOH	Vacuum unit	SPE column	Line Tube	ASW	ASW filter
INB	3.2	2.96	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
DCM		2.52	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Micropipette tips			2.96	0	0	2.96	2.96	2.96	2.96	2.96	0	2.96	2.96	2.96	2.96	2.96
Micropipette tips (Auto cleaved)			0	28.413												
GC vial						0	0									
Syringe						15.17	15.17									
Syringe filter						0	13.68									
Na ₂ SO ₄			2.22	2.22	2.22	2.22	2.22	1.11	1.11	1.11	1.11	1.11	1.11	1.11	1.11	1.11
Nitrogen Unit			2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Iso-octane																
Methanol											2.96	0	2.96			
Vacuum unit											2.05	2.05	2.05			
SPE cartridge													3.7			
Tube (silicon)														1.11	0	
ASW														0	10.25	
ASW filtered	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.76
Total	3.2	5.48	13.58	39.033	10.62	28.75	42.43	12.47	12.47	12.47	14.52	14.52	21.18	13.58	22.72	13.23

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table S2.7: The total mass of DnOPs (ng) in each sequence of blank testing for the corresponding materials added

DnOP	INB	DCM	Micro pipette tips	Micro pipette tips (Auto clave)	GC vial	Syringe	Syringe e filter	Na₂SO₄	N₂ Unit	Iso octane	MeOH	Vacuu m unit	SPE column	Line Tube	ASW	ASW filter
INB	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
DCM	0	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Micropipette tips			0.72	0	0	0.72	0.72	0.72	0.72	0.72	0	0.72	0.72	0.72	0.72	0.72
Micropipette tips (Auto cleaved)				8.316												
GC vial																
Syringe						3.33	3.33									
Syringe filter							4.18									
Na ₂ SO ₄			0.6	0.6	0.6	0.6	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Nitrogen Unit			0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Iso-octane																
Methanol											0.8		0.8			
Vacuum unit											0.55	0.55	0.55			
SPE cartridge													1.1			
Tube (silicon)														0.27		
ASW															2.5	
ASW filtered															0	0.24
Total	0.8	1.5	3.54	11.136	2.82	6.87	11.05	3.24	3.24	3.24	3.87	3.79	5.69	3.51	5.74	3.48

Chapter 2: Improved method for GC-MS analysis in seawater of phthalates from polyethylene following liquid-liquid and solid-phase extraction

Table S2.8: The list of PAEs study explained in corresponding studies listed in the figure of marits table in the main text (Table 2.8)

S.no	PAEs mix	List of PAEs in each mix
1	6PAEs	DMP, DEP, DnBP, BBzP, DEHP, DnOP
2	15PAEs	DMP, DEP, DiBP, DnBP, DMEP, BMPP, DEEP, DnPeP, DHxP, BBzP, DBEP, DcHxP, DEHP, DnOP, DNP.
3	6PAEs	DMP, DEP, DnBP, BBzP, DEHP, DnOP.
4	6PAEs	DMP, DEP, DnBP, BBzP, DEHP, DnOP.
5	DMP, DEP, DnBP, DEHP	
6	6PAEs	DMP, DEP, DnBP, BBzP, DEHP, DnOP.
7	7PAEs	DMP, DEP, DAIP, DnBP, BBzP, DcHxP, DEHP.
8	15 PAEs	DMP, DEP, DPrP, DiBP, DnBP, DAIP, DHxP, BBzP, DEHP, DnOP, BMEP, BMPP, DEEP, DBEP, DcHxP.
9	11PAEsg	DMP, DEP, DPrP, DnBP, DAIP, BBzP, DcHxP, DEHP, DnOP, BMPPrP, DMPPrP.
10	8PAEs	DMP, DEP, DnBP, BBzP, DEHP, DnOP, DiNP, DiDP.
11	DMP, DAIP, DnBP, BBzP,	
12	6PAEs	DMP, DEP, DnBP, BBzP, DEHP, DnOP.
13	6PAEs	DMP, DEP, DnBP, BBzP, DEHP, DnOP.
14	DEP and DEHP	
15	7PAEs	DMP, DEP, DiBP, DnBP, BBzP, DEHP, DnOP.
16	7PAEs	DnBP, DnPeP, BBzP, DEHP, DnOP, DnNP, DiNP.
17	PAEs	DMP, DEP, DiBP, DnBP, DMGP, DMPP, DEEP, DnAlP, DnHxP, BBzP, HEHP, DBEP, DcHxP, DEHP, DnNP, DnOP.
18	6 PAEs	DMP, DEP, DnBP, BBzP, DEHP, DnOP.
19	6 PAEs	DMP, DEP, DnBP, BBzP, DEHP, DnOP.

Chapter 3

The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

3. The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater.

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Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

Abstract

In this study, the leaching of six phthalic acid esters (PAEs) from three common consumer plastics was investigated: low and high density polyethylene (LDPE, HDPE) and recycled polyethylene (RP). The effects of salinity, temperature, and ultraviolet irradiation (UVR) on leaching were investigated. The study of leaching of phthalates in aqueous environments in batch experiments is challenging due to their readsorption by the high hydrophobicity of PAEs, and there are no standard methods to study release processes. Here with the experiments, leaching (A) and spiking (B) using six PAEs to study the readsorption in the leaching process. PAEs were identified and quantified using GC-MS. Dibutyl phthalate (DBP) and benzyl butyl phthalate (DEHP) showed considerable leaching during the 5-day incubation: 14 ± 1 to 128 ± 14 and 25 ± 2 to 79 ± 5 ng/cm², respectively, under UVR, corresponding approximately to (1.9-13%) and (12.4-22.4%) of the solvent extracted mass. The highest K_d values were measured for RP polymers (0.3-9.4), followed by LDPE (0.5-5.4) and HDPE (0.2-2.2) polymers. Thus, readsorption of PAEs at the surface removed 30-80% of the leached PAEs in the dissolved phase. For example in LDPE, the calculated total release of DBP was up to 54 ± 4 ng/cm², while the dissolved amount was 8.5 ± 1 ng/cm² during the 5-day incubation under freshwater conditions. Increasing salinity negatively affected the leaching rate, which decreased for DBP from 54 ± 4 ng/cm² in freshwater to 44 ± 3 and 38 ± 3 ng/cm² at salinity of 20 and 40 g/L, respectively, from LDPE during the 5-day incubation. Temperature and UVR had a positive effect on the leaching rate, with the release of DBP from LDPE increasing from 44 ± 3 ng/cm² at room temperature (25°C) to 60 ± 6 and 128 ± 14 ng/cm² at high temperature (40°C) and UVR, respectively. Overall, this study highlights the positive relationship between temperatures, UVR on the extent of leaching and surface adsorption on the leaching measurements.

Keywords: Leaching, Re-adsorption, Phthalate esters (PAEs), Saltwater, temperature, UVR, GC-MS, Mass balance calculation.

3.1. Introduction

Plastics are ubiquitous in our world, either in the form of products or as pollutants. The versatile properties of plastics make them ideal materials for a wide range of household and industrial applications [Gewert et al., 2015]. Chemical additives are an important reason for the success of plastics, as they impart certain material properties to plastics, including color, flexibility, stability, and resistance to degradation [J. N. Hahladakis et al., 2018]. Plastic additives are commonly used as plasticizers, flame retardants, stabilizers, antioxidants, and pigments. Phthalate esters (PAEs), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs) are widely used chemical additives that do not chemically bond with plastic polymers and therefore can be released from plastics into the environment through weathering processes [Oelschlaegel et al., 2018]. Phthalates, bisphenol A (BPA), nonylphenols (NPs), and brominated flame retardants (BFRs) are the most commonly observed additives in the environment [Hermabessiere et al., 2017] and pose a threat to ecosystems due to their toxicity [Gardon et al., 2020; Delilah Lithner et al., 2011; D. Lithner et al., 2012].

Over the last 60 years, average annual plastic production has increased from 1.5 to 311 million tons in 2014 and is expected to increase about 1800 million tons by 2050 [UNEP, 2016]. About 8 million tons of plastic waste enter into the world's oceans every year. The marine environment is severely threatened by plastics waste and additives (Jambeck, 2015). Plastic debris in the ocean contains a variety of polymers and particle sizes. Five major plastics commonly found in MP are thermoplastics: polypropylene (PP), polyethylene (PE), polystyrene [PS], polyvinyl chloride (PVC) and polyethylene terephthalate (PET) [Andrady, et al., 2017]. Plastics that are less dense than seawater float on the surface, while plastics that are denser than seawater sink to the ocean floor. During their time in the ocean, plastics are exposed to physical stress, UV radiation, heat, elevated salinity, oxidizing conditions, and colonization by a range of microorganisms, leading to fragmentation and decomposition of plastics and the release of chemical additives.

Many studies have reported that plastics are the source of chemical additives in the marine environment [Bandow et al., 2017; Bridson et al., 2021; Gallo et al., 2018; John N. Hahladakis et al., 2018] and these chemicals resist degradation and persist in the aquatic environment [Avio et al., 2017; Gallo et al., 2018]. Concentrations of additives in marine waters range from pg/L to µg/L, with phthalates being the most commonly reported compounds [Fries et al., 2013;

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

Hermabessiere et al., 2017; Keil et al., 2011; A. Paluselli and Kim, 2020]. Phthalate esters are among a growing list of putative endocrine disruptors, i.e., chemical compounds, either natural or synthetic, that disrupt normal endocrine functions [Heo et al., 2019; Liu et al., 2013; A. Paluselli and Kim, 2020; Rendell-Bhatti et al., 2021]. Several PAEs such as dimethyl phthalate (DMP), diethyl phthalate (DEP), di-(2-ethylhexyl) phthalate (DEHP), and dibutyl phthalate (DnBP) are listed as priority pollutants by the US Environmental Protection Agency (EPA) [Hermabessiere et al., 2017; Hu et al., 2016]. Importantly, PAEs are not covalently bonded to the plastic polymer and are therefore more likely to leach from plastics into the environment or into the stomach or tissues of organisms during abiotic/biotic aging, although little is known about the processes involved. Phthalate esters have been detected on plastic surfaces because they are re-adsorbed after leaching and absorption from the environment [John N. Hahladakis et al., 2018; Pittura et al., 2018]. This makes microplastics (MP) a vector for the transport of chemicals in the marine environment [Ašmonaitė et al., 2020; Ye et al., 2020].

In recent years, there has been increasing interest to understand the release of additives into seawater, the kinetics of their release from various plastics [Andrea Paluselli et al., 2019; Suhrhoff and Scholz-Boettcher, 2016; Ye et al., 2020]. Such as, the author Andrea Paluselli et al, 2019, studied the leaching behavior of PAEs from garbage bags made from a mixture of LDPE and HDPE and mostly recycled plastic. He proposed to extend the study to untreated LDPE and HDPE films. However, leaching behavior is often evaluated by analyzing the mass loss in the main sample or the dissolved concentration in the surrounding media [Bach et al., 2014; Bridson et al., 2021; Duflos et al., 2017; Rani et al., 2015; Suhrhoff and Scholz-Boettcher, 2016; Tuezuem Demir and Ulutan, 2013]. Studying the leaching of phthalates into aqueous environments in batch experiments is challenging due to their low solubility and high hydrophobicity, and there are no standard methods to study leaching processes. Re-adsorption of PAEs on plastics after leaching can significantly affect transport and dissolved concentrations in the marine environment. This type of measurement may overestimate or underestimate the compounds due to their adsorption behavior, which makes it difficult to explain genotoxic effects [Wei et al., 2019]. Therefore, it is necessary to improve the knowledge of the leaching process of additives in the marine environment. [Bridson et al., 2021; Rodrigues et al., 2019]. Whenever there is a possibility of adsorption by the polymer and the total leaching concentration is in the ultratrace (ng-μg) range,

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

the adsorption loss ratio should be determined to evaluate the total amount of additive leached [Zhang et al., 2017].

There are some dynamic or semi-dynamic approaches in which samples are subjected to continuous flow, such as column percolation (Bandow et al., 2017; Fikarov'a et al., 2019) (Sorensen et al., 2021; Suhrhoff and Scholz-Bottcher, 2016). Dynamic approaches, while somewhat more complex to implement and to achieving dynamic conditions is to use infinite solid phase sinks that have a high adsorption capacity for additives and column and other materials can introduce contamination with additives from the sink itself (Henkel et al., 2019; Sun et al., 2019; Ye et al., 2020). The need to recover the infinite sink from the leaching medium and extract the analyzes from the solid phase adds additional steps that further complicate this approach (Bridson, 2021 #193). The other hand, studies have investigated the direct adsorption and desorption kinetics of micro pollutant with plastics (POPs, PAHs, PAEs, Pesticides, etc.) and these studies have only measured the adsorption and desorption of the spiked compounds without considering leaching of similar compounds from the polymer itself [Razanajatovo et al., 2018]. Therefore, the current study was designed with the experiments, leaching (A) and spiking (B) using six phthalate esters (PAEs) to validate and evaluate the re-absorption in the leaching process to investigate the effects of the leaching process of LDPE, HDPE and RP in seawater. The influence of physical parameters such as salinity, temperature and UV radiation on the leaching and re-adsorption of PAEs was investigated.

3.2. Materials and methods

3.2.1 Reagents and Materials

Commercially available LDPE, HDPE, and RP were selected because of their importance to marine litter and their large production volume (30% of European plastic demand) (Plastics Europe 2018). Film grade polymers with thickness of (7.6, 5.7 and 8.7 μm) for LDPE, HDPE and RP, respectively, were purchased directly after production from one manufacturer (Panidan plastics Co. Ltd, Madurai, Tamilnadu, India). All polymer materials were analyzed by Fourier transform infrared spectroscopy (FTIR attenuated total internal reflection, Shimadzu Thermo Scientific Nicolet iS50 FT-IR, 4000-500 cm^{-1} , 20 scans per sample, 0.5 cm^{-1} resolution, Figure S3.1). Detected peaks were checked for polymer type using the IR spectra library (Shimadzu, IRs

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

Polymer 2). The polymers were stored in a dark and airtight container at room temperature for further use. A standard mixture of phthalic acid esters, including three low molecular weight (LMW) compounds; dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), and three high molecular weight (HMW) compounds; benzyl butyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DNOP) at a concentration of 20,000 mg/L in methanol (purity 99.8%) was obtained from Sigma Aldrich (Germany). Some properties of the PAEs are listed in Table 2.1 (Hermabessiere et al. 2017). Dichloromethane (DCM) and methanol (MeOH) were purchased from Sigma Aldrich (HPLC grade). Working solutions of PAEs were prepared in isooctane and stored in the dark at 4°C for a maximum of two weeks. All experiments were performed in artificial seawater (ASW) prepared by dissolving the following salts (3.0 mg/L NaF, 20 mg/L SrCl₂·6H₂O, 100 mg/L KBr, 700 mg/L KCl, 1 mg/L CaCl₂·2H₂O, 4000 mg/L Na₂SO₄, 10.8 mg/L MgCl₂·6H₂O, 23,500 mg/L NaCl, 20 mg/L Na₂SiO₃·9H₂O, and 200 mg/L NaHCO₃) in ultrapure water (Milli-Q, resistivity 18.2 MΩ). Phthalate contaminants in the ASW were removed by solid-phase extraction (SPE polypropylene column, Chromabond Easy, 200 mg, Macherey-Nagel, Germany) at a flow rate of 250 mL/h, and the ASW was sterilized in an autoclave (JASC-80JSR, Korea). To avoid cross-contamination, no plastic materials were used, and the glass materials were incinerated at 400°C for 4 hours.

3.2.2 Experimental setup

Batch experiments: The polymer surface was rinsed with ultrapure water and cut into squares of 1×1 cm. For each experiment, ~100 - 150 mg of the polymer (17 cm²) was added to 30 mL screw cap tubes containing 10 mL ASW. The tubes were tightly sealed with polytetrafluoroethylene (PTFE) septa, covered with aluminum foil, and shaken continuously at 85 rpm for up to 120 hours. The time-course experiment was performed in the dark with LDPE, HDPE, and RP added in ASW with different salt contents (0.1, 20, and 40 g/L) and temperatures (10, 25, and 40°C) and irradiated with UVR (359 nm). The temperature and UVR experiments were performed at an intermediate salinity (20 g/L), and the UVR experiment was performed at room temperature (25°C). All experiments were performed in triplicate.

In addition to the polymer incubations, a parallel experiment was performed with a 40 ng/mL spike of PAEs to evaluate adsorption to the polymer under all experimental conditions. In the following,

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

the experiments with polymer and polymer with spiked additives (PAEs) will be referred to as Experiment A and B, respectively. Experiment A was used to measure the leaching of PAEs from the polymers, while Experiment B determined the adsorption of additives and was used to correct the leaching data for the re-adsorption of the leached compounds. Parallel spike controls without plastics were also performed with 40 ng/mL PAEs to quantify their percent recovery. To exclude any external source of contamination, blank incubations were performed with ASW only. A water bath shaker was used for temperature control (GFL 1083 Thermo Labs, Germany), and low temperature conditions (10°C) were achieved using a refrigerated circulator (Julabo ED, Julabo GmbH, Germany). For the UVR experiment, a CCP-4V photoreactor (Luzchem, Canada) with a UVA (~ 350 nm) lamp was used. Since the current study focuses on the effects of microplastics in the Red Sea region, the UVR exposure intensity (UVA; 15.6 W/m², UVB; 1.26 W/m² and UVC; 0.86 W/m²) was chosen to represent the conditions in the region of Saudi Arabia (Mahfoodh et al. 2003).

3.2.3 Extraction and analysis

Total water samples (10 ml) were collected from the experimental tubes at regular intervals (0.25, 0.5, 1, 2, 3, and 5 days) and then filtered through a 0.45 µm glass fiber membrane that had previously been ashed at 400°C. The concentrations of dissolved PAEs in ASW were determined by liquid-liquid extraction with successive aliquots of 2 mL, 1 mL, and 1 mL DCM. The combined organic phases were dried with sodium sulfate (Na₂SO₄) and reduced to 0.2 mL under nitrogen (model N- EVAP 111, Organization, USA. To investigate the initial composition of PAEs, each plastic (LDPE; 133.2 ± 5.6 mg, HDPE; 125 ± 4.3 mg, and RP; 142 ± 8.2 mg) was extracted overnight with 5 mL toluene: dichloromethane (4:1 v/v) (Table S3.2). The sample was then sonicated twice) for 15 min followed by centrifugation at 3000 rpm for 5 min. The combined organic phases were dried with sodium sulfate and reduced to 1 mL under nitrogen. The extracted samples were stored at 4°C until further analysis.(Xu, et al., 2020)

The concentrations of PAEs in the extracts were analyzed by gas chromatography-mass spectrometry (GC-MS QP -2010 plus, Shimadzu, Japan) using a flame ionization detector (FID) and a mass spectrometer (MS, EI conditions). The initial temperature of the column oven was 250°C. A capillary column was used (30 m × 0.25 mm i.d. × 0.25 m, 5% phenylmethylsiloxane,

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

Agilent HP -5MS) with a temperature program of 60°C (2 min hold), ramp 5°C/min to 310, and 5 min hold. Helium was used as the carrier gas (2 mL/min). The ion source temperature was 220°C and the interface temperature was 250°C. The injection volume was 2 µL using an auto-sampler (AOC-5000, Shimadzu, Japan). Injection was performed in "splitless" mode, with a spitting time of 0.98 min and a purge flow of 30 mL/min. The Selected Ion Mode (SIM) was developed experimentally for each compound based on precursor and production ions, collision energies, and other parameters. Target compounds were positively identified by comparing their retention times and target ions to the specific reference ions.

3.2.4 Instrument calibration and method validation

Instrument performance was calibrated based on the European regulation, article number; 10/2011/EU (Commission 2011) and an eleven-point calibration curve was analyzed in triplicate at 0.005, 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4, 0.8, 1 and 2 µg/mL for all target PAEs. Quantification was performed using the external standard method in selected ion monitoring SIM mode. The regression coefficient of linearity was greater than 0.99 with RSD of 12-18%. The instrumental limit of detection (LOD) and limit of quantification (LOQ) were calculated by the signal-to-noise ratio (S/N) of the lowest concentration used. Extraction recovery was measured by the standard addition method (Frenna, Mazzola et al. 2012). Known concentrations of standard PAEs (0.01, 0.02, 0.04, 0.08, 0.16, 0.2 µg/mL) were spiked into 10 mL ASW and extracted using the same procedure. The average recoveries were in the range of 94 -107 % with a of RSD 11-14 %. The optimized parameters for GC-MS calibration, SIM ions, method validation, are given in Table 2.2.

3.2.5 Mass balance equations

The adsorption capacity (Q_e) of the additives by the polymer may affect the assessed dissolution concentration. A mass balance approach was used to determine the total leachate for each PE. For the polymer-only (P) treatment (Experiment A), the total mass of a leached PE includes both the dissolved mass and the mass re-adsorbed on the surface of the polymer. To determine the adsorbed mass, the P treatment (Experiment A) was compared to a treatment containing both polymer and a PE spike (P + S; Experiment B). We assume that the ratio of adsorbed leachate (L_{ads}) to dissolved leachate (L_{dis}) is the same as the ratio of adsorbed spike (S_{ads}) to dissolved spike (S_{dis}):

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

$$L_{ads}/L_{dis} = S_{ads}/S_{dis} \quad (3.1)$$

In experiment B (P + S), the total dissolved mass ($B_{Tot-dis}$) includes both the dissolved leachate and the dissolved spike:

$$B_{Tot-dis} = B_{L-dis} + B_{S-dis} \quad (3.2)$$

The mass of dissolved leachate in B (B_{L-dis}) is equal to that in A (A_{L-dis}), so by substituting and rearranging Eq. 2, the mass of the dissolved spike can be determined:

$$B_{S-dis} = B_{Tot-dis} - A_{L-dis} \quad (3.3)$$

Assuming no degradation, the mass loss of the dissolved spikes (S_{dis}) from the initial spiked value (S_{Int}) is equal to the adsorbed mass of the spikes (S_{ads}):

$$B_{S-ads} = B_{S-Int} - B_{S-dis} \quad (3.4)$$

Since the ratio of adsorbed to dissolved leachate is the same as the ratio of adsorbed spike (B_{S-ads} , Eq. 4) to dissolved spike (B_{S-dis} , Eq. 3.3), the mass of adsorbed leachate (L_{ads}) can be estimated by rearranging equation (3.1) to:

$$A_{L-ads} = A_{L-dis} * (B_{S-ads} / B_{S-dis}) \quad (3.5)$$

Therefore, the total leachate can be estimated by adding the dissolved and adsorbed mass of leachate to obtain the following equation:

$$A_{L-total} = A_{L-dis} + A_{L-dis} * (B_{S-ads} / B_{S-dis}) \quad (3.6)$$

Equation (3.6) can be rearranged to obtain the following expression:

$$A_{L-total} = A_{L-dis} [1 + (B_{S-ads} / B_{S-dis})] \quad (3.7)$$

3.3. Results

3.3.1 Dissolved concentration of leached PAEs (L_{dis})

The dissolved concentration of all PAEs from LDPE, RP and HDPE in experiment A (polymer only, no spike) for 5 days of incubation are shown in Figures (S3.2, S3.3, and S3.4). During the 5-day incubation period, the highest measured concentration in experiment A (Q_m ; A_{L-dis}) was observed between 24 and 48 hours after incubation, followed by a decrease. The Q_m ; A_{L-dis} values of the individual PAEs from LDPE, RP and HDPE polymer under all experimental conditions are shown in Table S3.1. The overall the concentration different on minimum to maximum leached PAEs (Q_m-A_{L-dis}) values under different experimental conditions from LDPE, HDPE and RP are shown in Table 3.1. For all the three polymer lowest concentration was observed in high salinity

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

water (S 40g/L) and the highest concentration in UVR experiment. Comparatively the highest sum of the six phthalic acid esters \sum_6 PAEs was observed from LDPE at both high salt concentration (\sum_6 PAEs: 40 ± 4 ng/L) and under UVR experimental conditions (\sum_6 PAEs: 163.0 ± 10 ng/mL). Followed by RP polymers (\sum_6 PAEs: 18.6 ± 3 and 39 ± 3 ng/mL) at high salt concentration and under UVR conditions, respectively. While HDPE showed the lowest average concentration (\sum_6 PAEs: 8.0 ± 1.5 ng/mL) at high salt concentration, the leaching increased up to (\sum_6 PAEs: 138 ± 10 ng/mL) by UVR.

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

Table 3.1: The dissolved leachate (ng/mL) at Q_m for LDPE, RP and HDPE polymer at different salinities (0.1, 20 and 40 g/L), temperature (10, 20 and 40°C), and UVR (354nm)

Names	LDPE (ng/mL)				RP (ng/mL)				HDPE (ng/mL)			
	min	max	Avg	(%)	min	max	Avg	(%)	min	max	avg	(%)
DMP	4.3± 0.3	11.6± 0.8	5.2 ±0.6	8.2	0.3±0	3.2±0.2	0.9±0.1	2.3	0.5±0	30.6±1.5	5.7±0.6	20.2
DEP	21± 1.9	31.5± 2.2	20±1.6	31.7	10.5±0.7	9.5±0.7	7.2±0.7	18.7	1.4±0.1	25.3±2	5±0.4	17.6
DBP	9±0.7	67.5± 4	20.9±2.5	33.1	4.4±0.3	9.8±0.9	9.2±0.9	23.9	3.3±0.2	10.7±0.5	4.9±0.4	17.5
BBP	0.2±0	11.4± 0.6	2.6±0.2	4.1	1.7±0.1	0.7±0	1.8±0.2	4.5	0±0	32.7±2.9	5.1±0.4	18.1
DEHP	4.6±0.4	35.7± 2.1	12.4±1.5	19.7	2.7±0.2	7.1±0.6	5.5±0.6	14.3	1.6±0.1	26.8±1.3	5±0.5	17.7
DnOP	0.5±0	7.2± 0.6	2±0.2	3.2	2.5±0.2	8.6±0.8	14±1.5	36.3	1.2±0.1	12.5±1	2.5±0.3	8.9
Σ₆ PAEs	39.4	164.8	63.1		18.6	38.8	38.7		7.9	138.6	28.3	

Among the six PAEs; DEP, DBP, and DEHP showed the greatest increase in concentration over DMP, BBP, and DnOP from LDPE and RP polymer under all conditions. The compounds DEP, DBP, and DEHP accounted for more than 84.4 % of the sum of the concentrations of Σ₆ PAEs, with DEP (31.7 and 18.7%), DBP (33.1 and 24.0%), and DEHP (19.7 and 14.3%) from LDPE and RP polymers, respectively. The other compounds (DMP, BBP and DnOP) reached low and variable proportions. Thus, the proportions of DMP, BBP and DnOP in LDPE were 8.2, 4.1 and 3.2 %, respectively, while in RP polymers a similar range was observed for DMP (2.3%) and BBP (4.5%), except for DnOP (36.3%).

