A journey into microplastic analysis using FTIR spectroscopy

Vianello, Alvise

Publication date:
2020

Document Version
Publisher's PDF, also known as Version of record

A JOURNEY INTO MICROPLASTIC ANALYSIS USING FTIR SPECTROSCOPY

BY
ALVISE VIANELLO

DISSERTATION SUBMITTED 2020

AALBORG UNIVERSITY
DENMARK

A JOURNEY INTO MICROPLASTIC ANALYSIS USING FTIR SPECTROSCOPY

by

Alvise Vianello

AALBORG UNIVERSITY
DENMARK

Dissertation submitted in 2020

.

Dissertation submitted: February 2020

PhD supervisor: Professor Jes Vollertsen
Aalborg University

PhD committee: Associate Professor Jesper Ellerbæk Nielsen (chairman)
Aalborg University

Senior Research Scientist Alessio Gomiero
Norwegian Research Centre AS

Senior Research Scientist Yvonne Shashoua
National Museum of Denmark

PhD Series: Faculty of Engineering and Science, Aalborg University

Department: Department of Civil Engineering

ISSN (online): 2446-1636
ISBN (online): 978-87-7210-602-1

Published by:
Aalborg University Press
Langagervej 2
DK – 9220 Aalborg Ø
Phone: +45 99407140
aauf@forlag.aau.dk
forlag.aau.dk

Printed in Denmark by Rosendahls, 2020

CV (EXTRACT)

RELEVANT WORK EXPERIENCE

2016 – Ongoing	Postdoc at Department of the Built Environment, Aalborg University, Denmark
2010 – 2016	Research assistant at the Institute for the Dynamics of Environmental Processes, Italian National Research Council (IDPA – CNR), Italy
2006 – 2007	Coordinated Collaboration Contract (CoCoCo), Faculty of Chemistry, University Ca' Foscari of Venice, Italy

EDUCATION

2011 – 2020	PhD of Environmental Engineering at Department of the Built Environment, Aalborg University, Denmark
2006 – 2009	Master of Chemical Sciences for Conservation and Restoration of Cultural Heritage (Chemistry), University Ca' Foscari of Venice, Italy
2002 – 2006	Bachelor of Chemical Sciences and Technologies for Conservation and Restoration of Cultural Heritage (Chemistry), University Ca' Foscari of Venice, Italy

KNOWLEDGE DISSEMINATION AT CONFERENCES

SETAC Europe 29th Annual Meeting, 2018

- Oral presentation of part of the PhD project (Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin)

- Poster presentation on research activities relevant in the topic of the PhD (First evidence of microplastics in the sediments of the Limfjord: hydrodynamic modelling-based sampling combined with FPA-µFTIR-Imaging automated analysis)

Micro 2018

- Oral presentation on research activities relevant in the topic of the PhD (Shipyards: a source of microplastic and micro-paint particles spreading to the marine environment)

- Oral presentation on research activities relevant in the topic of the PhD (Combining hydrodynamic modelling-based sampling with FPA-µFTIR-Imaging automated analysis: first evidence of microplastic pollution in the sediments of the Limfjord (Denmark))

SETAC Europe 28th Annual Meeting, 2018

- Poster presentation of part of the PhD project (Microplastic Indoor air pollution using a simulated breathing manikin - µFTIR Imaging quantification)
- Poster presentation on research activities relevant in the topic of the PhD (Detection of micro-paint particles and microplastics in harbour soil samples using FPA-µFTIR Imaging quantification)

IWA Sweeden Microplastic Conference, 2017

- Oral presentation on research activities relevant in the topic of the PhD (Analysis with FTIR and Image software)

ECSA 50, Today's science for tomorrow management, 2012

- Poster presentation of part of the PhD project (Occurrence and distribution of microplastic particles in the sediments of the lagoon of Venice, Italy: preliminary results)

ENGLISH SUMMARY

The use of plastic materials increased exponentially in the past seventy years, and the extensive use of this material in a wide range of applications, combined with the scarce degradability of polymers and the lack of strong policies on plastic recycling, led to the accumulation of plastic debris in the environment. Plastic pollution is nowadays a global threat to the environment, the wildlife, and ultimately to humans. Synthetic polymers are relatively inert materials, but abandoned plastic items are subjected to degradation, slowly becoming brittle and easily fractured by physical processes, until they turn into countless tiny microscopic pieces, defined as microplastics (MP). These microscopic particles are not only the result of degradation or larger items, but they can also be purposely manufactured for specific uses. Due to a combination of physical and chemical features, like size, colour, and content of toxic substances, as well as their presence in all environmental compartments, microplastics are also impacting the wildlife food chain, posing a serious risk related to potential contamination of human food. Water and beverages also seem to be potentially prone to microplastic contamination, contributing to increasing the risk of human exposure to this pollutant. Moreover, the health risk related to microplastic is not limited to an indirect human exposure through food, water, and beverages, but there is evidence of airborne synthetic particles polluting both outdoor and indoor environments, and potentially threatening humans by direct exposure.

The present awareness of microplastic pollution is based on a large number of scientific studies conducted and published in the last fifteen years, and only recently also amplified by media. It has been a gradual process, and it started from isolated studies carried out by researchers who were typically working in other fields of science, but who recognised the potential incoming threat.

The complexity and interdisciplinary of this emerging topic poses several challenges, here amongst related to the analytical methods used. Microplastic analysis is still in its early stages, and there is a lack of standardised and harmonised protocols, making the published studies hard to compare. Also, data on microplastic spatial and temporal distribution in different environments is fragmentary, and monitoring activities are of paramount importance to improve knowledge for further risk assessment. Furthermore, the analytical workflow and methodology strongly influence the outcome of a scientific study. Every single analytical step influences the others, and all of them have to be carefully designed to correctly asses the magnitude of an emerging pollutant like microplastics.

This PhD study provides scientific knowledge on analytical methods using FTIR spectroscopic techniques for MP analysis in different environmental matrices. At the same time, it also complements the knowledge on microplastic distribution in selected environments, like coastal waters and sediments, contributing to novel data on global

microplastic pollution. Moreover, the PhD study investigates another aspect of microplastic pollution, namely the occurrence of this emerging pollutant in indoor environments, and provides the first evidence of potential human direct exposure to airborne microplastics.

Microplastic pollution is predicted to impact the coastal areas globally, especially in highly populated and enclosed waters. That is due to a combination of heavy anthropogenic activities and a high amount of plastic waste produced and mismanaged, which together contribute to the spread of microplastics from land to sea. On the one hand, the Mediterranean coastal waters are suspected to be an important accumulation zone for plastic and microplastic debris, but the field data available are, even if increasing, still fragmentary. On the other hand, some remote areas (minor islands spread across the Mediterranean basin) are still perceived as virgin environments, mainly because of their distance from land-based sources. To fill the knowledge gaps on the presence of microplastic in coastal and transitional environments, providing at the same time new data from potentially "clean" areas, this PhD study investigated some specific areas along the Italian coast, namely the Lagoon of Venice, the coastal waters of the North Adriatic Sea, and open waters in proximity to some smaller Italian islands.

The microplastic contamination of the sediments of the Lagoon of Venice was assessed for the first time in 2012, choosing sites characterised by specific hydro-dynamic features (freshwater and seawater inputs), and differently impacted by anthropogenic activities, like urban and industrial discharge, as well as aquaculture. The collected samples were extracted from the matrix and analysed by μFTIR-chemical mapping. The sediments of the lagoon were extensively polluted by microplastics, with concentrations ranging from 672 to 2175 MP kg^{-1}. Higher concentrations were observed at landward sites related to lower hydrodynamics, where other micropollutants typically tend to accumulate. The results also highlighted a correlation with the sediment grain size, suggesting similar sinking mechanisms to the finer sediment particles. This study was one of the first to apply a novel spectroscopic FTIR approach for microplastic analysis in environmental samples based on FTIR-chemical mapping, avoiding the pre-sorting of material (a potential source of bias in the results).

The spatial and short-term temporal occurrence of microplastics in the coastal waters of the North Adriatic Sea was investigated for the first time in 2014, collecting samples (manta trawl sampling) from two transects, the first one outside the Lagoon of Venice, and the second one close to the Po river delta. Microplastics were isolated, quantified by stereo-microscopy and further identified using ATR-μFTIR spectroscopy, also investigating the degradation of the polymers by their FTIR spectra. All stations were affected by microplastic pollution, with the highest concentration measured in March (10.4 MP m^{-2} – Pellestrina; 4.3 MP m^{-2}). A high spatial and temporal variability was observed within the results; several factors, like

surface circulation in the basin and river discharge, both driven by specific weather conditions on a short time scale, possibly influenced specific accumulation patterns. The occurrence and distribution of microplastics were also determined at six "clean" sites located in the vicinity of minor islands and archipelagos (Tremiti Islands, Aeolian Islands, Ischia, Ventotene, Island of Elba, and Asinara), and compared with two polluted areas close to two of the largest Italian rivers (Po and Tiber). The samples were collected both with manta trawl (surface water), and WP2 FAO net (water column – river mouths excluded). Potential microplastics were extracted and quantified by stereo-microscopy and ATR-μFTIR, and samples were also analysed for several persistent organic pollutants (POPs) not directly linked to microplastics. The results revealed the ubiquity of microplastic pollution also at commonly perceived "clean" areas, with an average concentration of 0.3 MP m^{-3}, even though the contamination at the islands was significantly lower than the one measured close to the rivers. POPs analysis showed a higher concentration of PCBs in most of the surface water samples, but no other specific trends between MP and POPs concentrations were noticed. Overall, the uniformity of the results suggested that the hydrodynamics at the surroundings of these islands contribute to constantly mixing the waters, and possibly limiting the microplastic accumulation in these specific areas.

Human exposure to microplastic pollution is becoming an emerging topic due to the potential health implication connected to the interaction between microplastic particles and the organism. Besides the indirect exposure through food and beverages, recently documented by scientific literature, the potential direct exposure through inhalation and breathing has raised concern due to recent findings related to airborne microplastics in outdoor and indoor environments. However, the knowledge on this specific topic is still extremely limited. To provide new insight on airborne microplastic pollution, and evidence of potential human exposure to this pollutant, this PhD study investigated how a human simulacrum was exposed to microplastics present in indoor air.

The human exposure was assessed collecting samples from three apartments using a Breathing Thermal Manikin. It simulated the presence of a person thanks to specific features, such as internal heating generating a boundary layer flow (influencing the transport of particulates across the surface of the body), and simulated breathing through an artificial lung system (pump), powered according to a specific metabolic rate. The collected samples were minimally treated to extract the particulates and analysed using FPA-μFTIR-Imaging spectroscopy, in combination with automated analysis of the data. Microplastics were ubiquitous and mainly composed of polyester fragments and fibre (81% of synthetic items), and their concentration ranged between 1.7 and 16.2 MP m^{-3} accounting for 4% of the detected particles, a non-negligible percentage. Among nonsynthetic particles, protein-based material (mainly shed skin) accounted for 91% of the items, while cellulose was in the same range of microplastics (4%). This study provided valid proof of concept of human exposure to airborne microplastic, also highlighting that inhalable microplastics were generally smaller

than nonsynthetic items. These synthetic particles, even though mainly removed by clearance mechanisms in the upper airways (due to their size), could still pose a threat to human health.

DANSK RESUME

Brugen af plastmaterialer er steget eksponentielt i de sidste halvfjerds år, og den omfattende brug af dette materiale i en lang række anvendelser, kombineret med den lave nedbrydelighed af polymerer og en mangel på stærke initiativer for genanvendelse af plast, har ført til akkumulering af plastaffald i miljøet. Plastforurening er i dag en global trussel mod miljøet, dyrelivet og i sidste ende for mennesker. Syntetiske polymerer er relativt inerte materialer, men alle plastgenstande udsættes for nedbrydning og bliver langsomt sprøde og brudt ved fysiske belastning, indtil de bliver til utallige små mikroskopiske stykker, defineret som mikroplast (MP). Disse mikroskopiske partikler er ikke kun resultatet af nedbrydning af større genstande, men nogle bliver fremstillet med vilje til specifikke anvendelser. På grund af det store forbrug af plast og hermed en stor forekomst af mikroplast i miljøet, udgør mikroplast en alvorlig forureningsrisiko, der kan påvirke fødekæden og forurene de madvarer mennesker indtager. Vand og drikkevarer synes også at være udsat for mikroplast kontaminering, hvilket bidrager til at øge risikoen for menneskelig eksponering for dette forurenende stof. Derudover er sundhedsrisikoen i forbindelse med mikroplast ikke begrænset til en indirekte human eksponering gennem mad, vand og drikkevarer, men der er tegn på, at luftbårne syntetiske partikler forurener både udendørs og indendørs miljøer og potentielt truer mennesker ved direkte eksponering.

Den nuværende bevidsthed om mikroplastforurening er baseret på et stort antal videnskabelige undersøgelser, der er udført og offentliggjort i de sidste femten år, og for nylig også taget op af medierne. Det har været en gradvis proces, der startede med isolerede undersøgelser udført af forskere, der typisk arbejdede inden for andre videnskabelige områder, men som så den potentielle trussel.

Kompleksiteten og tværfagligheden i dette nye forskningsområde giver flere udfordringer, her iblandt relateret til de anvendte analysemetoder. Mikroplast analyse er stadig i sin vorden, og der er mangel på standardiserede og harmoniserede protokoller, hvilket gør de offentliggjorte undersøgelser vanskelige at sammenligne. Data om mikroplasts rumlig og tidsmæssig fordeling i forskellige miljøer er også fragmentariske, og moniteringsaktiviteter er af største vigtighed for at øge viden og for at kunne udføre bedre risikovurdering. Desuden påvirker den analytiske arbejdsgang og metodologi resultatet af en videnskabelig undersøgelse ganske væsentligt. Hvert enkelt analytiske trin påvirker de andre, og alle skal omhyggeligt udformes for korrekt at kunne vurdere forekomsten af mikroplast i naturen.

Dette ph.d.-projekt giver videnskabelig viden om analysemetoder basseret på FTIR-spektroskopiske teknikker til mikroplastanalyse i forskellige miljømatricer. Samtidig supplerer det også viden om mikroplastfordeling i udvalgte miljøer, som kystvande og sedimenter, og bidrager til nye data om global mikroplastforurening. Desuden undersøger ph.d.-studiet et yderligere aspekt af mikroplastforurening, idet det

vurderer forekomsten af dette forurenende stof i indendørs miljøer og giver det første bevis for mulig direkte human eksponering for indendørs luftbåren mikroplast.

Mikroplast forurening forventes at påvirke kystområderne globalt, især i stærkt befolkede og lukkede farvande. Dette skyldes en kombination af menneskeskabte aktiviteter og en stor mængde plastaffald produceret og i kombination med utilstrækkelig affaldshåndtering, hvilket sammen bidrager til spredning af mikroplast fra land til hav. På den ene side mistænkes Middelhavets kystfarvande for at være en vigtig akkumuleringszone for makro- og mikroplast, men de disponible feltdata er stadig fragmentariske. På den anden side opfattes nogle fjerntliggende områder (mindre øer spredt ud over middelhavsbassinet) stadig som jomfruelige miljøer, hovedsageligt på grund af deres afstand til landbaserede kilder. For at udfylde videnhuller om tilstedeværelsen af mikroplast i kyst- og overgangsmiljøer og samtidig give nye data fra potentielt "rene" områder, undersøgte dette ph.d.-studie nogle specifikke områder langs den italienske kyst, nemlig lagunen i Venedig, kystnære farvande i det nordlige Adriaterhav og åbne farvande i nærheden af nogle mindre italienske øer.

Mikroplast kontaminering af sedimenterne i lagunen i Venedig blev for første gang vurderet i 2012 ved at vælge steder, der er kendetegnet ved specifikke hydrodynamiske forhold (ferskvand og havvand input) og forskelligt påvirket af menneskeskabte aktiviteter, såsom by- og industriudledning, samt akvakultur. De indsamlede prøver blev ekstraheret fra matricen og analyseret ved µFTIR-kemisk kortlægning. Lagunernes sedimenter var i vid udstrækning forurenet med mikroplast med koncentrationer mellem 672 og 2175 MP kg^{-1}. Højere koncentrationer blev observeret på steder med lavere hydrodynamik, hvor andre mikroforureninger typisk også har en tendens til at ophobes. Resultaterne fremhævede også en sammenhæng med sedimentets kornstørrelse, hvilket tyder på lignende bundfældningsmekanismer som de finere sedimentpartikler. Denne undersøgelse var en af de første, der anvendte en ny spektroskopisk FTIR-metode til mikroplast analyse i miljøprøver baseret på FTIR-kemisk kortlægning, hvor man undgik præ-sortering af materiale (en potentiel kilde til systematisk fejl i resultaterne).

Den rumlige og tidsmæssige forekomst af mikroplast i kystfarvandet i det nordlige Adriaterhav blev for første gang undersøgt, idet man indsamlede prøver (manta trawl-prøveudtagning) i marts og april 2014 fra to transekter fra lagunen i Venedig og tæt på Po-floddeltaet. Mikroplast blev isoleret, kvantificeret ved stereomikroskopi og yderligere identificeret under anvendelse af ATR-µFTIR-spektroskopi, hvor også nedbrydningen af polymererne kunne vurderes via deres FTIR-spektre. Alle stationer var påvirket af mikroplastforurening med den højeste koncentration målt i marts (10.4 MP m^{-2} - Pellestrina; 4.3 MP m^{-2}). En høj rumlig og tidsmæssig variabilitet blev observeret for resultaterne; flere faktorer, såsom overfladecirkulation i bassinet og udledning af ferskvand fra floder, begge drevet af specifikke vejrforhold på en kort tidsskala, påvirkede muligvis specifikke akkumuleringsmønstre. Forekomsten og

distributionen af mikroplast blev også målt på seks "rene" steder beliggende i nærheden af mindre øer og øgrupper (Tremiti-øer, De æoliske øer, Ischia, Ventotene, øen Elba og Asinara) og sammenlignet med to forurenede områder tæt på to af de største italienske floder (Po og Tiber). Prøverne blev opsamlet både med manta trawl (overfladevand) og WP2 FAO-net (vandsøjle). Potentiel mikroplast blev ekstraheret og kvantificeret ved stereomikroskopi og ATR-uFTIR. Prøver blev endvidere analyseret for adskillige persistente organiske forurenende stoffer (POP'er), der ikke var direkte knyttet til mikroplast. Resultaterne afslørede forekomsten af mikroplastforurening også i områder der almindeligvis opfattes som "rene" med en gennemsnitlig koncentration på 0.3 MP m^{-3}, selvom forureningen ved øerne var markant lavere end den, der blev målt tæt på floderne. POPs-analyse viste en højere koncentration af PCB i de fleste af overfladevandsprøverne, men ingen andre specifikke tendenser mellem MP- og POP-koncentrationer blev bemærket. Generelt antydede ensartetheden af resultaterne, at hydrodynamikken i øernes omgivelser bidrager til konstant at blande vandene og muligvis begrænse mikroplast akkumulering i disse specifikke områder.