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

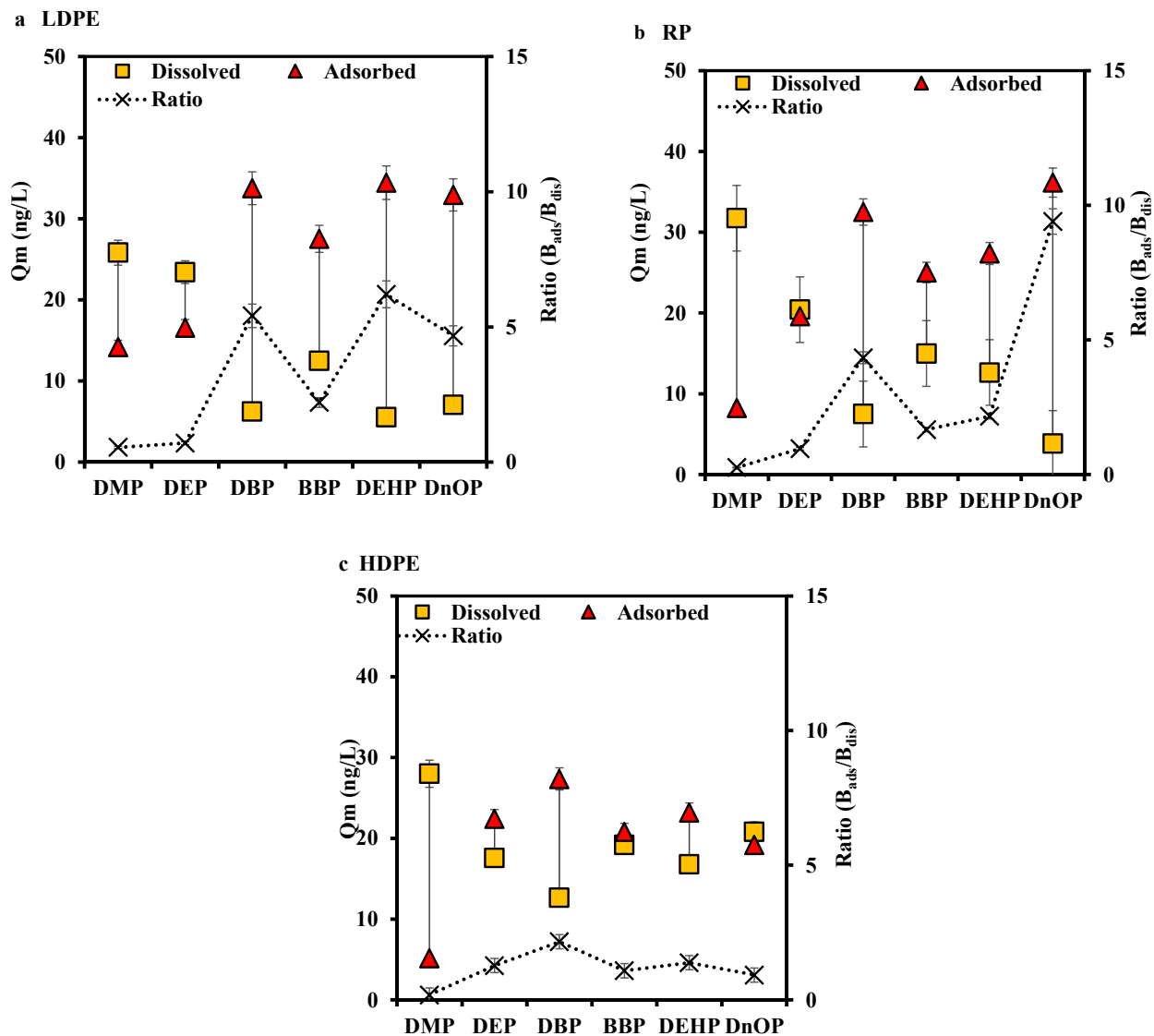


Figure 3.1: The average concentrations of dissolved, adsorbed, and the ratio of adsorbed to dissolved spikes PAEs in experiment B for LDPE (a), RP (b) and HDPE (c) under the conditions of the freshwater experiment (S0.1 g/L), respectively

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

In contrast, higher concentrations were leached from HDPE only upon UVR, and percentage of PAE ranged from 8.9 to 20.2%. (Table 3.1). Overall, the leached concentration PAEs leached from LDPE than from HDPE and RP polymer.

The mass of leached PAEs was compared with their initial solvent-extracted mass in each polymer. The initial polymer content of the measured Σ_6 PAEs ranged from 177 ± 12 , 243 ± 23 ng/mg and 162 ± 10 for LDPE, RP and HDPE polymers, respectively. Under all experimental conditions and the initial concentrations, the leaching percent of PAEs are given in Table S3.2. For example, in freshwater conditions (S 0.1 g/L), the major leaching products DMP, DEP, and DBP leached from LDPE ranged from 0.8% to 4.4%, while the values ranged from 0.3 - 3.99% and 0.09 to 1.10% from RP and HDPE, respectively.

3.3.2 Adsorption of spiked PAEs (S_{ads})

In addition to the polymer incubation, a parallel experiment was performed with a 40 ng/mL spike of PAEs to evaluate the adsorption capacity (Q_e) to the polymer under all experimental conditions (Experiment B). The dissolved concentration of spike PAEs for 5 days of incubation in the adsorption experiment (polymer only, no spike) ranged from 6.5 ± 0.5 to 29 ± 2 ng/mL, 3.8 ± 0.3 to 31 ± 2 ng/mL and 5.2 ± 1.2 to 34.5 ± 1.7 ng/mL and, respectively, for leachates from LDPE, RP and HDPE (Figure S3.5, S3.6 and S3.7). The adsorption capacity (Q_e) of the polymer was calculated from the mass loss over a 5-day period. The dissolved concentration of spiked PAEs in experiment B (Q_m ; B S_{dis}) measured at the time of maximum leachate observed in the experiment A (Q_m ; A L_{dis}), values of the individual PAEs from LDPE, RP and HDPE polymer under all experimental conditions are shown in Table S3.3. The resolved spikes (B S_{dis}) relative to the original spike value (B S_{Int}) as follows. (See Section 2.5; Equation 3: B $S_{ads} = B S_{Int} - B S_{dis}$). The further mass of leachate in Experiment B was normalized (see Section 2.5; Equation 2; B $S_{dis} = B Tot_{dis} - A L_{dis}$).

The average concentrations of dissolved and adsorbed PAEs under the freshwater experiment conditions are shown in Figure 3.1. The adsorbed PAE concentrations of LDPE, HDPE, and RP range from 5 to 36 ng/mL. For example, the average adsorption of DMP, DEP, DBP, BBP, DEHP and DnOP on LDPE (14.2, 16.6, 33.8, 27.5, 34.4, and 32.9 ng/mL), HDPE (5.16, 22.4, 27.4, 20.8,

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

23.2, and 19.2 ng/mL), and RP (8.3, 19.6, 32.5, 25.0, 27.4, and 36.2 ng/mL) under freshwater conditions (0.1 g/L) (Figures 3.1a, 3.1b, and 3.1c), respectively. The ratio of adsorbed to

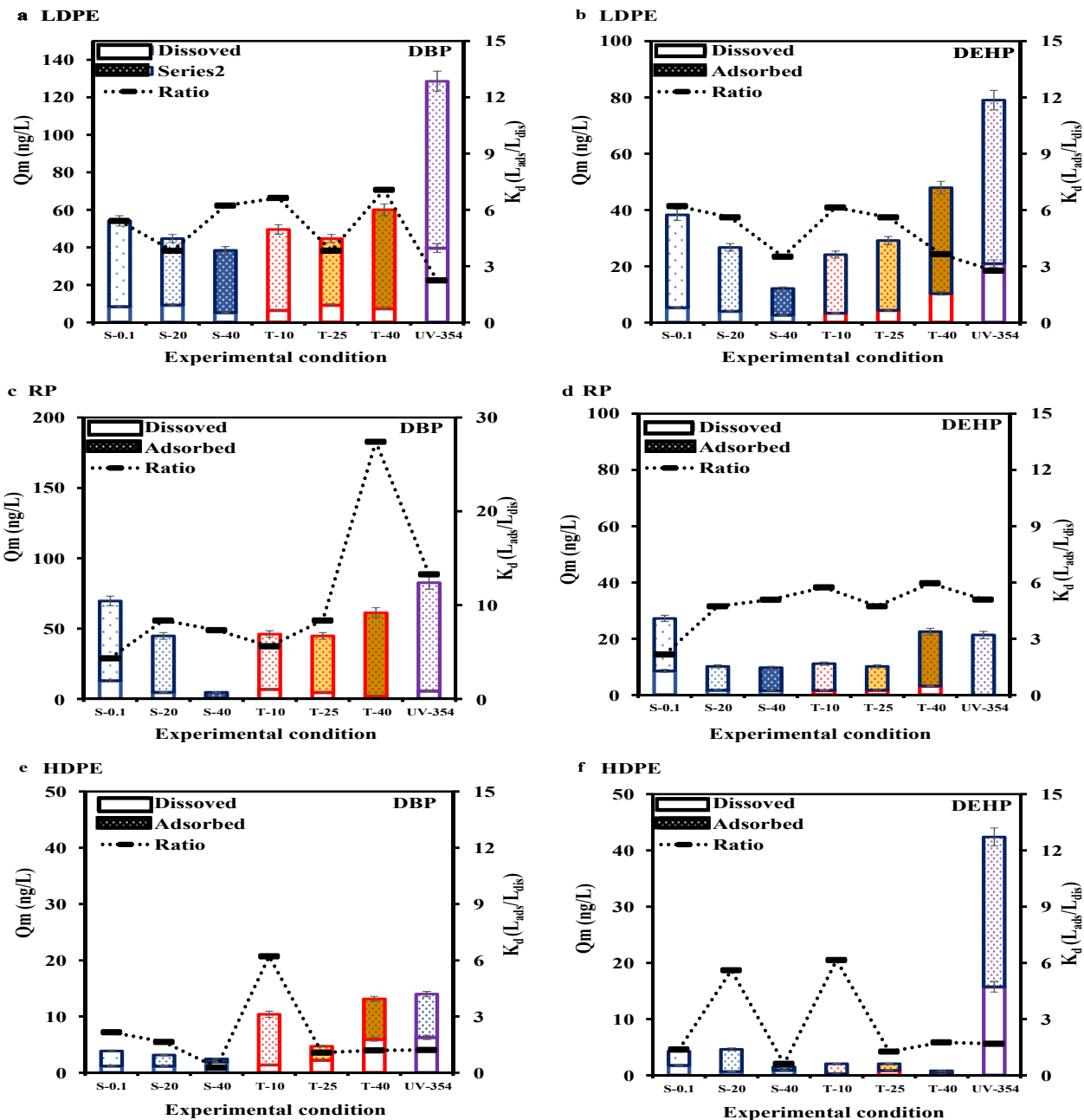


Figure 3.2: Compares the effects of the experimental conditions on the leaching of dissolved, adsorbed, and adsorbed-to-dissolved mass ratios of DBP and DEHP from LDPE (a & b), RP (c & d) and HDPE (e & f) polymers, respectively

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

dissolved mass of the spiked PAEs is defined as the plastic-water partition coefficient (K_d). The adsorption increased with increasing molecular weight of the PAEs. The K_d of the PAEs ranged from (0.5-6.2), (0.2-2.2), and (0.3-9.4) for LDPE, HDPE, and RP polymer, respectively at fresh water condition (S 0.1 g/l). Moreover, the results show that K_d increased linearly with the low molecular weight and linear chain of the compounds (DMP, DEP and DBP). Thus, LDPE showed a linear fit with K_d (slope = 0.0615; $r^2 = 0.953$), while the high molecular weight and branched chain compounds BBP, DEHP and DnOP did not increase linearly.

3.3.3 Total amount of PAEs leached ($A L_{\text{total}}$)

The effects of salinity (0.1, 20, and 40 g/L), temperature (10, 25, and 40 °C), and UV radiation (354 nm) on the adsorption ratio (K_d) of each PAE with LDPE, HDPE, and RP are shown in (Table S3.4). The highest K_d values were measured with the RP polymer, followed by LDPE and HDPE polymer. For the HMW compounds, a slight increase in K_d values was observed with increasing salinity of the medium. For example, alues for BBP, DEHP, and DnOP increased from 3.9 ± 0.4 to 4.2 ± 0.3 , from 4.7 ± 0.2 to 5.0 ± 0.2 , and from 9.6 ± 0.7 to 14.2 ± 0.7 , with an increase in salinity of 20 and 40 g/L with the RP polymer, respectively. In LDPE the K_d values of DBP and BBP increased from 3.8 ± 0.2 to 6.2 ± 0.5 and 3.1 ± 0.3 to 6.5 ± 0.5 , respectively. The temperature change from 25 to 40°C for the LDPE polymer increased the partition coefficient (K_d) for the LMW PAEs such as DBP (3.8 ± 0.2 to 7.0 ± 0.4), (8.4 ± 0.3 - 27.4 ± 2) (1.0 ± 0.1 to 1.2 ± 0.1) for LDPE, RP and HDPE polymer, respectively. There is not much difference in K_d values between treatments non-UVR and UVR.

The total amount of PAEs leached was calculated by balancing the adsorption losses from ASW (see Section 2.5; Equation 7: $A L_{\text{total}} = A L_{\text{-dis}} [1 + (B S_{\text{-ads}} / B S_{\text{-dis}})]$). Considerable adsorption was observed for the high molecular weight compounds BBP, DEHP, and DnOP, while the low molecular weight compounds DMP, DEP, and DBP showed lower adsorption. Therefore, the calculated total amount leached per unit area of HMW-PAEs showed the largest difference from the observed dissolved concentrations (Figures S3.8, S3.9, and S3.10). The comparison between the total dissolved and calculated amounts during the 5-day incubation under freshwater conditions (S 0.1 ng/L) for LDPE, HDPE, and RP is shown in Table 3.2. In LDPE, the calculated total amount

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

of leached DEHP released was 38 ± 3 ng/cm² while the observed dissolved amount was 5.3 ± 0.4 ng/cm², during the 5-day incubation in freshwater (S0.1 ng/L). The total leached amount of DEP and DBP increased from measured 9.6 ± 1 and 8.4 ± 0.7 ng/cm² to estimated 16.5 ± 1.2 and 54 ± 4 ng/cm², respectively. RP showed similar differences. For example, the estimated total dissolved DBP, DEHP, and DnOP increased from (13 ± 2 , 8.5 ± 1 , and 5.7 ± 1.5 ng/cm²) to (69 ± 8 , 27 ± 2 , and 59 ± 3 ng/cm²).

Table 3.2: The difference between dissolved and calculated total leachate (ng/cm²) at Q_m for LDPE, RP and HDPE polymer at freshwater condition (S 0.1 g/L)

Compounds	LDPE (ng/cm ²)		RP (ng/cm ²)		HDPE (ng/cm ²)	
	Dissolved	Total	Dissolved	Total	Dissolved	Total
DMP	2.9 ± 0.3	4.5 ± 0.4	0.4 ± 0	3.2 ± 0.2	0.2 ± 0	0.3 ± 0
DEP	9.7 ± 1	16.5 ± 1.2	3 ± 0.3	9.5 ± 0.7	0.1 ± 0	0.3 ± 0
DBP	8.4 ± 0.7	54 ± 4	69 ± 7	9.8 ± 0.9	1.2 ± 0.1	3.9 ± 0.4
BBP	1 ± 0.1	3.2 ± 0.2	3.2 ± 0.3	0.7 ± 0	0 ± 0	0 ± 0
DEHP	5.3 ± 0.4	38 ± 3	27 ± 2	7.1 ± 0.6	1.8 ± 0.2	4.3 ± 0.3
DnOP	1.3 ± 0.1	7.2 ± 0.5	59.2 ± 0.5	8.6 ± 0.8	0.8 ± 0.1	1.5 ± 0.2
Σ6 PAEs	28.56	123.76	30.35	162.7	4.18	10.24
Median	4.11	11.83	3.62	15.22	0.51	0.9

For PAEs that showed low leaching ($1\text{--}5$ ng/cm²), the high spike level (23.5 ng/cm²) means that the adsorbed and dissolved fractions can be a small difference between large numbers, potentially resulting in high uncertainty on the resultant ratio. Given that the uncertainty on most replicate incubations was relatively low ($\sim 12\%$), we believe this effect to be minor. However, the calculated difference for a few compounds had relatively high uncertainty (e.g., BBP (25%) and DnOP (32%) from HDPE under high temperature conditions).

3.3.4 Effect of salinity, temperature and UV radiation on the total amount of PAEs leached: (A L_{-total})

The effects of salinity (0.1, 20, and 40 g/L), temperature (10, 25, and 40 °C), and UVR (354 nm) on the leaching behavior of PAEs were investigated. The total leachate of PAEs at Q_m for each

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

condition are given in Table S3.5 for LDPE, RP, and HDPE respectively. The overall results show that increasing salinity has a negative effect, while temperature and UVR have positive effects on the leaching of PAEs. HDPE is the only polymer that exhibits higher leaching at low temperature (10°C) than LDPE and RP polymers. For example, Figure 3.2 compares the effects of the experimental conditions on the leaching of DBP and DEHP from LDPE, RP, and HDPE polymers. The total leachate of the LMW compound DBP in LDPE decreased from 54 ± 4 ng/cm² in freshwater to 44 ± 3 and 38 ± 3 ng/cm² at salinity of 20 and 40 g/L, respectively (Figure 3.2a.) and DEHP decreased from 38 ± 3 to 27 ± 3 and 12 ± 1 ng/cm² (Figure 3.2b) and was statistically significant ($p=0.0071$). Similar trends were observed in RP for DBP (69 ± 8 , 45 ± 5 , and 4.7 ± 0.4 ng/cm²; $p=0.0086$) (Figure 3.2c), whereas the HMW DEHP (27 ± 2 , 10.2 ± 0.7 , and 9.8 ± 0.8 ng/cm²) only small and non-significant decreases were measured at salinity from 0.1 g/L to 20 and 40 g/L, respectively (Figure 3.2d). DnOP were measured only with the RP polymer at concentrations of 59 ± 3 , 48 ± 3 , and 22 ± 2 ng/cm², respectively, under the same experimental conditions (0.1 g/L to 20 and 40 g/L; Table S3.5). For all PAEs from HDPE and other target compounds (DMP, BBP) from LDPE, the RP is below 4 ng/cm², which is very low compared to the ratio of adsorbed to dissolved spikes.

All temperature experiments were performed at an average salinity (20 g/L). Very little difference was measured when the temperature was increased from 10 to 25°C for LDPE and RP polymers. For example, the Q_m value for DBP was 49 ± 4 and 44 ± 3 ng/cm² for LDPE and 46 ± 3 and 45 ± 3 ng/cm² for RP polymers at 10 and 25°C, respectively (Figures 3.2a and 3.2c). Similar trends were also measured for the other major leachate DEHPs (LDPE; 24 ± 2 , 26 ± 3 and RP; 11 ± 1 , 10.2 ± 0.7 , ng/cm²) at 10 and 25°C, respectively (Figures 3.2b and 3.2d). For HDPE polymers, higher values were measured at low temperatures, e.g., for DBP (10.4 ± 0.8 ng/cm² at 10°C) than at 25°C (3.2 ± 0.6 ng/cm²) (Figure 3.3e). These two main leachable substances showed a significant difference (DBP: $p=0.0042$, DEHP; $p=0.0391$) between 10 and 25°C, only for HDPE polymers. When the temperature was further increased from 25 to 40°C, the Q_m of DBP and DEHP increased to 60.0 ± 6 and 44 ± 3 ng/cm² and 48 ± 4 and 26 ± 3 ng/cm² for LDPE and RP, respectively. This shows that increasing the temperature between 25 and 40°C resulted in an increase in the leaching rates of DBP and DEHP from LDPE and RP, respectively. The leaching of these compounds from HDPE was constant between 25 and 40°C. Similar trends were observed for the other compounds

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

such as DEP (LDPE; 27 ± 2 , 21.4 ± 1.5 and 42 ± 3 , HDPE; 5.7 ± 0.8 , 1.2 ± 0.1 and 1.8 ± 0.2 , RP; 3.4 ± 0.4 , 8.3 ± 0.7 and 40 ± 4 ng/cm²) at 10, 25 and 40°C, respectively. RP is the only polymer from which DnOP was leached ($42 - 49$ ng/cm²: (Table S3.5).

The UVR experiment was performed with a salinity of 20 g/L at 25°C to determine the effect of natural sunlight. The observed effect of UVR was an increase in the leaching rate, which exceeded even that observed in the experiments with different temperatures (10 to 40°C) and salinities (0.1 to 40 g/L). To evaluate the effect of irradiation itself, the results of the UVR experiment were compared only with the non-UV experiment conducted at a salinity of 20 g/L at 25°C (non-UVR). Among the three polymers, HDPE was more affected by UVR. Thus, the Q_m of PAEs from HDPE without UVR showed only (< 4 ng/cm²) for DBP and DEHP, respectively, which increased to (24 ± 2 to 42 ± 3 ng/cm²) under UVR; (Figure 3.2e and 3.2f). In LDPE, Q_m values for DBP (44 ± 3 to 128 ± 14 ng/cm²) and DEHP (26 ± 3 to 79 ± 5 ng/cm²) also increased by UVR (Figure 3.2a and 3.3b). In RP, the increases were observed for DBP (45 ± 5 to 82 ± 9 ng/cm²) and DEHP (10.2 ± 0.7 to 25 ± 2 ng/cm²). Q_m values for DnOP (48 ± 4 to 43 ± 3 ng/cm²) did not increase with UVR (Table S3.5).

3.3.5 Leaching rates

The leaching rate of each compound was calculated based on the slope of the linear range observed during the first 2-3 days of the experiment. The leaching rate of all PAEs under different experimental conditions with three polymers is shown in Table S3.6. Overall, the leaching rate of all PAEs decreased with increasing salinity and increased with increasing temperature and UVR for LDPE and RP polymers. The differences in rates under freshwater conditions (S 0.1g/L), high temperature (T 40°C), and UVR are shown in Table 3.3 for LDPE, RP and HDPE polymers, respectively. Since leaching is higher for HDPE than high temperature (40°C), low temperature (10°C) was used for comparison.

The leaching rate of PAEs from two polymers was greatly increased by increasing the temperature and UVR. For example, the average rate of DBP is 15 ± 2 ng/cm²/d under freshwater conditions. This value increases to 73 ± 9 and 70 ± 8 ng/cm²/d at high temperature (40°C) and UVR, respectively (Table 3. 3). Similarly, DEHP increases from 16.2 ± 0.2 to 30 ± 3 and 72 ± 7 ng/cm²/d

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

(Table 3.3). In contrast, the values reported in RP for DBP (11.3 ± 1.4 , 69 ± 8 and 39 ± 4 ng/cm²/d) and DEHP (4.8 ± 0.3 , 60 ± 3 , and 17.3 ± 2) under freshwater (S 0.1 g/L), high temperature (40°C), and UVR experimental conditions Table 3.4. However, the rate differences between the temperature and UVR treatments were not significant ($P > 0.01$). In contrast, HDPE was released more at low temperature (10°C) than at higher temperature (40°C). The average rate for DEP (40 ± 4 ng/cm²/d) was measured at low temperature (10°C), followed by UV irradiation (6.2 ± 0.7 ng/cm²/d) and freshwater (0.2 ± 0.01 ng/cm²/d). According to the rate plot, the leaching profile of PAEs from LDPE was faster than from RP and HDPE polymers.

3.3.6 Diffusivity of PAEs

Leaching rates were dependent on the diffusivity of the additives through the polymer matrix. The diffusion coefficient (D_e) of PAEs in the three polymer films was estimated from the slope (h) of the linear regression of the leaching mass (Q_t/Q_m) vs. $t^{0.5}$ (Supplementary Information). The D_e values of all PAEs at different experimental conditions with three polymers are listed in Table S3.8 (Supplementary Table S3.8). The relationship between leaching rate trends and the differences in D_e versus number among the polymers (LDPE and RP) at fresh water (S 0.1g/L), high temperature (T 40°C), and UV irradiation are shown in Figures 3.3a, and 3.3b, respectively. Since leaching is higher for HDPE than high temperature (40°C), low temperature (10°C) was used for the comparison. (Figure 3.3c). The D_e values were plotted against the number of carbons

The effective diffusion coefficients of the PAEs in all three polymers increased with temperature, as expected, and decreased with increasing molar mass of the PAEs. In LDPE, the D_e for DMP, DEP, DBP, DEHP, BBP and DnOP were 12.2, 14.4, 13.6, 6.9, 6.1 and 8.6×10^{-20} m²/d, respectively, at high temperature conditions (Figure 3.3a)

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

The D_e values were different for the different types of polymers. In RP polymer, the highest D_e value was measured for DBP and DEHP with 11.3 and $7.0 \times 10^{-20} \text{ m}^2/\text{d}$, respectively; (Figure 3.3b). Among the three polymers, HDPE showed low D_e values for all PAEs. The trends show that the highest D_e values were observed for the highly flexible polymer LDPE, followed by RP and HDPE. The polymer and water diffusion coefficients for the straight chain carbon compounds (DEP, DEP and DBP) showed a linear relationship with the number of carbon atoms, while the branched chain carbon compounds (BBP, DEHP and DnOP) were not linear with carbon number or molar mass.

Table 3.3: The leaching rate of PEs in fresh water (0.1 g/L), high temperature (40°C), and UVR (354 nm) treatments (i.e., fastest leaching conditions for each category) for LDPE, RP and HDPE polymer. In HDPE low temperature condition (10°C) were used to compare due its higher leaching then high temperature condition (40 °C)

Compounds	Total leaching rate (ng/cm ²)								
	LDPE			RP			HDPE		
	S0.1	T40	UVR	S0.1	T40	UVR	S0.1	T10	UVR
DMP	5±0.5	9.2±0.8	6.7±0.6	0.1±0	1.4±0.1	1.1±0.1	0±0	1.3±0.1	10.5±0.9
DEP	15.9±1.8	56±6.2	27.4±3	2.6±0.3	11.8±1.3	8.6±1	0.2±0	40.3±4.4	6.2±0.7
DBP	14.9±1.8	73.5±8.8	70.2±8.4	11.3±1.4	69.5±8.3	38.9±4.7	1.5±0.2	7.3±0.9	0.4±0
BBP	4.3±0.3	14.6±0.9	11.3±0.7	4.6±0.3	5.5±0.3	12.4±0.7	4.6±0.3	1.8±0.1	6.8±0.4
DEHP	16.2±0.2	30.3±0.3	72.8±0.7	4.8±0	60.2±0.6	17.3±0.2	1.1±0	1.1±0	13.1±0.1
DnOP	4±0.3	6.5±0.6	25.9±2.2	9.5±0.8	16.5±1.4	14.4±1.2	0.8±0.1	0.6±0.1	14.1±1.2

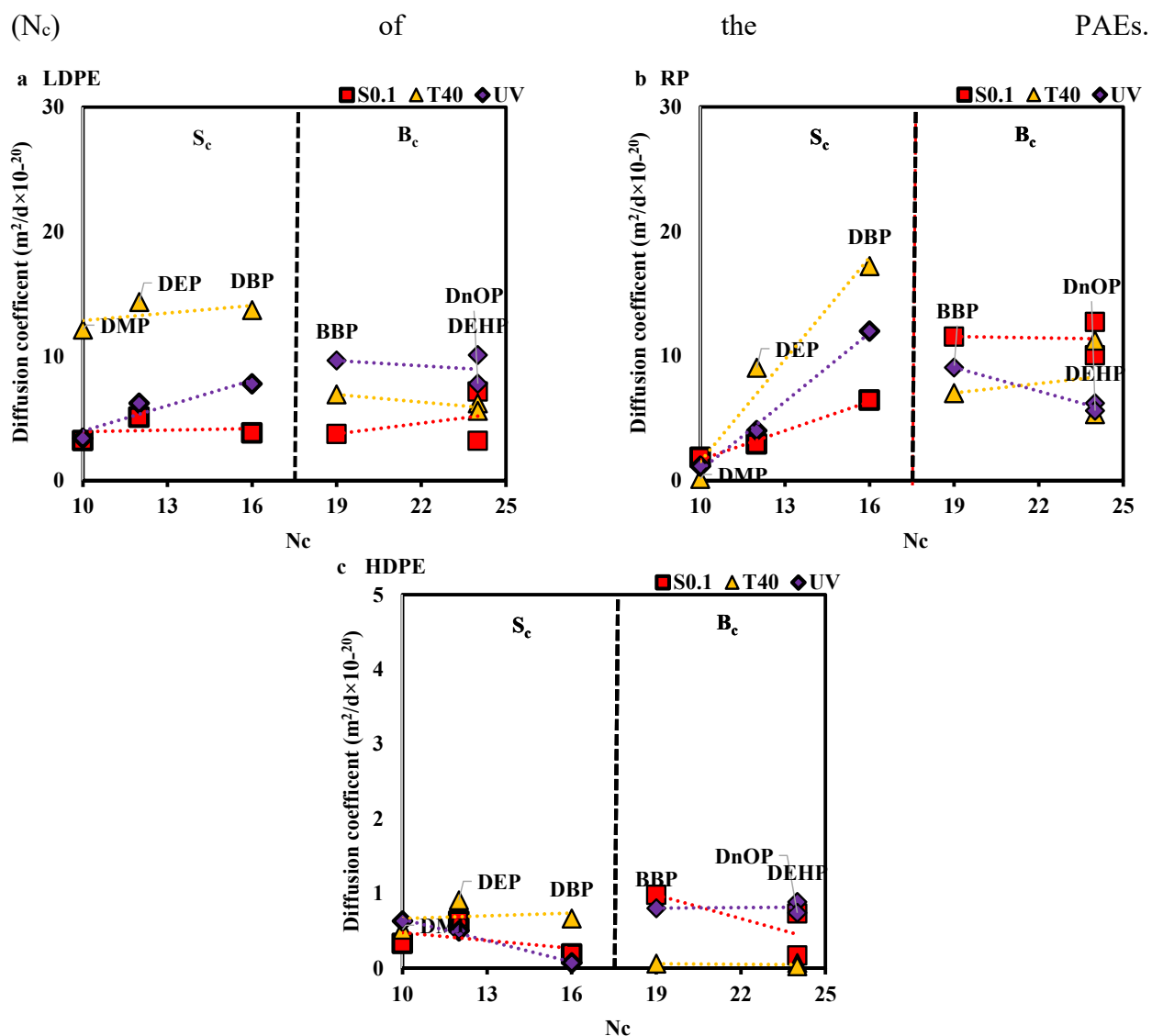


Figure 3.3: The diffusion coefficient (D_e) for PEs versus number of carbons (N_c) of PEs in fresh water (0.1 g/L), at high temperature (40°C), and UVR (354 nm) conditions for LDPE (a), RP (b) and HDPE (c). The D_e results of straight chain carbons PEs (S_c) and branched chain carbons PEs (B_c) were separated by dotted lines.

3.4. Discussion

All plastic PAEs examined in the current study released PAEs to seawater during the 5-day incubation period ($< 4 - 128 \pm 14 \text{ ng/cm}^2$), with the largest amounts of PAEs released by DBP ($14 \pm 1 - 128.0 \pm 14 \text{ ng/cm}^2$), DEHP ($25 \pm 2 - 79 \pm 5 \text{ ng/cm}^2$), and DnOP ($< 4 - 49 \pm 3 \text{ ng/cm}^2$). Indeed, DBP (LMW) and DEHP (HMW) are the most commonly used plasticizers and account for half of the total PE production in Western Europe (PlasticsEurope 2019). The absence of other target compounds (DMP, DEP, and BBP) is due to their low concentrations ($< 4 \text{ ng/mL}$) in the polymer, low leaching rate, high affinity to the polymer, or losses during the production process (Satkowski 1990). The greatest leaching was observed within the first 24 to 48 hours. This is consistent with the fact that plasticizer leaching is concentration dependent, with the rate decreasing with decreasing concentration (Wei et al. 2019). Leaching is initially related to the release of substances near to the surface of the polymer, controlled primarily by the dissolved concentration in the surrounding media. Later, the additives are leached from the interior of the polymer layer by diffusion processes, which occur more slowly (Hanspach and Pinno, 1992). In the current study, the polymer with the highest PAE mass (i.e., DEHP, DEP and DBP, with 13.6 ± 2.5 to $156.2 \pm 23.6 \text{ ng/mg}$ respectively) were leached only between 0.09 - 4.5% in the dissolved phase, while the other PAEs (DMP, BBP and DnOP) with low levels in the polymers (0.36 - 5 ng/mg) were leached between 3.0 – 55 %. This indicates that DMP, BBP and DnOP are not mixed with the polymer but associated with the polymer surface. The diffusion process (rate of delivery of additives to the surface) can be influenced by many other factors such as the polymer (size and shape of additives, polymer structure, diffusivity of additives in the polymer, dissolved concentrations in the surrounding media, temperature, UV irradiation, salinity) [Wei et al., 2019]. Previous studies analyzing additives after leaching from plastics in solutions were determined by measuring the mass loss of the plastic sample or the dissolved concentrations in the surrounding media (Tuezuem Demir and Ulutan 2013, Bach, Dauchy et al. 2014, Rani, Shim et al. 2015, Suhrhoff and Scholz-Boettcher 2016, Duflos, Dehaut et al. 2017, Bridson, Gaugler et al. 2021). For example, reported DBP levels from PE ranged from [1.2 ng/cm^2 over 1-78 days; Suhrhoff and Scholz-Boettcher 2016] to [8 ng/cm^2 over 1-12 weeks] (Paluselli, Fauvelle et al. 2018). These values were lower than the leaching limits set by the European Commission (EC) for various plasticizers based on their toxicity, e.g., 8.2 ng/cm^2 (1.5 mg kg/L) for DEHP 1.76 ng/cm^2 (0.3 mg kg/L) for DBP (EU

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

2011, Groh, Backhaus et al. 2019). Although the partition coefficient is often defined as the ratio (K_d) between the concentration of additive in the polymer and the concentration in the system that has leached or migrated from the polymer [Bridson et al., 2021, Li, et al., 2019], i.e., it is a direct relationship between the initial concentration of additive in the polymer and the leached or migrated concentration in food, seawater, etc. In these studies, the adsorption losses of the polymer during the experiments were not considered [Li et al., 2019; Razanajatovo et al., 2018]. According to previous many studies [Rodrigues et al., 2019, (Li et al. 2019) and our data in the current study, the adsorption efficiency of phthalates is up to (80-96%) by polymers which affects the dissolution of leachate and leading to an underestimation of the total leaching concentration. When the adsorption rate is greater than the leaching rate, the release to the environment is minimal. On the other hand, when the leaching rate exceeds the adsorption rate, the release to the environment is higher (Tourinho, Kočí et al. 2019). Especially for compounds with low water solubility, such as PE, there are debates about the extent and rate of release of additives from plastics in marine waters (Bridson et al. 2021). Therefore, the method used in this study to measure PAEs considered the adsorption loss of each target compound during the leaching process at each experimental condition. Such as in the current study the dissolved concentrations for DBP (8.4 ± 0.7 , 1.2 ± 0.1 , and 13.1 ± 0.6 ng/cm²) and DEHP (5.6 ± 3.2 ng/cm², 1.8 ± 0.1 ng/cm², and 8.6 ± 0.4 ng/cm²) for LDPE, HDPE, and RP, respectively and considerable adsorption was observed for all PAEs in all polymers. Therefore, the calculated total amount leached per unit area differed most from the dissolved concentrations for HMW PAEs. In the current study, the calculated total leached amount increased to (54 ± 4 , 3.9 ± 0.4 , and 69 ± 8 ng/cm²) for DBP and (38 ± 3 , 4.3 ± 0.3 , and 27 ± 2 ng/cm²) for DEHP from LDPE, HDPE, and RP polymers, respectively, when adsorption was considered. Marine organisms are known to ingest plastic articles, which can transfer the adsorbed chemicals to the organism. Once ingested, plastics are exposed to gut surfactants, acidic conditions, and elevated temperatures (in warm-blooded animals), all of which can promote chemical desorption. Similarly, leaching of DEP and DEHP was increased by changing the medium from seawater to gut conditions (seawater with 2 g/L pepsin enzyme, pH 0.5) (Coffin et al. 2019). This could be due to the reduction of adsorption in the intestinal medium, as adsorption is also controlled by the solubility of the additives in the medium (Poças, Oliveira et al. 2008, Fikarová, Cocovi-Solberg et al. 2019, Liu, Zhu et al. 2019). It is important to understand the hazards and magnitude of additive exposure from plastic pollution. Using the current method, we

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

have successfully measured both solute phase and adsorbed polymer concentrations at six time points with three replicates including uncertainties (~12%), which improves the reliability of the current results, although they are still based on indirect assumptions. The potential limitation of this method is in the leaching concentration. PAEs that showed low leaching ($< 5 \text{ ng/cm}^2$), the high spike level (23.5 ng/cm^2) means that the adsorbed and dissolved fractions can be a small difference between large numbers, potentially resulting in high uncertainty on the resultant ratio. The uncertainty was relatively higher (25-32%) for the lower leaching concentration ($< 5 \text{ ng/cm}^2$). The higher uncertainty values for the limited leaching compounds indicate that the estimated partition coefficients for these compounds should be considered with some caution.

In the current experiment, a correlation was observed between the adsorption ratio and the molecular weight of the compounds. The adsorption ratio increased with increasing molecular weight. This suggests that the structural properties of the phthalates are partly responsible for their different concentrations in the environment, which could also be the reason for their different adsorption abilities. The structural difference between DEHP and DEP is the size of the alkyl chains. The longer alkyl chain of DEHP seems to be responsible for the stronger adsorption via van der Waals forces and hydrophobic interactions. This is consistent with previous studies in which phthalates (DMP, DEP, DPRP, DBP, DnBP, DMEP, DHP, BBP, DEHP, DOHP, DnOP, DNP, DiDP) were detected in fish at concentrations of (0.2 to 1.23 mg/g). This could explain the elevated concentrations of HMW compounds such as DEHP in organisms consuming MP, and the highest concentration of PAEs in seawater was reported for DEHP (1.21 mg/g) (Cheng, Nie et al. 2013, Heo H 2020).

In addition, our experiments showed a strong relationship between the experimental conditions, e.g., salinity has a negative effect on the leaching rates, which could be due to the fact that increasing ionic strength of the medium most likely decreases the solubility of the nonionic phthalic esters. The highly ionic medium around the plastics may reduce the diffusion of the nonionic phthalic acid esters. Phthalic acid esters are nonionic molecules that do not contain hydrogen atoms that could bind to electronegative atoms. Therefore, electrostatic attraction and hydrogen bonding can be excluded as important bonding mechanisms (Tourinho, Kočí et al. 2019, Bridson, Gaugler et al. 2021). Early studies also reported that leaching of PAEs was lower in saltwater (Rani, Shim et al. 2015, Suhrhoff and Scholz-Boettcher 2016, Paluselli, Aminot et al.

2018) than in freshwater (Liu, Zhan et al. 2020). As reported, the leaching of PAEs increased with temperature (Audouin, Dalle et al. 1992, Tuezuem Demir and Ulutan 2013). The effect with temperature change from 10 to 25°C was lower for DBP and DEHP and higher between 25 and 40°C. This can be explained by the fact that increasing temperature makes the polymer chain more flexible and causes greater segmental movement compared to low temperatures, resulting in a larger free volume and providing more opportunities for the additive molecule to move out of the polymer matrix (Zhang et al., 2013).

In the case of HDPE, leaching decreased with increasing temperature, with a higher leaching rate observed at 10°C than at 25°C and 40°C (Figure 3.3b). As the fracture toughness (brittleness) of polymers increases with decreasing temperature, it exhibits glassy properties (such as hardness, stiffness, transparency, and brittleness) and loses its interaction with additives (Kanthabhabha Jeya and Bouzid 2018, Salakhov, Shaidullin et al. 2021) which contributes to the loss of PAEs from HDPE. In a previous study, the effect of temperature on creep strain (fracture toughness) of HDPE under constant pressure (14 MPa) was investigated. Lowering the temperature from 75-24°C reduced the creep strain to (0.10 to 0.025 strain/hour), which meant a loss of flexibility or hardness. In addition, the fracture stress increased with increasing density and crystallinity (K.kito, 1997). According to Lu et al (1995), the breaking stress (K_c) decreased from 120-60 MPa with increasing crystallinity from 39.2-81.0%. HDPE has a higher percentage of crystalline regions (70-80%) than LDPE (40-50%). This could contribute to the fact that HDPE leaches more PAEs at low temperatures (10°C) than at 25 and 40°C. The amount of leachate released increased sharply with UV irradiation for the three polymers (LDPE, HDPE, and RP) (Figures 1c, 1f, and 1i). In general, UV radiation is adsorbed by chromophoric sites ($C=C$ or $C-O$) in the polymer. Pure PAEs usually have no chromophoric sites other than carbonyl groups ($C=O$), but impurities and defects introduced during the manufacturing process of the polymers or PAEs can provide more chromophoric sites. The carbonyl groups and conjugated $C=C$ bonds absorb energy above $\lambda=200$ nm with absorption maxima between 200 nm and 300 nm. When the electrons transition to lower energy states, energy is released in the form of vibrational modes. When the energies released exceed the dissociation energy of the bond, bond breakage occurs and radicals are formed (Kamweru, Ndiritu et al. 2009). This can lead to stretching of the porous structure in the polymer

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

and absorption of water into the polymers, which can facilitate the leaching process (Suhrrhoff and Scholz-Boettcher 2016, Coffin, Dudley et al. 2018).

A similar process has been reported for leaching of PAEs from PE, PVC by UVR (Paluselli, Fauvelle et al. 2018, Rummel, Escher et al. 2019). In general, additives were higher in LDPE than in RP polymers because they are more flexible and RP is a recycled polymer, so the probability of impurities (chromophoric sites) is higher in these polymers, which could be a reason for the higher leaching rates. Since pure polyethylene contains fewer impurities than processed products, the UV-induced degradation results for pure polymer resins are much lower than for processed products or environmental samples of the same polymer. This could be the reason that environmentally contaminated samples in marine surface waters are more susceptible to UVR.

In general, leaching depends on the diffusivity of the additive and migration through the voids in the polymer. Thus, the leaching rate depends on the size and shape of the additives as well as the internal structure of the polymer (Basfar 2002, Tuezuem Demir and Ulutan 2013). Among the three polymers (LDPE, RP and HDPE), higher leaching rates were observed for LDPE. This is consistent with the fact that the diffusivity of additives within the polymer is related to their proportion in the amorphous regions of the polymer (Satkowski 1990, Jordan, Casem et al. 2016). This refers to the ratio of random (amorphous) and composite (crystalline) arrangements of molecules in the internal structure of polymers. Thus, the structure with high random content is loose and flexible and more like a liquid.

In the present study, a strong correlation was found between the percentage of amorphous region of LDPE (40-70%), RP (30-43%) and HDPE (25-30%) and their respective diffusivities, such as for DMP with 3.8, 0.18 and 3.4 for LDPE, RP and HDPE, respectively (Figure 3.3). The size and shape of the additives also affected the diffusivity. Additives with larger molecular size have lower diffusivity and branching structure can also slow down the diffusivity of additives in the polymer (Satkowski 1990, Davis 2013). The results of the current study are consistent with the decrease in the diffusion coefficient of PAEs with increasing molecular weight and the higher diffusivity of straight-chain PAEs (DMP, DEP, and DnOP) compared to branched-chain PAEs (BBP, DEHP, and DnOP) (Figure 3.3), indicating the relationship between the leaching process and the structure of the additives and polymers.

3.5. Conclusions and future perspectives

All plastics used here (LDPE, RP, and HDPE) were leached in seawater over a 5-day incubation period ($> 4 - 128 \pm 14 \text{ ng/cm}^2$). The highest leaching rates were observed in the initial phase (24 - 48 h) of incubation, indicating that the leaching process is concentration dependent. Overall, fewer PAEs were leached from HDPE than from LDPE and RP polymers. Low molecular weight compounds were leached from LDPE (i.e. DMP, DBP and DEP), while HDPE and RP released more high molecular weight compounds (HMW; DEHP and DnOP). This highlights that the diffusivity of additives in the polymer is also an important factor in the leaching process. The highest values of diffusion coefficient (D_e) were observed for the highly flexible polymer LDPE, followed by RP and HDPE. Thus, the structure of the polymer and the additive is one of the most important factors in the leaching process. Considerable read sorption from PE was observed for all three polymers, which can reduce the amount of actual/total leachate in the dissolved phase by up to 30-80%. The approach used in this study to measure PAEs accounts for the adsorption loss of each target compound during the leaching process, with some limitation on leaching concentration ($> 5 \text{ ng/cm}^2$). This is an important step in understanding the hazards and extent of exposure to additives from plastic pollution. The long-chain PAEs (HMW), i.e., DEHP, DnOP, and BBP, have the highest adsorption ratio (K_d) values, followed by the short-chain compounds, implying that the longer chain length compounds are responsible for greater adsorption to the polymer. This could be the reason for the high concentration of DEHP detected in plastic samples in the environment. Our experiments have shown a strong relationship between the experimental conditions and the leaching processes. Increasing salinity has a negative effect on leaching, while temperature and UV radiation increase the leaching rate. The leaching of PAEs from LDPE and RP increases with temperature. This suggests that leaching varies at different times of the year and that hot weather conditions increase additive release rates. When organisms have ingested MP, the additives it contains are more likely to be released when the internal temperature of the gut is relatively high and poses a threat to marine animals. In contrast, HDPE shows a higher leaching rate at low temperatures (10°C), suggesting that the cold climate may increase the leaching potential of PAEs from HDPE polymers. The amount of leachate increased sharply with UV irradiation. The higher impact on LDPE and RP suggests that additive concentration and recycling of plastics increase the impurities (chromophores) in the polymer and they are very sensitive to UV irradiation.

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

CRediT authorship contribution statement

All authors made significant contributions to this study. Jeyakumar dhavamani: Performed all analyses and writing- original draft preparation, Aaron J. Beck: Co-supervision: Data curation, , Mohammad El-Shahawi Soror: Reviewing and editing, Martha Gledhill: Co-supervision, Eric P Achterberg; Supervision. Mohammad W. Kadi and Iqbal MI Ismail: Resources, funding acquisition.

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Appendix. Supplementary informations

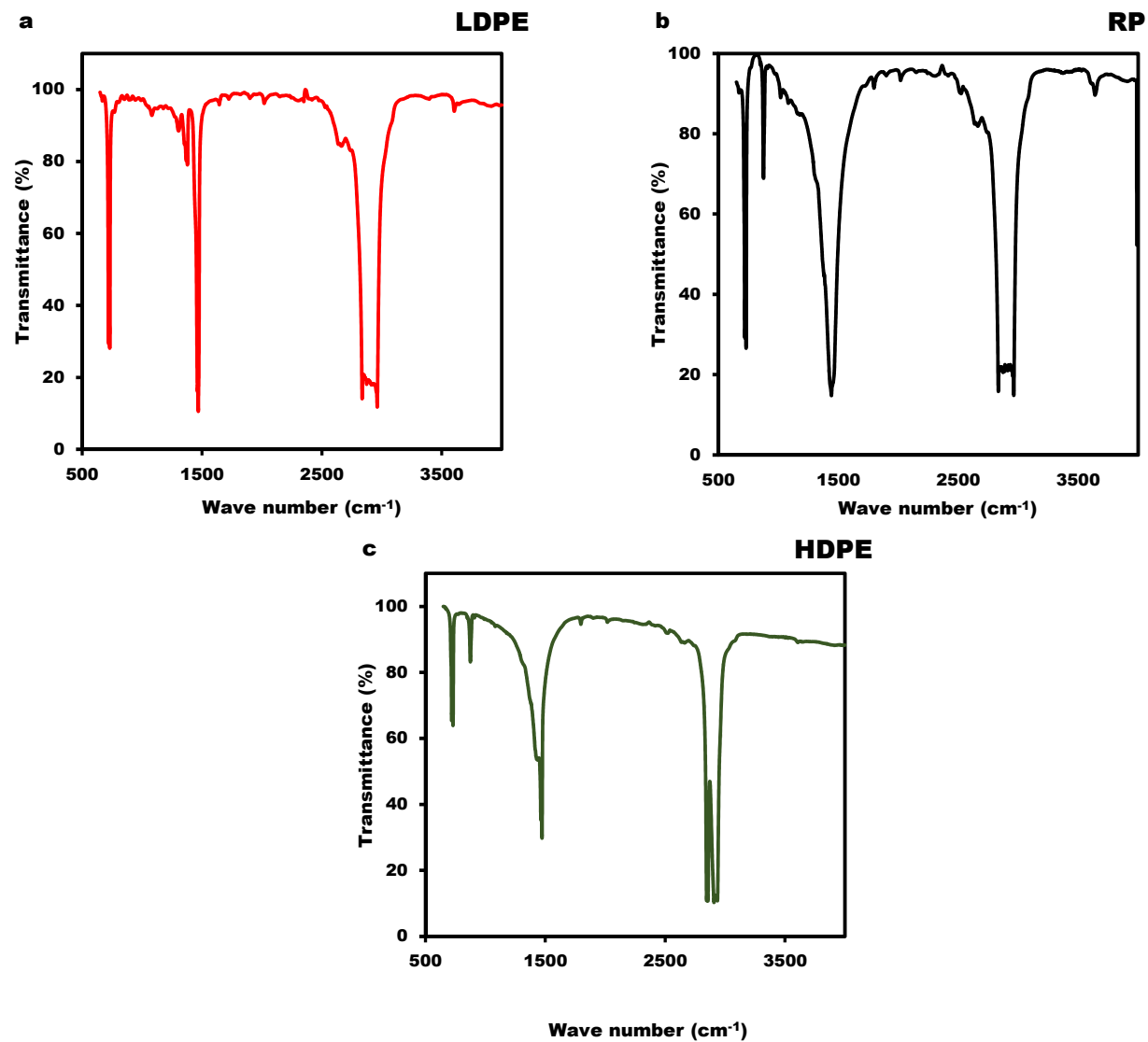


Figure S3.1: The FTIR spectrum of raw plastic films as (a) LDPE, (b) RP, and (c) HDPE.

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

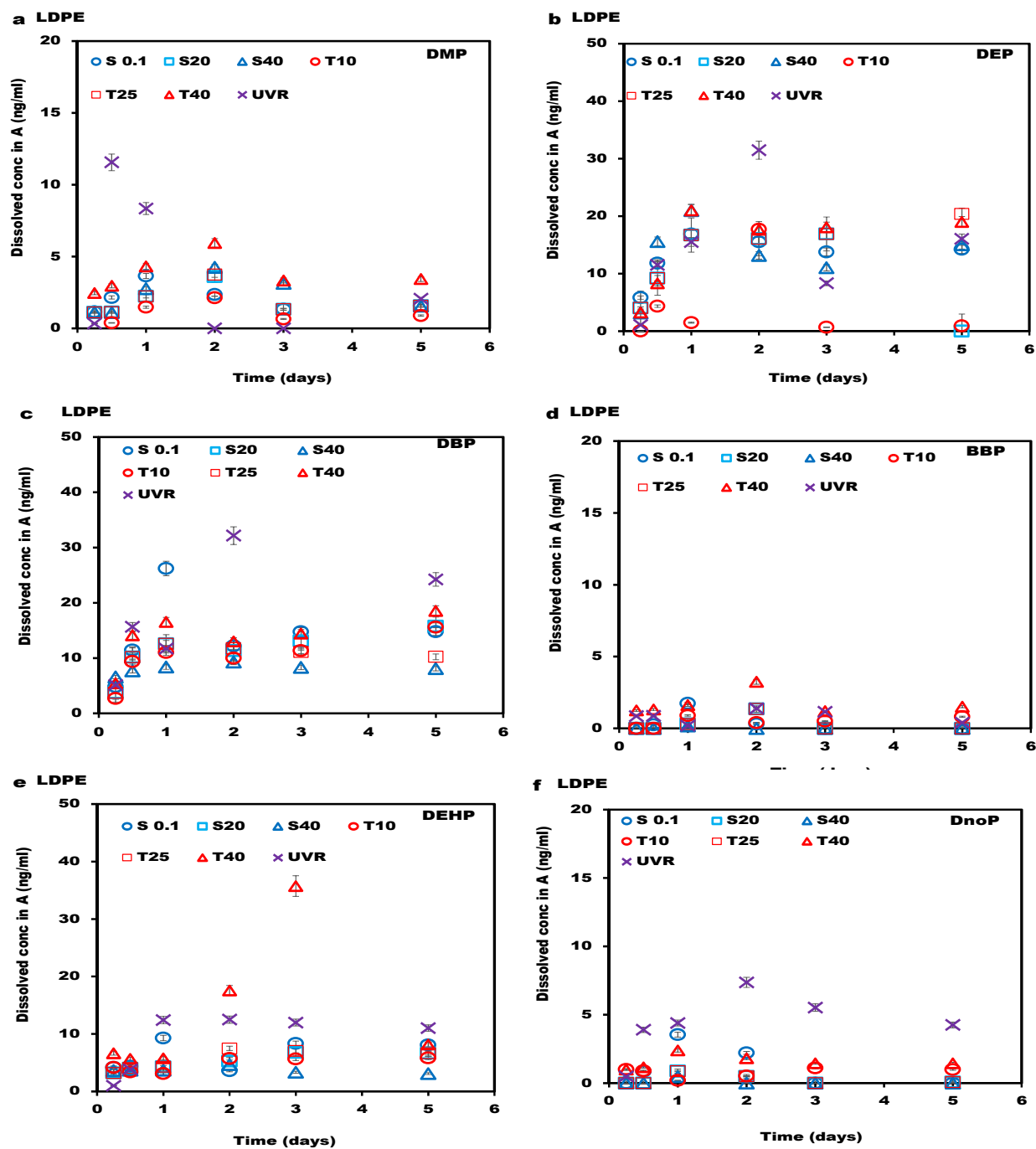


Figure S3.2: Dissolved Concentration of PAEs from experiment A, in artificial seawater at different conditions; 1. salinities (0.1, 20, and 40 g/L), 2. Temperature (10, 25 and 40°C) and, 3. UVR (354 nm) from LDPE over the 5 days incubation period

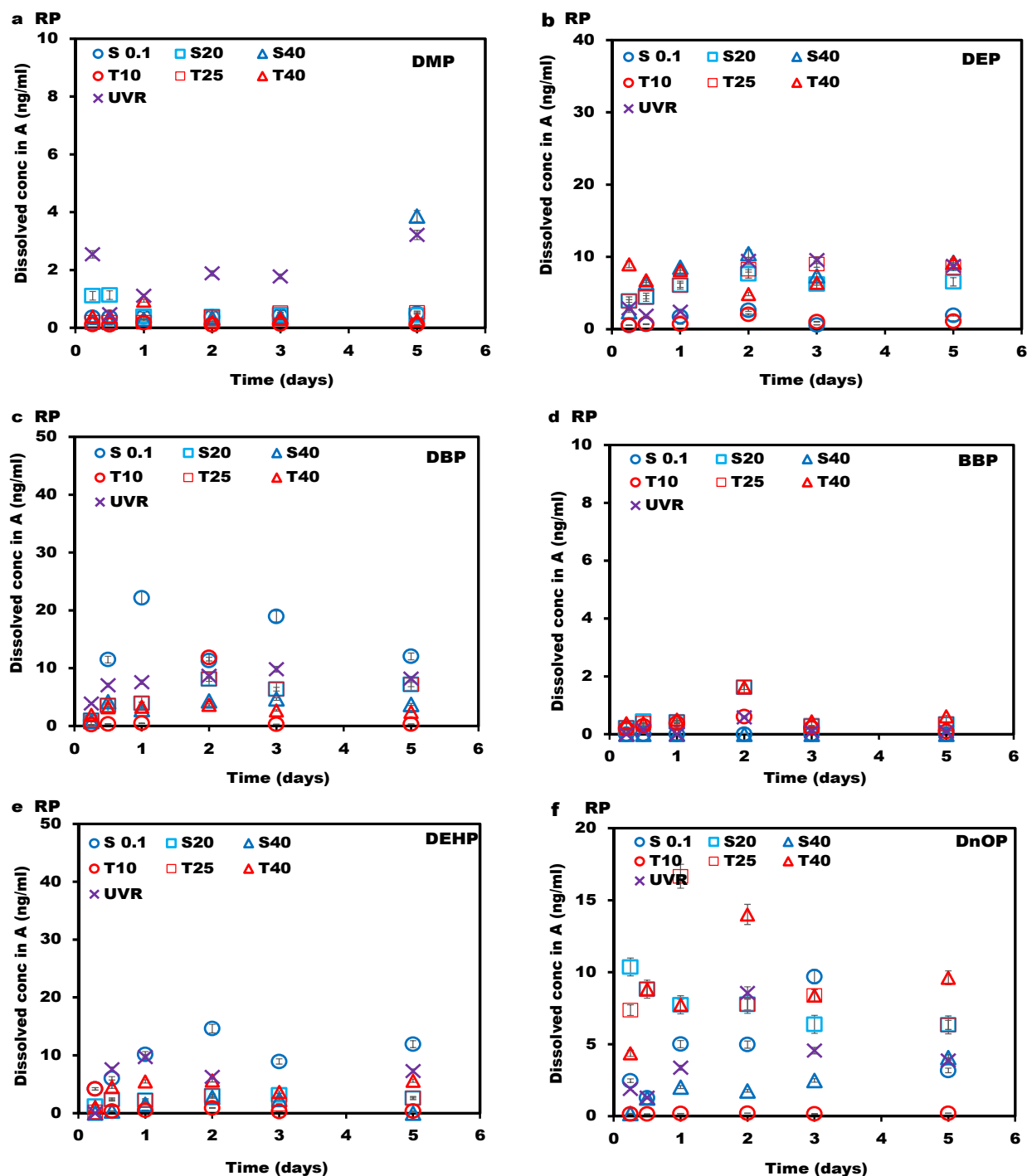


Figure S3.3: Dissolved Concentration of PAEs from experiment A, in artificial seawater at different conditions; 1. salinities (0.1, 20, and 40 g/L), 2. Temperature (10, 25 and 40°C) and 3.UVR (354 nm) from RP over the 5 days incubation period.