Menneskelig eksponering for mikroplast forurening er ved at blive et stor emne på grund af den potentielle sundhedsmæssige implikation forbundet med samspillet mellem mikroplast partikler og den menneskelige organisme. Udover den indirekte eksponering gennem mad og drikkevarer, der for nylig er dokumenteret i videnskabelig litteratur, har den potentielle direkte eksponering gennem indånding og vejrtrækning rejst bekymring på grund af nylige fund, der er relateret til luftbåren mikroplast i udendørs og indendørs miljøer. Viden om dette specifikke emne er dog stadig ekstremt begrænset. For at give ny indsigt i luftbåren mikroplastforurening og bevis for potentiel menneskelig eksponering for dette forurenende stof, undersøgte dette ph.d.-studie, hvordan et humant simulacrum blev udsat for mikroplastik til stede i indeluft.

Den menneskelige eksponering blev vurderet ved at indsamle prøver fra tre lejligheder ved hjælp af en Manikin, der simulerede indånding. Manikinen simulerede tilstedeværelsen af en persons specifikke funktioner, såsom intern opvarmning, der genererer en grænselagstrøm (påvirker transporten af partikler over kroppens overflade), og simulerede vejrtrækning gennem et kunstigt lungesystem (pumpe), drevet i henhold til en specifik metabolisk rate. De opsamlede prøver blev minimalt behandlet for at ekstrahere partiklerne og analyseret under anvendelse af FPA-µFTIR-spektroskopi i kombination med automatiseret analyse af dataene. Mikroplast var allestedsnærværende og bestod hovedsageligt af polyesterfragmenter og -fibre (81% af alle de syntetiske partikler), og deres koncentration varierede mellem 1.7 og 16.2 MP m^{-3} svarende til 4% af de detekterede partikler, en ikke-ubetydelig procentdel. Blandt ikke-syntetiske partikler tegnede proteinbaseret materiale (hovedsageligt død hud) sig for 91% af partiklerne, medens cellulose var tilstede i samme omfang som mikroplast (4%). Denne undersøgelse leverede bevis for menneskelig eksponering for luftbåren mikroplast, hvilket også fremhævede, at indhalerbar mikroplast partikler

generelt var mindre end ikke-syntetiske partikler. Selvom mange af disse syntetiske partikler vil blive fjernet fra de øvre luftveje ved fx hoste, kan de, på grund af deres størrelse, formentlig stadig udgøre en trussel mod menneskers sundhed.

ABSTRACT ITALIANO

L’utilizzo della plastica è aumentato in modo esponenziale negli ultimi settant'anni, e l'applicazione estensiva di questo materiale in una vasta gamma di applicazioni, combinato con la scarsa degradabilità dei polimeri e la mancanza di efficienti politiche sul riciclaggio della plastica, ha portato all'accumulo di rifiuti sintetici nell'ambiente. Oggi l'inquinamento da plastica è una minaccia globale per l'ambiente, la fauna selvatica e, in definitiva, per l'uomo. I polimeri sintetici sono materiali relativamente inerti, ma gli oggetti in plastica abbandonati sono soggetti a degradazione, diventando gradualmente fragili e si frammentandosi a causa di processi chimico-fisici, fino a trasformarsi in innumerevoli microscopici frammenti, definiti microplastiche (MP). Queste particelle microscopiche non sono solo il risultato del degrado o di oggetti più grandi, ma possono anche essere prodotte appositamente per usi specifici. A causa di una combinazione di caratteristiche, come le dimensioni, il colore e la grande disponibilità, le microplastiche stanno inquinando anche la catena alimentare della fauna selvatica, comportando un grave rischio legato alla potenziale contaminazione delle risorse alimentari destinate alla dieta umana. Anche l'acqua e le bevande sembrano potenzialmente soggette a contaminazione da microplastiche, contribuendo ad aumentare il rischio di esposizione umana a questo inquinante. Inoltre, il rischio per la salute correlato alle microplastiche non si limita a un'esposizione umana indiretta attraverso cibo, acqua e bevande, ma ci sono prove dell’esistenza di particelle sintetiche trasportate dall'aria che contaminano sia gli ambienti esterni che quelli interni, e potenzialmente minacciano l'uomo a causa di fenomeni di esposizione diretta.

L'attuale consapevolezza sull'inquinamento da microplastiche si basa su una serie di studi scientifici condotti e pubblicati negli ultimi quindici anni, e solo recentemente amplificati anche dai media. È stato un processo graduale ed è partito da studi isolati condotti da ricercatori che, in genere, lavoravano in altri rami scientifici, ma che hanno riconosciuto la potenziale minaccia in arrivo.

La complessità e l'interdisciplinarietà di questo nuovo campo di ricerca pone diverse sfide, principalmente legate ai metodi analitici utilizzati. L'analisi delle microplastiche è ancora nelle sue fasi iniziali e mancano protocolli standardizzati e armonizzati, il che rende difficile confrontare gli studi pubblicati. Inoltre, i dati sulla distribuzione spaziale e temporale delle microplastiche in diversi ambienti sono frammentari e le attività di monitoraggio sono di fondamentale importanza per migliorare le conoscenze e per poter quindi fare un'ulteriore valutazione del rischio. Inoltre, il flusso di lavoro analitico e la metodologia influenzano fortemente il risultato di uno studio scientifico. Ogni singolo passaggio analitico influenza gli altri e tutti devono essere attentamente progettati per valutare correttamente la magnitudo di un inquinante emergente come le microplastiche.

Questo studio di dottorato fornisce conoscenze scientifiche su metodi analitici che utilizzano tecniche spettroscopiche FTIR per l'analisi delle microplastiche in diverse matrici ambientali. Allo stesso tempo, integra anche le conoscenze sulla distribuzione della microplastica in ambienti selezionati, come le acque costiere e i sedimenti, contribuendo a fornire nuovi dati sull'inquinamento globale da microplastiche. Inoltre, lo studio di dottorato indaga un altro aspetto dell'inquinamento da microplastica, valutando il la presenza di questo inquinante emergente negli ambienti interni e fornendo le prime prove della potenziale esposizione umana diretta a microplastiche in sospensione nell'aria.

Si prevede che l'inquinamento da microplastiche abbia un impatto sulle aree costiere a livello globale, specialmente in acque molto popolate e con poco ricambio (chiuse). Ciò è dovuto a una combinazione di elevate attività antropogeniche e una grande quantità di rifiuti di plastica prodotti e mal gestiti, che contribuiscono sinergicamente alla diffusione delle microplastiche dalle aree continentali a quelle costiere e conseguentemente al mare aperto. Da un lato si sospetta che le acque costiere del Mediterraneo costituiscano un'importante zona di accumulo per i detriti di plastica e microplastica, ma i dati speriemtentali sono, anche se in aumento, ancora frammentari. D'altra parte, alcune aree remote (isole minori sparse nel bacino) sono ancora percepite come ambienti vergini, principalmente a causa della loro distanza dalle sorgenti terrestri. Allo scopo di colmare i vuoti di conoscenza sulla presenza di microplastiche negli ambienti costieri e di transizione, fornendo allo stesso tempo nuovi dati provenienti da aree potenzialmente “pulite”, questo dottorato ha studiato alcune aree specifiche lungo la costa italiana, nello specifico la Laguna di Venezia, le acque costiere del Mare Adriatico settentrionale e le acque in prossimità di alcune isole italiane più piccole.

La contaminazione da microplastiche nei sedimenti della Laguna di Venezia è stata determinata per la prima volta nel 2012, scegliendo siti caratterizzati da specifiche caratteristiche idro-dinamiche (input di acqua dolce e di mare) e diversamente influenzati da attività antropogeniche, come scarichi urbani e industriali, ma anche da attivitá di acquacoltura. I campioni raccolti sono stati estratti dalla matrice ambientale ed analizzati mediante μFTIR-chemical mapping. I sedimenti della laguna sono risultati ampiamente inquinati da microplastiche, con concentrazioni comprese tra 672 e 2175 MP kg^{-1}. Concentrazioni più elevate sono state osservate in siti interni alla laguna, caratterizzati da parametri idrodinamici con valori inferiori, dove anche altri micro-inquinanti tendono generalmente ad accumularsi. I risultati hanno anche messo in evidenza una correlazione con la granulometria dei sedimenti, suggerendo per le plastiche meccanismi di affondamento simili alle particelle di sedimento più fini. Questo studio è stato uno dei primi ad applicare un nuovo approccio spettroscopico FTIR per l'analisi delle microplastiche basatio su FTIR-chemical mapping in campioni ambientali, evitando la pre-selezione delle particelle da analizzare (approccio che introduce una potenziale fonte di errore nell’analisi).

La distribuzione spaziale e temporale a breve termine di microplastiche nelle acque costiere del Mare Adriatico settentrionale è stata studiata per la prima volta nel 2014, raccogliendo campioni (campionamento con manta trawl) da due transetti, il primo all'esterno dalla Laguna di Venezia e il secondo vicino al delta del fiume Po. Le microplastiche sono state isolate, quantificate mediante stereo-microscopia e ulteriormente identificate utilizzando la spettroscopia ATR-μFTIR, studiando anche la degradazione dei polimeri utilizzandone gli spettri FTIR. Tutte i siti indagati sono risultati contaminati da inquinamento da microplastiche, con la massima concentrazione misurata a marzo (10.4 MP m^{-2} - Pellestrina; 4.3 MP m^{-2}). I risultati hanno evidenziato un'elevata variabilitá spaziale e temporale; diversi fattori, come la circolazione superficiale nel bacino e l'input fluviale, entrambi influenzati da condizioni meteorologiche specifiche su una scala temporale breve, hanno probabilmente determinato specifici modelli di accumulo. La presenza e la distribuzione di microplastiche sono state misurate anche in sei siti "incontaminati" situati in prossimità di isole minori e arcipelaghi (Isole Tremiti, Isole Eolie, Ischia, Ventotene, Isola d'Elba e Asinara) ed i risultati sono stati confrontati con due aree inquinate vicine a due dei più grandi fiumi italiani (Po e Tevere). I campioni sono stati raccolti sia con manta trawl (acque superficiali) che con retino WP2 FAO (colonna d'acqua - foci dei fiumi escluse). Le potenziali microplastiche sono state estratte e quantificate mediante stereo-microscopia e ATR-μFTIR, e i campioni sono stati analizzati anche per determinare il livello di diversi inquinanti organici persistenti (POPs) non direttamente collegati alle microplastiche. I risultati hanno rivelato l'ubiquità dell'inquinamento da microplastiche anche nelle aree comunemente percepite come "incontaminate", con una concentrazione media di 0.3 MP m^{-3}, anche se la contaminazione nelle isole è risultata significativamente inferiore a quella misurata vicino ai fiumi. L'analisi dei POPs ha evidenziato una concentrazione piú elevata di PCB nella maggior parte dei campioni di acque superficiali, ma non sono state osservate altre relazioni specifiche tra le concentrazioni di microplastiche e POPs. Nel complesso, l'uniformità dei risultati ha suggerito che l'idrodinamica nei dintorni di queste isole contribuisce a mescolare costantemente le acque, e probabilmente a limitare l'accumulo di microplastiche in queste specifiche aree.

L'esposizione umana all'inquinamento da microplastiche sta diventando un argomento emergente a causa delle potenziali implicazioni per la salute connesse all'interazione tra particelle sintetiche e organismo. Oltre all'esposizione indiretta attraverso alimenti e bevande, recentemente documentata dalla letteratura scientifica, la potenziale esposizione diretta per inalazione e respirazione ha destato preoccupazione a causa dei recenti risultati relativi alla presenza di microplastiche nell'aria di ambienti esterni e interni. Tuttavia le conoscenze su questo argomento specifico sono ancora estremamente limitate. Per fornire nuove informazioni sull'inquinamento da microplastiche nell'aria e prove della potenziale esposizione umana a questo inquinante, questo studio di dottorato ha indagato la potenzial espozione umana a microplastiche nell'aria di ambienti indoor con uno speciale manichino.

Il livello di espozione è stato valutato raccogliendo campioni da tre appartamenti usando un manichino termico respiratorio (Breathing Thermal Manikin). L'apparato ha simulato la presenza di una persona grazie a caratteristiche specifiche, come il riscaldamento interno che genera un flusso di strato limite (il quale influenza il trasporto di particelle attraverso la superficie del corpo) e simula la respirazione attraverso un sistema polmonare artificiale (una doppia pompa), alimentato secondo un tasso metabolico specifico. I campioni raccolti sono stati trattati minimamente per estrarre il particolato e analizzati usando spettroscopia FPA-μFTIR-Imaging, in combinazione con l'analisi automatizzata dei dati. Le microplastiche erano onnipresenti e composte principalmente da frammenti e fibre di poliestere (81% di articoli sintetici) e la loro concentrazione variava tra 1.7 e 16.2 MP m^{-3}, rappresentando il 4% delle particelle rilevate (una percentuale non trascurabile). Tra le particelle non sintetiche, i materiale a base proteica (principalmente pelle secca) rappresentavano il 91% delle particelle, mentre la percentuale di cellulosa era nella stesso range delle microplastiche (4%). Questo studio ha fornito prove valide del concetto di esposizione umana alla microplastica presente nell'aria, evidenziando anche che le microplastiche inalabili erano generalmente più piccole rispetto alle particelle non sintetiche. Le microplastiche, sebbene rimosse principalmente dai meccanismi di eliminazione delle vie aeree superiori (a causa delle loro dimensioni), potrebbero comunque costituire una minaccia per la salute umana.

PREFACE

This thesis has been submitted for assessment in partial fulfilment of the PhD degree. As part of the assessment, co-author statements have been made available to the assessment committee and are also available at the Faculty.

This PhD study covers an extended time frame, from 2011 to 2019, and it contains studies which were published from 2013 to 2019 and were conducted in both Italy and Denmark. The title "A journey into microplastic analysis using FTIR spectroscopy" stresses the main meaning of the study, viewing it as a long-range tour across different aspects of microplastic science, a complex and interdisciplinary research field. In fact, this PhD study shows how FTIR spectroscopic techniques have become more and more important in microplastic analysis over time, a reliable tool to enhance the analytical potential available to the scientific community. This evolution is clearly noticeable, especially by comparing the analytical approaches of **Paper I** and **Paper IV**, as both rely on chemical mapping by imaging-based FTIR technologies. Besides, this PhD study provides scientific knowledge on different sampling methodologies and sampling processing techniques used for specific environmental matrices. This PhD study also contributes to filling knowledge gaps on occurrence and distribution of microplastic in the Mediterranean area, which is an important region due to its specific geographical and oceanographic features, as well as the related anthropogenic pressure. Finally, this PhD study explores new insights into microplastic research, providing novel scientific knowledge on indoor airborne microplastic pollution, and related human exposure.

The thesis is based on four published scientific papers (**Paper I**, **II**, **III**, **IV**). The PhD candidate is the first author of three of the papers (**Paper I**, **II**, **IV**), and second author of **Paper III**. Contents of the papers are briefly discussed in the extended summary of the thesis.

ACKNOWLEDGEMENTS

Studying a PhD is challenging. It is a period of your life in which you learn to deal with stress because it is not much you can do otherwise…It is part of the process. If then you do a PhD as "side activity", the challenge can be even more demanding, as you have to keep your PhD stuff a bit on the side, but without diminishing its importance. I wanted to study a PhD for several years without having the chance of doing it…and it was something I always missed. But to make it happen, one has to find the right conditions, the "right chemistry" to light the inner spark. Well, here in Aalborg, I finally found the right chemistry, and that is how I have got my PhD…eventually.

First of all, I want to thank all the members of the Assessing Committee for the great job done evaluating my PhD thesis and defence with great care and competence.

When I say that need you the "right chemistry", I am talking about the people, because none of my recent achievements would have been possible without my supervisor, all my colleagues of the group, and at the department.

My deepest gratitude first and foremost goes to Professor Jes Vollertsen, my supervisor and mentor during this whole adventure. Working with him opened a new world of possibilities and opportunities for me. He always finds the way of inspiring and supporting with tireless optimism and endless energy, giving me the freedom and the space to mature and improve, while being helpful and present whenever it is needed. He is the spark I needed, and I will always be grateful for the chance he gave me and the amazing experience of these years.

I want to thank Asbjørn Nielsen, first because he was with Jes when they interviewed me, and I guess he gave his "blessing" for hiring me, but foremost because he is always helpful and incredibly resourceful, amazing me with a broad knowledge which ranges over science, and the same time with a calm and humble attitude which irradiates serenity and positive vibes.

Nothing could work out in our lab without Jytte Denker and Henrik Koch…Jytte is by far one of the best multi-talented lab technicians I ever met, combining structured organisation with reliability and expertise. Henrik a miracle worker: engineer, technician, designer. He can build whatever sort of device, no matter of the complexity of the project, seeing beyond ordinary people's imagination. However, besides their solid-rock professional expertise, Jytte and Henrik are both fantastic people, always committed and helpful, kind and funny, and always there for you. Thank you, guys!

Thanks to my colleagues because they made me feel part of a family. We had good and bad days, and we shared a whole spectrum of experiences. I want to thank Diana

for her exquisite kindness and empathy and the quality time spent together, and Nikki for sharing her vast knowledge of FTIR with me, it helped me to improve and reach new goals. Thanks to Kristina, one of the first people I met in the lab, and a trustable colleague along the way. I want to thank Márta for the quality time spent working together. I truly believe she is professional and practical, bright, pragmatic and at the same time funny, sarcastic and kind. It is an honour to work together with you. Speaking about kindness and brightness, I want to thank Fan, who shared with me countless hours of quality hard work and good vibes, always helping me when I was tired and negative, telling me the right word at the right moment…like a "little sister – cit.". Talking about working hard, I cannot forget to thank Claudia, who since joined the group shared with me quite some workload, but also quite some cool experience, always been committed, kind, and helpful. When I need to discuss some important stuff, I can always count on Inga, always inspiring me with her strong determination and extremely effective organisation, combined with funny humour and willing to chat.

I want to thank Lucian sincerely because he is one of my best friends ever, who also became one of my closest colleagues. Everybody appreciates his versatility and commitment, supported by great kindness and enjoyable humour. I am personally proud to have a friend and colleague like him on board. Thanks to Konstantinos, who recently joined us and he's already sharing the everyday workload with enthusiasm and renewed energy. I also want to thank Lasse, former master student and part of our team since then, sharing with me quite some workload, including some "paintful" but also funny moments. Another tireless hard worker with a smile on her face and the joy in her eyes is Rupa, who always remind me how nice is to work here…So thanks, Rupa for your positive vibes! Our group is expanding so fast, that we are always meeting new people! Jeanette and Fides…Our new entries, who brought us new energy to face new challenges. Thanks, girls! A special thank also to Amelia, my Danish colleague from the section who always encouraged me to try to learn Danish, and she still does it despite the results…I admire her. Another Dane who changed my life here is Rasmus, bright mind and great sarcasm, a perfect mix for a good colleague…and an even better friend! Besides my closest colleagues, I want to thanks all the people working at the department, starting with my Italian colleagues and friends, Elena, Lucia and Francesco, and continuing with Sorin, Charles, Kristopher, Thomas, Søren, Jesper, Guohan, Michael, Per, and all the colleagues of the section and the department. Special thanks to Pernille and all the secretaries, who are always helpful, kind, and funny.

And what about life outside the department…well, I have to admit I met my best friends in connection with the working environment, since my first good friend, Damian, was a former colleague of mine. I will always thank him for being a great friend and introducing me to the best group of people I could ever find here: the Aalborg Family. I thank you all my friends, without writing any name, because I know you I know I am thinking of you.

As many of you know, my studies started in Italy, where I was working at CNR. I want to thank my former colleagues Alberto Sturaro and Rocco Rella, who took great care of me for six years and taught me most of the things I know about analytical chemistry. Thanks also to Liliana and Gilberto, for sharing great moments during my CNR adventure. With them, I also want to thank Umberto Casellato for inspiring me by sharing his vast scientific knowledge and experience. I also thank my former Director Carlo Barbante and all the colleagues from IDPA-CNR.

Every story has an origin. I started to work in microplastic research thanks to Luisa Da Ros, a talented researcher, a great person and mentor to me. I owe her a lot. She helped me to believe in myself, improve, and find my way in this new community, even when the future was doubtful and unclear. Thank you, Luisa. I'll never forget what you've done for me. And thanks also to the other colleagues and from ISMAR, Alfredo, Vanessa, who shared with me most of the research done in Italy and taught me how all I know about marine sampling and marine science in general. Together with my Venetian colleagues, I also want to thank my former colleagues from IAS-CNR and ISPRA: Andrea, Mine, Stefania, Luca and Marco. You are all so friendly and funny, but also professional. And you helped me so much, keeping our collaboration and friendship active for so many years.

Back in Italy, I want to thank all my friends, who shared so many experiences with me along all these years, helped me during hard times, and supported me when I decided to move to Aalborg. Thank you guys, you helped me to raise again.

Finally, I want to thank my family, especially my mother and my father, because things would have never been the same without their constant and unwavering support. Their love for culture has always been a stimulating substrate from where I could draw new knowledge, no matter if it were an on-site geology lesson "for dummies" to keep me entertained during a family hiking, or a "tailored historical description" while visiting a "boring" ancient church. Every day has been part of a learning process on principles and values which I assimilated and used as foundations to build my future life. Thank you for being my polar star for all these years. I now think I learned how to use the compass…I guess I'll find my way.

TABLE OF CONTENTS

TABLE OF FIGURES

LIST OF SCIENTIFIC PAPERS

I. Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., Da Ros, L., 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuarine, Coastal. Estuar. Coast. Shelf Sci. 130, 54–61. https://doi.org/http://dx.doi.org/10.1016/j.ecss.2013.03.022

II. Vianello, A., Da Ros, L., Boldrin, A., Marceta, T., Moschino, V., 2018. First evaluation of floating microplastics in the Northwestern Adriatic Sea. Environ. Sci. Pollut. Res. 25, 28546–28561. https://doi.org/10.1007/s11356-018-2812-6

III. de Lucia, G., A., Vianello, A., Camedda, A., Vani, D., Tomassetti, P., Coppa, S., Palazzo, L., Amici, M., Romanelli, G., Zampetti, G., Cicero, A., Carpentieri, S., Di Vito, S., Matiddi, M., 2018. Sea Water Contamination in the Vicinity of the Italian Minor Islands Caused by Microplastic Pollution. Water 10, 1108. https://doi.org/10.3390/w10081108

IV. Vianello, A., Jensen, R.L., Liu, L., Vollertsen, J., 2019. Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Sci. Rep. 1–11. https://doi.org/10.1038/s41598-019-45054-w

OTHER SCIENTIFIC PAPERS AND REPORTS

I. Mazzocchin, G.A., Vianello, A., Minghelli, S., Rudello, D., 2010. Analysis of Roman wall paintings from the thermae of 'Iulia Concordia'. Archaeometry 52, 644–655. https://doi.org/10.1111/j.1475-4754.2009.00501.x

II. Sturaro, A., Vianello, A., Denti, P., Rella, R., 2013. Fire debris analysis and scene reconstruction. Sci. Justice 53, 201–205. https://doi.org/10.1016/j.scijus.2012.08.002

III. Rocchia, M., Ruff, I., Vianello, A., 2017. Microplastic identification and characterization by Raman imaging spectroscopy. White paper, ThermoScientific

IV. Borg Olesen, K., van Alst, N., Simon, M., Vianello, A., Vollertsen, J., Liu,

F., 2017. Analysis of Microplastics using FTIR Imaging. Identifying and quantifying microplastics in wastewater, sediment and fauna. Application note, Environmental, Agilent Technologies, pp. 6

V. Simon, M., Vianello, A., Vollertsen, J., 2019. Removal of > 10 μm microplastic particles from treated wastewater by a disc filter. Water (Switzerland) 11. https://doi.org/10.3390/w11091935

VI. Liu, F., Vianello, A., Vollertsen, J., 2019. Retention of microplastics in sediments of urban and highway stormwater retention ponds. Environ. Pollut. 255, 113335. https://doi.org/10.1016/j.envpol.2019.11333

VII. Wander, L., Vianello, A., Vollertsen, J., Westad, F., Braun, U., Paul, A., 2020. Exploratory analysis of hyperspectral FTIR data obtained from environmental microplastics samples. Anal. Methods. https://doi.org/10.1039/c9ay02483b

CHAPTER 1. INTRODUCTION

Plastic is essential for modern human life. Thanks to its broad range of properties, it is easily applicable in many fields. Since plastic made its first appearance, the world plastic production increased from 1.7 x 10^6 ton (1950) to 3.5 x 10^8 ton in 2017 (Plastics Europe, 2018). Today plastic is widely used in everyday life, providing an incredible range of features combined with low costs, which makes this material suitable for manufacturing disposable items. The extended use of these synthetic materials, combined with an irresponsible policy of waste management, has, however, lead to plastic accumulation in natural environments, which has caused plastics to be ubiquitously present in nature (Barnes et al., 2009). The combination of physical, biological and photo-chemical degradation eventually alters the plastic surface, which becomes weathered, producing a large amount of micro- and nano- fragments (Andrady, 2011; Koelmans et al., 2015; Song et al., 2018).

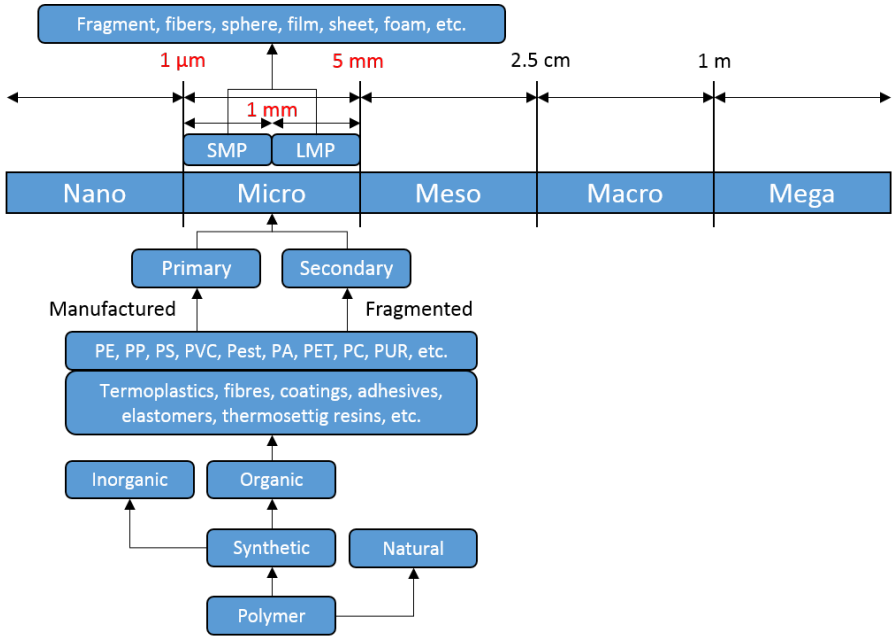

Figure 1-1. Schematic chart of microplastic definitions and classification concerning polymer type, shape, size, chemical origin, application and source (the diagram is slightly modified from Shim et al., 2018).

Although some authors pointed out the need for finding better consensus on the definition of microplastics, the fragments with size between 0.001 and 5 mm are defined microplastics (MP) (GESAMP, 2015), while plastic particles < 0.001 mm are

defined nanoplastics, and those bigger than 5 mm are considered mesoplastic (5-25 mm); even bigger fragments are defined as macroplastics and megaplastics (> 1m). A more detailed definition of microplastic discriminates between large microplastics (LMP – range 5 – 1 mm) from small microplastics (SMP – range 0.001 mm – 1 mm) (Imhof et al., 2012). Microplastics can be further divided considering their origin (GESAMP, 2015). Small and micro-sized plastics, such as those used for resin pellet pre-production, microbeads (abrasives in cosmetic, toothpaste and blasting, microsized powder for industrial coatings, drug delivery media) are called primary microplastics (Boucher et al., 2016; CONTAM, 2016), while the fragmented particles derived from any synthetic polymer, including the ones found in the environment, like solid fragments, microfibers, coatings, foams, debris from tire wear, are defined as secondary microplastics (Shim et al., 2018). Microplastics includes an almost infinite variety of polymers and copolymers, and these materials have a broad range of densities which span from 0.8 to > 2 g cm^{-3}. Therefore, they are divided into floating and non-floating MP according to the density of seawater.

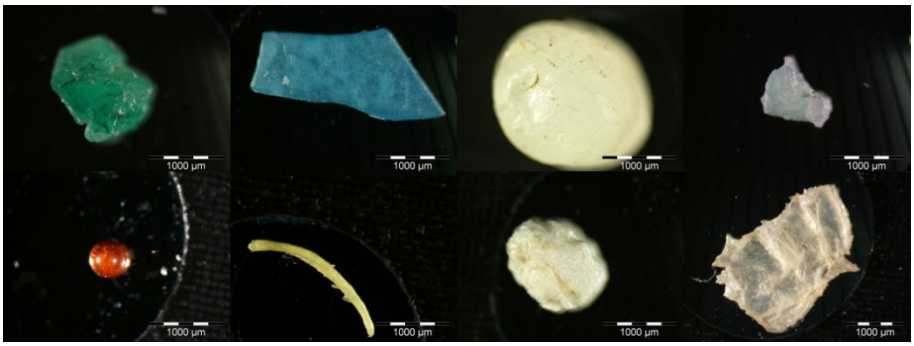

Figure 1-2. Images of microplastics of different size, shape, and polymer type. Photo-Credits: Alvise Vianello.

Due to their size, these particles can be ingested by several animal species (Lusher et al., 2015; Van Cauwenberghe et al., 2015) potentially causing negative effects on these organisms, even though the translocation, bioaccumulation and trophic accumulation are still under investigation (Farrell and Nelson, 2013). Plastic items contain a wide range of additives (softeners, flame retardants, metals, and antioxidants) to modify the polymers' features according to specific applications. Due to the type of chemical bonding involved, these substances can leach into the aquatic environment and interact with the organisms present (Ziajahromi et al., 2016), or be released after ingestion by marine organisms (Rochman et al., 2013) constituting a severe threat to the environment. As wildlife has been recognized to be affected by plastic and microplastic pollution, it is highly likely that also humans are potentially threatened by exposure to micro- and nanoplastics via consumption of contaminated seafood (Van Cauwenberghe and Janssen, 2014; Barboza et al., 2018; Cox et al., 2019). The main focus of microplastic research has hitherto been the marine

environment (Barboza and Gimenez, 2015, van Sebille et al., 2015; Villarrubia-Gómez et al., 2018), which has been identified as the ultimate endpoint of most of the lost plastic. The main sources to the marine MP pollution have, however, been recognised to be land-based (Jambeck et al., 2015). Albeit the continental contribution to the spreading of these micro-particles is still under-investigated, several potential sources have already been identified. It is worth to mention laundry machine discharges as a source of microplastics, due to the high amount of synthetic fibres released during washing cycles (Browne et al., 2011; Napper and Thompson, 2016). These fibres can also contaminate sewage sludge, which is used as an agricultural fertilizer in many countries (Zubris and Richards, 2005). Another potential MP source is recognised to come from car tires, which are suspected to contribute heavily to the MP land-based pollution (Unice et al., 2013; Lassen, 2015; Jan Kole et al., 2017). Land-based polymeric particles can be transported to aquatic environments via different pathways, like incomplete MP removal in conventional WWTPs (Carr et al., 2016; Mintenig et al., 2017; Simon et al., 2018; Simon et al., 2019), road runoff, stormwater systems (Liu et al., 2019), combined and misconnected sewer systems and so forth. MP atmospheric fallout driven by wind transportation is also suspected to contribute significantly to the spreading of MP in the environment (Dris et al., 2016; Dris et al., 2017; Gasperi et al., 2018; Klein and Fischer, 2019), and is undeniably an under-investigated area, which clearly requires further investigation.

The distribution of microplastics in time and space, as well as their distribution on parameters, such as polymer type, size, shape, in different environmental matrices, is a key factor to understand the environmental exposure level to this pollutant and to provide reliable data for further ecological risk-assessment and hazard identification (Shim and Thomposon, 2015). In recent years, several studies pointed out how wide the variety of environments affected by microplastic pollution is, introducing new research questions and, consequently, new challenges for this still-young scientific field, which are interdependent and clearly bounded to an inter-disciplinary approach.

Validated and trustable analytical methods are the key-factor to correctly assess the magnitude of a specific pollutant in the environment and to produce reliable and comparable data. Although the study of microplastic pollution tracks back to 2004, with the ground-breaking article published by Richard Thompson and co-workers (Thompson et al., 2004) or even further back in time, the studies published so far are hardly comparable, due to a substantial lack of standardized and validated methodologies (Hidalgo-Ruz et al., 2012; Rocha-Santos and Duarte, 2014; Song, Kyoung et al., 2015; Shim et al., 2017; Zhang et al., 2019). Microplastic analysis represents a constantly growing scientific challenge, albeit it can still be considered in its infancy if compared to other topics (e.g. PAHs and PCBs analysis in environmental matrices, which have specific standardised methods). In recent years a joint effort under the JPI-OCEANS umbrella funded 4 European projects focused on microplastic research, among which JPI-OCEANS-BASEMAN coordinated a consortium focused on harmonisation and validation of analytical methods for

microplastic analysis (Gerdts, 2019). Even though standardized operative protocols are still lacking, many improvements have been done since this topic started to attract scientific interest. MP analysis is a difficult task, mainly due to the main characteristics of this pollutant. Microplastics are particulate pollutants: they are inhomogeneous in regard to chemical (wide variety of polymeric composition) and physical properties (density, shape, size), and they typically are at very low concentration if compared to other particles contained in environmental matrices. The combination of these features makes the analytical procedures rather demanding, and in some cases not so efficient, especially when the matrix is a very complex one (e.g. muddy sediments, organic-rich soils, sewage sludge, etc.).

MP analysis involves mainly three steps: sampling, sample preparation, analysis. Several methods are used, according to the parameters involved in the investigation, like e.g. environmental matrix, size range, polymer type and availability of different equipment, affecting the analytical approach. As a result, MP analysis provides different types of information, typically complementary and equally important to provide useful data.

1.1. SAMPLING TECHNIQUES

Microplastics have been found in different environments and matrices, and recently the potential food contamination by MP has been pointed out, as well as the presence of this contaminant in drinking water as well as outdoor and indoor air (Lusher et al., 2015; Van Cauwenberghe et al., 2015; Dris et al., 2016; Dris et al., 2017; Mason et al., 2018). Assessing the magnitude of the issue is of paramount importance, but considering the difference in sample matrix that this entails, different sampling techniques and equipment need to be used to collect microplastics. This first step is often under-considered and described in less detail in the literature, although it is essential and defines limits and thresholds of further analytical procedures, such as MP extraction, analysis and, therefore, the type of data which can then be obtained and reported. Many factors influence the sampling methods, and the type of matrix (water, sediment, soil, biota, wastewater, air, etc.) is probably the most important parameter to consider when performing MP sampling. The size range addressed is also a key factor to scrutinize, as it strongly influences the further steps and the analytical output. Although there are no universally accepted methods (GESAMP, 2015), a carefully designed sampling strategy is a key factor to provide a representative sample to be further processed. Replicability and reproducibility, together with accuracy and minimal contamination, are also important factors to consider. Finally, a balance between costs and effectiveness needs to be considered, especially when aiming to routine monitoring programs over specific research activities.

The sampling methods strongly influences the type of sample collected, and therefore the type of data obtained at the end of the analytical workflow. The size range of the collected debris depends on the sampling technique (e.g. the mesh size used), by the processing method (Duis and Coors, 2016), and ultimately by the analytical method used. Three main sampling methods are used to collect MP from the environment: selective sampling, volume-reduced sampling, and bulk sampling (Hidalgo-Ruz et al., 2012). Each of these approaches comes with advantages and drawbacks, and improvements are applied continuously to protocols and procedure as the research provides new inputs.

Selective sampling directly extracts items from the environment, and it is applied to particles typically bigger than 1 mm, such as primary plastic pellets or fragments and fibres of similar size. It is a rather simple and coarse approach, which tends to overlook smaller and more heterogeneous items, especially when mixed with other debris. Despite the simplicity of the technique, selective sampling has been applied to several studies (Hidalgo-Ruz et al., 2012), and it is widely used in citizen science.

Volume reduced sampling reduces the size of the sample by discarding the bulk of the sample and saving only the specific items of interest for further analysis. Although applicable also to solid matrices using sieving procedures (sediment, soil), this type of sampling is widely used for liquid and airborne samples, and the volume reducing is typically achieved by filtering the bulk through a filter or a mesh. On the one hand, this type of sampling is very useful and allows to collect a sample of larger volume. On the other hand, it systematically discharges part of it, limiting the possibility of determining the concentration of plastics smaller than the mesh/pore size, and potentially underestimating the sample's particles content due to the potential loss of items. Bulk sampling collects the sample in its entirety, without any size/volume reduction. This approach has its main limitation related to the amount of sample that can be collected, stored and subsequently processed, but it is, in theory, the approach which ensures the best sample integrity, as all the microplastics contained in the sample are theoretically recovered. Moreover, limiting the sample handling helps to decrease the contamination due to the surrounding environment (Hidalgo-Ruz et al., 2012).

Environmental sampling is prone to external contamination coming from MP particles from the surrounding environment, which does not belong to the sample itself. It is a major issue documented by several studies (Imhof et al., 2012; Nuelle et al., 2014; Duis and Coors, 2016) and therefore great care is required to mitigate unwanted contamination and avoid to hamper the results of the analysis. The best way to address this specific issue is to limit the use of plastic items when handling the sample, and at the same time introduce the use of air-blanks, collecting potential airborne plastics on Petri dishes (or other containers) while handling the sample.

When sampling in the environment, it is always important to record prevailing weather parameters affecting not only the sampling period but also the previous days, as it could strongly affect the quality of the samples collected (Fok and Cheung, 2015). Wind direction and speed (e.g. Beaufort scale), sea conditions (e.g. Douglas scale) and time are important variables to consider, as well as the time of the day concerning the tidal height, especially in specific environments, like marine and estuarine environments (Moreira et al., 2016).