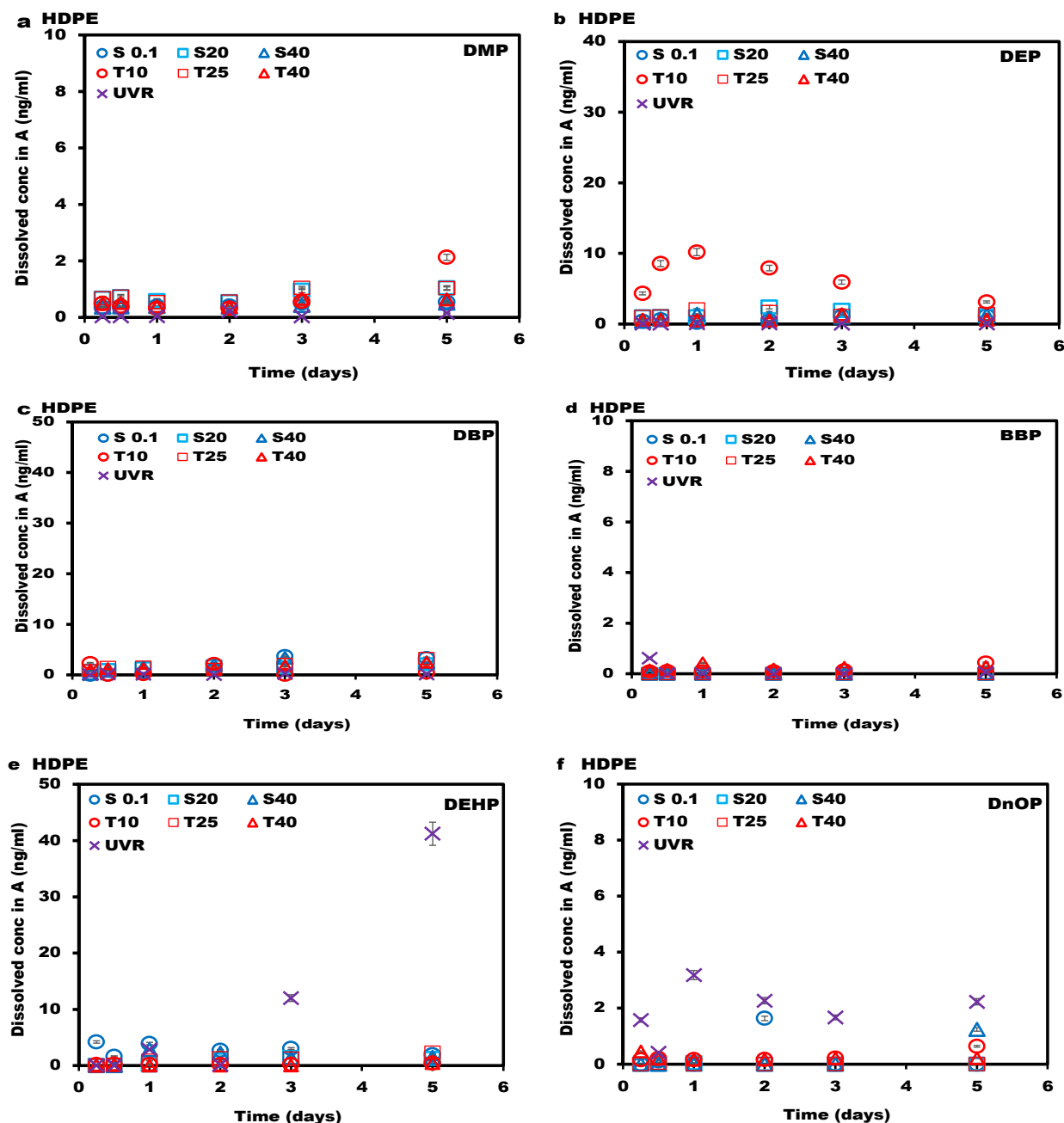


Figure S3.4: Dissolved Concentration of PAEs from experiment A, in artificial seawater at different conditions; 1. salinities (0.1, 20, and 40 g/L), 2. Temperature (10, 25 and 40°C) and, 3.UVR (354 nm) from HDPE over the 5 days incubation period.

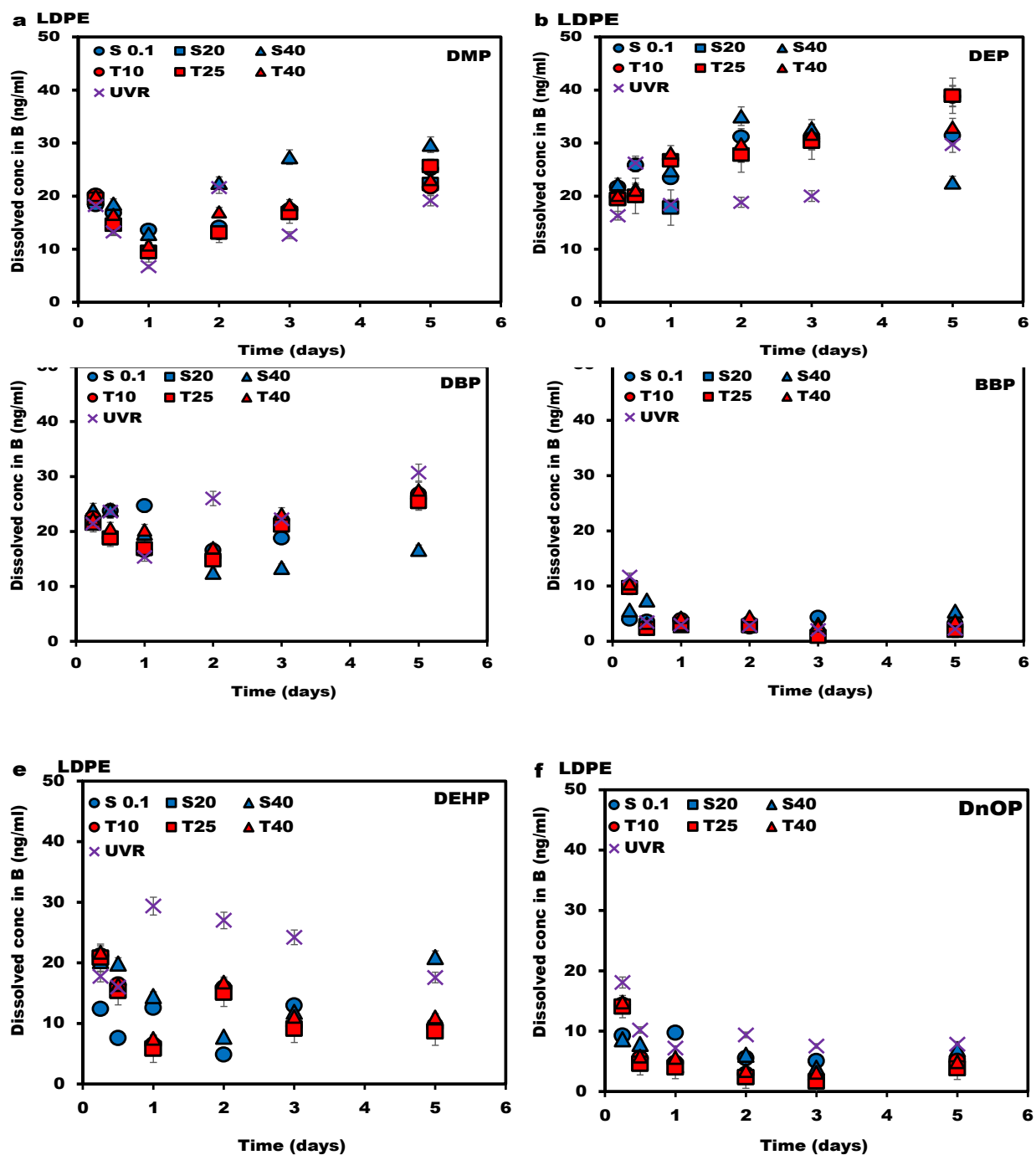


Figure S3.5: Dissolved Concentration of PAEs from experiment B, in artificial seawater at different conditions; 1. salinities (0.1, 20, and 40 g/L), 2. Temperature (10, 25 and 40°C) and, 3.UVR (354 nm) from LDPE over the 5 days incubation period.

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

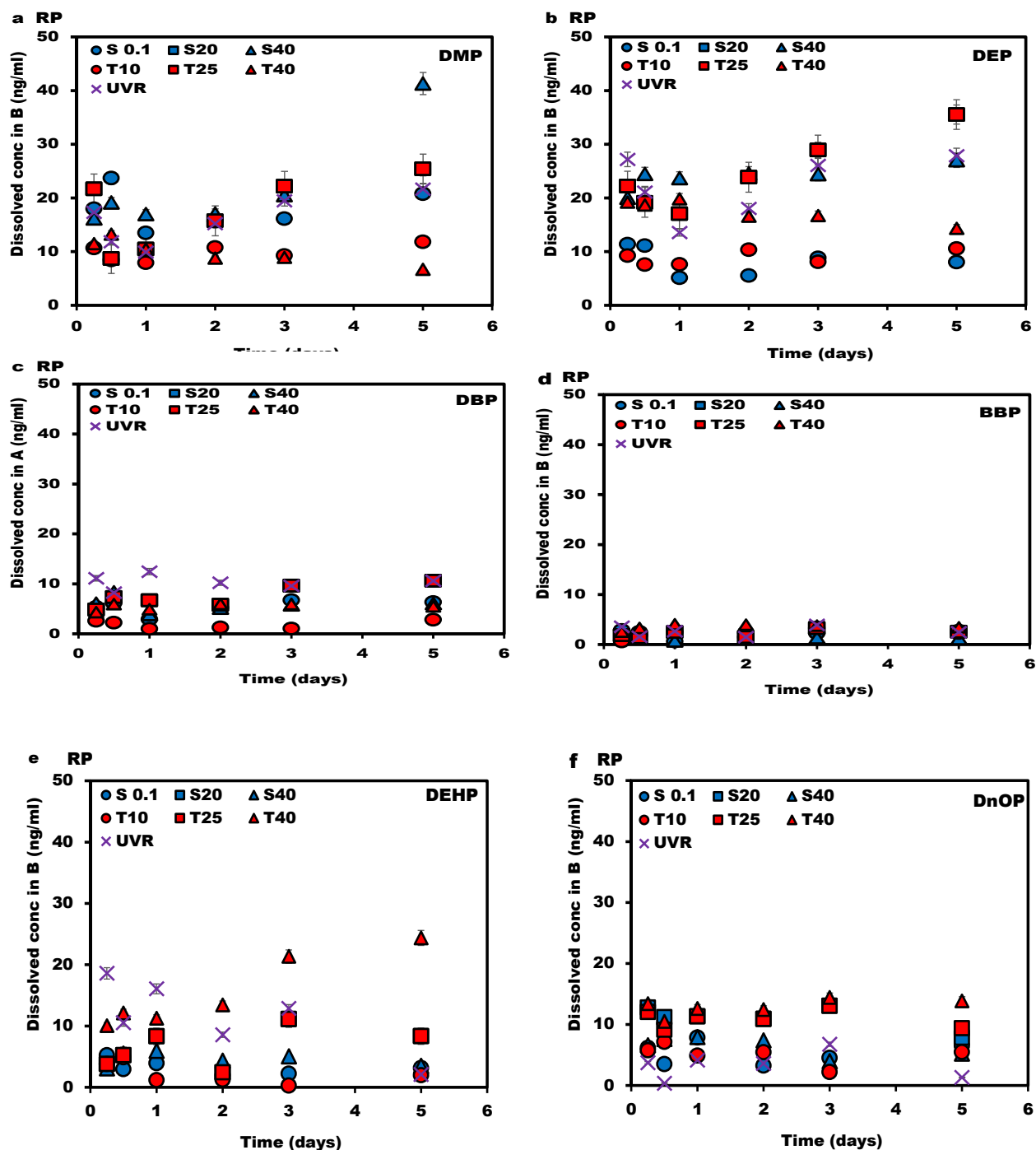


Figure S3.6: Dissolved Concentration of PAEs from experiment B, in artificial seawater at different conditions; 1. salinities (0.1, 20, and 40 g/L), 2. Temperature (10, 25 and 40°C) and, 3.UVR (354 nm) from RP over the 5 days incubation period.

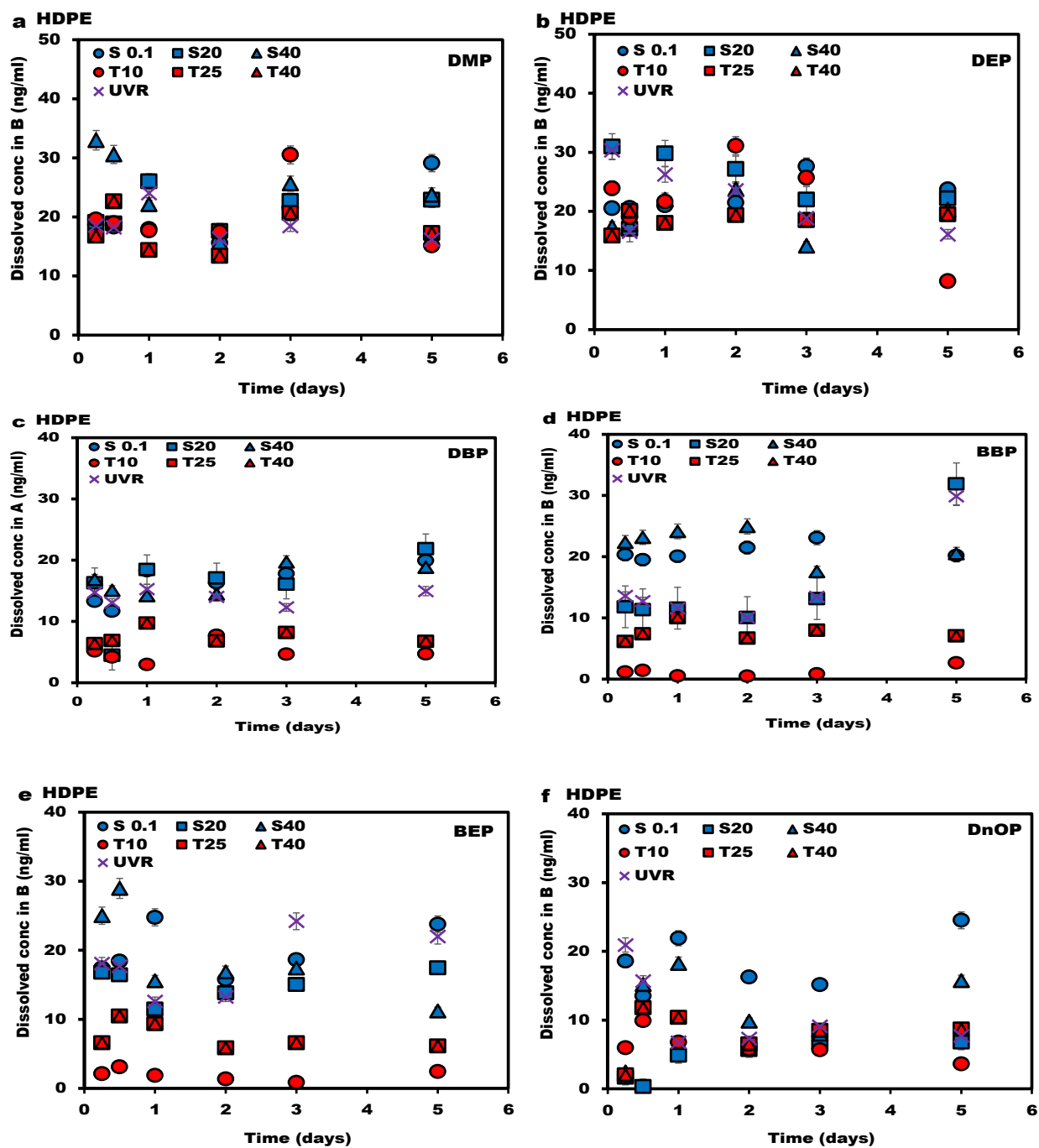


Figure S3.7: Dissolved Concentration of PAEs from experiment B in artificial seawater at different conditions; 1. salinities (0.1, 20, and 40 g/L), 2. Temperature (10, 25 and 40°C) and, 3.UVR (354 nm) from HDPE over the 5 days incubation period.

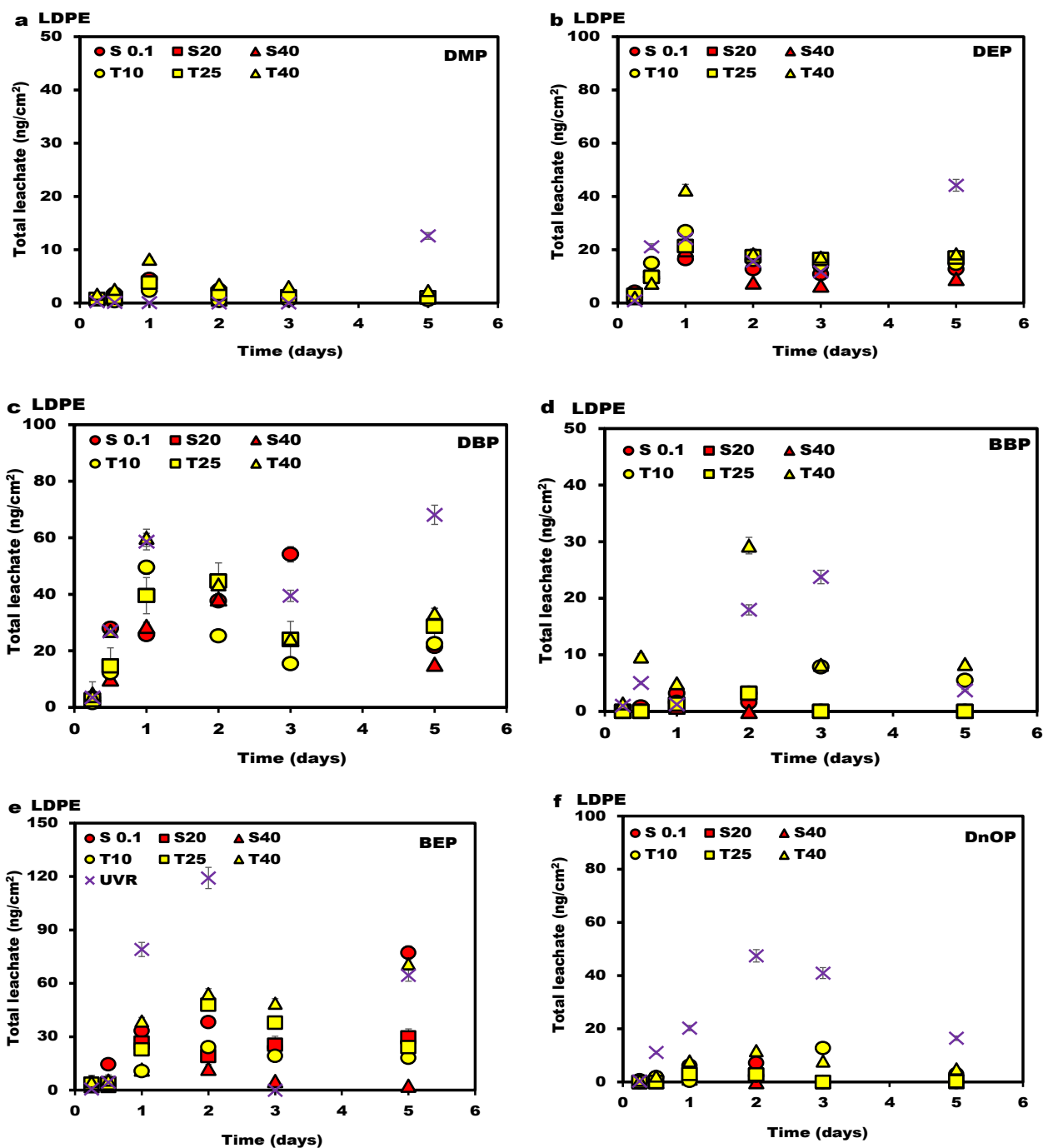


Figure S3.8: The calculated value of total leaching concentration (ng/cm²) for experiment A at different salinities (0.1, 20 and 40g/L), temperature (10, 25 and 40°C) and UVR (354 nm) from LDPE.

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

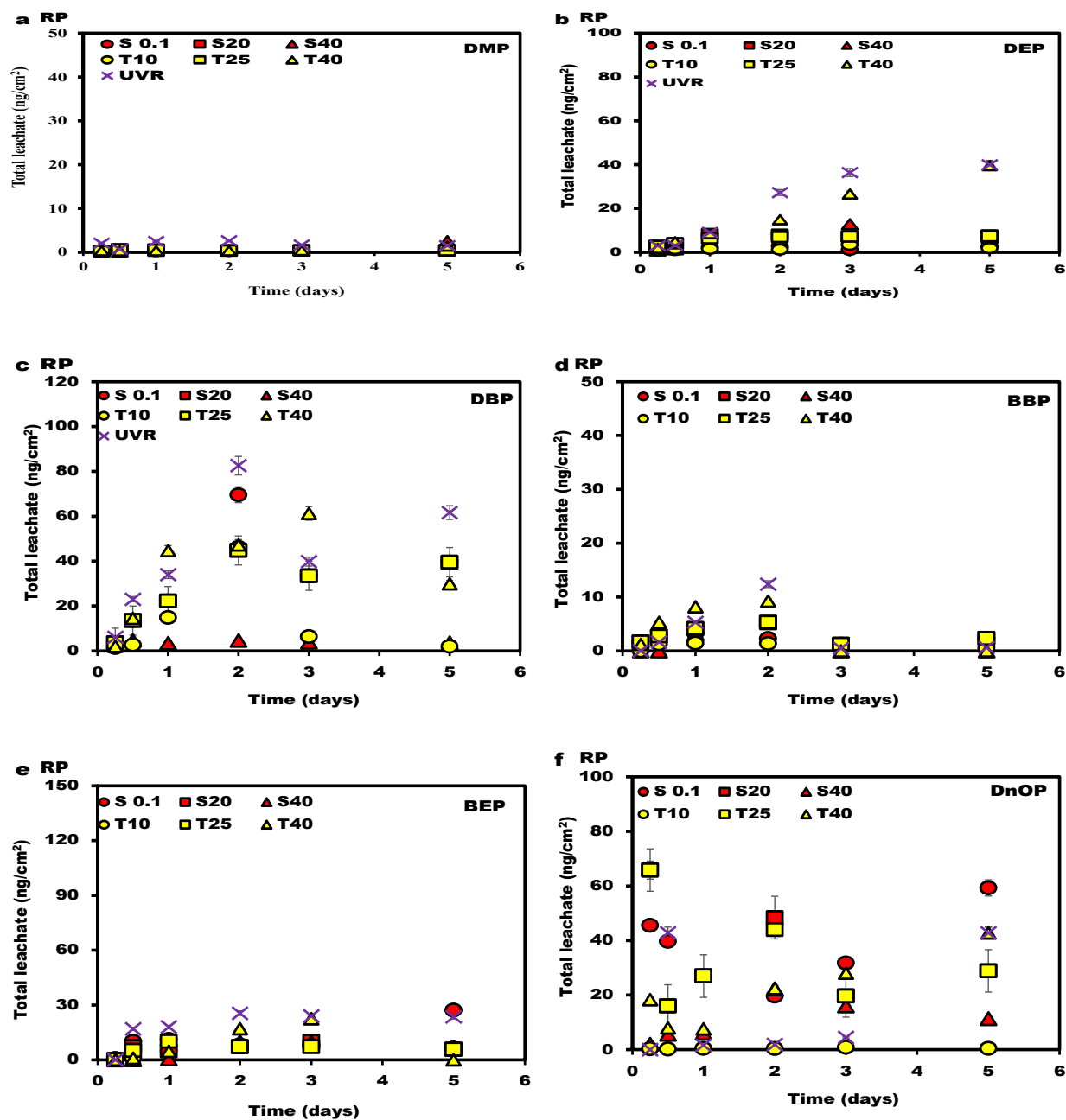


Figure S3.9: The calculated value of total leaching concentration (ng/cm²) for experiment A, at different salinities (0.1, 20 and 40g/L), temperature (10, 25 and 40°C) and UVR (354 nm) from RP.