1.1.1. SAMPLING LIQUID MATRICES

Due to their different densities and sizes, including the influence by weathering and biofouling, microplastics have been found in most water bodies, and are present in all the water column, including the surface layer and the bottom one. The most used sampling approach for liquid matrices involves volume reducing sampling, as typically, large volumes of water need to be collected to obtain representative samples. The sample collection can be performed horizontally (water surface or sub-surface) or vertically (and obliquely, water column), according to the specific compartment which is investigated. The most common sampling devices are mainly derived from neustonic nets, typically used to collect zoo- and phytoplankton samples in marine and freshwater environments. When sampling surface waters, the most used sampling device is the Manta trawl (Eriksen et al., 2009; Doyle et al., 2011; Collignon et al., 2012; Eriksen et al., 2013), a modified neuston net where the metal frame is equipped with floating wings, to keep the device afloat and stabilizing it while sampling. The water passes through a front opening and is collected from a rear one, filtered through a mesh (typically 330 μm) and concentrated in the cod end, hard plastic or soft-net bucket connected to the net. A flowmeter (mechanical or digital), typically at the front opening of the net, records the volume of water filtered during the trawling. Water column sampling is typically carried out by using WP2 net (de Lucia et al., 2018) and bongo net (Hidalgo-Ruz et al., 2012). The WP2 (UNESCO Working Party 2) is a vertical plankton net with messenger-operated closing mechanism, which can also be used to collect particulate vertically or obliquely from the water column. The bongo is used to perform sub-surface horizontal sampling of microplastics. It generally has two twin aluminium circular frames connected to a central rod. Two nylon nets are collected to the frames, and they can be of the same mesh (duplicate sample) or a different one (addressing different size ranges). The device is often used with a flowmeter to record the volume filtered. The net is deployed to a certain depth and towed at this depth at a set speed for a specific time; then it is recovered, typically performing an oblique trajectory. A bongo net can also be used to carry out vertical sampling by pulling it vertically after deploying it to a certain depth. In the last years also pumping equipment started to be introduced as sampling equipment for microplastics. Underwater vessel pumping systems can provide large volume samples from sub-surface waters (Enders et al., 2015; Kanhai et al., 2017), while custom-made filtering/pumping devices were recently used to collect microplastics in freshwater, wastewater and pond water (Mintenig et al., 2017;

Liu et al., 2019; Simon et al., 2019). Using an active pumping/filtering system allows to reduce the mesh size (typically down to 10 µm) and still be able to filter a quite large volume of water (from hundreds of litres to 1 m^3, depending on the specific matrix). Although this sampling is more suitable for particles smaller than 500 µm, due to the potentially limited amount of large MP likely to be found at this sample size, the use of pumping/filtering devices is increasing and provides more interesting samples to assess the small MP pollution in environmental matrices.

1.1.2. SAMPLING SOLID MATRICES

The most common solid matrix investigated for microplastic pollution is by far sediments, due to the chronological development of this research field (Browne et al., 2010; Claessens et al., 2011), and to the fact that sediments are considered an ideal medium for long-term accumulation of environmental micro-pollutants (Chapman and Wang, 2001). However, the ubiquity of microplastic pollution has recently expanded the range of matrices investigated, including other types of solid and semi-solid matrices besides sediments, like soils, sewage sludge, road dust, etc. As in the case of liquid matrices, there is a substantial lack of standardised sampling protocols (Rocha-Santos and Duarte, 2014), besides the recommendation derived by JPI-OCEANS BASEMAN (Gerdts, 2019). Also for solid matrices, it is possible to carry out bulk sampling or volume reduced sampling, and as previously mentioned for liquid matrices, the advantages and drawbacks of the two approaches are similar also in this case. Bulk sampling is the best method to obtain a representative sampling when aiming to investigate small MP (e.g. down to 10 µm), as the sample is entirely preserved (Hidalgo-Ruz et al., 2012). On the other hand, when performing large scale studies, possibly involving volunteers and non-trained personnel, it is easier to apply a volume reduce sampling by using sieves and pre-select a specific range to investigate (typically between 5 mm – 1 mm). This type of sampling is typically used when collecting beach sand samples.

Focussing on bulk sampling, which is the most suitable method for detailed monitoring, solid samples can be collected using different devices. Sediments are typically collected using grabs (e.g. Van Veen grab, Ekman grab) (Browne et al., 2011; Claessens et al., 2011) and/or corers (e.g. Haps corer, Box corer) (Van Cauwenberghe et al., 2013; Vianello et al., 2013; Van Cauwenberghe et al., 2015b). Grabs only provide a surface sample and can perturb the sediments during the sampling (especially the Van Veen grab). Corers can provide a less disturbed and also a stratified sample, potentially allowing to investigate the MP deposition over time (Löder and Gerdts, 2015). Both grabs and corers are typically deployed from a boat/ship, impacting the bottom of the water body where the sample is collected. The device is then closed by typically using a messenger (a weight sent down through the wire/rope which triggers a mechanism) and retrieved on board. Haps corers can also be used manually in very shallow water (e.g. marshes, lagoons, and ponds) mounting them on a stick. Soil samples are typically collected by using metal spoons, shovels

or corers, after defining the sampling area and dividing it by using a grid and/or performing a randomised sampling. A similar approach can be used when collecting sand samples from beaches, always taking into account the longshore drift (MP transportation to the shore at a specific angle) and the swash (MP are transported to the beach at the same angle as the waves) and backwash (MP are carried back down at right angles) phenomena (Crawford and Quinn, 2017a). Sludge samples are collected after dewatering at WWTPs, and the tools used are similar to the ones employed for soil. A more recent type of solid sample is road dust. It can be swiped using a natural fibre swipe and collected using a metal shovel, or by using more sophisticated machinery, such as modified sweepers.

1.1.3. AIRBORNE PARTICULATES SAMPLING

Recently, some studies documented the presence of airborne microplastics both in outdoor and indoor environments (Dris et al., 2016; Dris et al., 2017). It is a rather new research field, and so far, the sampling techniques used are mainly derived from aerosol science and related technologies. The sampling can be carried out passively or actively. The first approach uses either glass Petri dishes left open to accumulate airborne deposition (small sample size, suitable for short sampling periods or indoor air deposition) or custom made atmospheric fallout samplers (Dris et al., 2015) consisting of a funnel and a large-volume glass or metal bottle where the sample is accumulated (both dry and wet deposition). Active aerosol sampling can be carried out using different types of devices, most of those based on active filtration (e.g. air pumps connected to a filtering device) (Dris et al., 2017) or cascade impactors, operating on the principle of curvilinear motion of particles in an aerosol stream (Papastefanou, 2008). This sampling equipment is typically related to air quality analysis, but so far no dedicated sampling devices have been developed, e.g. to address potential human exposure to airborne microplastics. Microplastic sampling has to take into account the synthetic composition of the particles and fibres which have to be collected, and this detail narrows down the range of materials that can be employed during the sample collection, excluding, e.g. all the polymer-based filtering membranes, as well as filter holders.

1.2. SAMPLE EXTRACTION AND PREPARATION

Sample preparation for microplastic analysis is a crucial and complex step, as it strongly influences the results of further analysis, but at present standardized operative protocols are substantially lacking. Microplastics are typically present in very low concentration if compared to the environmental matrix where they are contained. It is therefore paramount to extract MP from the matrix and concentrate them before submitting them to analysis, and also to effectively reduce the matrix effect, which can cause severe interferences during the analysis. Although there are differences

when treating different matrices, some common procedures and techniques are used for the extraction and purification of microplastics from organic and inorganic matrices, here amongst visual sorting under a microscope, sieving, filtration, density separation, chemical oxidation, etc. (Hidalgo-Ruz et al., 2012; Rocha-Santos and Duarte, 2014; GESAMP, 2015; Duis and Coors, 2016).

The early studies on microplastic pollution used basic techniques to extract the MP from the environmental matrices, and the sample treatment was minimal, if not absent (Ng and Obbard, 2006; Browne et al., 2010; Claessens et al., 2011). The MP extraction from sediment was carried out mainly using Sodium Chloride (NaCl), following the protocol published by Thompson and co-workers (Thompson et al., 2004), but this approach limited the extraction to only to the low-density MP, as most of the plastic denser than 1.2 g cm^{-3} were not recovered. The absence of chemical treatment hampered the analysis, and the identification of potential MP was difficult and uncertain.

In the last years, several improvements have been achieved both regarding the extraction of microplastics from solid matrices (typically sediments) (Imhof et al., 2012; Claessens et al., 2013; Nuelle et al., 2014), typically a crucial and problematic step to carry out successfully, and in sample purification, introducing the use of enzymes (Cole et al., 2014). Following these improvements, the most accurate and validated protocol for MP sample extraction so far has been published by Löder and co-workers (Löder et al., 2017), introducing a sort of universal protocol which can be applied to most of the matrices and setting a sort of state of the art. The sample preparation derived from this protocol can be summarised as a sequence of steps, involving a density separation as first step (for solid matrices, and sometimes a pre-oxidation step is also required), which is typically carried out by using a high-density media (e.g. Sodium Iodide - NaI, Zinc Chloride - $ZnCl_2$, and Sodium Polytungstate). Microplastic particles are typically less dense (typical of a density from 0.8 to 2.0 g cm^{-3}) than inorganic sediments (typical density 1.7 - 2.9 g cm^{-3}) and soil particles (average density around 2.6 g cm^{-3}), so they tend to float when immersed in a high-density liquid. This step can be performed both manually (by shaking a bottle or a beaker containing the solid matrix and the flotation media) and using dedicated equipment like, e.g. the MPSS (Imhof et al., 2012; now available at Hydro-Bios, Kiel, Germany) or other custom made devices (Claessens et al., 2013). Extracted samples are then typically treated with a range of chemicals to remove the organic matter, as it otherwise can hamper the results of further analysis. The most advanced sample extraction protocols involve multi-step sample clean-up, by using a combination of surfactants, enzymatic digestion and oxidation. The use of surfactants (SDS, TWEEN, etc.) is connected to their capability to partially digest the organic matter and opening the cells walls and membranes, preparing the matrix for further enzymatic digestion. Enzymatic digestion is nowadays considered a very effective and important sample preparation step, and it typically consists of a multi-enzymatic treatment, typically involving a proteolytic enzyme (protease in TRIS buffer), followed by a cellulolytic

one (cellulase or a combination of cellulase blend in acetate buffer). In some cases, also other enzymes are used, like chitinase, when the matrix contains high amounts of chitin (marine water samples rich in biota, marine sediment), or lipase during the preparation of samples containing high amounts of lipids (e.g. biota samples). A final digestion step involves oxidation using a hydrogen peroxide solution (Imhof et al., 2012; Nuelle et al., 2014), which can also be used in combination with other chemicals (e.g. iron sulphate – Fe(II)), to enhance speed efficiency of the reaction via catalysis (Fenton reaction - Tagg et al., 2017; Simon et al., 2018; Liu et al., 2019). This latter step is becoming more and more used among the scientific community working on microplastic analysis, and in some cases, it allows to skip the enzymatic treatment. After oxidation, the sample is typically re-submitted to flotation to eliminate the residual inorganic particles and then concentrated for further analysis.

1.3. MICROPLASTIC IDENTIFICATION AND QUANTIFICATION

The final stage of microplastic analysis involves the identification and quantification of the particles in the sample. It is not a trivial task, considering that the amount of different polymers produced over the decades is extremely extensive, and many of these plastics are blended with fillers, additives, while others exist as copolymers. Moreover, the residence time in the environment can affect their chemical and physical composition through oxidation and biofouling (GESAMP, 2015). Also, the size is an important parameter which causes further complications when trying to identify MP in a sample, especially for particles < 1 mm.

1.3.1. VISUAL INSPECTION

The simplest method to identify MP is based on manual sorting and visual identification of potential MP by using optical microscopy, typically a stereo-microscope. The single candidate particles are manually picked, observed, and classified following a list of selection criteria established by experts and based on morphological and chromatic features, like the absence of cellular structure, the consistent diameter of fibres etc. (Crawford and Quinn, 2017b). This approach has been extensively used (Hidalgo-Ruz et al., 2012) and is still a very important part of microplastic analysis nowadays, being an acceptable compromise when limited resources are available. On the other hand, this approach is prone to subjectivity, and potential bias derived from individual interpretation by the operator and can lead to severe bias in the results (Song, Kyoung et al., 2015), ranging from 20% and 70% with decreasing size (Hidalgo-Ruz et al., 2012). To overcome this limitation, a range of analytical techniques can be used, namely Pyrolysis-Gas Chromatography Mass Spectrometry (Py-GCMS), Fourier Transform Infrared Spectroscopy (FTIR), Near Infrared Spectroscopy (NIR), and Raman Spectroscopy.

1.3.2. PYROLYSIS – GAS CHROMATOGRAPHY MASS SPECTROMETRY (PY-GCMS)

Pyrolysis-Gas Chromatography Mass Spectrometry (Py-GCMS) is a hyphenated technique where the sample is thermally decomposed in an inert atmosphere (typically between 500 – 600˚C), typically Helium, and the products obtained (pyrolysates) are separated inside the chromatographic column according to their interaction with the stationary phase of the column itself. Subsequently, these molecules are further fragmented and detected using mass spectrometry, probably the most powerful analytical technique to determine the presence of a vast range of compounds, providing specific information about the structural composition of the molecules contained in the sample, therefore allowing for tracking back the composition and the mass-based concentration of several polymers. This technique has been recently introduced to carry out MP analysis (Fries et al., 2013; Nuelle et al., 2014), and it is quickly developing, allowing the simultaneous detection of at least eight to ten polymers (Fischer and Scholz-Böttcher, 2017; Hermabessiere et al., 2018; Gomiero et al., 2019; Fischer and Scholz-Böttcher, 2019), also allowing to identify and quantify pollution from car tire treads (Lachowicz et al., 2012; Lachowicz et al., 2013; Unice et al., 2013), which is otherwise impossible with most of the spectroscopic approaches.

1.3.3. MOLECULAR SPECTROSCOPY

The most established analytical methods for microplastic analysis are based on molecular spectroscopy, a group of techniques which are based on the interactions that occur between molecules and electromagnetic (EM) radiation (Larkin, 2011).

1.3.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful analytical tool for MP analysis (Rocha-Santos and Duarte, 2014; Löder and Gerdts, 2015; Shim et al., 2017). This technique relies on specific absorption of infrared light by different material and substances. When a material is irradiated with IR light, it can partially absorb it and partially transmit it, and this can cause a variation in the dipole moment of the molecules, which will be IR active depending on the symmetry of the molecule itself. This change of dipole moment causes some distortions within the molecular bonds (bending and stretching) occurring at specific wavelengths and generating distinct bands that can be recorded as FTIR spectra, which are characteristic of each substance and material. Unknown spectra can then be compared to reference databases. FTIR can be applied to a quite broad range of materials (both organic and inorganic substances) and particle size range, but different approaches and equipment must be used. The three main collection modes are Transmission, Reflection and Attenuated Total Reflection (ATR). In transmission mode, the IR beam passes through the samples, upon which it reaches the detector. This approach is applied to thin samples,

as Transmission-FTIR is a thickness-dependent technique, and produces saturated spectra when the thickness of the sample exceeds around 100 µm. High-reflective samples and powders can be analysed in reflection, where the beam hits the sample and it is reflected (specular reflection – useful for coating analysis) and/or scattered (diffuse reflection – DRIFT) by the sample surface. Focussing on MP analysis, the bigger size range (5 mm – 500 µm) is usually analysed using a bench instrument equipped with an ATR accessory, which consists of a high refractive index crystal (typically diamond, germanium or zinc selenide). The particle is put in optical contact with the crystal, and a certain pressure is applied using a clamp. The IR beam passes through the crystal, and it extrudes its surface penetrating for few micrometres (0.2 – 5 µm) inside the sample (evanescent wave), being selectively absorbed. The beam is then recollected in the detector where the IR absorption is recorded. The detection of small particles (500 µm – 20 µm) can be carried out by using an infrared microscope combined to the spectrometer (µFTIR). The analysis can be performed in single-point mode, focussing on a single particle by adjusting the microscope aperture (slit), or by using mapping/imaging techniques (µFTIR-Imaging), where a portion of the whole sample, typically deposited on an IR suitable surface (IR window or specific filter) is automatically scanned, producing an IR image superimposed to the visible one. Harrison et al (2012) made the first attempt of µFTIR mapping for MP analysis with a single detector instrument, but part of this PhD also provides one of the first studies carried out using µFTIR mapping (Vianello et al., 2013).

Today, FPA-µFTIR-Imaging spectroscopy (Focal Plane Array-Fourier Transform-Infrared-micro-spectroscopy) has been proven to be an efficient approach for quantifying the smaller MP fraction in a sample, avoiding pre-sorting of potential microplastic candidates, and hence limiting the bias potentially related to each different analysist (Loder et al., 2015; Primpke et al., 2017). This technique provides at the same time a magnified optical image, and the relative infrared map, stitching FPA generated tiles into a single image. This technique is useful to map the sample's surface, obtaining the chemical information on the particles at a high spatial resolution (typically 10 µm). The FPA (Focal Plane Array) is a multi-detector which detects up to several thousands of spectra at the same time. Every pixel of the IR map holds an FTIR spectrum, allowing to identify a multitude of organic and inorganic materials by comparing the spectrum behind the pixels to spectral databases. The handling of the large dataset produced by µFTIR-Imaging applied for MP analysis can be quite challenging using the commercial software provided by the main suppliers of analytical instruments. The analyst is forced to manually conduct a number of interpretation steps, potentially introducing bias in the results, which could nullify the benefit of this technique. To overcome this issue, and fully automatize MP detection by µFTIR-Imaging, a software called siMPle (Systematic Identification of MicroPlastics in the Environment (siMPle, 2019) has been developed by Aalborg University (AAU) and Alfred Wegener Institut (AWI). It is the combination of the AWI automatic pipeline (Primpke et al., 2017) and MPhunter (Liu et al., 2019). The software automatically compares each single spectrum in the IR map (each pixel) with

a database using Pearson's correlation coefficient analysis, assigning specific materials to the pixels above a certain correlation threshold, hence re-constructing every identified particle and providing a 2D map of the identified materials in the sample, also including particle size measurement and mass estimation.

1.3.3.2 Near Infrared Spectroscopy (NIR)

Near Infrared spectroscopy (NIR) is a spectroscopic technique that uses the near infrared region of the EM spectrum (750 nm – 3000 nm), and can identify a broad range of chemical compounds and different materials, including polymers. The sample interacts with the incident radiation, specifically absorbing it and producing molecular overtones and combination vibrations. This technique has the advantage of penetrating more of the sample than FTIR, but on the other hand, it is not particularly sensitive. It is hence useful to analyse bulk samples of plastic with no sample preparation, and it is widely diffused because of the availability of portable devices. Hyperspectral Imaging can be applied in NIR spectroscopy, and a few attempts to use this specific upgrade to analyse MP have been made (Karlsson et al., 2016; Serranti et al., 2019), but its application remains very limited due to the complexity of the data sets, which requires the use of chemometrics.

1.3.3.3 Raman Spectroscopy

Raman spectroscopy relies on the interaction between a monochromatic laser light (incident beam) and the sample, which is partially absorbed, reflected or scattered. The scattered light, which is the component of interest in this technique, is the result of photons interacting with the molecules of the sample. Although the most intense component of scattered light (elastic Rayleigh scattering) is not useful to this method, a weaker part of it, called Raman scattering (inelastic) can be used to identify molecular bonds. The energy change in the incident photons when interacting with molecules is characteristic of those types of bonds and provides accurate molecular structural information producing characteristic Raman spectra, which can be compared with reference ones to identify a wide range of organic and inorganic compounds (Smith and Dent, 2005; Crawford and Quinn, 2017b). Raman spectroscopy is typically used in combination with microscopy (μRaman spectroscopy) to identify microplastics (Rocha-Santos and Duarte, 2014), and it can also address very small MP and even sub-micrometric MP, thanks to its high spatial resolution resulting from the monochromatic incident beam (laser). μRaman spectroscopy can also provide Imaging analysis like FTIR-Imaging, providing even a better spatial resolution (Rocchia et al., 2017), but with the disadvantage of longer scanning time (Raman is a dispersive technique), which makes this approach not so suitable for the analysis of large sample batches. There are other solutions to largely automatize μRaman analysis avoiding the full scan of the sample. These approaches are based on particle identification via optical microscopy, as μRaman systems are equipped with a standard optical microscope (with glass lens objectives, not

Cassegrains), followed either by single point Raman analysis (GEPARD - Fischer et al., 2019) or multiple micro-sized imaging carried out around the single particles or regions of interest of the sample.

1.4. MICROPLASTIC POLLUTION IN SPECIFIC COASTAL AREAS AND TRANSITIONAL ENVIRONMENTS OF ITALY

Highly populated, as well as shallow, and enclosed waters, are expected to be highly impacted by plastic pollution. The Mediterranean Sea, which shows all the characteristics mentioned above, is suspected to be the sixth greatest accumulation zone for marine litter (after the oceanic gyres) (Cózar et al., 2015). Around 10% of the world's coastal population lives along the Mediterranean area, which is globally recognised as an essential intersection for maritime routes and activities. The basin receives waters from highly populated freshwater systems, and has a very long water residence time (up to one hundred years), as the Strait of Gibraltar represents the only connection to the Atlantic Ocean (Lacombe et al., 1981).

Albeit anthropogenic activities strongly influence the majority of the Mediterranean coastal waters, a few areas are still commonly considered as virgin and clean sites, especially those located in the vicinity of the numerous minor islands spread across the basin, situated reasonably far from land-based sources (e.g. Balearic Islands, Spain; Aeolian Islands, Italy; Cyclades Islands, Greece; etc.). Italy has one of the most extended coastline in the Mediterranean area (7,600 km; Field Listing, Coastline, The World Factbook, Central Intelligence Agency, 2019) with several minor islands located along the coastline. Some of these islands are important touristic destinations (therefore potentially prone to anthropogenic pressure), while others appear comparatively virgin environments. Lebreton and co-workers (Lebreton et al., 2012) modelled how floating debris are transported and distributed across the oceans, and they identified a relevant area of accumulation in the Mediterranean Sea, with an estimated plastic debris load up to 23,150 tons (Eriksen et al., 2014). Although these data strongly point in the direction of ubiquitous plastic pollution affecting the Mediterranean Sea, it is still unclear whether these partially isolated areas are also prone to MP pollution or, on the contrary, it is possible to observe differences between urbanised and partially isolated areas.

The Adriatic Sea lies in the central-eastern part of the Mediterranean basin, and it is enclosed by the Italian east coast and the Balkan peninsula. The northern part of the basin is also the shallower one, on average some 30 m deep with no parts deeper than 70 m. Its morphology, together with seasonally variable meteorological, thermal and saline conditions, strongly influences the oceanography of this area. A substantial amount of fresh water enters the basin from the Po river, the largest in Italy with an average flow of 1,540 m^3/s. Po flows into the north-eastern part of the Adriatic Sea,

together with some other minor rivers. Substantial marine activities characterise this area of the Adriatic Sea, due to active commercial and merchant routes, as well as offshore installations and active fishing industry. Based on previous modelling studies (Liubartseva et al., 2016), a predicted plastic accumulation zone stretches along the Italian coastline in this region. Local inputs, as well as the general basin circulation, appear to influence the occurrence and accumulation zones of floating debris strongly.

In the north-western part of the Adriatic Sea is located the Lagoon of Venice. The lagoon is among the most extended Mediterranean transitional environments, with a surface of 550 km^2. This area holds several specific habitats, where tidal flats and marshes surround islands, typically connected by canals. The city of Venice is located in the centre of the Lagoon, while a few other minor towns have access to the inner shore; the city of Chioggia defines the southern lagoon's borders. Although this unique habitat has been extensively explored over the years, and many studies have addressed the effects induced by extensive human activities impacting this delicate environment, no data were available on the occurrence of microplastics in this specific area until 2012. The Lagoon is potentially prone to multiple MP sources, mainly related to the specific morphology of this environment, the hydro-dynamical features, and the presence of a massive anthropogenic footprint. The tides affect the hydrodynamics of the Lagoon, which exhibit a particular water exchange regime, with the seawater inflowing through three separate inlets. On the other side, there is a significant freshwater run-off because a large drainage basin (2,000 km^2) discharges into the lagoon via several tributaries (Gačić et al., 2002). The combination of these features reduces the hydrodynamics from the inlets to the inner areas, suggesting a potential accumulation area for plastic debris. Other potential sources are linked to spillage of raw plastic material from the polymer industries located in Porto Marghera, as well as urban and industrial discharge.

1.5. AIRBORNE MICROPLASTIC POLLUTION AND POTENTIAL HUMAN EXPOSURE

Microplastic pollution affects not only the environment but is a potential direct threat to human health. As highlighted by recent studies, microplastic pollution could directly affect humans through food, drink, and air (Dris et al., 2016; Dris et al. 2017; Waring et al., 2018), and the scientific knowledge related to the latter pathway is almost absent. Airborne microplastic could be inhaled, even affecting the lower airways, when the particles are smaller than 5 μm or of fibrous morphology (Gasperi et al., 2018). These are defined as breathable particles, while the bigger ones are (inhalable particles) are mostly removed by mucociliary clearance (Gasperi et al., 2018; WHO, 1997). However, some of these particles, especially the fibres, can escape clearance (Warheit et al., 2001), showing high persistence in the organisms after inhalation, as also pointed out by Eschembacher and co-workers who found

fibres of nylon in lung tissue of patients related to the textile industry (Eschembacher et al., 1999).

It appears that only particles and fibres smaller than 5 μm can deposit in the deeper part of the lungs (breathable particles), as the biggest ones (inhalable particles) are mostly removed by mucociliary clearance (Gasperi et al., 2018; WHO, 1997). However, some of these particles, especially the fibres, can escape clearance (Warheit et al., 2001), showing high persistence in the organisms after inhalation, as also pointed out by Eschembacher and co-workers who found evidence of synthetic fibres in lung specimens of patients related to the textile factories (Eschembacher et al., 1999). Secondary toxic effects might also occur when these particles are retained (Greim et al., 2001), even if the mechanisms are still poorly explained. Also, the particles removed by mucus can have a negative side effect, because, when not spat out or evacuated by coughing or sneezing the nose, they enter the digestive tract, potentially affecting the organism like MP ingested from food and beverages. Another aspect related to synthetic particles inhaled and breathed in is connected to the potential micropollutants adsorbed (PAHs and metals) to their surface, especially in urban environments, as well as additives, dyes and unreacted monomers which can also have a negative implication for human health. Despite a few studies (Dris et al., 2015; Dris et al., 2017), there is a lack of knowledge on the potential exposure to airborne microplastics and the deriving effects. Airborne microplastic is not only a potential threat but also represents a new analytical challenge. The studies conducted so far only addressed a certain size range because of technical limitation. It is hence important to increase the sensitivity of the detection methods to address the lower size ranges, developing new approaches valid for MP detection down to a few micrometres, and possibly expand to the sub-micrometric range.

CHAPTER 2. OBJECTIVES OF THE PHD THESIS

The research described in this thesis has been studied over a timeframe from 2011 to 2019. Some activities have been conducted in different chronological periods, even if they present several similarities regarding the research topic. Therefore, project objectives are presented according to the chronological order of the related publications. Due to the time span, the techniques and methodologies used in the PhD project vary and represent a "natural" evolution of the analytical tools used in microplastic analysis.

To address this aim, the study was divided into several research questions, and each one was addressed and answered to complete the specific objective.

Research question 1*: Are the sediments of the Lagoon of Venice contaminated by microplastic particles? Is it possible to backtrack potential sources according to the MP concentration? Is there any relationship between the MP concentration and the sediment grain size? Is it possible to develop and apply a μFTIR method to avoid the pre-sorting of potential MP particles?*

This objective was addressed examining the occurrence of MP in the sediments of the Lagoon of Venice applying a novel analytical method, which allows avoiding visual sorting of potential MP. Fourier-Transform Infrared micro-Spectroscopy-chemical mapping (μFTIR – chemical mapping) was used to identify and quantify microplastic pollution, providing information on polymer type, size and shape of the analysed particles. Scanning electron microscopy (and energy-dispersive X-ray spectroscopy) was also used to provide additional details on particles morphology and surface appearance.

Research question 2*: Does microplastic pollution contaminate the surface water along the Italian coast? Which is the actual concentration and which are the main drivers for microplastic spreading in this area? Is it still possible to find comparatively virgin areas, especially when comparing them to others which are highly impacted by anthropogenic activities?*

This objective was addressed by investigating a total of 8 coastal stations located along two transects, collecting surface water with a Manta trawl and analysing the samples combining stereo-microscopy and ATR-μFTIR spectroscopy. Additionally, the seawater contamination in the vicinity of some minor Italian islands was studied applying a similar method, and the results were compared to those obtained from samples collected in suspected polluted areas located in the surrounding of the mouth of two main Italian rivers.

Research question 3*: Are humans directly exposed to airborne indoor microplastics? To which extent? How can human exposure be measured?*

This objective of the PhD study focussed on the potential human exposure to indoor airborne microplastics, combining a novel sampling method based on Breathing Thermal Manikin (BTM), a human-shaped device capable of simulating human breathing activity, with FPA-μFTIR-Imaging spectroscopy for MP analysis. The study provided novel insights into potential human exposure to indoor airborne MP, expanding the analytical size range (lower size range of 11 μm) and the knowledge on MP occurrence and chemical composition of these airborne particulates.

CHAPTER 3. METHODOLOGY

The PhD study was carried out focusing on 3 main sub-topics, one per research question:

- Occurrence and distribution of microplastics in the sediments of the Lagoon of Venice, Italy
- Spatial distribution of floating microplastics in the North-Adriatic Sea and spatial distribution of floating microplastics in the vicinity of Italian minor islands
- Simulated human exposure to indoor airborne microplastics

Each topic was addressed using approaches suitable for the specific environmental matrix, as well as customized devices were developed or adapted when suitable devices were not available otherwise. Sampling protocols, as well as sample preparation procedures and analysis, reflected the state of the art of the analytical field at the time the specific study was conducted, also describing the analytical developments in this specific emerging research field.

3.1. OCCURRENCE AND DISTRIBUTION OF MICROPLASTICS IN THE SEDIMENTS OF THE LAGOON OF VENICE, ITALY

The survey aimed to investigate the occurrence of MP in the sediment of the Lagoon of Venice and study any possible relation to the sediment grain size. At the same time, the study intended to improve the analytical method used to determine the MP concentration in the samples, avoiding pre-sorting of the particles before the analysis. To achieve the goals mentioned above, the main analytical steps described in chapter 1.1., 1.2., and 1.3. were followed.

3.1.1. SAMPLING

The sediment samples were collected in duplicate using a box corer and at ten stations inside the Lagoon of Venice. Some stations were located in inner areas affected by freshwater inputs and low hydrodynamics (1-Palude della Rosa; 7-Lago Rivolta; 8-Valle di Bon), while others showed higher dynamics (2-Sant'Erasmo; 9-Ca'Roman) as they were positioned close to the lagoon's inlets. The other stations were influenced by urban activities (3-Fondamente Nuove; 5-Sacca Sessola), aquaculture (6-mussel farm), and industrial (4-San Giorgio in Alga) inputs.

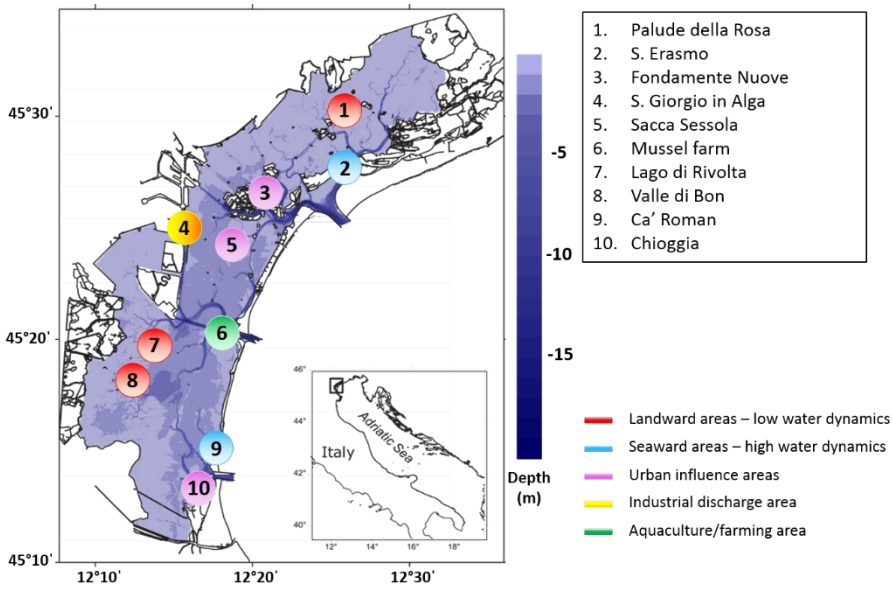

Figure 3-1. Sampling station for the sediment sampling collection in the Lagoon of Venice, Italy (Modified from Vianello et al., 2013)

3.1.2. SAMPLE PREPARATION

A sub-sample (250 g w.w.) was collected from each bulk sediment sample, placed in a glass bottle, and submitted to density separation using a concentrated NaCl solution (1.5 minutes shaking followed by 1.5 h settling time). The obtained supernatant was extracted and filtered onto a 32 µm steel micro-sieve (3 times), and the extracted particulates were resuspended in Milli-Q water and eventually filtered onto a glass fibre filter, then dried overnight at 50˚C. A more detailed description of the sample preparation procedure can be found in **Paper I**.

3.1.3. SAMPLE ANALYSIS

Polymer identification was conducted using Fourier Transform-Infrared-micro-Spectroscopy (µFTIR), using a Thermo Fisher Scientific (Madison, Wi, USA) Nicolet iN10 infrared microscope. The analysis was performed in reflection directly on selected sub-areas of the filter and constituted one of the first attempts of µFTIR mapping for microplastic analysis. The acquired spectral range was 4000 – 675 cm^{-1}, and the spectral resolution was set at 8 cm^{-1}; the spectra were collected using 8 co-added scans, using an aperture of 50 x 50 µm and 50 µm step-size scanning. The 12 obtained areas were then manually inspected using the features of the instrument's software, highlighting characteristic IR absorptions of the most common polymers. When necessary micro-Attenuated-Total-Reflection-Fourier Transform-Infrared

Spectroscopy (µATR-FTIR) was applied to re-analyse specific particles. The identified MP were also characterized by shape (fragments, fibres, films, pellets), and some selected particles were analysed using scanning electron microscopy and X-ray dispersive spectroscopy, acquiring high-resolution pictures and analysing the atomic elemental composition of selected microplastics (previously identified by µFTIR).

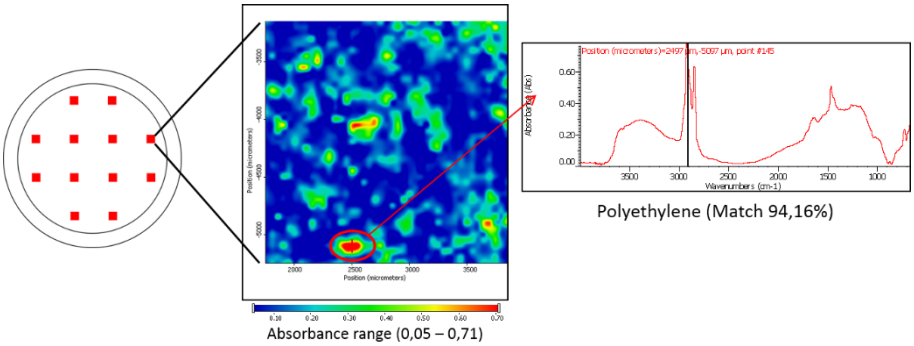

Figure 3-2. Filter surface, with 12 sub-areas analysed by µFTIR chemical mapping (aperture 50 x 50 µm, step-size 50µm); an example of a 4.5 mm2 area and a polyethylene particle (red spot at the bottom and relative FTIR spectrum) (after Vianello et al., 2013)

Further details on the study area and sampling stations, as well as sample preparation procedure and analytical methods, can be found in **Paper I**.

3.2. SPATIAL DISTRIBUTION OF MICROPLASTICS IN THE NORTH ADRIATIC SEA AND IN THE VICINITY OF SOME OF THE ITALIAN MINOR ISLANDS

The monitoring survey performed in the North-Adriatic Sea (a) was part of a regional and national initiative to provide qualitative and quantitative data on the MP spatial distribution along the Italian coastline. The survey related to the Veneto region was carried out by the Italian National Research Council (CNR), combining the expertise of the Institute of Marine Sciences (ISMAR), which led the sampling activities, and the Institute for the Dynamics of Environmental Processes (IDPA), in charge of the analytical procedures. The study related to the spatial distribution of MP in waters close to some Italian islands and two of the largest rivers was part of a joint effort between Legambiante ONLUS, The Superior Institute for the Environmental Research in Italy (ISPRA), and the Italian National Research Council (CNR). In both cases, similar approaches in sampling, sample preparation and analysis were used.

3.2.1. SAMPLING

(**Paper II**) The sampling activities linked to the North Adriatic Sea were conducted along two transects located along the coast of Veneto, close to specific potential sources of microplastic. The first transect was situated in front of the Lagoon of Venice, in front of the island of Pellestrina – connected with the Lagoon, an area affected by high anthropic pressure; the second transect was off the Po river delta, an area strongly affected by the river Po, the biggest river in Italy, which flows along the Pianura Padana (Po Valley), the most industrialized area of the country. Four sites were chosen on each transect at 0.5, 3, 0, 20 km from the shoreline to represent the area of the Gulf of Venice (Figure 3-3a). The sampling was carried out during two monitoring campaigns (March and April 2014), according to weather conditions and Po river discharge. The sampling was carried out with a manta net (330 µm mesh-size) in calm conditions, lowering the manta laterally to the vessel (outside the boat's wake) and filtering water for 20 min at 2-3 kt speed. GPS positions were acquired before and after each sample collection and every 2 minutes. The sampled area was calculated geometrically from the length of the sampling transect (GPS coordinates) and the width of the opening of the manta frame (the "mouth"), hence providing the area-specific particle abundances (particles m^{-2}).