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

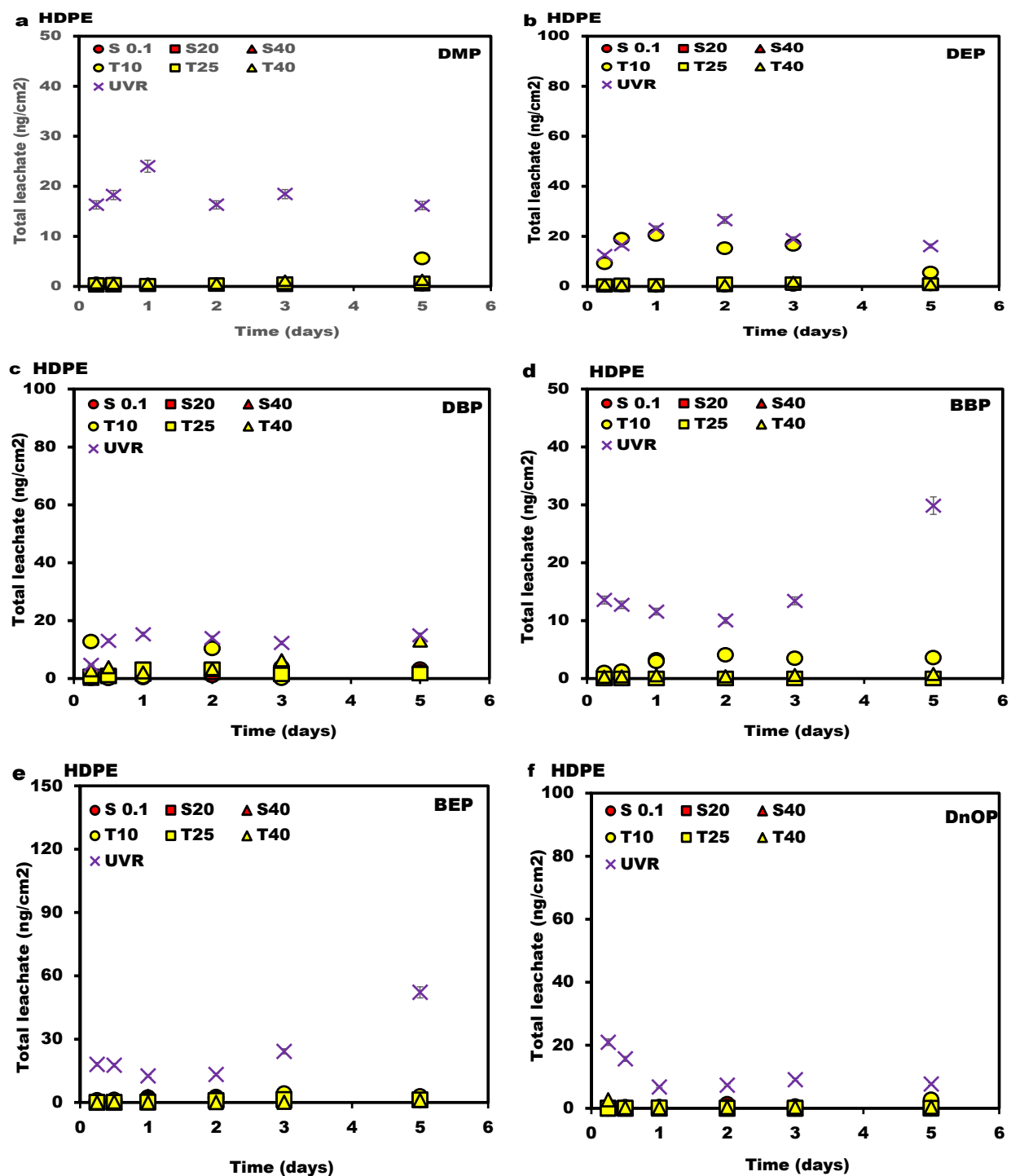


Figure S3.10: The calculated value of total leaching concentration (ng/cm²) for experiment A, at different salinities (0.1, 20 and 40g/L), temperature (10, 25 and 40°C) and UVR (354 nm) from HDPE.

Table S3.1: The dissolved concentration of leached PAEs at Q_m from experiment A from LDPE, RP, and HDPE in different salinities (0.1, 20 and 40 g/L), temperature (10, 25 and 40°C) and UVR experiment.

Compounds	Dissolved concentration A ng/mL						
	S-0.1	S-20	S-40	T-10	T-25	T-40	UV-354
LDPE							
DMP	4.9±0.3	3.6±0.3	4.3±0.3	2.1±0.1	3.6±0.3	6±0.4	11.6±0.8
DEP	16.4±1.3	16.2±0.8	21±2	17±1	16.2±0.8	21±1	31.5±2.2
DBP	14.3±1.3	15.8±0.8	9±0.7	11±1	15.8±0.8	12.6±1.2	67.5±4
BBP	1.7±0.4	1.3±0.2	0.2±0.05	0.9±0.2	1.3±0.2	1.6±0.3	11.4±0.6
DEHP	9±0.6	6.9±0.4	4.6±0.4	5.8±0.4	6.9±0.4	17.6±1.2	35.7±2
DnOP	2.1±0.6	0.9±0.2	0.5±0.2	0.5±0.2	0.9±0.2	2.3±0.3	7.2±0.6
RP							
DMP	0.5±0.1	0.4±0.1	0.3±0	0.2±0.3	0.4±0.1	1.3±0.1	3.2±0.2
DEP	2.6±0.2	7.6±0.6	10.5±0.7	2±0.1	7.6±0.6	9.3±0.7	9.5±0.7
DBP	22±1.1	8.1±0.5	4.4±0.3	11.8±0.6	8.1±0.5	3.7±0.2	9.8±0.9
BBP	2±0.2	1.8±0.3	1.7±0.1	0.7±0.3	1.8±0.3	1.8±0.2	0.7±0
DEHP	14.6±0.7	3±0.4	2.7±0.2	2.8±0.2	3±0.4	5.5±0.5	7.1±0.6
DnOP	9.7±0.8	7.8±0.4	2.5±0.2	0.2±0	7.8±0.4	14±0.7	8.6±0.8
HDPE							
DMP	0.4±0.2	0.3±0.2	0.5±0.2	5.8±0.5	13.6±0	2±0.1	30.6±1.5
DEP	0.2±0.1	1±0.1	1.4±0.1	3.9±0.2	13.6±0.1	1.6±0.1	25±2
DBP	2.1±0.1	2±0.3	3.3±0.2	2.5±0.2	18.7±0.2	10.2±0.8	10.7±0.5
BBP	0±0	0±0	0±0	0.5±0.3	0±0	0.5±0.3	33±3
DEHP	3.1±0.2	1.2±0.1	1.6±0.4	0.5±0.1	18.7±0.1	0.5±0.2	26.8±1.3
DnOP	1.3±0.3	0±0	1.2±0.3	0.7±0.2	0±0	2±0.2	12.5±1

Table S3.2: The concentration PAEs by solvent extraction (ng/mg) and the percentage of dissolved leachate in the ASW from LDPE, RP and HDPE polymer at different salinities (0.1, 20 and 40 g/L), temperature (10, 25 and 40°C) and UVR experiment.

Compounds	Solvent extracted mass ng/mg	Percentage of dissolved leachate in artificial sea water (ASW)						
		S-0.1	S-20	S-40	T-10	T-25	T-40	UV-354
LDPE								
DMP	12±1	3.1±0.2	2.3±0.2	2.7±0.2	1.3±0.1	2.3±0.2	3.8±0.2	7.3±0.8
DEP	28±2	4.4±0.2	4.4±0.5	5.7±0.4	4.8±0.4	4.4±0.5	5.7±0.5	8.5±0.1
DBP	54±3	2±0.2	2.2±0.2	1.2±0.1	1.5±0.1	2.2±0.2	1.7±0.1	9.4±0.1
BBP	1.5±0.4	8.5±2	6.5±1	1±0.28	4.5±1	6.5±1	8±1	57 ±13
BEP	80±8	0.8±0.1	0.7±0.07	0.4±0.1	0.5±0.1	0.7±0.07	1.7±0.1	3.4±0.3
DnOP	1.86±0.1	8.5±0.4	3.6±0.4	2±0.3	2±0.2	3.6±0.4	9 ±2	29 ± 4
Σ ₆ PAE	177± 12	RP						
DMP	0.26±0	13±1	10±2	8±0.5	5.3±0.2	10±2	34 ± 2	55 ±4
DEP	13±0.8	1.4±0.11	4.1±0.2	5.6±0.4	1.1±0.1	4.1±0.2	5±1	5 ±0.6
DBP	39±4	4±0.1	1.5±0.2	0.8±0.1	2.1±0.1	1.5±0.2	0.7±0.1	1.8±0.1
BBP	0.36±0.1	39±7	35±8	33±8	13±3	35±8	35 ± 6	14 ±2
BEP	157±17	0.6±0.1	0.1±0.05	0.1±0.1	0.1±0.05	0.1±0.05	0.2±0.02	0.3±0.1
DnOP	34±5	2.0±0.5	1.6±0.3	0.5±0.1	0±0.01	1.6±0.3	2.9±0.1	1.8±0.3
Σ ₆ PAE	243±23	HDPE						
DMP	12±2	0.5±0.08	0.4±0.1	0.7±0.2	7.9±0.7	0.4±0.1	2.7±0.2	41 ±3
DEP	33±3	0.1±0	0.5±0.1	0.7±0.1	1.8±0.1	0.5±0.1	0.8±0.1	12 ±2
DBP	30±3	1.1±0.7	1±0.1	1.7±0.2	1.3±0.2	1±0.1	5.3±0.4	5.6 ± 0.3
BBP	0.20±0	0±0	0±0	0±0	39 ±10	0±0	39 ±9	54 ± 18
BEP	87±9	0.6±0.04	0.2±0.1	0.3±0.1	0.1±0.05	0.2±0.1	0.1±0.01	4.8 ± 0.4
DnOP	0.34±0	59±6	0±0	55 ± 3	32 ±3	0±0	91 ± 25	54 ± 22
Σ ₆ PAE	162±10							

Table S3.3: The dissolved concentration of spiked PAEs from experiment B at Q_m from LDPE, RP, and HDPE in different salinities (0.1, 20 and 40 g/L), temperature (10, 25 and 40°C) and UVR experiment.

Compounds	Dissolved spikes in B (ng/mL)						
	S-0.1	S-20	S-40	T-10	T-25	T-40	UV-354
LDPE							
DMP	25.8±1.5	22.3±1.3	29.7±1.8	21.6±1.5	22.3±0.9	17±1.2	21.6±1.1
DEP	23.4±0.9	17.9±0.7	24.9±1.5	15.5±1.1	17.9±0.7	11.6±0.8	16.8±0.7
DBP	6.2±0.3	8.3±0.6	5.5±0.3	5.2±0.4	8.3±0.5	5±0.3	12.3±0.6
BBP	12.5±0.6	9.7±0.5	5.3±0.2	6.4±0.3	9.7±0.7	5.6±0.3	11.3±0.6
DEHP	5.6±0.2	6.1±0.4	8.9±0.6	5.6±0.3	6.1±0.4	8.6±0.4	10.6±0.7
DnOP	7.1±0.4	6.6±0.4	8.2±0.5	5±0.2	6.6±0.4	5±0.3	7.6±0.5
RP							
DMP	32±2	18.7±0.9	20.8±1	10.8±0.4	18.7±1.3	22.3±0.9	28.8±1.4
DEP	20.4±0.8	21.6±0.9	19.2±1.3	14±1	21.6±0.9	5.4±0.3	4.8±0.2
DBP	7.5±0.4	4.3±0.2	4.8±0.3	6±0.3	4.3±0.2	1.4±0.1	2.8±0.1
BBP	15.2±0.7	8.2±0.6	7.6±0.5	11.2±0.8	8.2±0.3	4.7±0.3	1.3±0.1
DEHP	12.6±0.9	7±0.4	6.6±0.4	5.9±0.4	7±0.5	5.7±0.3	6.6±0.5
DnOP	3.8±0.3	3.8±0.2	2.6±0.2	5.7±0.4	3.8±0.2	6.7±0.4	4.7±0.2
HDPE							
DMP	35±2	22.1±0.9	33±2	24.5±1	22.1±1.3	36±2	30±1.2
DEP	17.6±0.7	20.1±1.4	32±2	16.1±0.6	20.1±1	20±1.4	22.4±1.6
DBP	12.6±0.8	15.1±0.8	31.1±1.6	5.5±0.3	15.1±0.9	18.2±0.7	18±0.7
BBP	19.2±1	9.7±0.7	26±1.8	12.8±0.6	9.7±0.7	14.8±1	22±1.5
DEHP	16.8±1	6.1±0.3	24.8±1.7	5.6±0.2	6.1±0.4	14.5±0.6	14.9±0.9
DnOP	20.8±1.5	6.6±0.4	27.8±1.9	6.4±0.3	6.6±0.3	17.7±1.2	14±0.6

Table S3.4: The K_d Values of adsorbed leachate to dissolved spikes at Q_m in different salinities (0.1, 20 and 40 g/L), temperature (10, 25 and 40°C) and UVR (354 nm) of LDPE, RP, and HDPE.

Compounds	Ratio (K_d)						
	S-0.1	S-20	S-40	T-10	T-25	T-40	UV-354
LDPE							
DMP	0.5±0.2	0.8±0.2	0.3±0.1	0.8±0.2	0.8±0.2	1±0.2	0.9±0.2
DEP	0.7±0	1±0.1	0.6±0	1.6±0.3	1±0.1	2.4±0.3	1±0.2
DBP	5.4±0.2	3.8±0.2	6.2±0.3	6.6±0.3	3.8±0.2	7±0.4	2±0.1
BBP	2.1±0.3	3.1±0.3	6.5±0.5	5±0.2	3±0.2	6±0.2	2.5±0.1
DEHP	6.2±0.4	5.6±0.3	3.5±0.1	6±0.4	5.6±0.3	3.6±0.2	2.8±0.2
DnOP	4.7±0.5	5±0.3	3.9±0.2	7±0.3	5±0.3	7±0.4	4.3±0.2
RP							
DMP	0.3±0.2	1.1±0.05	0.9±0.1	2.7±0.2	1.1±0.1	0.8±0.2	0.4±0.1
DEP	1.0±0.2	0.8±0.1	1.1±0.1	1.9±0.1	0.8±0.1	6.3±0.3	7.3±0.4
DBP	4.3±0.2	8.4±0.3	7.3±0.4	5.6±0.4	8.4±0.3	27±2	13±0.9
BBP	1.7±0.1	3.9±0.4	4.3±0.3	2.6±0.1	3.9±0.2	7.6±0.5	30.9±1.5
DEHP	2.2±0.2	4.7±0.2	5.1±0.2	5.7±0.2	4.7±0.2	6±1.0	5.1±0.4
DnOP	9.4±0.5	9.6±0.7	14.2±0.7	6±0.3	9.6±0.7	5±0.3	7.5±0.4
HDPE							
DMP	0.1±0.05	0.8±0.1	0.2±0.05	0.6±0.1	0.8±0.2	0.1±0.1	0.3±0.1
DEP	1.3±0.1	1±0.1	0.2±0.1	1.5±0.1	1±0.1	1±0.1	0.8±0.1
DBP	2.0±0.2	1.6±0.1	0.3±0.1	6.2±0.3	1.0±0.1	1.2±0.1	1.2±0.1
BBP	1.0±0.1	3.1±0.2	0.5±0.1	2.1±0.1	3.1±0.2	1.7±0.1	0.8±0.1
DEHP	1.4±0.1	5.6±0.3	0.6±0.05	6.1±0.4	5.6±0.3	1.8±0.1	1.7±0.1
DnOP	0.9±0	5.1±0.4	0.4±0.1	5.3±0.3	5.1±0.4	1.3±0.1	1.9±0.1

Table S3.5: The calculated total leachate concentration (ng/cm²) at Q_m from experiment B from LDPE, RP, and HDPE in different salinities (0.1, 20 and 40 g/L), temperature (10, 25 and 40°C) and UVR experiment.

Compounds	Total leachate concentration (ng/cm ²)						
	S-0.1	S-20	S-40	T-10	T-25	T-40	UV-354
LDPE							
DMP	4.5±0.4	3.8±0.4	3.4±0.2	2.3±0.3	3.8±0.4	8.2±0.6	12±0.9
DEP	16.5±1.2	21.4±1.5	19.8±2	27±2	21.4±1.5	42±3	44±3
DBP	54±4.3	44 ±3	38.4±3.5	49 ±4	44 ±3.6	60±6	128±14
BBP	3.2±0.3	3.2±0.6	0.7±0.1	3.2±0.4	3.2±0.6	6.6±0.7	23.8±2.4
DEHP	38±3	27±3	12 ±1	24 ±2	26±3	48±4	79±5.5
DnOP	7.2±1.2	3.1±0.5	1.3±0.1	2.4±0.3	3.1±0.5	11±1	22±2.4
RP							
DMP	0.4±0.2	0.6±0.2	0.4±0.05	0.4±0.2	0.6±0.2	1.4±0.1	2.6±0.3
DEP	3±0.5	8.3±0.7	12.8±0.9	3.4±0.4	8.3±0.7	40±4	46.3±3.2
DBP	69±8	45±5	4.7±0.4	46±44	45±5	61±5	82±9
BBP	3.2±0.5	5.3±0.8	5.2±0.4	1.4±0.1	5.3±0.8	9.2±0.6	12.4±0.9
DEHP	27 ±2	10.2±0.7	9.8±0.8	11.2±1	10.2±0.7	22.5±2.3	25.5±2
DnOP	59±3	48±4	22 ± 2	0.9±0.1	48.4±0	49.3±4.4	43 ±3.4
HDPE							
DMP	0.3±0.2	0.3±0.1	0.4±0	5.6±0.6	0.3±0.1	1.3±0.1	24±2
DEP	0.3±0.2	1.2±0.1	1±0.2	5.7±0.8	1.2±0.1	1.8±0.2	26±3
DBP	3.9±0.4	3.2±0.3	2.5±0.2	10±0.7	3.2±0.3	13.2±1.4	14±1.2
BBP	0±0	0±0	0±0	0.9±0.2	0±0	0.8±0.2	35±3
DEHP	4.3±0.3	4.7±0.5	1.5±0.3	2±0.2	4.7±0.5	0.8±0.2	42±4
DnOP	1.5±0.2	0±0	1±0.3	2.6±0.3	0±0	2.6±0.2	20.9±2.3

Table S3.6: The leaching rate of PAEs from LDPE, RP, and HDPE at different salinities (0.1, 20 and 40 g/L), temperature (10, 20 and 40°C), and UVR (354nm).

Names	Total leaching rate (ng/cm ²)						
	S 0.1 g/L	S 20 g/L	S 40 g/L	T 10°C	T 20°C	T 40°C	UVR
LDPE							
DMP	5±0.5	4±0.4	3.8±0.3	4±0.4	4±0.4	9±0.8	6.7±0.6
DEP	16±2	24±3	23±2.5	32±3.5	24±3	56±6	27±3
DBP	15±2	23±3	19±2	13±1.6	23±3	73±9	70±8
BBP	4.3±0.5	2±0.2	1±0.2	3±0.2	2±0.2	14.6±0.9	11±0.7
DEHP	16.2±0.2	9.6±0.1	9.6±0.2	13.2±0.1	9.6±0.1	30 ±3	72.8± 7
DnOP	4±0.3	4.4±0.8	1.9±0.2	6.1±0.5	4.4±0.8	6.5±0.6	25.9±2.2
RP							
DMP	0.1±0.1	0.3±0.1	0.1±0.1	0.2±0.1	0.3±0.1	1.4±0.1	1.1±0.1
DEP	2.6±0.3	7.5±0.8	8.6±1	0.9±0.1	7.5±0.8	12±1	8.6±1
DBP	11.3±1.4	22.6±2.7	22.2±2.7	26.6±3.2	22.6±2.7	69.5±8	38.9±4
BBP	4.6±0.3	0.5±0.2	0.05±0.1	4.9±0.3	0.5±0.2	5.5±0.3	12.4±0.7
DEHP	4.8±0.3	7.8±0.1	10±0.1	13.7±0.1	7.8±0.1	60.2±3	17.3±2
DnOP	9.5±0.8	9.1±0.8	9.8±0.8	0.2±0.1	9.1±0.8	16.5±1.4	14.4±1.2
HDPE							
DMP	0.1±0.05	0.3±0.2	0.1±0.2	1.3±0.1	0.3±0.2	0.2±0.05	10.5±1
DEP	0.2±0.1	5.2±0.6	7.9±0.9	40.3±4.4	5.2±0.6	0.7±0.1	6±0.7
DBP	1.5±0.2	17.7±2.1	21.9±2.6	7.3±0.9	17.7±2.1	2.8±0.3	0.4±0.1
BBP	4.6±0.3	3.2±0.2	0.1±0.05	1.8±0.1	3.2±0.2	0.1±0.1	6.8±0.4
DEHP	1.1±0.2	9.9±0.8	0.1±0.05	1.1±0.2	9.9±0.8	0.4±0	13±0.1
DnOP	0.8±0.3	2.4±3.6	2.7±0.4	0.6±0.1	2.4±3.6	0.1±0.2	14±1.2

Table S3.7: The D_e from LDPE, RP, and HDPE at different salinities (0.1, 20 and 40 g/L), temperature (10, 20 and 40°C), and UVR (354nm).

Names	Diffusion co efficient (m^2/d) $\times 10^{-20}$						
	S 0.1 g/L	S 20 g/L	S 40 g/L	T 10°C	T 20°C	T 40°C	UVR
LDPE							
DMP	3.2 \pm 0.3	2.7 \pm 0.2	2.2 \pm 0.2	8.3 \pm 0.7	2.7 \pm 0.2	12 \pm 1.1	3.4 \pm 0.3
DEP	5 \pm 0.6	3.6 \pm 0.4	2.6 \pm 0.3	8 \pm 1	3.6 \pm 0.4	14.4 \pm 1.6	6.2 \pm 0.7
DBP	3.8 \pm 0.5	2.2 \pm 0.3	1.4 \pm 0.2	5.4 \pm 0.6	2.2 \pm 0.3	13.7 \pm 1.6	7.8 \pm 0.9
BBP	3.7 \pm 0.2	3.3 \pm 0.2	2.9 \pm 0.2	5.5 \pm 0.3	3.3 \pm 0.2	6.9 \pm 0.4	4.6 \pm 0.3
DEHP	3.2 \pm 0.1	2.9 \pm 0.1	2.4 \pm 0.1	4.9 \pm 0.2	2.9 \pm 0.1	6.2 \pm 0.1	5.6 \pm 0.1
DnOP	7.1 \pm 0.6	6.6 \pm 0.6	6 \pm 0.5	6.9 \pm 0.6	6.6 \pm 0.6	8.6 \pm 0.7	3.2 \pm 0.3
RP							
DMP	1.8 \pm 0.2	1.5 \pm 0.1	1.2 \pm 0.1	0.1 \pm 0.8	1.5 \pm 0.1	0.1 \pm 0.05	1 \pm 0.7
DEP	2.9 \pm 0.3	1.9 \pm 0.1	1.3 \pm 0.1	8.8 \pm 0.7	1.9 \pm 0.1	11 \pm 0.8	4 \pm 1
DBP	3.4 \pm 0.3	2.4 \pm 0.3	1.7 \pm 0.3	8.4 \pm 1.2	2.4 \pm 0.3	11 \pm 1.3	8 \pm 0.7
BBP	1.5 \pm 0.1	1.3 \pm 0.1	1.2 \pm 0.1	6.1 \pm 0.3	1.3 \pm 0.1	7 \pm 0.3	9.3 \pm 0.3
DEHP	2.7 \pm 0.2	2.4 \pm 0.1	2.1 \pm 0.1	9.7 \pm 0.3	2.4 \pm 0.1	11.2 \pm 1	6.2 \pm 0
DnOP	1.2 \pm 0.1	1.1 \pm 0.05	1 \pm 0.1	4.6 \pm 0.5	1.1 \pm 0.05	5.3 \pm 0.3	5.6 \pm 0
HDPE							
DMP	0.3 \pm 0.03	0.3 \pm 0.1	0.2 \pm 0	0.6 \pm 0.2	0.3 \pm 0.1	0.5 \pm 0.2	0.6 \pm 0.1
DEP	0.6 \pm 0.1	0.5 \pm 0.1	0.3 \pm 0.1	1.1 \pm 0.1	0.5 \pm 0.1	0.9 \pm 0.1	0.7 \pm 0.1
DBP	0.2 \pm 0.1	0.1 \pm 0.05	0.1 \pm 0.05	1.2 \pm 0.1	0.1 \pm 0.05	0.9 \pm 0.1	0.5 \pm 0.1
BBP	0.1 \pm 0.05	0.1 \pm 0.05	0.1 \pm 0.05	0.1 \pm 0.05	0.1 \pm 0.05	0.1 \pm 0.05	0.2 \pm 0.05
DEHP	0.1 \pm 0.05	0.1 \pm 0.05	0.1 \pm 0.05	0.1 \pm 0.05	0.1 \pm 0.05	0.1 \pm 0.05	0.3 \pm 0.1
DnOP	0.2 \pm 0.1	0.2 \pm 0.1	0.1 \pm 0.05	0.1 \pm 0.05	0.2 \pm 0.1	0.1 \pm 0.05	0.2 \pm 0.1

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

Calculation:

Diffusion coefficient calculation:

The total leaching process from the bulk to the surface of the polymer can be described by Fick's law diffusion (Wei, Linde et al. 2019). Diffusion of relatively small molecules through rubbery polymers in one dimension can be expressed by the following equation:

$$\delta CA/at = D \times (\delta^2 CA / \delta^2 X) \quad (3.8)$$

Where D is the diffusion coefficient, C is the concentration of the diffusion substance, A is the migrating species, t is the time, and X is the coordinate perpendicular to the section. For the diffusion of an additive from the bulk film which has two faces open to the ambient, the coordinate perpendicular to the section is taken as half-thickness (l) of the film (Macdonald 1977) have shown that if $Q_t/Q_m < 0.5$ at short times, hence the diffusion coefficient (De) can be calculated from the slope (h) of the initial portion of Q_t/Q_m versus square root of time plots using the following equation:

$$De = \Pi \times (l \times (\theta/4))^2 \quad (3.9)$$

The fractional loss was calculated from the linear range observed in the initial days by (Q_t/Q_∞) using eq. (10) where the Q_0 and Q_t are initial mass and the mass at any instant time t , respectively. The Q_m is the mass at equilibrium. This amount can be calculated from the presented data in the article.

$$Q_t/Q_m = Q_0 - Q_t / Q_0 - Q_m$$

Chapter 3: The effects of salinity, temperature, and UV irradiation on leaching and adsorption of phthalate esters from polyethylene in seawater

Chapter 4

Partitioning of phthalate esters and plastic debris and their ecological risk level in surface waters of the Red Sea and the bay of Sharm Obhur

4. Partitioning of phthalate esters and plastic debris and their ecological risk level in surface waters of the Red Sea and the bay of Sharm Obhur

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Abstract

The abundance of plastics debris (PDs) and phthalic acid esters (PAEs), a class of contaminants associated with plastics, in the marine environment is not well constrained and very few studies make a link between these pollutants in seawater. We investigated the abundance of PDs and PAEs in surface sea waters of the bay Sharm Obhur and the Red Sea. Sharm Obhur is a semi-enclosed bay on the eastern shore of the Red Sea, near Jeddah, Saudi Arabia, and is heavily impacted by human activity. The PAEs concentrations in the study area ranged from 0.8 to 1224 ng/L. Among the six PAEs studied, diethyl phthalate (DEP) (22.4 - 1124 ng/L), di-n-butyl phthalate (DBP) (8.9 - 346 ng/L) and di (2-ethylhexyl) phthalate (DEHP) (61.6 - 639.5 ng/L) were the predominant additives detected. The $\sum_6\text{PAE}$ was lower at Sharm Obhur (587 ± 82 ng/L) and on the Red Sea shelf (677 ± 182 ng/L) compared to the slope of the open waters of the Red Sea (1266 ± 354 ng/L). This suggests that degradation and adsorption of PAEs were higher in Sharm Obhur and on the shelf than slope waters. In contrast, there was no difference in abundance of PDs between Sharm Obhur (0.0374 PDs/m³) and the Red Sea (0.0301 PDs /m³). Polyethylene (32%) and polypropylene (8%) were dominant, mostly smaller than 5 mm (78%), with the majority consisting of white (52%) and black (24%) fragments (39%), fibers (35%) and films (24%). A positive correlation between PAE concentration and abundance of PDs, suggesting either a common source or a causal link through leaching. The ecological risk of $\sum_4\text{PAEs}$ (DMP, DEP, DBP and BEP) ranged from (0.206 to 0.796), indicating a low to moderate risk for the Red Sea. The pollution index of PDs ranged from (0.14 - 0.36), showing that the Red Sea near Jeddah suffered relatively low pollution.