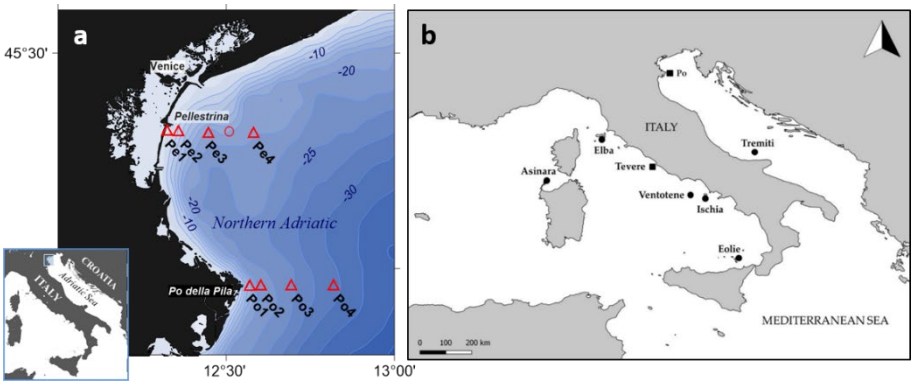

Figure 3-3. (a) Sampled transects at the northern Adriatic coast. Pellestrina transect: stations Pe 1 – Pe 4; Po Delta transect: stations Po 1 – Po 4. The circle indicates the oceanographic platform "Acqua Alta", at which meteorological data, surface currents, and waves were measured (figure and caption from Vianello et al., 2018). (b) Map of the study area: the minor islands are marked by circles, while the river mouths are marked by squares (figure and caption from de Lucia et al., 2018)

(**Paper III**) The sampling survey in the proximity of some smaller Italian islands was carried out in 2015 in collaboration with Legambiente ONLUS, collecting samples close to six minor islands/archipelagos (Tremiti Islands, Aeolian Islands, Ischia, Ventotene, Island of Elba, and Asinara). It was also decided to take samples from highly polluted areas, like the vicinity of the Po river delta and the Tiber river to use these samples for comparison with areas where land-based microplastic sources are limited (the small islands: Tremiti, Eolie, Ischia, Ventotene, Asinara, Elba; Figure 3-3b). Four replicate samples were collected by manta trawl and four by WP2 FAO net (both equipped with 333 µm mesh net) at each station, sampling for 20 min at an average speed of 2 kt. The manta filtered the top water layer, while the WP2 net was deployed at a depth of 20 m and slowly retired back to the surface, ending point of the sampling. The volume of water filtered by both the nets was monitored using a mechanical flowmeter.

3.2.2. SAMPLE PREPARATION

Samples collected along the coast of Veneto (**Paper II**) were processed following a protocol developed by the Italian environmental ministry, even if small modification were applied to optimize it. Samples were sieved (5 mm and 300 µm mesh) to eliminate items not included in the analytical procedure, and the containers were rinsed with filtered water to detach particles. The fraction retained between the sieves was submitted to density separation (NaCl, density = 1.2 g cm^{-3}), diving the buoyant from non-buoyant material. Both fractions were then sorted by microscopic inspection, and MP candidates were filtered on 100 µm mesh size nylon filters and weighed. The sample preparation relative to samples collected at the minor islands (**Paper III**) was kept at a minimum to lower the time and cost of the survey. The

sample extraction was performed similarly to the one carried out for the samples collected in Veneto in 2014 (**Paper II**) and previously described, but the weight of the isolated particles was not recorded in this specific survey. The collected samples (**Paper III**) were also analysed for several POPs (Persistent Organic Pollutants), specifically polychlorinated biphenyls (PCBs) and organochlorine pesticides (OC). The sample extraction was performed following the US Environmental Protection Agency (EPA) methods (USEPA, 1987).

3.2.3. SAMPLE ANALYSIS

Particles were placed in Petri dishes and counted and classified according to colour and shape. The results were expressed as item m^{-2} and mg m^{-2} (**Paper II**) and in item m^{-3} (**Paper III**). Both units have been used in microplastic analysis (Hidalgo-Ruz et al., 2012), even if in recent years item m^{-3} has become more popular. Unfortunately, the lack of a flowmeter during the survey back in 2014 required to report MP concentration in item m^{-2}. The chemical composition of the sorted MP candidates was assessed using ATR-μFTIR in both the surveys (Samples from the Veneto coast, **Paper II**; samples from the Italian minor island, **Paper III**) (Figure 3-4).

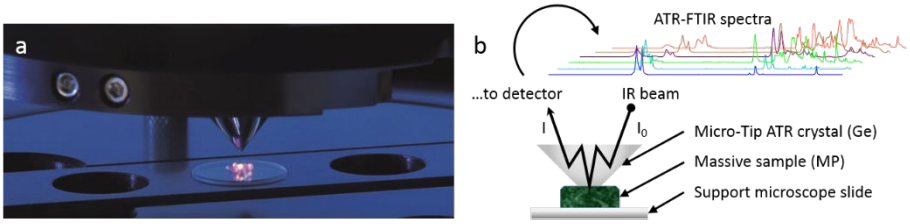

Figure 3-4. (a) ATR-μFTIR measurement of a particle, before the contact between the ATR crystal and the sample; (b) schematics of the ATR-μFTIR analysis. Photo Credits (a): Thermo Fisher Scientific (Madison, WI, USA)

The instrument used was a Nicolet iN10 IR microscope (Thermo Fisher Scientific, Madison, WI, USA). The signal was acquired in ATR mode, using a slide-in micro-ATR (tip diameter = 350 μm) in Germanium (Refractive index = 4). The spectra were collected in the spectral range of 4000 – 675 cm^{-1} and at 4 cm^{-1} resolution, acquiring 64 co-added scans both for the background and the samples. The chemical composition of the analysed items was assessed using multiple spectral databases, further manually checking the spectra peak by peak. The infrared spectra obtained from the samples collected along the Veneto coast were also used to assess the relative ageing of the most common polyolefins (polyethylene and polypropylene) by determining the Carbonyl Index (Nagle et al., 2010; Veerasingam et al., 2016), a parameter widely used to evaluate the photo-degradation of polymers, typically related to the environmental exposure (Cooper and Corcoran, 2010; Matsuguma et al., 2017) (detailed description in **Paper II**). The samples related to **Paper III** were also analysed by High-Resolution Gas Chromatography - Electron Capture Detection

(HRGC-ECD, Agilent 6890 equipped with ECD detectors) to investigate the presence of specific organic pollutants (PCBs and OC pesticides) previously prepared for POPs analysis.

A detailed description of the study area and sampling stations, as well as sample preparation procedure and analytical methods, can be found in **Paper II** and **Paper III**.

3.3. SIMULATED HUMAN EXPOSURE TO INDOOR AIRBORNE MICROPLASTIC

The simulation of human exposure to indoor air microplastics was part of a Master Thesis Project conducted in 2017 – 2018 by three Master's students at Aalborg University, who collected the exposure samples in their flats.

3.3.1. SAMPLING

The sampling of indoor air was carried out in three four-room apartments (age and type of building differ among the three locations) located in Aarhus, Denmark, in 2017. The sampling was done on three consecutive days in each flat (a total of nine samples were collected), recording the microplastic concentration linked to the residents' daily activities.

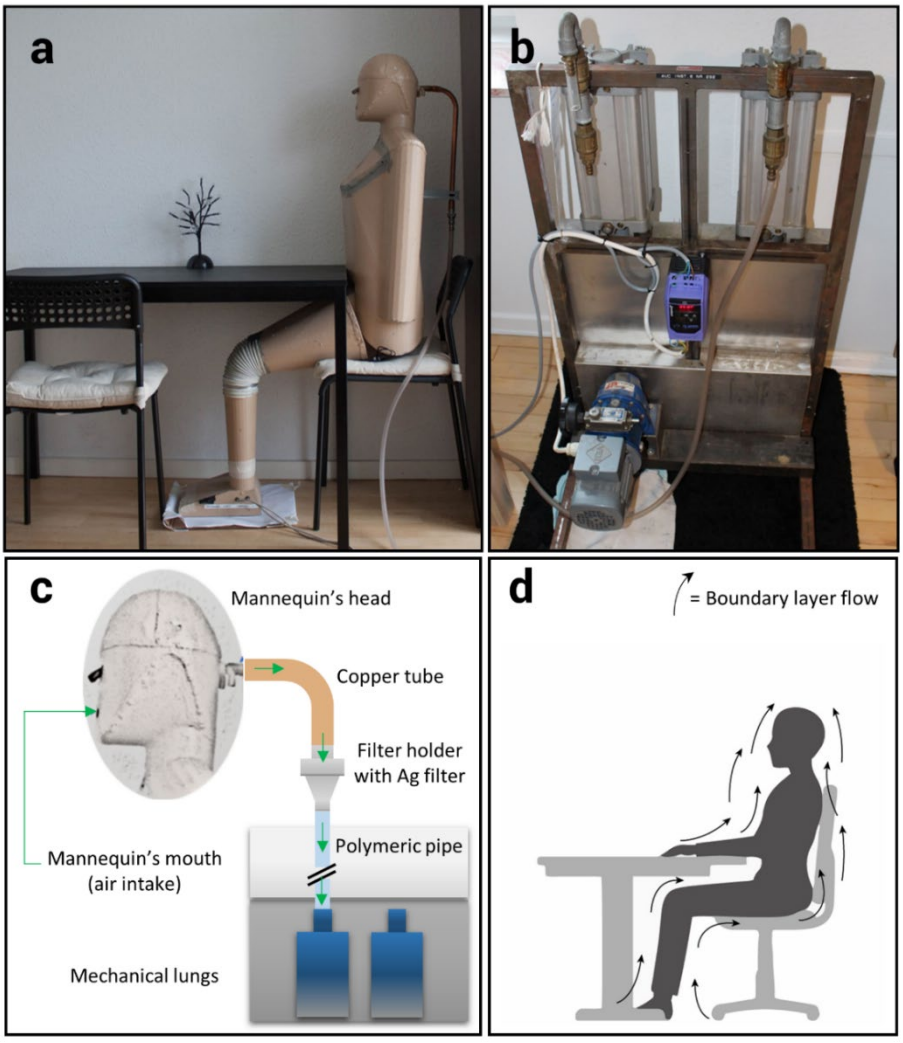

Figure 3-5. Sampling device setup: (a) manikin in seated position ready for sampling; (b) twin adjustable pistons connected to the motor to simulate breathing in and out; (c) sampling setup diagram; (d) Illustration of human boundary layer flow (figure and caption taken from Vianello et al., 2019)

The sampling was carried out using a Breathing Thermal Manikin (BTM), a human-shaped device (Figure 3-5) heated similarly to a human body, to create a boundary layer flow, which transports air along the body to the upper area of the body (Bivolarova et al., 2017; Nielsen et al., 2009; Xu et al., 2018). The BTM was powered according to 1.5 Met (Metabolic Rate) (Ainsworth et al., 2000; Ghiaus et al., 2005; Xu et al., 2015a; Xu et al., 2015b; ISO 7730, 2005) (**Paper IV**, SI 3), and connected to a custom made double piston pump, which produced an airflow simulating

breathing. The sampling was carried out from the air intake of the mannequin, its "mouth" (diameter of 9 mm), in periods of 24 hours, filtering air through a silver filter (0.8 µm pore size) in a metal filter holder connected to the back of the manikin's head. The volume of air filtered per sample was 16.8 m^3. A more detailed description of sampling and sampling equipment is presented in **Paper IV** and the relative SI.

3.3.2. SAMPLE PREPARATION

Each enriched membrane was sonicated (5 min) and flushed with ethanol to detach the sampled particles. The resulting liquid was deposited on a zinc selenide (ZnSe) window secured in a compression cell and dried at 55˚C for two days prior analysis. Three blanks were analysed to assess the sample contamination occurring during the sample preparation and analysis. Further details are presented in **Paper IV** and relative SI.

3.3.3. SAMPLE ANALYSIS

The samples were analysed by FPA-µFTIR-Imaging spectroscopy, a reliable tool for identification and quantification of unsorted small MP (Löder et al., 2015; Primpke et al., 2017) (See Chapter 1.3.3.1.).

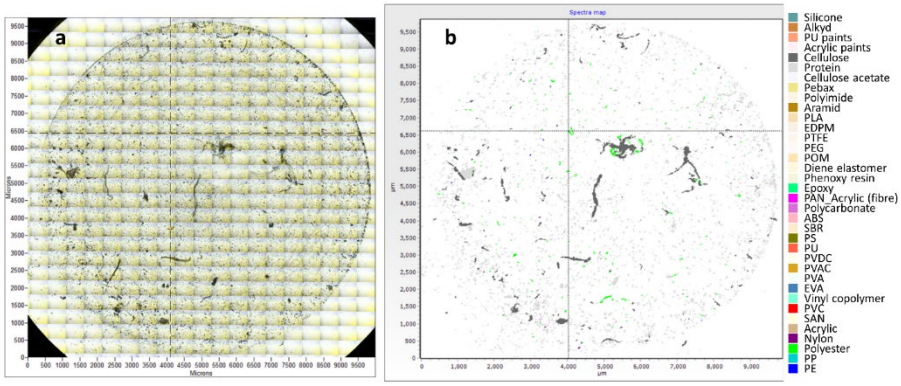

Figure 3-6. (a) Visual stitched image of a sample and corresponding MPhunter map. Each polymer group is highlighted in a different colour. Cellulose and protein-based fragments and fibres are shown in grey colours (figure and caption taken from Vianello et al., 2019)

The analysis was performed using an Agilent Cary 620-670 µFTIR system with a 128x128 pixel FPA detector (Agilent Technologies, Santa Clara, CA, USA) by scanning each ZnSe window in transmission mode, and the collected FPA-µFTIR-Imaging data were analysed using a custom-developed software called MPhunter (Liu et al., 2019). The software automatically detects the particles on the scanned surface, identifying their composition thanks to a custom spectral database (Figure 3-6b), and

measuring the main size parameters of each particle. MPhunter is described in detail in **Paper IV** (SI 1). The simulated exposure to MP was presented as the number of MP per unit volume (N_{MP} m^{-3}) inhaled by the manikin (MP items per unit volume - N_{MP} m^{-3}) during the exposure time of 24 hours. Nonsynthetic items identified as protein-based, as well as cellulose-based particles, were also quantified. Size distributions and morphological parameters of the identified items were recorded and used to distinguish between particles and fibres. A detailed description of the methodology can be found in **Paper IV** and relative SI.

CHAPTER 4. RESEARCH OUTCOMES

The PhD study is a collection of studies which give a substantial contribution to the research in microplastic pollution, highlighting the complexity of the analytical challenges involved and providing an insight into the evolution of the analytical methodologies. Although the studies conducted are not directly connected, they provide an overview of the most suitable analytical IR-spectroscopic methods applied in this field.

Four papers were published where the main research topics of the PhD study have been addressed.

- Occurrence and distribution of microplastics in the sediments of a transitional environment (the Lagoon of Venice, Italy)
- Spatial distribution of floating microplastics in the North-Adriatic Sea, and spatial distribution of floating microplastics in the vicinity of Italian minor islands
- Simulated human exposure to indoor airborne microplastics

4.1. OCCURRENCE AND DISTRIBUTION OD MICROPLASTICS IN THE SEDIMENTS OF THE LAGOON OF VENICE, ITALY

The monitoring survey conducted on the sediments of the Lagoon of Venice provided the first insight on microplastic contamination in this complex transitional environment (**Paper I**). The study highlighted diffuse microplastic pollution in the sediments (up to 2175 MP kg^{-1}), recording higher values than those previously published (Claessens et al., 2011). The MP concentration was influenced by specific hydrodynamics of the water bodies from where they were sampled, suggesting that microplastics accumulate at sites affected by low water dynamics (landward areas), where also finer sediments and associated contaminants tend to concentrate. The MP spatial distribution was positively correlated with the Metal Pollution Index (MPI), which is used to determine the overall metal pollution in environmental compartments (Usero et al., 1996). The correlation was more evident in areas affected by industrial activities, suggesting a potential link between MP and other micropollutants. The significant correlation between MP spatial distribution and the mud percentage measured at the sampling sites suggested that MP sink and accumulate similarly to the finer sediment fraction. The analytical approach used in this study was one of the first attempts to use μFTIR chemical mapping for MP analysis, as only one other study previously explored a similar approach (Harrison et al., 2012). This innovative method provided novel features to avoid the visual sorting of the particles, which has

been demonstrated to introduce bias in the analytical workflow (Hidalgo-Ruz et al., 2012). The results also demonstrated the applicability of this technique to successfully identify and quantify small MP in environmental matrices, which is a crucial aspect to assess for the feasibility of a specific analytical technique. This study provided the foundation and inspiration for further research, which consolidated the approach, establishing μFTIR-Imaging as a reliable analytical method for small MP analysis (Loder et al., 2015; Mintenig et al., 2017; Primpke et al., 2017; Simon et al., 2018; Liu et al., 2019).

4.2. SPATIAL DISTRIBUTION OF MICROPLASTICS IN THE NORTH ADRIATIC SEA AND IN THE VICINITY OF SOME OF THE ITALIAN MINOR ISLANDS

The coastal waters of Italy are affected by heavy anthropogenic pressure, and the coast of the Veneto region is influenced by the presence of several potential microplastic land-based sources, including the Po discharge, the biggest river in Italy which flows to the sea at the southern borders of this Region. The study included in this PhD thesis (**Paper II**) provided the first insight on the floating MP pollution in the North-West Adriatic Sea, contributing to increasing the available data on the occurrence and distribution of microplastics in the Adriatic Sea, and on a bigger scale, the Mediterranean Sea. All the investigated stations were contaminated by MP pollution, highlighting high spatial and temporal variabilities. Indeed, the MP concentration measured in March (2.2 ± 3.6 MP m^{-2}) were higher than what was reported in other studies carried out in the Mediterranean Sea, while the values recorded in April (0.2 ± 0.1 MP m^{-2}) were closer to other findings (Suaria et al., 2016; Avio et al., 2017). This variability could be associated with different environmental and hydrological features affecting the study area during the period investigated. March showed weather instability with rainfall contributing to increase the freshwater input (Braga et al., 2017) and therefore the mainland MP contribution via river discharge, mainly affecting the MP concentration at landward stations of the related transect. The high MP concentration measured at the remote station off Pellestrina might be influenced by passive transport of debris from the eastern shore of the Adriatic Sea, as well as from other materials previously discarded by the North-western part of the basin. The phenomenon is caused by a specific cyclonic circulation occurring in this area, driven by strong NE winds recorded immediately before the sampling, which generate a counter-clockwise gyre in the Northern Adriatic Sea. In April, the recorded hydrological parameters were more stable, possibly due to more stable weather conditions, leading to a lower input of riverine water (lower flow from Po River) as well as a more constant salinity. These factors combined can explain the lower and more constant MP concentration recorded in the second part of the survey. Overall, it appears that a transient convergence area occurs outside the borders of the Lagoon of Venice, and together with the Po river discharge, it contributes to spread MP pollution

in the surrounding waters, showing occasional peaks of concentration which are higher than those previously recorded in the same area by other studies.

Besides the coastal areas heavily affected by anthropogenic activities, where microplastic pollution is likely to derive mainly from landward sources, Italy has also several Minor Island spread all over the coast, and some of them are considered remote and "clean" areas, probably less affected by the plastic sources coming from the coast, but more likely subjected to sea-based microplastics. **Paper III** contributes with the first data on microplastic pollution from the Italian Minor Islands, comparing the result with two polluted sites (Po river delta and Tiber estuary). All the sites were contaminated by microplastics, even if a high spatial variability was observed among the samples, both in terms of polymer type and concentration. The mean value of MP concentration was 0.30 ± 0.04 MP m^{-3}, which falls inside the range of concentrations already recorded by other studies (Suaria et al., 2016; Avio et al., 2017). The MP concentration quantified by manta trawl was higher than the WP2 one at most of the sites, but no significant difference was recorded. Also, the polymer distribution between Manta and WP2 samples was quite patchy, with no specific trends recognizable. Black MP, which tends to adsorb more POPs than the other colours (Frias et al., 2010; Antunes et al., 2013), were generally the most abundant in the samples. POPs analyses (PCB and OC pesticides) confirmed the presence of organic micropollutants, showing that PCBs were the more abundant compounds, and their relative percentages were partially comparable with other previous studies (Endo et al., 2005). PCBs concentration in most of the surface (Manta) samples (2504 ng g^{-1} – 9.89 ng g^{-1}) was higher than in the deep water (WP2 FAO) samples (849.3 ng g^{-1} and 5.78 ng g^{-1}), but the concentration of these contaminants was not connected to the MP abundance. Although the surface samples were generally slightly more contaminated than the deepwater ones (MP and POPs), the overall uniformity of the results obtained between these two sampling techniques is an interesting finding. It suggests that the hydrodynamics at the surroundings of the minor islands are constantly mixing the water and, therefore its particulates, as well as limiting the accumulation of plastic debris in these specific areas (Browne et al., 2011). At the same time, the significantly higher MP load found at the river mouths confirmed that plastic litter density is strongly influenced by human activities (Cheung et al., 2018), and the most impacted areas are the closest to land-based sources, such as river systems.

4.3. SIMULATED HUMAN EXPOSURE TO INDOOR AIRBORNE MICROPLASTIC

Direct exposure to airborne microplastic pollution is a potential threat to human health. The outcomes of **Paper IV** clearly show how MP down to 11 μm were ubiquitous at the three locations studied, with a mean concentration value of 9.3 ± 5.8 MP m^{-3} and a maximum value of 16.2 MP m^{-3}, which is equal to 11.3 MP inhaled per hour. At this rate, a person could accumulate in the upper airways up to 272 MP over 24 hours. Although most of the particles identified likely derived from shed skin, the MP percentage was around 4% of the inhaled organic materials detected, which is a non-negligible fraction. Of these plastic particles, polyester dominated the polymer composition. Its concentration was of the same order of magnitude as cellulose-based particles, reflecting the massive use of this synthetic material in nowadays textile industry. Other polymers were found in low concentrations, but it is important to note that also polyurethane and paint particles were identified. Both these polymer types could have adverse effects on human health, due to the chemicals contained (Flame retardants in polyurethanes and biocides in paints). Although ubiquitous, the MP concentration was quite different among the samples and among apartments. This study also addressed the size distribution of the microplastics, measuring both their major and minor dimension and trying to distinguish particles from fibres on the assumption that a fibre has a ratio major/minor dimension > 3. Although with some method limitations (further details in **Paper** IV, SI), the results showed that fragments were the majority of the identified items (not fibres), suggesting that when size decreases, it is likely to find more of this type of item instead of elongated ones. An interesting finding was also related to the size of the identified particles, as the overall size of MP items was smaller than the nonsynthetic particles. Although the methodology developed in this study allowed to detect only particles typically removed by clearance mechanisms and probably not reaching the deep airways, **Paper IV** introduces novel aspects in microplastic science and constitutes a valid proof of concept of human exposure to airborne microplastic pollution. Moreover, it highlights that inhaled MP cannot be excluded from harming human health, also suggesting that smaller particles are likely to be found in indoor air.

CHAPTER 5. CONCLUSIONS

The PhD study was divided into three main research questions, which were answered following the established objectives. The following conclusions were drawn for each question.

1. *Are the sediments of the Lagoon of Venice contaminated by microplastic particles? Is it possible to track back potential sources according to the MP concentration? Is there any relationship between the MP concentration and the sediment grain size? Is it possible to develop and apply a µFTIR method to avoid the pre-sorting of potential MP particles?*

The sediments of a transitional environment like the Lagoon of Venice are massively contaminated by MP pollution, which is likely influenced by specific anthropogenic sources, such as urban and industrial discharge, even if it is not possible to clearly backtrack the specific sources. The specific local hydrodynamics related to tides, and riverine discharge, are likely to also play an active role in spreading the microplastic pollution in the study area. MP tend to concentrate in areas influenced by low water dynamics, which are quite common in the inner parts of the lagoon, where also finer sediments and associated contaminants tend to accumulate. The pre-sorting of small MP is avoidable applying µFTIR-chemical mapping to the filter surface, even with some limitation. This method was one of the first examples of semi-automated spectroscopic analysis for MP detection and quantification, inspiring further research which led to the µFTIR-Imaging for small MP analysis, considered at present one of the most suitable analytical approaches for unbiased MP detection.

2. *Does microplastic pollution contaminate the surface water along the Italian coast? Which is the actual concentration and which are the main drivers for microplastic spreading in this area? Is it still possible to find comparatively virgin areas, especially when comparing them to others which are highly impacted by anthropogenic activities?*

The coastal waters of Italy are heavily affected by MP pollution, and the coast of the Veneto region is one of the most impacted by potential land-based sources of plastic debris. The MP concentration detected was highly variable in spatial and temporal distribution, likely influenced by several hydrological and geographical features, which all combined contributes to a very complex scenario. The Po River discharge contributes to the MP land-based sources, as the biggest river of Italy flows to the sea at the southern borders of this Region through the Po Valley (Pianura Padana), collecting water from the most heavily industrialised regions through hundreds of tributaries. Finally, a transient convergence area located in the surroundings of the

Lagoon of Venice, and possibly driven by a cyclonic circulation induced by specific weather conditions, could increase the accumulation of floating debris in this specific area.

Besides highly impacted coastal areas, where microplastic pollution is likely to derive mainly from landward sources, microplastics were also found at several Minor Islands along the coasts of the country, some of them previously considered remote and clean areas. It appears that the major MP contribution in these areas is likely sea-based, and the constant mixing of the surface and subsurface water, driven by specific hydrodynamics is limiting the accumulation of plastic debris.

3. *Are humans directly exposed to airborne indoor microplastics? To which extent? How can human exposure be measured?*

Human exposure to airborne microplastics has been proven, and indoor environments seem to highly contribute to airborne microplastic pollution, due to the multiple sources available and the limited exchange of air which occurs at some indoor sites. Although the data available are limited, the study conducted in the framework of this PhD thesis clearly showed the ubiquity of MP pollution in the investigated sites. The percentage of this synthetic particulate pollutant was non-negligible and comparable to other nonsynthetic particles (cellulose-based items), and it will probably increase in the future, due to the massive production of polymeric based fabrics and furniture, which are gradually substituting the natural materials, such as cotton and wool in the textile industry, and wood in the furniture industry. Even though there are still several limitations which need to be addressed, the novel sampling approach, as well as the analytical method applied, provided valid proof of concept of direct human exposure to airborne microplastic pollution. Inhaled MP cannot be excluded from harming human health, also because smaller particles are likely to pollute the indoor air.

CHAPTER 6. MAIN CONTRIBUTION TO THE SCIENCE AND FUTURE PERSPECTIVES

Microplastics are considered an emerging pollutant, but the scientific knowledge, even if still limited, is contributing to quickly changing the perception towards this topic, which is nowadays already considered a real threat to our environment, and ultimately, to human welfare. Significant progress has been achieved in MP science since the first evidence of MP pollution were discovered, thanks to a joint effort which involves many different branches of science.

Analytical techniques are the key to assess the magnitude of an environmental threat correctly, and the methods described in this PhD study clearly show how different approaches must be applied to different environmental compartments and matrices, to understand better the dynamics involved in this complex scenario.

Paper I was a study conducted in 2012, and published in 2013, introducing novel aspects related to the identification and quantification of microplastics via infrared spectroscopy in complex environmental matrices, such as sediments. It also served as a spark to further develop more reliable and trustable methods, which are nowadays considered the most suitable to obtain unbiased outputs to assess the level of pollution in environmental samples. The study also highlighted the need for further research to assess the uptake of microplastics by local benthic-feeders (especially bivalves), which are living at the water-sediment interface, and considered an important economic resource in this particular area.

Paper II and **Paper III** contribute to improving the knowledge on microplastic pollution in the coastal areas of Italy, one of the European Countries with the largest coastal extension and the most prominent industrial activities, and which is responsible for a large percentage of the discharged plastic litters in the Mediterranean Sea. The sampling techniques, as well as the analytical approach, provide a useful portfolio of information that can be used for future large scale monitoring surveys, also highlighting the complexity of the variables at stakes when investigating the spatial and temporal distribution of floating microplastic debris. It is of paramount importance to integrate hydrodynamic modelling combined to field studies in future investigations, as the key to understand such dynamics and complex phenomena.

The study carried out on human exposure to indoor microplastic pollution (**Paper IV**) is by far the most innovative in this PhD thesis. It opens new paths to the investigation of microplastic pollution, especially considering how this emerging contaminant has

shown to be ubiquitous. The MP threat is not only linked to indirect human exposure, but it could directly affect humans in everyday life, while we do the most natural action of all: breathing. It is of paramount importance to increase the knowledge on airborne MP pollution, and focus the effort to assess the potential human exposure to this emerging pollutant better. It can be done developing dedicated sampling methodologies which take into account the variables involved in the complex mechanisms of inhalation and breathing of suspended particulates, as well as improving the analytical methodologies. The traditional FTIR technology is limited by diffraction limits; therefore, other approaches need to be explored. The technology to push the analytical size range even further down is somehow available, even confined to high-end research-grade instrumentation, like Imaging-μRaman, ESEM-μRaman spectroscopy, Optical Photothermal IR spectroscopy and Nano-FTIR. It is up to the scientific community to develop such technologies and make them finally available for the analysis of environmental samples.

LITERATURE LIST

Ainsworth, B.E., Haskell, W.L., Whitt, M.C., Irwin, M.L., Swartz, A.M., Strath, S.J., O'Brien, W.L., Bassett, D.R., Schmitz, K.H., Emplaincourt, P.O., Jacobs, D.R., Leon, A.S., 2000. Compendium of Physical Activities: an update of activity codes and MET intensities. Med. Sci. Sport. Exerc. 32, S498–S516. https://doi.org/10.1097/00005768-200009001-00009

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Antunes, J.C., Frias, J.G.L., Micaelo, A.C., Sobral, P., 2013. Resin pellets from beaches of the Portuguese coast and adsorbed persistent organic pollutants. Estuar. Coast. Shelf Sci. 130, 62–69. https://doi.org/10.1016/j.ecss.2013.06.016

Avio, C.G., Gorbi, S., Regoli, F., 2017. Plastics and microplastics in the oceans: From emerging pollutants to emerged threat. Mar. Environ. Res. 128, 2–11. https://doi.org/https://doi.org/10.1016/j.marenvres.2016.05.012

Barboza, L.G.A., Dick Vethaak, A., Lavorante, B.R.B.O., Lundebye, A.K., Guilhermino, L., 2018. Marine microplastic debris: An emerging issue for food security, food safety and human health. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2018.05.047

Barboza, L.G.A., Gimenez, B.C.G., 2015. Microplastics in the marine environment: Current trends and future perspectives. Mar. Pollut. Bull. 97, 5–12. https://doi.org/10.1016/j.marpolbul.2015.06.008

Barnes, D.K. a, Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205

Bivolarova, M., Kierat, W., Zavrl, E., Popiolek, Z., Melikov, A., 2017. Effect of airflow interaction in the breathing zone on exposure to bio-effluents. Build. Environ. 125, 216–226. https://doi.org/10.1016/j.buildenv.2017.08.043

Boucher, C., Morin, M., Bendell, L.I., 2016. The influence of cosmetic microbeads on the sorptive behavior of cadmium and lead within intertidal sediments: A laboratory study. Reg. Stud. Mar. Sci. 3, 1–7. https://doi.org/10.1016/j.rsma.2015.11.009

Braga, F., Zaggia, L., Bellafiore, D., Bresciani, M., Giardino, C., Lorenzetti, G., Maicu, F., Manzo, C., Riminucci, F., Ravaioli, M., Brando, V.E., 2017. Mapping turbidity patterns in the Po river prodelta using multi-temporal Landsat 8 imagery. Estuar. Coast. Shelf Sci. 198, 555–567.

https://doi.org/https://doi.org/10.1016/j.ecss.2016.11.003

Browne, M. a, Galloway, T.S., Thompson, R.C., 2010. Spatial patterns of plastic debris along Estuarine shorelines. Environ. Sci. Technol. 44, 3404–9. https://doi.org/10.1021/es903784e

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: Sources and sinks. Environ. Sci. Technol. 45, 9175–9179. https://doi.org/10.1021/es201811s

Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. 91, 174–182. https://doi.org/10.1016/j.watres.2016.01.002

Chapman, P.M., Wang, F., 2001. Assessing sediment contamination in estuaries. Environ. Toxicol. Chem. 20, 3–22. https://doi.org/10.1002/etc.5620200102

Cheung, P.K., Fok, L., Hung, P.L., Cheung, L.T.O., 2018. Spatio-temporal comparison of neustonic microplastic density in Hong Kong waters under the influence of the Pearl River Estuary. Sci. Total Environ. 628–629, 731–739. https://doi.org/10.1016/J.SCITOTENV.2018.01.338

Claessens, M., De Meester, S., Van Landuyt, L., De Clerck, K., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62, 2199–204. https://doi.org/10.1016/j.marpolbul.2011.06.030

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2013.03.009

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4, 4528. https://doi.org/10.1038/srep04528

Collignon, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–4. https://doi.org/10.1016/j.marpolbul.2012.01.011

CONTAM, 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA J. 14, 4501–4531. https://doi.org/10.2903/j.efsa.2016.4501

Cooper, D. a, Corcoran, P.L., 2010. Effects of mechanical and chemical processes on the degradation of plastic beach debris on the island of Kauai, Hawaii. Mar. Pollut. Bull. 60, 650–4. https://doi.org/10.1016/j.marpolbul.2009.12.026

Cox, K.D., Covernton, G.A., Davies, H.L., Dower, J.F., Juanes, F., Dudas, S.E., 2019. Human Consumption of Microplastics. Environ. Sci. Technol. 53. https://doi.org/10.1021/acs.est.9b01517

Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J. I., Ubeda, B. J., Gálvez, Á., Irigoien, X., Duarte, C.M., 2015. Plastic Accumulation in the Mediterranean Sea. PLoS One 10. https://doi.org/e0121762. doi:10.1371/ journal.pone.0121762 Academic

Crawford, C.B., Quinn, B., 2017a. 8 - Microplastic collection techniques, in: Crawford, C.B., Quinn, B.B.T.-M.P. (Eds.), . Elsevier, pp. 179–202. https://doi.org/https://doi.org/10.1016/B978-0-12-809406-8.00008-6

Crawford, C.B., Quinn, B., 2017b. 10 - Microplastic identification techniques, in: Crawford, C.B., Quinn, B.B.T.-M.P. (Eds.), . Elsevier, pp. 219–267. https://doi.org/https://doi.org/10.1016/B978-0-12-809406-8.00010-4

de Lucia, G.A., Vianello, A., Camedda, A., Vani, D., Tomassetti, P., Coppa, S., Palazzo, L., Amici, M., Romanelli, G., Zampetti, G., Cicero, A., Carpentieri, S., Di Vito, S., Matiddi, M., 2018. Sea Water Contamination in the Vicinity of the Italian Minor Islands Caused by Microplastic Pollution. Water 10, 1108. https://doi.org/10.3390/w10081108

Doyle, M.J., Watson, W., Bowlin, N.M., Sheavly, S.B., 2011. Plastic particles in coastal pelagic ecosystems of the Northeast Pacific ocean. Mar. Environ. Res. 71, 41–52. https://doi.org/10.1016/j.marenvres.2010.10.001

Dris, Rachid, Gasperi, Johnny, Mirande, Cecile, Mandin, C., Guerrouache, Mohamed, Langlois, Valerie, Tassin, B., 2017. A fi rst overview of textile fi bers , including microplastics , in indoor and outdoor environments * 221, 453–458. https://doi.org/10.1016/j.envpol.2016.12.013

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic contamination in an urban area: A case study in Greater Paris. Environ. Chem. 12, 592–599. https://doi.org/10.1071/EN14167

Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2016. Synthetic fi bers in atmospheric fallout : A source of microplastics in the environment ? MPB 104, 290–293. https://doi.org/10.1016/j.marpolbul.2016.01.006

Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects.

Environ. Sci. Eur. 28, 1–25. https://doi.org/10.1186/s12302-015-0069-y

Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics ≥ 10 μm in the Atlantic Ocean and their modelled vertical distribution. Mar. Pollut. Bull. 100, 70–81. https://doi.org/10.1016/J.MARPOLBUL.2015.09.027

Endo, S., Takizawa, R., Okuda, K., Takada, H., Chiba, K., Kanehiro, H., Ogi, H., Yamashita, R., Date, T., 2005. Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: Variability among individual particles and regional differences. Mar. Pollut. Bull. 50, 1103–1114. https://doi.org/10.1016/j.marpolbul.2005.04.030

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLoS One 1–15. https://doi.org/10.1371/journal.pone.0111913

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., Amato, S., 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar. Pollut. Bull. 77, 177–182. https://doi.org/10.1016/j.marpolbul.2013.10.007

Eriksen, M., Moore, C., Lattin, G.L., Wilson, S., 2009. Three easy-to-ship and easy-to-use aluminium neuston trawls designed and fabricated for use on different vessels of opportunity . Results of field tests and preliminary intercallibra > on efforts . 26, 2244.

Eschenbacher, W.L., Kreiss, K., Lougheed, M.D., Pransky, G.S., Day, B., Castellan, R.M., 1999. Nylon Flock–Associated Interstitial Lung Disease. Am. J. Respir. Crit. Care Med. 159, 2003–2008. https://doi.org/10.1164/ajrccm.159.6.9808002

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046

Field Listing :: Coastline — The World Factbook - Central Intelligence Agency [WWW Document], n.d. URL https://www.cia.gov/library/publications/the-world-factbook/fields/282.html (accessed 1.3.20).

Fischer, D., Käppler, A., Fischer, F., Brandt, J., Bittrich, L., Eichhorn, K.-J., 2019. Identification of Microplastics in Environmental Samples. G.I.T. Lab. J. 23, 43–45.