Keywords: Phthalate esters, Chemical Additives, Micro plastics, Correlation, Ecological risk level, Sharm Obhur and Red Sea.

4.1. Introduction

Plastic debris is the main source of chemical additives to the marine environment (Hahladakis et al. 2018a, Zhang et al. 2021). The most common additives recovered from the marine environment are phthalates or phthalic acid esters (PAEs), bisphenol A (BPA), nonylphenols (NPs) and brominated flame retardants (BFRs) (Alimi et al. 2018). Additives observed the marine environment, including PAEs, have recently received increasing attention (Baini et al. 2017). PAEs are widely used in the manufacture and processing of plastic products, for example, as plasticizers to improve their processability and flexibility (Hahladakis et al. 2018b). Phthalate esters account for about 92% of plasticizers produced and are the most produced and consumed plasticizers worldwide (Stenmarck and et al. 2013). Phthalate esters are endocrine disrupting chemicals with detrimental effects on reproduction of marine species (Andrady 2011), and on the development of obesity and human cancer (Heudorf et al. 2007, Zhang et al. 2021). Given their potential environmental and health risks, six PAEs - dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl benzyl phthalate (BBzP), di(2-ethylhexyl) phthalate (BEP), and di-n-octyl phthalate (DnOP) - have been identified as priority pollutants by the US EPA and European Commission (U.S 2014)(Directive 2005/84/EC). Recent years have seen an upsurge of interest in the distribution of dissolved PAEs in the marine environment (Gao and Wen 2016, Liu et al. 2020c, Sun et al. 2021, Zhang et al. 2021). However, recent studies showed that additives can be efficiently absorbed and accumulated on plastic residues, due to their hydrophobic properties and the large surface area to volume ratio of plastic particles (Liu et al. 2019, Liu et al. 2020c). Surface-adsorbed PAEs concentrations can be up to six orders of magnitude higher than ambient seawater (Bakir et al. 2016, Hahladakis et al. 2018b), due to hydrophobic interactions with plastic surfaces (Pittura et al. 2018, Rodrigues et al. 2019). Therefore, the distribution of PAEs in the environment depends not just on their source from plastic waste, but also their removal by adsorption (Dhavamani et al. 2022)

Plastic debris in the ocean may continuously release PAEs during aging and degradation (Paluselli et al. 2019), suggesting that PAEs can be used as an indicator of plastic debris pollution in the ocean. (Baini et al. 2017), found a significant correlation between some PAEs and micro plastics (MPs) in plankton samples, and suggested that PAEs levels detected in organisms could be used

as markers of exposure to PDs. Levels of PAEs in PDs and seawater samples have been studied mainly in the western and central Mediterranean Sea. Recently, Paluselli and colleagues (2018) reported that PAEs concentrations in water samples from the northwestern Mediterranean Sea were higher above the seafloor than in subsurface waters. However, to our knowledge, no similar data are available for the Red Sea (Paluselli et al. 2018b).

The Red Sea is characterized by low annual precipitation, the absence of permanent rivers in its catchment, and high evaporation. Red Sea is a warm and shallow marine basin with surface temperatures between 20 and 34°C and salinity between 36.5 and 41. Jeddah is located on the east coast of Red Sea, and is the second largest city in Saudi Arabia, with a population of 3.4 million. Jeddah produces two million tons of solid waste annually and plastic is the second most common waste in Saudi Arabia after organic waste (food, meat, fat etc.,) (Miandad et al. 2016, Nizami et al. 2015), and only 10-20% of plastic waste is reused or recycled (Miandad et al. 2015). An inefficient disposal strategy for plastics is a clear driver of the pollution on beaches and in surface waters clear along the entire Red Sea coastline of Saudi Arabia. Data on the occurrence of PDs and their plastic additives (such as PAEs) in the marine environment of the Red Sea are limited, although there are some studies on MPs pollution of Red Sea fish and sediments (Sanchez-Avila et al. 2012). To the best of our knowledge, no studies have reported the distribution of individual phthalate esters in Red Sea water. Therefore, the purpose of the current study focuses on to decide the level of PAEs and PDs in the bay of Sharm Obhur and the Red Sea near Jeddah. The correlation, if any, between PAEs, PDs and environmental parameters are reported, and the ecological risk level (ERL) of PDs and PAEs were calculated.

4.2. Materials and methods

4.2.1. Reagents and materials

A standard mixture of PAEs including three low molecular weight (LMW) compounds, dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), and three high molecular weight (HMW) compounds, benzyl butyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP/BEP) and di-n-octyl phthalate (DnOP) at a concentration of 20,000 mg/L in methanol (purity 99.8%) was obtained from Sigma Aldrich (Germany). The most common properties of the PAEs are listed in Table 2.1. Dichloromethane (DCM) and methanol (MeOH) were bought from Sigma Aldrich (HPLC grade). Working solutions of PAEs were prepared in isooctane and stored

in the dark at 4°C for a maximum of two weeks. To avoid cross contamination, plastic materials were not used, and glass materials were ashed at 400°C for 4 h prior to use.

4.2.2 Sampling

4.2.2.1 Net sampling

The Jeddah coast of the Red Sea was selected for sampling, representing areas influenced by wastewater discharges. Floating PDs were collected during two consecutive cruises, which took place on 16th December 2020 from the shelf to the slope of the Red Sea, and in 22nd February 2021 in the bay of Sharm Obhur (Figure 4.1 and Table S4.1 for details of sampling locations). Sharm Obhur is connected to the Red Sea. Samples were collected using a Neuston net with an aperture of 0.4 × 0.6 m and a mesh size of 150 µm, and at each station the net was towed for ~15 min at a speed of 1.5-2 knots (0.7-1 m s⁻¹).

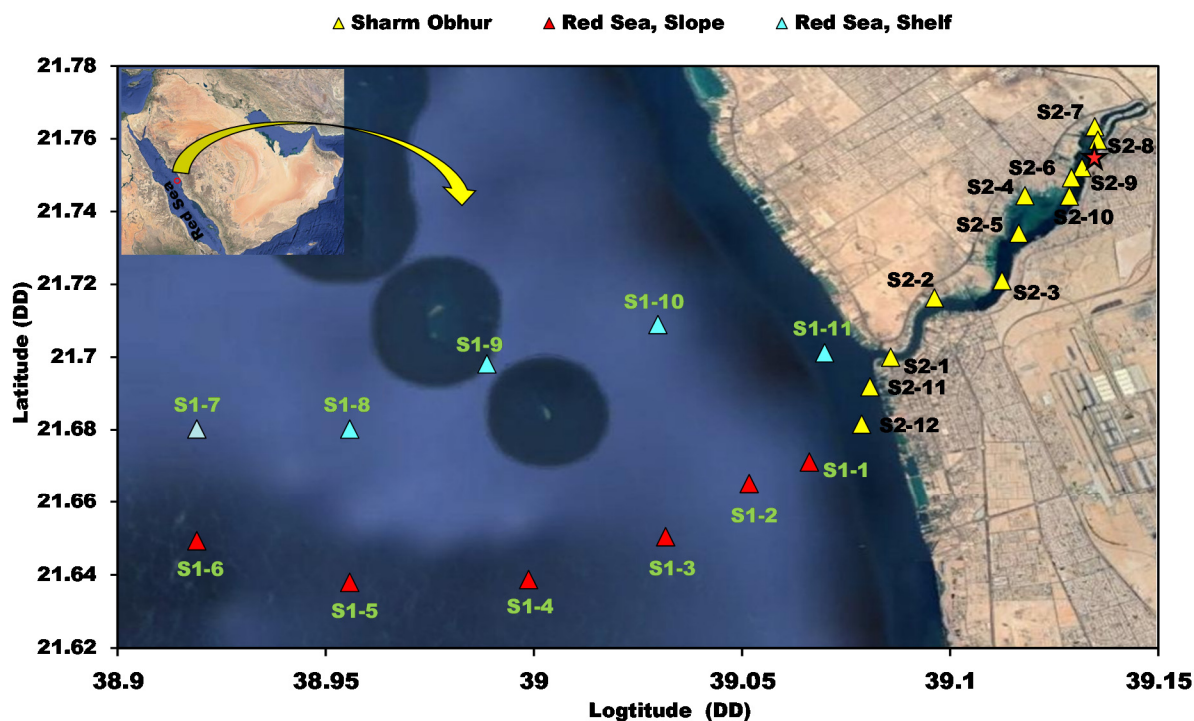


Figure 4.1. The map shows the sampling area (Red Sea; Jeddah region in the insert) and enlarged (yellow arrow) locations of the sampling stations in the bay of Sharm Obhur to the open waters of the Red Sea. *The wastewater input is highlighted (★)

All deployments were from the starboard side of the boat (Ananta, Model II,) beyond the bow wave to avoid wake turbulence. The net was rinsed on board with seawater and the contents of the

cod end were emptied through a 50 μm metal sieve. Samples were transferred to 200 mL bottles (Schott-Duran), fixed with ethanol to a final concentration of 80%, and stored for laboratory analysis. The ship's data logger was used for recording sea surface temperature and salinity continuously and averaged over the duration of each tow. Tow distance was calculated using ship speed and tow time (distance ~ 810 m). The volume of water sampled was calculated using a mechanical flow meter positioned at the center of the net mouth (Hydro-Bios). The sampled area and filtered volume ($\sim 90.3 \pm 10.2$ m³) of each trawl were calculated using the diameter of the frame (300 mm), the towing distance (~ 810 m).

4.2.2.2 Surface water samples

At each station, water samples of about 2 L were collected in clean amber glass bottles previously rinsed with distilled water and DCM (Merck, Darmstadt, Germany), and baked at 400°C to remove organics. To avoid over handling samples and to minimize external contamination during sampling and extraction, water was transported, stored and analyzed in the same bottle used for sampling. Transport and storage of the samples were at 4°C. All samples were extracted within 48 hours of collection to avoid degradation of the target compounds.

4.2.3. Polymer identification of net-collected plastic particles

Net samples were separated by sequentially sieving through stainless steel sieves (0.1 mm). The sieves were then dried in an oven at 60°C for 10 minutes. The plastic particles were carefully removed using laboratory metal tweezers and transferred to glass vials. All samples were double checked by two different researchers to ensure that all particles were captured and to avoid operator bias during sorting. In the laboratory, samples were examined under a dissecting stereo microscope (Kruss Optronic, MBL 2000, and Germany).

Microplastic polymer types were identified using a near-infrared hyperspectral imaging system. The hyperspectral imaging system used in the current study was a Specim FX17 camera (Specim Spectral Imaging Ltd.; Oulu, Finland) mounted on a Specim linear lab bed scanner. The FX17 linescan camera has a spectral range of 900 – 1700 nm, with 224 spectral bands and a spatial sampling of 640 pixels. A macro lens was used to achieve a field of view about 1200 μm , giving a pixel dimension of approximately 2-4 μm . Samples were illuminated overhead by two halogen lights at approximately 45°C from the front and back of the camera target field. The hyperspectral

camera and lab scanner were controlled using Specim's LUMO software suite. A combination of an edge-finding algorithm (Sobel) and a segmentation algorithm (Watershed) from Scikit-image (van der Walt et al. 2014), was used to identify particles in the images. Particle dimensions were calibrated with a 1 cm white reference bar on each scan. A series of virgin plastic polymer beads obtained during the JPI Oceans project BASEMAN (Gerdtz 2019), were used to establish the reference spectra for polymer identification. The polymers included polystyrene (PS), high- and low- density polyethylene (HDPE and LDPE), polyethylene terephthalate (PET), and polypropylene (PP). The spectra for these polymers are markedly different from those for natural materials. The presence of peaks and troughs in the observed particle spectra were identified using the Scipy peak finding algorithm. This information was then one-hot encoded for each particle for input to the classification algorithm. The Scikit-learn random forest algorithm (Pedregosa et al. 2011) was used to classify the particle polymer types.

4.2.4 Extraction

Concentrations of dissolved PAEs in seawater (2 L) were determined by solid phase extraction (SPE, Chromabond Easy, 6 ml, 200 mg, Macherey-Nagel, Germany). Surrogate standards of PAEs (10 ng/L) were added to samples before extraction. SPE cartridges were conditioned with 10 mL of methanol, followed by 10 mL of dichloromethane and 15 mL of ultrapure water, all at a flow rate of 5 mL/min. Samples were aspirated under vacuum through the cartridges at a flow rate of 10 mL/min. The cartridge was finally rinsed with three 5 mL aliquots of ultrapure water and dried under vacuum for 30 min. Elution was performed with 10 mL of DCM. The blank controls were performed with artificial sea water (ASW). To avoid background contamination in the experiments and standard solutions, as well as in the extraction procedures, all materials used were plastic-free except in cases where the material was not replaceable. To determine the method blank value, the contamination of materials and instruments was measured as blank value.

4.2.5. GC-MS analysis

The concentrations of PAEs in the extracts were analyzed by gas chromatography mass spectrometry (GC-MS QPplus-2010, Shimadzu, Japan) using a flame ionization detector (FID) and a mass spectrometer (MS, EI conditions). We used the same experimental conditions as in our previous study (Dhavamani et al., 2021). The initial temperature of the column oven was 250°C. A HP 5MS, 30 m capillary column was used (30 m × 0.25 mm i.d. × 0.25 m, 5%

phenylmethylsiloxane, Agilent HP -5MS) with a temperature program of 60°C (2 min hold), ramp 5°C/min to 310°C, and 5-min hold. Helium was used as the carrier gas (2 mL/min). The ion source temperature was 220°C and the interface temperature was 250°C. The injection volume was 2 µL using an auto-sampler (AOC-5000, Shimadzu, Japan). Injection was performed in "splitless" mode with a spitting time of 0.98 min and a flush flow rate of 30 mL/min. Selected Ion Mode (SIM) was developed experimentally for each compound based on precursor and production ions, collision energies, and other parameters. Target compounds were positively identified by comparing their retention times and target ions to the specific reference ions.

4.2.6. Method validation

Instrument performance was calibrated based on European regulation, Article number; 10/2011/EU (Commission 2011), and an eleven-point calibration curve was analyzed in triplicate at 0.005, 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4, 0.8, 1 and 2 µg/mL for all target PAEs. Quantification was performed using the external standard in the selected ion monitoring mode SIM. The regression coefficient of linearity was greater than 0.99 with an RSD of 18%. The instrumental detection limit (LOD) and quantitation limit (LOQ) were calculated using the signal-to-noise ratio (S/N) of the lowest concentration used. The method's detection limits were measured using the standard addition method (Frenna et al. 2012). Known concentrations of standard PAEs (0.01, 0.02, 0.04, 0.08, 0.16, 0.2 µg/mL) were spiked into 10 mL of ASW and extracted using the same procedure. The average recoveries were in the range 94 - 107% and the RSD was ±14%. The optimized parameters for GC-MS calibration, SIM ions, method validation, are listed in Table 2.2.

4.2.7 Risk assessment related to PAEs and PDs

The risk quotient (RQ) of a single PAEs was used as a representative model in the risk assessment of PAEs. The value of RQ was calculated using the following equation (Sun et al. 2021):

$$RQ = MEC/PNEC \quad (4.1)$$

Where MEC is the Measured Environmental Concentration of PAEs, and PNEC is the Predicted No Effect Concentration, based on the relevant European Commission regulations (EC, 2003). According to the common classification, there are three levels of RQs: $RQ < 0.1$ for low risk, $0.1 < RQ < 1$ for medium risk, and $RQ > 1$ for high risk. The PNEC values of DMP, DEP, DBP, and

BEP are 96, 96, 0.50 and 1.54 µg/L, respectively (Li et al., 2017). The total potential Hazard Index of PAEs (HI) was calculated as the sum of the individual RQs for the individual PAEs (Liu et al., 2017):

$$\text{PAEs HI} = \sum \text{RQ}_i \quad (4.2)$$

Plastic debris are relatively new marine pollutants, and there is not yet a systematic and standardized model developed specifically for assessing their potential ecological risk. Their potential risk can be based on the chemical toxicity and hazard classification of individual polymers (Lithner et al. 2011). The PDs polymer risk index (PRI) was computed using the following equation:

$$\text{PRI} = \sum P_n \times S_n \quad (4.3)$$

The percentage of each polymer type in the samples is P_n and the hazard score of each type is S_n , taken from (Lithner et al. 2011) (Table S4.5). The pollution load index method (PLI) (Tomlinson et al., 1980) was used to assess the pollution degree/index of PDs. PLI is considered a standardized rule for monitoring pollution levels between different areas (Sun et al. 2021). The evaluation model can be expressed as follows:

$$CF_i = C_i / C_{0i} \quad (4.4)$$

$$\text{PLI} = \sqrt{CF_i} \quad (4.5)$$

$$\text{PLI}_{\text{zone}} = \sqrt[n]{CF_1 CF_2 \cdots CF_n} \quad (4.6)$$

Where CF_i (PDs concentration factor) is the ratio at each sampling point of the observed PDs concentration (C_i) to the minimum PDs concentration (C_{0i}). C_{0i} was assigned a specific value (0.5 pc/m³) based on the minimum mean concentration in available documentation (Isobe et al. 2014). The PLI was calculated by taking the n-root of the n- PLI of all sampling sites.

4.3. Results

4.3.1 Blank test experiment

Blank tests are an essential part of laboratory quality control, where precise data are extremely important to increase the precision of the results. In the process of preparing and determining PAE levels, the PAE contamination ranged from 0.22 to 2.53 ng/L. For example, the blank contamination for DEP, DBP and DEHP were respectively 2.5, 0.97 and 0.98 ng/L. Other PAEs (DMP, BBP, DnOP) are measured with 0.68, 0.49, to 0.23 ng/L respectively. The blank values of each PAE are subtracted from the actual test sample values.

4.3.2 Concentration of PAEs in surface seawater

The concentrations of PAEs in surface water of the bay Sharm Obhur and the adjacent coastal Red Sea are presented in Figure 4.2a. The individual PAEs concentrations ranged from 1 to 1124 ng/L, while the arithmetic average individual PAEs concentration ranged from 25 to 263 ng/L (Table 4.1). High levels of DEP, DBP and BEP were detected in all samples, ranging from 22 - 1124, 8.9 - 346 and 61 - 651 ng/L, respectively. In contrast, DMP (4.5-76.0 ng/L), BBP (3.6-24.7 ng/L) and DnOp (0.5-80.1 ng/L) were generally lower in most samples, except in samples S1-6 (DMP 245 ng/L, BBP 205 ng/L) and S1-3 (BBP 241.8 and DnOP 245.6 ng/L). (Table S4.2; supplementary information). The compounds DEP, DBP and BEP accounted for more than 87% of the sum of concentrations of \sum_6 PAEs, with DEP (34%), DBP (22%) and BEP (33%). The concentration of the other three compounds represented between 4 - 6% of the total concentration, with the proportion for DMP (3%), BBP (6%) and DnOP (3%), as summarized in Table 4.1

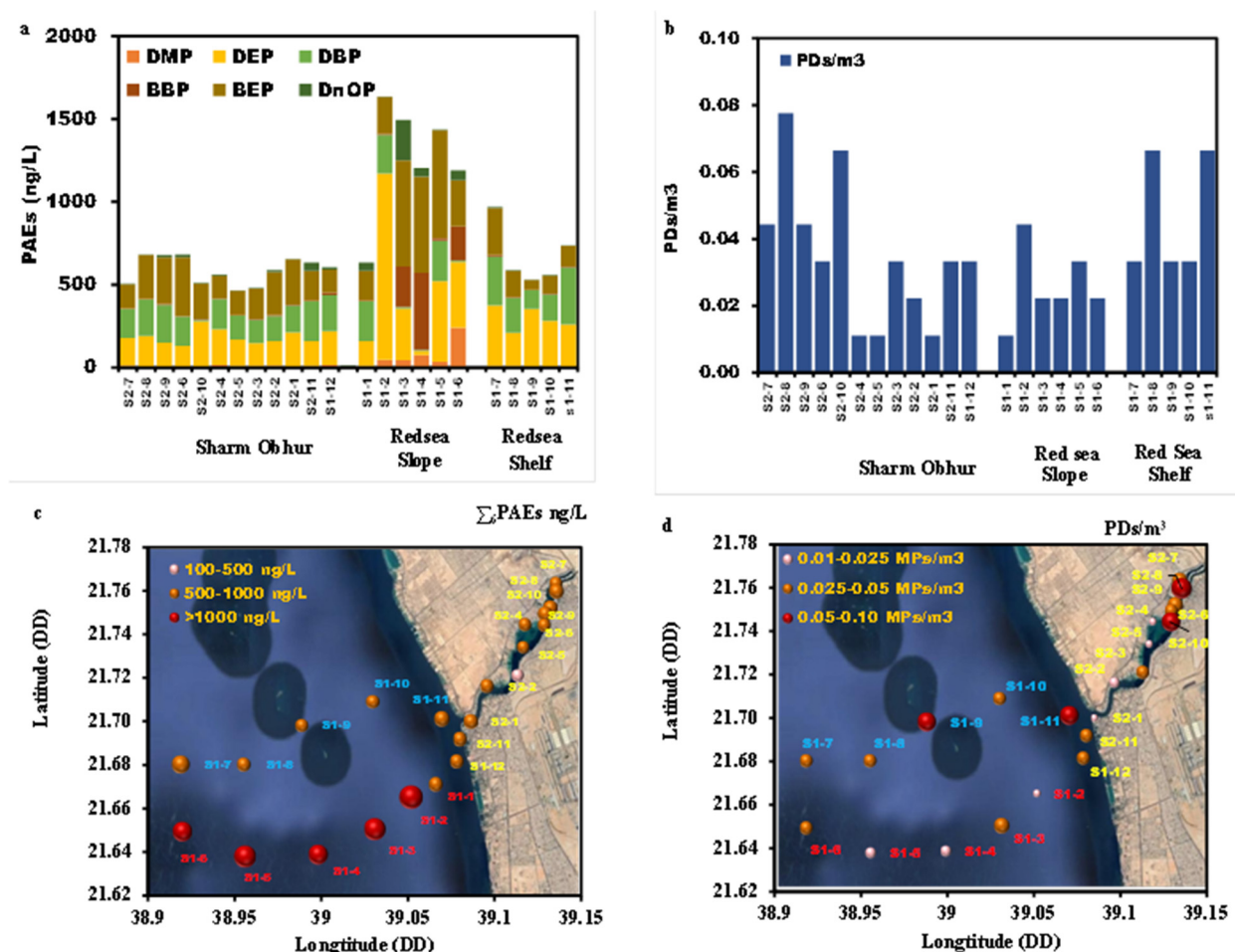


Figure 4.2: The concentrations of all PAEs (a) and plastic debris (b), distributions of Σ_6 PAEs (c) and plastic debris (d) in samples of surface waters from Sharm Obhur (yellow labeled), Red Sea slope (red labeled) and Red Sea shelf (blue labeled).

The distribution of PAEs in the study area varied depending on the total water column depth (Figure 4.2c). For example, the dissolved concentration of Σ_6 PAEs in Sharm Obhur (S2-7 to S1-12; depths 10-30 m) averaged 587 ± 82 ng/L. In the Red Sea, dissolved concentrations at stations on the shelf (S1-7 to S1-11; depths 42-20 m) averaged 677 ± 182 ng/L for Σ_6 PAEs, similar to observations in the bay of Sharm Obhur. In comparison, the highest concentrations of Σ_6 PAEs 1377 ± 354 ng/L were found on the slope of the Red Sea (S1-1 to S1-6; depths 30-503 m). In addition, among the major detected compounds (DEP, and BEP). The average concentration of low molecular weight

compounds (LMW-DEP) was lower in Sharm Obhur (177.3 ± 75 ng/L) and on the Red Sea shelf (290 ± 65 ng/L). The highest concentration DEP (458 ng/L) was found in the Red Sea slope region (depths 30-503 m). The high molecular weight HMW-BEP were also lower in Sharm Obhur and shelf waters than in slope waters, (215, 148 and 474 ng/L, respectively: Table 4.1).

4.3.3 Plastic debris abundance in surface seawater

In the current study, a total of 23 neuston samples were collected and plastic-like particles were found in all samples. The concentration of the plastic debris was 0.035 ± 0.019 PDs/ m³ (n= 23), (Figure 4.2b). Most of the particles were visually classified as irregularly shaped fragments, fibers, films, and pellets (Figure 4.S1). The abundances of PDs at sites near the head of Sharm Obhur (S2-7 and S2-8) ranged from 0.044-0.076 PDs/m³ and were higher than in the open waters at stations S1-6 (0.022 PDs/m³), which are 18.2 km away from the head of Sharm Obhur (Figure 4.2d). The abundance of PDs in the bay Sharm Obhur varied from 0.011 to 0.0776 PDs/m³, and compared to 0.022 to 0.044 PDs/m³ in the Red Sea. Overall, the average PDs abundance in Sharm Obhur (0.0374 ± 0.018 PDs/m³) and Red Sea (0.0301 ± 0.012 PDs/m³) were similar (Table S4.2).