Fischer, M., Scholz-Böttcher, B.M., 2019. Microplastics analysis in environmental

samples-recent pyrolysis-gas chromatography-mass spectrometry method improvements to increase the reliability of mass-related data. Anal. Methods 11, 2489–2497. https://doi.org/10.1039/c9ay00600a

Fischer, M., Scholz-Böttcher, B.M., 2017. Simultaneous Trace Identification and Quantification of Common Types of Microplastics in Environmental Samples by Pyrolysis-Gas Chromatography-Mass Spectrometry. Environ. Sci. Technol. 51, 5052–5060. https://doi.org/10.1021/acs.est.6b06362

Fok, L., Cheung, P.K., 2015. Hong Kong at the Pearl River Estuary: A hotspot of microplastic pollution. Mar. Pollut. Bull. 99, 112–118. https://doi.org/10.1016/j.marpolbul.2015.07.050

Frias, J.P.G.L., Sobral, P., Ferreira, a M., 2010. Organic pollutants in microplastics from two beaches of the Portuguese coast. Mar. Pollut. Bull. 60, 1988–92. https://doi.org/10.1016/j.marpolbul.2010.07.030

Fries, E., Dekiff, J.H., Willmeyer, J., Nuelle, M.-T., Ebert, M., Remy, D., 2013. Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. Environ. Sci. Process. Impacts 15, 1949–56. https://doi.org/10.1039/c3em00214d

Gačić, M., Kovačević, V., Mazzoldi, A., Paduan, J., Arena, F., Mosquera, I.M., Gelsi, G., Arcari, G., 2002. Measuring water exchange between the venetian lagoon and the open sea. Eos (Washington. DC). 83. https://doi.org/10.1029/2002EO000147

Gasperi, J., Wright, S.L., Dris, R., Collard, F., Mandin, C., Guerrouache, M., Langlois, V., Kelly, F.J., Tassin, B., 2018. Microplastics in air: Are we breathing it in? Curr. Opin. Environ. Sci. Heal. 1, 1–5. https://doi.org/10.1016/j.coesh.2017.10.002

Gerdts, G., 2019. Defining the Baselines and Standards for Microplastics Analyses in European Waters (JPI-O BASEMAN).

GESAMP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment". Reports Stud. GESAMP 90, 96. https://doi.org/10.13140/RG.2.1.3803.7925

Ghiaus, C., Allard, F., Wilson, M., 2005. Natural Ventilation in the Urban Environment Assessment and Design.

Gomiero, A., Øysæd, K.B., Agustsson, T., van Hoytema, N., van Thiel, T., Grati, F., 2019. First record of characterization, concentration and distribution of microplastics in coastal sediments of an urban fjord in south west Norway using

a thermal degradation method. Chemosphere 227, 705–714. https://doi.org/10.1016/j.chemosphere.2019.04.096

H. Greim, P. Borm, R. Schins, K. Do, 2001. Toxicity of fibers and particles. Report of the workshop held in Munich, Germany, 26 – 27 October 2000. Inhal. Toxicol., null 13, 737–754. https://doi.org/10.1080/08958370118273

Harrison, J.P., Ojeda, J.J., Romero-González, M.E., 2012. The applicability of reflectance micro-Fourier-transform infrared spectroscopy for the detection of synthetic microplastics in marine sediments. Sci. Total Environ. 416, 455–63. https://doi.org/10.1016/j.scitotenv.2011.11.078

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environ. Sci. Technol. 46, 3060–3075.

Imhof, H.K., Schmid, J., Niessner, R., Ivleva, N.P., Laforsch, C., 2012. A novel , highly efficient method for the separation and quantification of plastic particles in sediments of aquatic. Limnol Ocean. Methods 524–537. https://doi.org/10.4319/lom.2012.10.524

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science (80-.). 347, 768–771. https://doi.org/[doi: 10.1126/science.1260352

Jan Kole, P., Löhr, A.J., Van Belleghem, F.G.A.J., Ragas, A.M.J., 2017. Wear and tear of tyres: A stealthy source of microplastics in the environment. Int. J. Environ. Res. Public Health 14. https://doi.org/10.3390/ijerph14101265

Kanhai, L.D.K., Officer, R., Lyashevska, O., Thompson, R.C., O'Connor, I., 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. Mar. Pollut. Bull. 115, 307–314. https://doi.org/10.1016/j.marpolbul.2016.12.025

Karlsson, T.M., Grahn, H., Bavel, V., Geladi, P., 2016. Hyperspectral imaging and data analysis for detecting and determining plastic contamination in seawater filtrates 149, 141–149. https://doi.org/10.1255/jnirs.1212

Klein, M., Fischer, E.K., 2019. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Sci. Total Environ. 685, 96–103. https://doi.org/10.1016/j.scitotenv.2019.05.405

Koelmans, A.A., Besseling, E., Shim, W.J., 2015. Nanoplastics in the Aquatic Environment . Critical Review 325–340. https://doi.org/10.1007/978-3-319-16510-3

Lachowicz, T., Kościelniak, P., Zięba-Palus, J., 2012. Analysis of rubber samples by PY-GC/MS for forensic purposes. Z Zagadnien Nauk Sadowych 91, 195–207.

Lachowicz, T., Zieba-Palus, J., Kościelniak, P., 2013. Chromatographic Analysis of Tire Rubber Samples as the Basis of Their Differentiation and Classification for Forensic Purposes. Anal. Lett. 46, 2332–2344. https://doi.org/10.1080/00032719.2013.800536

Lacombe, H., Gascard, J.C., Cornella, J., Béthoux, J.P., 1981. Response of the Mediterranean to the water and energy fluxes across its surface, on seasonal and interannual scales. Oceanol. Acta 4, 247–255.

Larkin, P., 2011. Chapter 1 - Introduction: Infrared and Raman Spectroscopy, in: Larkin, P.B.T.-I. and R.S. (Ed.), . Elsevier, Oxford, pp. 1–5. https://doi.org/https://doi.org/10.1016/B978-0-12-386984-5.10001-1

Lassen, C., 2015. Microplastics Occurrence , effects and sources of releases.

Lebreton, L.C.M., Greer, S.D., Borrero, J.C., 2012. Numerical modelling of floating debris in the world's oceans. Mar. Pollut. Bull. 64, 653–661. https://doi.org/10.1016/j.marpolbul.2011.10.027

Liu, F., Olesen, K.B., Borregaard, A.R., Vollertsen, J., 2019. Microplastics in urban and highway stormwater retention ponds. Sci. Total Environ. 671, 992–1000. https://doi.org/10.1016/j.scitotenv.2019.03.416

Liubartseva, S., Coppini, G., Lecci, R., Creti, S., 2016. Regional approach to modeling the transport of floating plastic debris in the Adriatic Sea. Mar. Pollut. Bull. 103, 115–127. https://doi.org/10.1016/j.marpolbul.2015.12.031

Löder, M.G.J., Gerdts, G., 2015. Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal BT - Marine Anthropogenic Litter, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), . Springer International Publishing, Cham, pp. 201–227. https://doi.org/10.1007/978-3-319-16510-3_8

Löder, M.G.J., Imhof, H.K., Ladehoff, M., Löschel, L.A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I., Laforsch, C., Gerdts, G., 2017. Enzymatic Purification of Microplastics in Environmental Samples. Environ. Sci. Technol. 51, 14283–14292. https://doi.org/10.1021/acs.est.7b03055

Löder, M.G.J., Kuczera, M., Claudia, G.G.M.S.L., 2015. Focal plane array detector-based micro-Fourier-transform infrared imaging for the analysis of microplastics in environmental samples. CSIRO Environ. Chem. https://doi.org/http://dx.doi.org/10.1071/EN14205 RESEARCH

Lusher, A.L., Hernandez-Milian, G., Brien, J.O., Berrow, S., Connor, I.O., Officer, R., 2015. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean : The True's beaked whale Mesoplodon mirus. Environ. Pollut. 199, 185–191. https://doi.org/10.1016/j.envpol.2015.01.023

Mason, S.A., Welch, V.G., Neratko, J., 2018. Synthetic Polymer Contamination in Bottled Water. Front. Chem. 6. https://doi.org/10.3389/fchem.2018.00407

Matsuguma, Y., Takada, H., Kumata, H., Kanke, H., Sakurai, S., Suzuki, T., Itoh, M., Okazaki, Y., Boonyatumanond, R., Zakaria, M.P., Weerts, S., Newman, B., 2017. Microplastics in Sediment Cores from Asia and Africa as Indicators of Temporal Trends in Plastic Pollution. Arch. Environ. Contam. Toxicol. 73, 230–239. https://doi.org/10.1007/s00244-017-0414-9

Mintenig, S.M., Int-Veen, I., Löder, M.G.J., Primpke, S., Gerdts, G., 2017. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Res. 108, 365–372. https://doi.org/10.1016/j.watres.2016.11.015

Moreira, F.T., Prantoni, A.L., Martini, B., de Abreu, M.A., Stoiev, S.B., Turra, A., 2016. Small-scale temporal and spatial variability in the abundance of plastic pellets on sandy beaches: Methodological considerations for estimating the input of microplastics. Mar. Pollut. Bull. 102, 114–121. https://doi.org/10.1016/j.marpolbul.2015.11.051

Nagle, D.J., George, G.A., Rintoul, L., Fredericks, P.M., 2010. Use of micro-ATR/FTIR imaging to study heterogeneous polymer oxidation by direct solvent casting onto the ATR IRE. Vib. Spectrosc. 53, 24–27. https://doi.org/10.1016/J.VIBSPEC.2010.01.018

Napper, I.E., Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. Mar. Pollut. Bull. 112. https://doi.org/10.1016/j.marpolbul.2016.09.025

Ng, K.L., Obbard, J.P., 2006. Prevalence of microplastics in Singapore's coastal marine environment. Mar. Pollut. Bull. 52, 761–767. https://doi.org/https://doi.org/10.1016/j.marpolbul.2005.11.017

Nielsen, P. V., Jensen, R.L., Litewnicki, M., Zajas, J., 2009. Experiments on the Microenvironment and Breathing of a Person in Isothermal and Stratified Surroundings. 9th Int. Conf. Exhib. 1–4.

Nuelle, M.-T., Dekiff, J.H., Remy, D., Fries, E., 2014. A new analytical approach for monitoring microplastics in marine sediments. Environ. Pollut. 184, 161–9. https://doi.org/10.1016/j.envpol.2013.07.027

Organisation, I., Standardization, F.O.R., Normalisation, O.I.D.E., 1988. International Standard Iso.

Organization, W.H., 1997. Determination of airborne fibre number concentrations: a recommended method, by phase-contrast optical microscopy (membrane filter method). https://doi.org/ISBN 92 4 154496 1

Papastefanou, C., 2008. Chapter 6 Aerosol sampling and measurement techniques, in: Papastefanou, C.B.T.-R. in the E. (Ed.), Radioactive Aerosols. Elsevier, pp. 113–159. https://doi.org/https://doi.org/10.1016/S1569-4860(07)12006-4

Plastics Europe, 2018. Plastics - the Facts 2018. An analysis of European plastics production , demand and waste data. Belgium.

Primpke, S., Lorenz, C., Rascher-Friesenhausen, R., Gerdts, G., 2017. An automated approach for microplastics analysis using focal plane array (FPA) FTIR microscopy and image analysis. Anal. Methods 8, 1–224. https://doi.org/10.1039/x0xx00000x

Rocchia, M., Ruff, I., Vianello, A., 2017. Microplastic identification and characterization by Raman imaging spectroscopy. ThermoScientific.

Rocha-Santos, T., Duarte, A.C., 2014. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC Trends Anal. Chem. 65, 47–53. https://doi.org/10.1016/j.trac.2014.10.011

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 1–7. https://doi.org/10.1038/srep03263

Serranti, S., Fiore, L., Bonifazi, G., Takeshima, A., Takeuchi, H., Kashiwada, S., 2019. Microplastics characterization by hyperspectral imaging in the SWIR range, in: Proceedings of SPIE. p. 38. https://doi.org/10.1117/12.2542793

Shim, W.J., Hong, S.H., Eo, S., 2018. Chapter 1 - Marine Microplastics: Abundance, Distribution, and Composition, in: Zeng, E.Y.B.T.-M.C. in A.E. (Ed.), . Elsevier, pp. 1–26. https://doi.org/https://doi.org/10.1016/B978-0-12-813747-5.00001-1

Shim, W.J., Hong, S.H., Eo, S.E., 2017. Identification methods in microplastic analysis: A review. Anal. Methods. https://doi.org/10.1039/c6ay02558g

Shim, W.J. oon, Thomposon, R.C., 2015. Microplastics in the Ocean. Arch. Environ. Contam. Toxicol. 69, 265–268. https://doi.org/10.1007/s00244-015-0216-x

Simon, M., van Alst, N., Vollertsen, J., 2018. Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging. Water Res. 142, 1–9. https://doi.org/10.1016/j.watres.2018.05.019

Simon, M., Vianello, A., Vollertsen, J., 2019. Removal of > 10 µm microplastic particles from treated wastewater by a disc filter. Water (Switzerland) 11. https://doi.org/10.3390/w11091935

Smith, E., Dent, G., 2005. Modern Raman Spectroscopy - A Practical Approach. Mod. Raman Spectrosc. - A Pract. Approach 5, 1–210. https://doi.org/10.1002/0470011831

Song, Kyoung, Y., Hee, S., Jang, M., Myung, G., Rani, M., Lee, J., Joon, W., 2015. A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples 1–8.

Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Jung, S.W., Shim, W.J., 2018. Corrections to " Combined E ff ects of UV Exposure Duration and Mechanical Abrasion on Microplastic Fragmentation by Polymer Type " 7, 3831–3832. https://doi.org/10.1021/acs.est.8b00172

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup: Synthetic polymers in Mediterranean surface waters. Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep37551

Systematic Identification of MicroPlastics in the Environment (siMPle) [WWW Document], 2019. URL https://simple-plastics.eu/ (accessed 1.9.20).

Tagg, A.S., Harrison, J.P., Ju-Nam, Y., Sapp, M., Bradley, E.L., Sinclair, C.J., Ojeda, J.J., 2017. Fenton's reagent for the rapid and efficient isolation of microplastics from wastewater. Chem. Commun. 53, 372–375. https://doi.org/10.1039/c6cc08798a

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304, 838. https://doi.org/10.1126/science.1094559

Unice, K.M., Kreider, M.L., Panko, J.M., 2013. Comparison of Tire and Road Wear Particle Concentrations in Sediment for Watersheds in France, Japan, and the United States by Quantitative Pyrolysis GC/MS Analysis. Environ. Sci. Technol. 47, 8138–8147. https://doi.org/10.1021/es400871j

USEPA, 1987. The Risk Assessment Guidelines of 1986-EPA/600/8-87/045.

Usero, J., González-Regalado, E., Gracia, I., 1996. Trace metals in the bivalve mollusc Chamelea gallina from the Atlantic coast of southern Spain. Mar. Pollut. Bull. 32, 305–310. https://doi.org/10.1016/0025-326X(95)00209-6

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015a. Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats. Environ. Pollut. 199, 10–17. https://doi.org/10.1016/j.envpol.2015.01.008

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015b. Microplastics in sediments: A review of techniques, occurrence and effects. Mar. Environ. Res. 111, 5–17. https://doi.org/10.1016/j.marenvres.2015.06.007

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010

Van Cauwenberghe, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. Environ. Pollut. 182, 495–9. https://doi.org/10.1016/j.envpol.2013.08.013

van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., van Franeker, J.A., Eriksen, M., Siegel, D., Galgani, F., Law, K.L., 2015. A global inventory of small floating plastic debris. Environ. Res. Lett. 10, 124006. https://doi.org/10.1088/1748-9326/10/12/124006

Veerasingam, S., Mugilarasan, M., Venkatachalapathy, R., Vethamony, P., 2016. Influence of 2015 flood on the distribution and occurrence of microplastic pellets along the Chennai coast, India. Mar. Pollut. Bull. 109, 196–204. https://doi.org/10.1016/J.MARPOLBUL.2016.05.082

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., Da Ros, L., 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuarine, Coastal. Estuar. Coast. Shelf Sci. 130, 54–61. https://doi.org/http://dx.doi.org/10.1016/j.ecss.2013.03.022

Vianello, A., Da Ros, L., Boldrin, A., Marceta, T., Moschino, V., 2018. First evaluation of floating microplastics in the Northwestern Adriatic Sea. Environ. Sci. Pollut. Res. 25, 28546–28561. https://doi.org/10.1007/s11356-018-2812-6

Vianello, A., Jensen, R.L., Liu, L., Vollertsen, J., 2019. Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Sci. Rep. 1–11. https://doi.org/10.1038/s41598-019-45054-w

Villarrubia-Gómez, P., Cornell, S.E., Fabres, J., 2018. Marine plastic pollution as a

planetary boundary threat – The drifting piece in the sustainability puzzle. Mar. Policy 0–1. https://doi.org/10.1016/j.marpol.2017.11.035

Warheit, D.B., Hart, G.A., Hesterberg, T.W., Collins, J.J., Dyer, W.M., Swaen, G.M.H., Castranova, V., Soiefer, A.I., Kennedy, G.L., 2001. Potential pulmonary effects of man-made organic fiber (MMOF) dusts. Crit. Rev. Toxicol. 31, 697–736. https://doi.org/10.1080/20014091111965

Waring, R.H., Harris, R.M., Mitchell, S.C., 2018. Plastic contamination of the food chain: A threat to human health? Maturitas 115, 64–68. https://doi.org/10.1016/j.maturitas.2018.06.010

Xu, C., Nielsen, P. V., Gong, G., Jensen, R.L., Liu, L., 2015a. Influence of air stability and metabolic rate on exhaled flow. Indoor Air 25, 198–209. https://doi.org/10.1111/ina.12135

Xu, C., Nielsen, P. V., Gong, G., Liu, L., Jensen, R.L., 2015b. Measuring the exhaled breath of a manikin and human subjects. Indoor Air 25, 188–197. https://doi.org/10.1111/ina.12129

Xu, C., Nielsen, P. V., Liu, L., Jensen, R.L., Gong, G., 2018. Impacts of airflow interactions with thermal boundary layer on performance of personalized ventilation. Build. Environ. 135, 31–41. https://doi.org/10.1016/j.buildenv.2018.02.048

Zhang, S., Wang, J., Liu, X., Qu, F., Wang, Xueshan, Wang, Xinrui, Li, Y., Sun, Y., 2019. Microplastics in the environment: A review of analytical methods, distribution, and biological effects. TrAC - Trends Anal. Chem. https://doi.org/10.1016/j.trac.2018.12.002

Ziajahromi, S., Neale, P.A., Leusch, F.D.L., 2016. Wastewater treatment plant effluent as a source of microplastics: review of the fate, chemical interactions and potential risks to aquatic organisms. Water Sci. Technol. wst2016414. https://doi.org/10.2166/wst.2016.414

Zubris, K.A. V, Richards, B.K., 2005. Synthetic fibers as an indicator of land application of sludge. Environ. Pollut. 138, 201–211. https://doi.org/https://doi.org/10.1016/j.envpol.2005.04.013

ISSN (online): 2446-1636
ISBN (online): 978-87-7210-602-1 AALBORG UNIVERSITY PRESS