4.3.4 Morphological and color characteristics of PDs in surface seawater

The morphological characteristics such as color, shape, polymer type and size of plastics debris at each sampling site and their average proportions (%) in the bay Sharm Obhur and Red Sea are shown in Figure 4.3. Different colors of PDs were observed in the samples, including white, black, green, yellow, and brown. White and black were the dominant colors and accounted for 43-60%, and 17-32% of the observed particles in the Red Sea and Sharm Obhur, respectively; whilst green, yellow, and brown accounted for (0-5 %) for Sharm Obhur and (6-17%) for Red Sea (Figure 4.3a, inserts). The total plastic waste from Sharm Obhur and the Red Sea was divided into fragments, fibers, films, and granules based on their morphological characteristics (Hartmann et al. 2019). Typical examples of PDs samples are shown in Figure S4.1. Fibers were found more often in Sharm Obhur (45%), than in the Red Sea (26%). In contrast, fragments were found more frequently found in the Red Sea (48%) than Sharm Obhur (29%). The percentage of films was similar in both

Chapter 4: Partitioning of phthalate esters and plastic debris and their ecological risk level in surface waters of the Red Sea and the bay of Sharm Obhur

study zones (23-26%). A single granule was found in open water in the Red Sea, out of all the types of plastic (Figure 4.3b).

Polymer typology was determined by comparing the spectrum of the most common commercial polymers (PE, PP, PC and PVC). Polyethylene (30 and 34%) and PP (7 and 11%) were the two most common plastics in Sharm Obhur and the Red Sea, respectively, with 55 and 63% of the particles not attributable to any of these common plastics (Figure 4.3c). Polyethylene was identified in almost all samples, followed by PP only in four samples (S2-3, S2-4, S2-1 and S1-5), (Figure 4.3c). Particles were classified into five categories according to their size: $< 1 \text{ mm}^2$, $1-5 \text{ mm}^2$, $5-10 \text{ mm}^2$, $10-25 \text{ mm}^2$, and $25-50 \text{ mm}^2$. Particles with size less than 5 mm (microplastics) were found more often than particles ($> 20 \text{ mm}^2$). In both sampling sites, the size of $1-5 \text{ mm}^2$ particles was abundant between 40 and 47%. The sizes of $5-10 \text{ mm}^2$, $10-25 \text{ mm}^2$ and $>25 \text{ mm}^2$ were more common in Sharm Obhur (16, 13 and 3%), than in the Red Sea (3, 3 and 6%). In contrast the particles with a size less than 1 mm^2 were found more frequently in the Red Sea (48%) than in Sharm Obhur (21%) (Figure 4.3d).

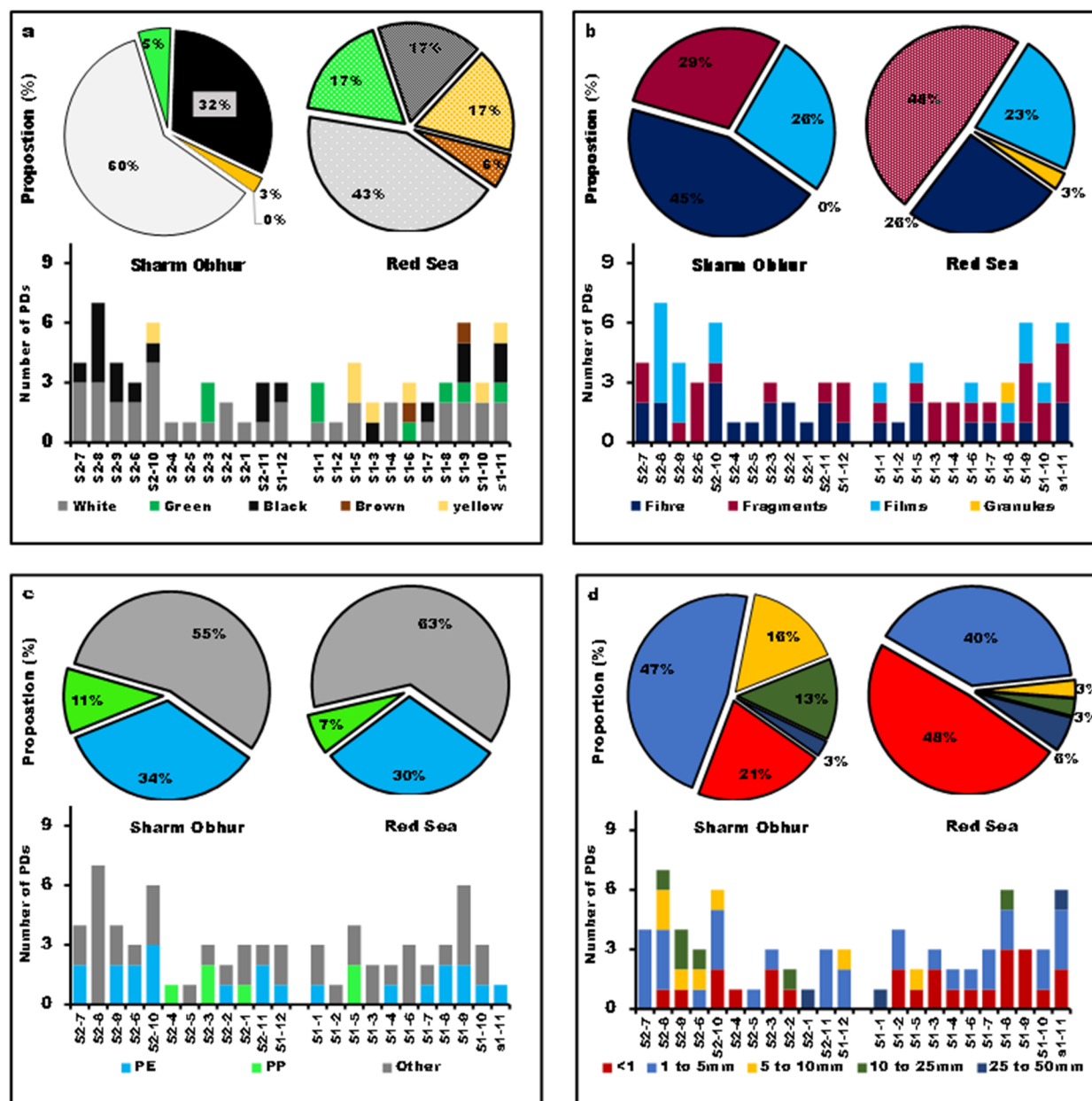


Figure 4.3: Abundance of plastic debris (PDs/m³) in different colors (a), shape (b), polymer type (c) and Sizes (d) of PDs at each sampling site and in inserts the average proportion (%) of Sharm Obhur and Red Sea surface seawater

4.3.5 Correlation between PDs, PAEs and environmental parameters

We examined the correlation factors between the PDs and PAEs in order to explore possible relationships between the pollutants. We compared the sum of the surface areas (mm^2/m^3) of all PDs found at each sampling site with the sum of six PAEs concentrations ($\sum_6 \text{PAEs}$, $\mu\text{g}/\text{m}^3$) and

Chapter 4: Partitioning of phthalate esters and plastic debris and their ecological risk level in surface waters of the Red Sea and the bay of Sharm Obhur

other environmental parameters (temperature, salinity, water depth, pH, total dissolved solids (TDS), and specific conductance (SPC)), for the surface waters of Sharm Obhur and the Red Sea. The results shows that a positive correlation between PAEs and PDs within the sampling area, which differed between the sampling regions. For example, the rate of increase in PAEs concentration with PDs was lower at within Sharm Obhur ($r^2=0.844$, slope= 4.496 ug/mm^2 of PDs surface area, $n=16$, $p < 0.05$; S2-7 to S1-12; depth 10-30 m) (Figure 4.4a) and in shelf waters of the Red Sea ($r^2=0.01$, slope= 1.37 , ($\mu\text{g/mm}^2$), $p < 0.05$, $n=5$; S2-7 to S1-12; depth 42-20 m) (Figure 4.4b). In comparison, a steeper positive slope ($r^2=0.88$, slope= $110.98 \text{ (}\mu\text{g/mm}^2\text{)}$, $n=6$, $p < 0.05$) was found in Red Sea slope waters (S1-1 to S1-6; depth 30-503 m) (Figure 4.4c). The relationship between total water column depth and the concentration of \sum_6 PAEs and PDs was investigated separately. A positive correlation was observed between station water depth and PAEs concentration ($r^2 = 0.678$, slope = 4.29 , $n = 23$) (Figure S4.2a). However, the number of plastic debris did not vary significantly with station water depth (Figure S4.2b). No other correlations were observed

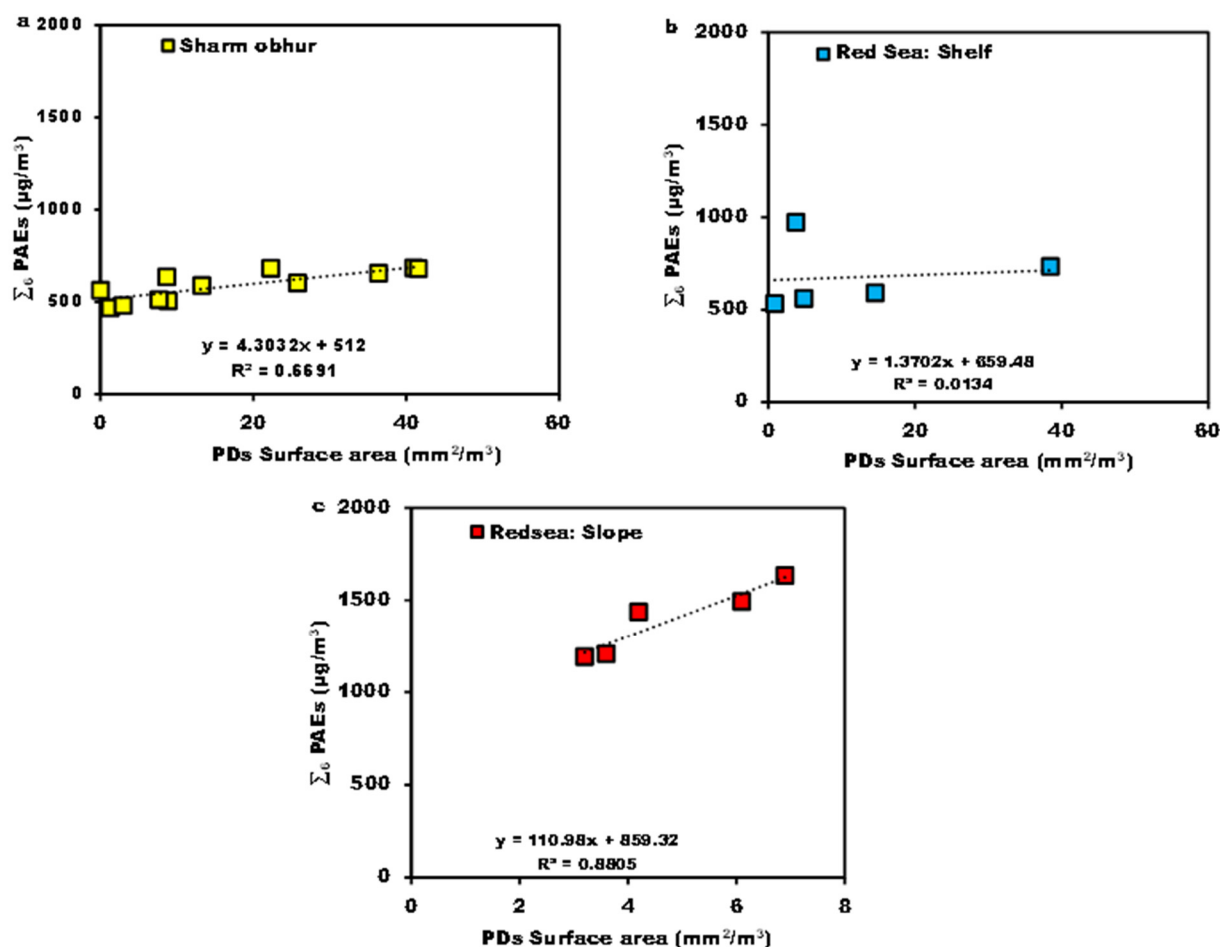


Figure 4.4: Correlations between PDs abundance and Σ₆ PAEs for Sharm Obhur (a), Red Sea, Shelf (b), Red Sea, Slope (c).

4.3.6 Potential ecological risk of PAEs and PDs

We assessed the ecological risk level (ERL) of the four PAEs for Red Sea surface waters for which PNEC values are available (DMP, DEP, DBP and BEP) (Liu et al. 2020c). The ERL of the PAEs was evaluated based on the risk quotient (RQ) of each PAE (Table S4.3). The values of the PAEs are shown in Figure 4.5a. Among all PAEs, DBP (0.017-0.6920) and BEP (0.04-0.4229) have low to medium RQs, while DMP and DEP have negligible risk, showing that the overall RQ is mainly determined by DBP and BEP. Relatively high average RQ values were found for DBP (0.3242 ± 0.01) due to their low predicted non-effect concentration ($0.5 \mu\text{g/L}$) than other compounds (DMP ($96 \mu\text{g/L}$), DEP ($96 \mu\text{g/L}$) and BEP ($1.54 \mu\text{g/L}$)). The hazard index (HI) of each sampling site was

calculated from the sum of the risk quotients (RQ) of all PAEs. The HI ranged from 0.206 to 0.796, indicating low to moderate risk in the surface waters of the Red Sea. The average health index for Sharm Obhur and Red Sea were similar, 0.47 and 0.48, respectively (Table S4.3), indicating that the ecological risk in the study region ranged from low to moderate and the data suggest that PAEs are not likely a serious ecological threat in the Red Sea.

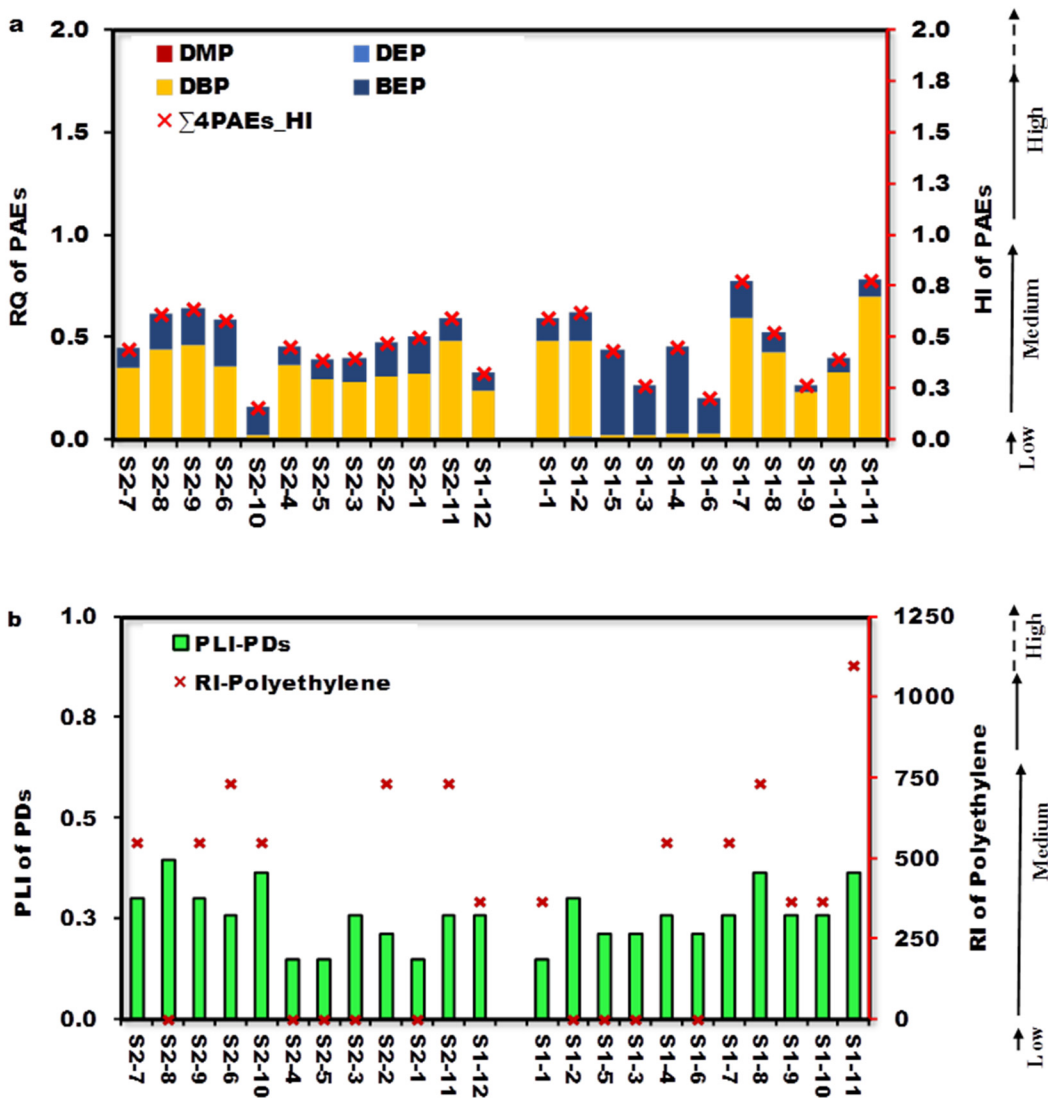


Figure 4.5: Risk quotient and health index of PAEs (a) and pollution load index (PLI) PDs and HI index of polyethylene polymer (b) for each sampling and the average (insert table) in Red Sea surface water samples.

The pollution index (PLI) of PDs ranges from 0.14 to 0.36 (Figure 4.5b), which means that all sites had relatively low to medium PD pollution index (PLI: < 0.1 low; 0.1-0.5 medium and > 0.5 high). The average PLI index in Sharm Obhur (0.2612) and in the Red Sea (0.2433) was similar (Figure 4.5c). The polymer risk index (PRI) by PDs pollution is also based on the type of polymer at a given sampling site. Previously, ecological risk level of PDs was classified according to the respective percentage (P_n) and hazard level of each polymer. Since polyethylene was the most frequently detected polymer in these samples, its ecological risk level was evaluated. The polyethylene risk index (PE-RI) varied from low to medium (0-1100), (polymer risk index: < 10 very low; 10-100 low; 100-1000 medium; > 1000 high) with an uneven distribution (Figure 4.5b). The highest PE-RI values were measured in Sharm Obhur (423.6) with lower values in the Red Sea (209.52) (Figure 4.5c).

4.4. Discussion

The total concentrations of six PAEs (Σ_6 PAEs) in surface waters of the study area ranged from 0.8 to 1124 ng/L, (Table 4.1). This concentration range is consistent, but on the low end, with data reported for other regions. For example, Σ_6 PAEs have been reported in the Mediterranean Sea: 17.4- 8442 ng/L (Sanchez-Avila et al. 2012), 130-1330 ng/L (Paluselli et al. 2018b) and 168-689 ng/L (Paluselli and Kim 2020) and northern Europe: 76-1440 ng/L (Turner and Rawling 2000). With the rapid development of the marine economic zones along the Red Sea coast near Jeddah, the concentration of PAEs could increase. The total amount of municipal solid waste (MSW) in Saudi Arabia is expected to double from 15 to 30 million tons per year by 2030, and plastic is the second most common part of MSW (5-17%) (Nizami et al. 2017). PAEs are the major additives of plastics and are expected to enter the environment through slow volatilization and/or leaching.

Overall, the relative contribution of the studied PAEs decreased in the order DEP > BEP > DBP > BBP > DMP > DnOP in water samples. Among them, DEP, BEP and DBP were the dominant additives in surface waters with mean relative contributions of 33%, 32% and 21% respectively (Table 4.1). This PAE composition is consistent with the proportion of BEP (22-75%), DEP (7-8%), and DBP (14-26%) in the total dissolved PAE observed in the Mediterranean Sea (Paluselli et al. 2018b). This could be result of the higher use (50-60%) of these additives compared with

other additives (DMP, BBP, DnOP) (Benson and Fred-Ahmadu 2020). The second reason for the high concentration of DBP in the environment is their high leaching rate from plastics (Dhavamani et al. 2022). The diffusivity of PAEs from plastic decreases with increasing molecular weight, such as for DMP, DEP, DBP, BBP, BEP and DnOP (14.2, 13.6, 14.6, 6.1, 6.9 and 5.6 (m^2/d) $\times 10^{-20}$), respectively (Bradlee 2003). In addition to the slow leaching, HMW PAEs (e.g., BEP) are relative resistant to degradation by photolysis, hydrolysis, and biological processes (Gao and Wen 2016), implying a higher input by leaching and longer persistence in terms of degradation. Therefore, it is understandable that seawater concentrations of DBP and BEP are higher than those of the other PAEs.

Table 4.1: Average concentration (ng/L) pattern of PAEs in the surface seawater of Sharm Obhur and Red Sea. “ \sum PAEs” are the sum of each PAE from all 22 sampling sites. “Proportion” is the ratio of each \sum PAE to the total \sum PAE concentration

Comp ounds	Min ng/L	Max ng/L	Avg (ng/L)	Av g	\sum PAEs (ng/L)	Pro- portio n (%)	Sharm Obhur (Avg ng/L)	Red Sea Shelf Avg ng/L	Red Sea Slope Avg ng/L
DMP	5	242	26	9	591	3	9	7	76
DEP	22	1124	264	203	6068	34	177	290	468
DBP	9	346	170	177	3912	22	170	225	133
BBP	2	465	44	5	1023	6	5	5	157
BEP	62	651	255	214	5870	33	215	148	474
DnOP	1	245	25	6	573	3	11	4	69
Sum							588	677	1377

The average dissolved concentration of \sum_6 PAEs in Sharm Obhur (587 ± 82 ng/L) and on the Red Sea shelf (677 ± 182 ng/L) was comparatively lower than the slope of open water in Red Sea (1396 ± 354 ng/L). These results could be related to the higher degradation rate in the lagoon and the adsorption of PAEs on suspended sediments, plastic particles, and other particles (Paluselli and

Kim 2020). In particular, the concentration of low molecular weight PAEs (DEP) was lower in Sharm Obhur (177 ng/L; depth ~10-30 m) and Red Sea shelf (290 ng/L; depth ~17-40 m) than in open water of the Red Sea (468 ng/L; depth ~30-503 m) (Table 4.1). A possible explanation would be a higher degradation rate of LMW-DMP in these waters higher bacterial abundances related to domestic waste, industrial effluents, and sewage treatment plant discharges, as suggested by Yuan et al. (2010). This is also consistent with earlier studies that the concentration of polyaromatic hydrocarbons (PAHs) increases from the head to the mouth of Sharm Obhur (PAHs; 0.3 µg/L to 1.73 µg/L), while the concentration of nutrients (nitrite, nitrate and ammonium) decreases (R. Al-Farawati, et al., 2008). The low BEP concentration in Sharm Obhur (215 ng/L) and Red Sea shelf waters (148 ng/L) were lower than in slope waters (474 ng/L), which could be related to adsorption onto particles. This suggests that the high molecular weight (BEP) compounds are more likely to adsorb onto suspended particles and settle to the seafloor due to their high hydrophobicity and high octanol/water partition coefficient (Kow; Table 2.1) (Cao et al. 2022). The results were consistent with those of a previous study in Marseille bay, in which high concentrations of BEP were detected at 30 m depth (296.5 ng/L) and then at DEP (50 ng/L) (Paluselli et al. 2018b). The distribution of contaminants from Sharm Obhur to the Red Sea is likely due to mixing of the surface and bottom water flows. Sharm Obhur has a two-layer flow at the mouth, with higher salinity (30.22‰) water flowing in from the Red Sea at the surface (0-10 m) and intermediate depths (10-20 m), and more saline (39.22 ‰) water flowing from the bay at depth (> 20 m) into the Red Sea (Abdulla and Al-Subhi 2020, Al saafani et al. 2017, Albarakati 2009). Contaminated sediments from Sharm Obhur may be carried out into deep waters of the Red Sea by the subsurface outflow of the dense saline water, forming contaminated sediment deposits that are potential sources of dissolved PAEs in the Red Sea.

This observation reflects the correlations between PDs and PAEs. We found differences in correlation within the sampling area with respect to water column depth. The slope of PAEs concentrations versus abundance of PDs was less steep in Sharm Obhur and shelf waters than in slope waters, likely due to the higher degradation and adsorption of PAEs in the bay and shelf waters. In contrast, the steeper slope of the slope stations was potentially the result of lower adsorption and degradation. A positive correlation was found between the water column depth and PAE concentration ($r^2 = 0.678$, slope = 4.29, $n = 13$) (Figure S4.1a), suggesting a lower removal

Chapter 4: Partitioning of phthalate esters and plastic debris and their ecological risk level in surface waters of the Red Sea and the bay of Sharm Obhur

by degradation adsorption in deep waters. A similar correlation between water level and PAEs concentration ($r^2 = 0.512$, $P < 0.05$) was found in Jiazhou Bay, China. (Liu et al. 2020b). We observed a positive correlation between PDs and PAEs in surface seawaters. Similar correlations between microplastics (MPs) and PAEs have been reported for Jiaozhou Bay (Liu et al. 2020b), northwestern Mediterranean Sea (Baini et al. 2017) and Mediterranean Sea (Fossi et al. 2016).

The concentrations of floating microplastics in our Red Sea samples (0.011 - 0.066 PDs/m³) are somewhat lower than levels found in other surface waters, e.g., Mediterranean Sea (0.15 pieces/m³; (de Lucia et al. 2014) and East China Sea (0.029-0.305 pieces/m³; (Zhao et al. 2014). This may be the low occurrence of floating plastic fragments in the current study may be due to a low input of plastic debris into the Red Sea and a lack of surface runoff (rivers, rain) into the Red Sea. Surface runoff is the major conduit for plastic input into the ocean (Lebreton et al., 2017). The Red Sea is characterized by low annual rainfall, lack of permanent rivers in its catchment area and high evaporation. The explanation for this discrepancy could lie in large differences in the movement of misdirected plastic waste from land to sea and/or a higher loss rate of floating plastic (Martí et al. 2017).

The high concentration of fragments (38.4%) and films (24.7%) in the study area indicates the high efficiency of photodegradation in the Red Sea due to high solar radiation throughout the year. The average monthly index of ultraviolet radiation (UVR) in the Red Sea is 14.4 W/m², and the temperature is 25°C or higher throughout the year. Summer temperatures can exceed 35-39°C. The large amount of white colored particles (52%) found in the surface water could also reflect the high intensity of sunlight. The color of the PDs usually comes from the original plastic products that have been bleached by photodegradation and other processes. This agrees with studies from the same area showing a high percentage of fragments (74%), films (17%) and white color particles (50-60%) (Al-Lihaibi et al. 2019).

Fibres can be formed from fisheries, surface runoff, and direct input of industrial waste like sewage treatment plants. This is perhaps why Sharm Obhur, a hot spot for boating, fishing, and sewage discharges, has a higher percentage of fiber (23.7%) than the Red Sea (12.3%). Plastic pellets, which are considered primary plastic debris (Law et al. 2010), were detected in only two samples in our study area. The dominance of PE and PP, accounting for 90% of the total plastic found, is in close agreement with the dominance of these materials in floating plastic fragments reported for

Chapter 4: Partitioning of phthalate esters and plastic debris and their ecological risk level in surface waters of the Red Sea and the bay of Sharm Obhur

the Mediterranean Sea (Suaria et al, 2016) and Red Sea (Al-Lihaibi et al. 2019). Polyethylene (density 0.86-0.96 g/cm³) and PP (0.85-0.91 g/cm³) are easily transported by surface currents due to their lower specific gravity compared to seawater (Jalón-Rojas et al. 2019, Zhang et al. 2018).

Concentrations of PAEs and PDs in the environment that exceed the ecological risk level (ERL) may negatively affect ecosystems and human health (Wang et al. 2021). We applied the risk quotient method to evaluate the ecological risk of PAEs (Gani and Kazmi 2016, Sirivithayapakorn and Thuyviang 2010). The risk quotient of PAEs (DMP (0.001), DEP (0.001), DBP (0.324), and BEP (0.139)) was lower than the high-risk quotient (High RQ; 0.1) for the 23 sampling stations, indicating that the PAEs concentrations in Sharm Obhur and the Red Sea poses a low (RQ < 0.1) to moderate risk (RQ 0.1-1.0). The ecological risk level of plastic waste was determined using the pollution index method (Liu et al. 2020c). The pollution index of PDs in Sharm Obhur and the Red Sea ranges from 0.07 to 0.08 (Figure 4.5b). This indicates that the risk of PDs pollution to the Red Sea surface water is not dramatic. The whole area is characterized by intermediate pollution levels, as the average concentration of PDs (0.035/m³) is lower than the minimum risk level of MPs (0.5/m³). Since PDs are considered a vector for pollutant transfer, the mobility of PDs with PAEs and other pollutants needs to be well documented. Therefore, more comprehensive studies on the behavior, fate, and exposure risks of PAEs and PDs should be conducted in future.

4.5. Conclusion and future perspectives

This study reports the recent information's regarding the partitioning of phthalate esters, and plastic debris and their ecological risk levels in the surface waters of the Red Sea and the bay of Sharm Obhur. The PAEs concentrations (0.8 to 1124 ng/L,) in Sharm Obhur and Red Sea were similar to reported values from other regions (e.g. Mediterranean: 30-1330 ng/L; and Northern Europe: 76-1440 ng/L). However, microplastic pollution (0.011 - 0.066 PDs/m³) was lower than that in other waters (e.g. Mediterranean Sea; 0.15 pcs/m³). This indicates that additional PAE inputs to Sharm Obhur and the Red Sea from domestic waste, industrial effluents, and sewage treatment plant discharges is higher than from rivers and surface runoff in other locations. The low concentration of LMW-DMP in Sharm Obhur also indicates a higher organic load and microbial degradation in Sharm Obhur than in the Red Sea. The high concentration of pollutants in Sharm Obhur and Red Sea is probably due to the mixing of surface and bottom waters transect. The high concentration of fragments and white colored plastics in the study area indicates a high

Chapter 4: Partitioning of phthalate esters and plastic debris and their ecological risk level in surface waters of the Red Sea and the bay of Sharm Obhur

photodegradation efficiency in the Red Sea. A positive correlation between the abundance of PDs and concentration of PAEs suggests that in situ leaching may contribute a major portion of the dissolved PAE pool. The calculated ERL due to PAEs and PDs for Sharm Obhur and the Red Sea is currently at a low to moderate level. The presence of PAEs in water is major cause of marine water pollution. Thus, effective controls of PAEs in various matrices will hamper the global water pollution and diseases. The overall suggestion and future study most likely will be focused on lateral flow protocols for the rapid extraction of these chemicals are model strategies, applicable on-site, and timely determination is desired. Despite the technological advances for PAEs, proper selection of starter culture plays an essential role in prevention of high levels of PAEs in water. Therefore, in making new policies on all aspects should be covered

CRediT authorship contribution statement

All authors made significant contributions to this study. Jeyakumar dhavamani: Performed all analyses and writing- original draft preparation, Aaron J. Beck: Co-supervision: Data curation, , Mohammad El-Shahawi Soror: Reviewing and editing, Martha Gledhill: Co-supervision, Eric P Achterberg; Supervision. Mohammad I. Oriff and Iqbal MI Ismail: Resources, funding acquisition.

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Appendix: Supplementary information Figures

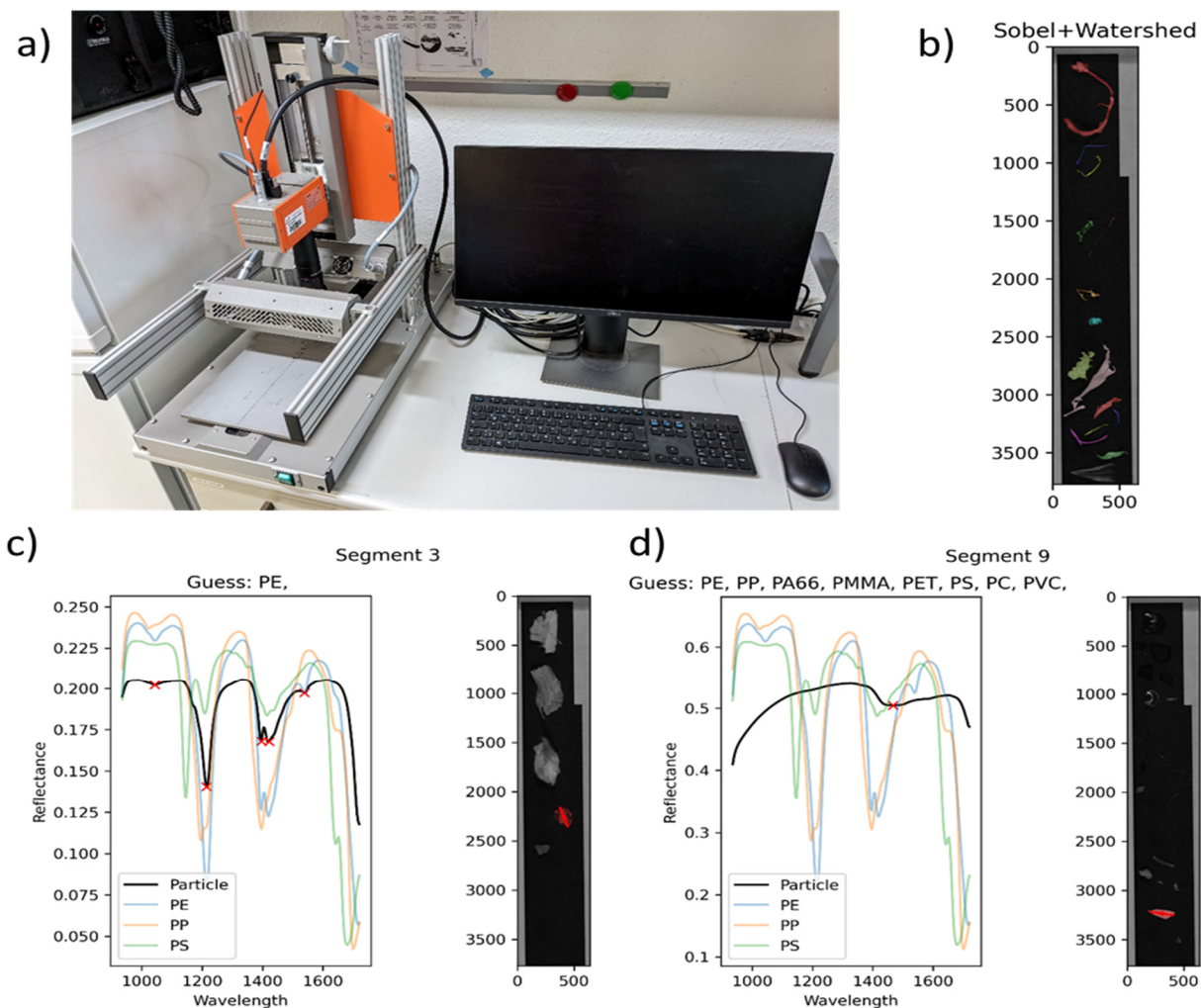


Figure S4.1: The images of identified plastic debris and the hyperspectral camera. a) Near infrared (NIR) hyperspectral camera and lab scanner. b) Results of particle edge-finding process for one scan. NIR spectra are averaged within each particle area for polymer identification. c) Spectrum (black) for an identified polyethylene particle. The colored spectra are from reference polymer particles. The “guess” is identification from a simple decision tree based on peaks/valleys, and does not reflect the machine learning identification. d) Spectrum for a non-plastic particle, likely of plant origin. Symbols as in panel c.

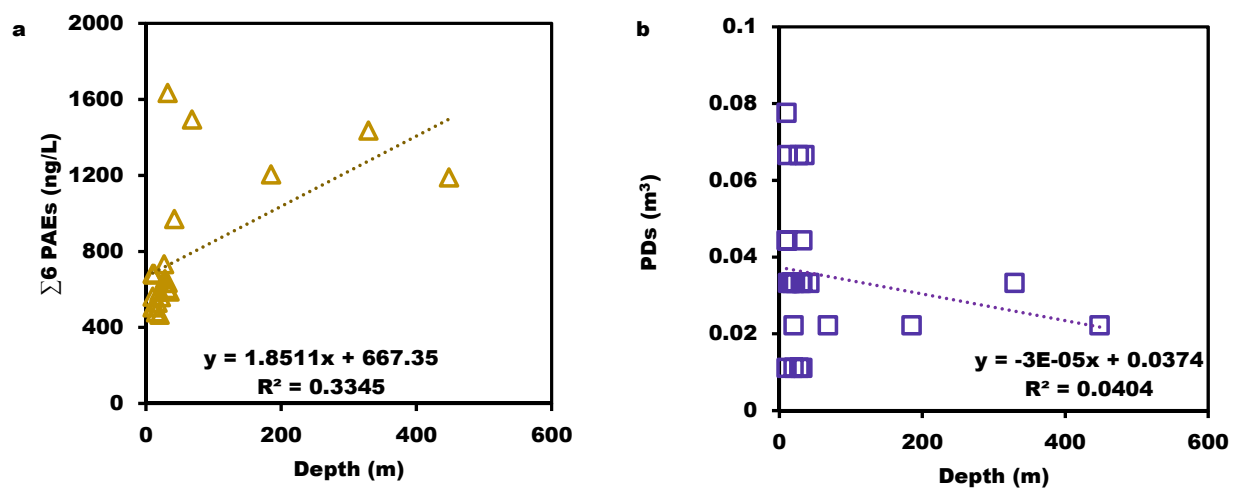


Figure S4.2: The correlation between water depth at all stations and $\Sigma 6 \text{ PAEs}$ (c) and PDs (d).

Table S4.1: GPS coordinate of the sampling stations in the Sharm Obhur to open water in the Red Sea near Jeddah

Sampling Zone	Sampling location		
	Sample Name	Latitude	Longitude
Sharm Obhur	S2-7	21°45'47.94"N	39°8'5.02"E
	S2-8	21°45'34.63"N	39°8'7.68"E
	S2-9	21°45'7.52"N	39°7'54.03"E
	S2-6	21°44'57.47"N	39°7'44.84"E
	S2-10	21°44'39.32"N	39°7'43.16"E
	S2-4	21°44'39.68"N	39°7'4.70"E
	S2-5	21°44'2.55"N	39°6'59.23"E
	S2-3	21°43'15.64"N	39°6'44.81"E
	S2-2	21°42'58.53"N	39°5'46.32"E
	S2-1	21°42'41.00"N	39°5'41.40"E
	S2-11	21°42'05.04"N	39°05'05.38"E
	S2-12	21°41'08.44"N	39°05'06.56"E
Red Sea, Slope	S1-1	21°41'30.61"N	39°4'50.60"E
	S1-2	21°40'16.32"N	39°4'36.19"E
	S1-3	21°39'30.26"N	39°2'29.91"E
	S1-4	21°38'40.88"N	39°1'4.11"E
	S1-5	21°38'16.50"N	38°59'19.34"E
	S1-6	21°38'57.48"N	38°56'23.28"E
Red Sea, Shelf	S1-7	21°41'10.30"N	38°56'20.55"E
	S1-8	21°41'28.30"N	38°58'32.70"E
	S1-9	21°42'14.80"N	39°0'6.74"E
	S1-10	21°42'32.10"N	39°2'41.32"E
	S1-11	21°42'18.06"N	39°04'29.63"E

Table S4.2: The concentration (ng/L) pattern of each PAEs and PDs in the surface seawater of Sharm Obhur and Red Sea, east coast Jeddah.

Sampling Zone	Stations	DMP	DEP	DBP	BBP	BEP	DnOP	\sum_6 PAEs	PDs/m ³
Sharm obhur	S2-7	10.5	168.9	174.3	3.6	144.9	3.3	505.5	0.044
	S2-8	6.3	184.0	220.0	3.3	265.0	4.5	683.1	0.078
	S2-9	7.0	143.7	227.8	5.3	282.1	12.9	678.9	0.044
	S2-6	5.3	125.8	176.6	3.6	353.7	16.3	681.3	0.033
	S2-10	3.4	276.5	8.9	3.7	213.7	5.7	511.8	0.067
	S2-4	15.5	215.6	181.6	3.3	138.9	7.0	561.9	0.011
	S2-5	8.0	160.2	146.3	1.6	148.7	2.2	466.9	0.011
	S2-3	9.2	139.5	140.1	5.0	182.0	3.9	479.8	0.033
	S2-2	10.5	149.2	152.4	6.1	257.6	13.0	588.9	0.022
	S2-1	3.0	210.3	161.3	4.6	273.4	2.4	655.1	0.011
	S2-11	9.3	150.8	238.3	4.4	182.0	49.8	634.6	0.033
	S1-12	16.6	203.5	217.7	14.7	138.4	14.7	605.5	0.033
Avg		8.7	177.3	170.4	4.9	215.0	11.3	587.8	0.035
Red Sea, Slope	S1-1	9.3	150.8	238.3	4.4	182.0	49.8	634.6	0.011
	S1-2	46.8	1124.4	233.0	5.4	223.7	3.1	1636.5	0.044
	S1-3	44.0	311.4	9.1	245.7	639.2	245.3	1494.7	0.022
	S1-4	76.0	22.0	9.1	465.0	579.5	54.0	1205.6	0.022
	S1-5	35.3	487.6	242.8	14.7	651.3	4.3	1436.1	0.033
	S1-6	241.8	395.6	10.1	205.8	276.6	60.1	1190.0	0.022
Avg		75.5	415.3	123.8	156.8	425.4	69.4	1266.3	0.026
Red Sea, Shelf	S1-7	9.7	365.1	294.2	9.5	284.1	8.4	971.0	0.033
	S1-8	9.5	201.2	210.5	4.5	156.8	6.2	588.7	0.067
	S1-9	3.1	350.9	112.4	2.0	61.6	0.8	530.8	0.033
	S1-10	6.3	276.0	161.4	3.3	109.2	3.5	559.8	0.033
	s1-11	4.5	255.0	346.0	3.3	126.0	2.1	736.9	0.067
Avg		6.6	289.6	224.9	4.5	147.5	4.2	677.4	0.047

Table S4.3: Risk quotient and health index of PAEs (a) and pollution load index (PLI) PDs and HI index of polyethylene polymer (b) for each sampling and the average (insert table) in Red Sea surface water samples.

Sampling Zone	Samples	RQ				HI Σ_4 PAEs	PLI	RI Polyethylene
		DMP	DEP	DBP	BEP			
Sharm Obhur	S2-7	0.000	0.002	0.349	0.094	0.444	0.298	550.0
	S2-8	0.000	0.002	0.440	0.172	0.614	0.394	0.0
	S2-9	0.000	0.001	0.456	0.183	0.640	0.298	550.0
	S2-6	0.000	0.001	0.353	0.230	0.584	0.258	733.3
	S2-10	0.000	0.003	0.018	0.139	0.159	0.365	550.0
	S2-4	0.000	0.002	0.363	0.090	0.456	0.149	0.0
	S2-5	0.000	0.002	0.293	0.097	0.391	0.149	0.0
	S2-3	0.000	0.001	0.280	0.118	0.400	0.258	0.0
	S2-2	0.000	0.002	0.305	0.167	0.474	0.211	733.3
	S2-1	0.000	0.002	0.323	0.178	0.502	0.149	0.0
	S2-11	0.000	0.002	0.477	0.118	0.596	0.258	733.3
	S1-12	0.000	0.001	0.235	0.090	0.326	0.258	366.7
	Avg	0.000	0.002	0.324	0.140	0.466	0.254	351.389
Red Sea, Slope	S1-1	0.00	0.00	0.48	0.12	0.60	0.15	366.7
	S1-2	0.00	0.01	0.47	0.15	0.62	0.30	0.0
	S1-5	0.00	0.00	0.02	0.42	0.44	0.21	0.0
	S1-3	0.00	0.00	0.02	0.25	0.27	0.21	0.0
	S1-4	0.00	0.01	0.03	0.42	0.45	0.26	550.0
	S1-6	0.00	0.00	0.02	0.18	0.21	0.21	0.0
	Avg	0.0008	0.0043	0.1708	0.2546	0.4305	0.2227	152.78
Red Sea, Shelf	S1-7	0.00	0.00	0.59	0.18	0.78	0.26	550.0
	S1-8	0.00	0.00	0.42	0.10	0.52	0.36	733.3
	S1-9	0.00	0.00	0.22	0.04	0.27	0.26	366.7
	S1-10	0.00	0.00	0.32	0.07	0.40	0.26	366.7
	S1-11	0.00	0.00	0.69	0.08	0.78	0.36	1100.0
	Avg	0.00	0.00	0.45	0.10	0.55	0.30	623.33

Table S4.4: The chemical toxicity and hazard Score of individual polymer

Polymer score							
Polymer type	Abbreviation	Monomer	Density (g/cm ³)	Main applications	Items (total)	Percentage	Score ^a
Polyethylene	PE	Ethylene	0.91–0.96	Film product, bottles, pipes, etc.	119.63	46.71%	11
Polypropylene	PP	Propylene	0.85–0.94	Woven bag, Food packaging, etc.	9.38	3.66%	1
Polyvinyl chloride	PVC	Vinyl chloride	1.41	Plastics doors and windows, pipes, etc.	8.57	3.35%	10551
Polyamide (nylon)	PA	Adipic acid	1.14–1.15	Wear part, bearings, etc.	6.67	2.60%	47
Polystyrene	PS	Styrene	1.05	Thermal insulation material, insulation parts, etc.	2.78	1.09%	30
Polyethylene terephthalate	PET	Terephthalic acid and glycol ester	1.38	Electric products, automotive applications, etc.	68.79	26.86%	4
Rayon ^b	-	Cellulose	-	Clothing, interior decoration, etc.	37.92	14.80%	-
Polyester ^b	-	-	-	Bottles, film product, tapes, etc.	2.37	0.92%	-
Total				-	256.11	100%	-

Chapter 5

Conclusions, Future Perspectives and limitations

5. Conclusions, Future Perspectives and limitations

Within the frame of the current thesis the following remarks and future perspectives can be outlined:

5.1 General conclusions

- i.** My work reports on the effects of the leaching process of six phthalic acid esters (PAEs) from three common consumer plastics, low and high density polyethylene (LDPE, HDPE) and recycled polyethylene (RP). The impact of salinity, temperature and ultraviolet irradiation (UVR) on the leaching of the PAEs was critically studied and properly assigned.
- ii.** The relationship between PAEs and plastic wastes in Red Sea water was investigated. One of the main challenges of this study was that quantifying PAEs with good precision and reliability is a real challenge due to blank contamination from the materials used in sampling and analysis. Great attempts to measure and control the problems of blank contamination and extend the method to quantify PAEs from three common consumer plastics such as LDPE, HDPE, and RP polymers as well as from real seawater were critically assessed. So far, the results of the work have been divided into three chapters: Chapters 2, 3, and 4.
- iii. Chapter 2** a detailed strategy for measuring and controlling the blank contamination of PAEs was developed, and the experimental conditions, such as extraction time and temperature, were optimized. The conditions of GC-MS were successfully optimized and the developed method was applied to the determination of PAEs in real environmental samples. The analysis times were shortened for LLE (1-2 h) and SPE (6-7 h), and the peak resolution was good in most cases.
- iv.** The average recoveries of PAEs in LLE (90-97%) and SPE (86-90%). From the results of the quality assurance/blank contamination carried out for the analysis of PAEs in this study, it was revealed, that the blank contamination of the selected materials ranged from (3 ± 0.7 to 35 ± 6 ng) for liquid-liquid extraction (LLE) and from (5 ± 1.8 to 63 ± 15 ng) for solid-phase extraction (SPE). A few materials showed higher mass of PAEs contamination like sterile tips, PP syringe and filter and un filtered ASW (96.3, 72.2, 110.0 and 57.0 ng, respectively) and these have been removed from the material list for experimental and analytical work. Therefore, it was necessary to standardize the methodology of sampling, separation and identification of blank values. This

Chapter 5: Conclusions, Future Perspectives and limitations

information may in particular be useful for analysis of PAEs where it can be of great importance to obtain precise determination of plastic additives in complex matrices.

v. Chapter 3 reports on the leaching of six PAEs from three common consumer plastics: low- and high-density polyethylene (LDPE, HDPE) and recycled polyethylene (RP) was investigated by using the optimized LLE method. The effects of salinity, temperature, and ultraviolet irradiation (UVR) on leaching were studied. The effects on the overall leaching process of re-adsorption of released PAES back onto plastic surfaces were considered. The highest leaching rates were observed in the initial phase (24 - 48 h) of incubation, indicating that the leaching process is concentration dependent. Overall, fewer PAEs were leached from HDPE than from LDPE and RP polymers.

vi. Low molecular weight compounds were leached from LDPE (i.e. DMP, DBP and DEP); while HDPE and RP released more high molecular weight compounds (HMW; DEHP and DnOP). Considerable re-adsorption from PE was observed for all three polymers, which can reduce the amount of actual/total leachate in the dissolved phase by up to 30-80%. The approach used in this study to measure PAEs accounts for the adsorption loss of each target compound during the leaching process. This is an important step in understanding the hazards and extent of exposure to additives from plastic pollution.

vii. The leaching of PAEs from LDPE and RP increases with temperature. This suggests that leaching varies at different times of the year and that hot weather conditions increase the rate of additive release. When organisms have ingested MP, the additives it contains are more likely to be released when the internal temperature of the gut is relatively high and poses a threat to marine animals. In contrast, HDPE shows a higher leaching rate at low temperatures (10°C), suggesting that the cold climate may increase the leaching potential of PAEs from HDPE polymers. The amount of leachate increased sharply with UV irradiation. The higher effect on LDPE and RP suggests that additive concentration and recycling of plastics increase the impurities (chromophores) in the polymer and they are very sensitive to UV irradiation.

viii. Chapter 4 reports the relationship between PAEs and plastic debris (PDs) in the marine environment; we studied the abundance of PDs and PAEs in the surface waters of Sharm Obhur Bay and the Red Sea. A positive correlation was found between the abundance of PDs and the concentrations of PAEs, suggesting that in situ leaching may account for a large fraction of the

dissolved PAE pool. We found differences in correlation within the sampling area with respect to water column depth. The slope of PAE concentrations versus PDs abundance was less steep in Sharm Obhur and shelf waters than in slope waters, likely due to greater degradation and adsorption of PAEs in the bays and shelf waters. In contrast, the steeper slope of the slope stations may have been the result of less adsorption and degradation. A positive correlation was found between water column depth and PAE concentration ($r^2 = 0.678$, slope = 4.29, $n = 13$) (Fig. S1a), suggesting lower removal by degradation and adsorption in deep waters. The positive correlation between the abundance of PDs and the concentration of PAEs suggests that in situ leaching accounts for a large fraction of the dissolved PAE pool. The calculated ERL due to PAEs and PDs for Sharm Obhur and the Red Sea is currently at a low to moderate level.

5.2 Future perspectives and limitations

Based on the obtained results from the current study, the following outlook and perspectives can be outlined:

- i. The current study investigates the leaching process of phthalate esters from the three common consumer films (LDPE, HDPE and RP). However, limited data on the leaching process are available for many other polymers (PVC, PP, PC, etc.) and plastic products (toys, nylon ropes, etc.) that are strongly represented in as part of marine debris, and need to be studied.
- ii. The study of chemical additives used in plastics needs to be extended to polybrominated diphenyl ethers (PBDEs), bisphenol A (BPA), nonylphenols (NPs) and brominated flame retardants (BFRs) and polychlorinated biphenyls (PCBs), are the most commonly observed chemical additives in the marine environment. However, in the current study, we only focused on the leaching process of PAEs. So, it is important to conduct the studies with other additives.
- iii. The current showed a clear correlation between the PAEs and PDs, further studies on the effects of PDs on the transport of PAEs in the Red Sea need to be conducted. The correlation study needs to examine different types of samples, such as PAEs in soils, marine biota, plants, algae, etc. This could help to improve the baseline data for source-to-sink study of pollutants in the Red Sea. Comprehensive modeling of the relationships between PAE pollutants and PDs in the marine environment needs to be developed.

v. Proper understanding of the transport dynamics of PAEs and PDs in a heavily built-up estuary of the Red Sea is of prime importance to be assigned. Data on many other chemical concentrations in and on marine plastic debris are needed to evaluate the effects of the matrix on the relationship between PAEs and PDs. This study will help our understanding of the factors affecting PAEs and can serve as proper indicators for predicting the risk of plastic debris pollution to marine biota.

vi. The overall goals of the current study was to achieve higher limits of quantification in LLE and SPE extraction, but this study also needs to be extended to other extraction and quantification methods to address the contamination of many laboratory materials with PAEs. Thus, standard protocols for plastic-associated chemicals are needed, from sampling to quality control procedures.

vii. The use of certified reference materials for different chemical groups and polymers, as well as a laboratory inter-comparison program is of great importance to help in validation of the established analytical methods and improve data quality. It is not easy to collect sufficient quantities of plastic waste for on-site chemical analyses, especially when they are getting smaller, resulting in high detection limits and low detection frequencies of target analytes.

Another potential limitation of the method used to calculate the total leaching is in the leaching concentration. PAEs that showed low leaching ($< 5 \text{ ng/cm}^2$), the high spike level (23.5 ng/cm^2) means that the adsorbed and dissolved fractions can be a small difference between large numbers, potentially resulting in high uncertainty on the resultant ratio. The uncertainty was relatively higher (25-32%) for the lower leaching concentration ($< 5 \text{ ng/cm}^2$). The higher uncertainty values for the limited leaching compounds indicate that the estimated partition coefficients for these compounds should be considered with some caution. Therefore, to overcome these practical difficulties, sensitive multi-residue analytical methods that are cost and time effective are needed.

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Statement of Declaration

I, Jeyakumar dhavamani, born on 23rd November 1985 in India, hereby declare that I have written this Ph.D. thesis independently, under compliance of the rules for good scientific practice of the German Research Foundation. I declare that I used only the sources, the data and the support that I have clearly mentioned. Moreover, I assure that this Ph.D. thesis has not been submitted for the conferral of a degree elsewhere, neither in part nor as a whole, and that none of my academic degrees has ever been withdrawn. Published manuscripts are identified at the relevant places.

Signature

Jeyakumar Dhavamani

Kiel, Date: