JPI OCEANS

BASEMAN
MICROPLASTICS ANALYSES IN EUROPEAN WATERS

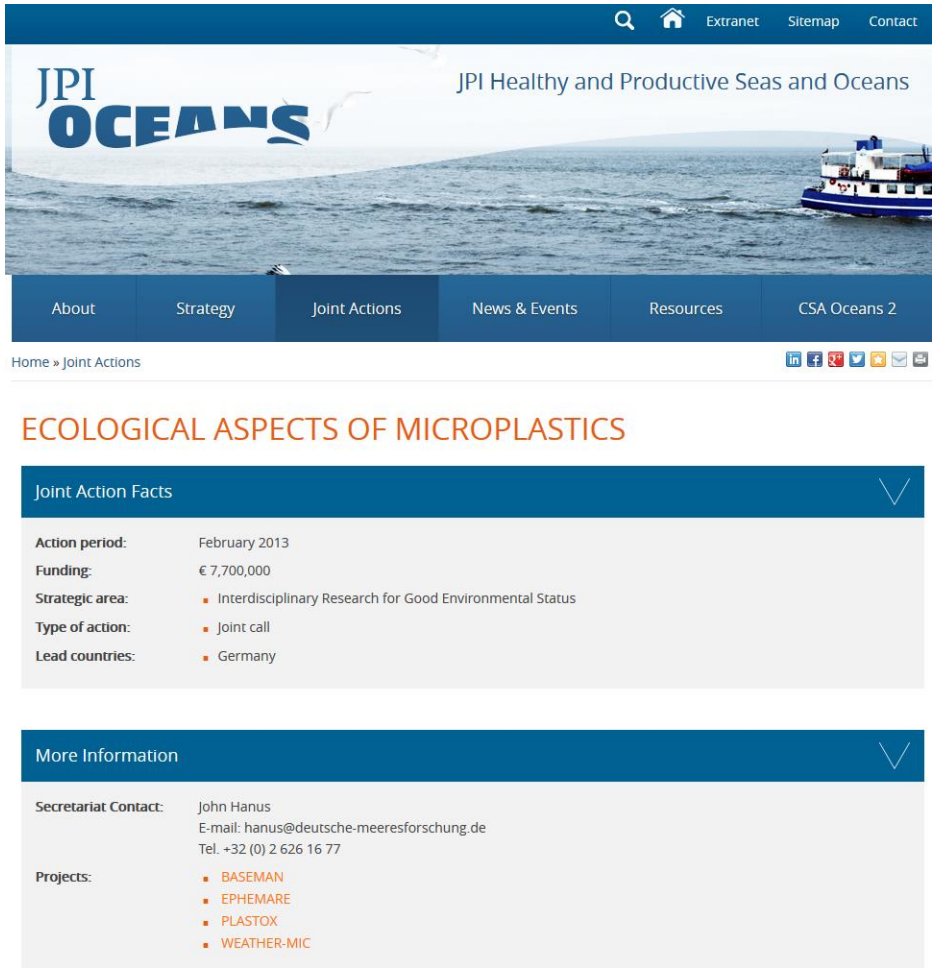
ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG

The Science of Microplastics in the World Ocean
An International Workshop to Formulate Next Steps in Understanding the Fate, Distribution, Impacts, and Technology Development Necessary to Push the Science

Defining the Baselines and Standards for Microplastics Analyses in European Waters (BASEMAN), lessons learned (?)

Gunnar Gerdts and the BASEMAN consortium
Shelf Seas Systems Ecology
Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research

The JPI-O pilot action Ecological Aspects of Microplastics



- Proposed in 2013
- Management Board (MB) **defined the scope of this pilot action as comprising methods, monitoring and effects of microplastics**
- Four projects were selected (funding 1/2016)
  - **BASEMAN** - Defining the baselines and standards for microplastics analyses in European waters
  - **EPHEMARE** - Ecotoxicological effects

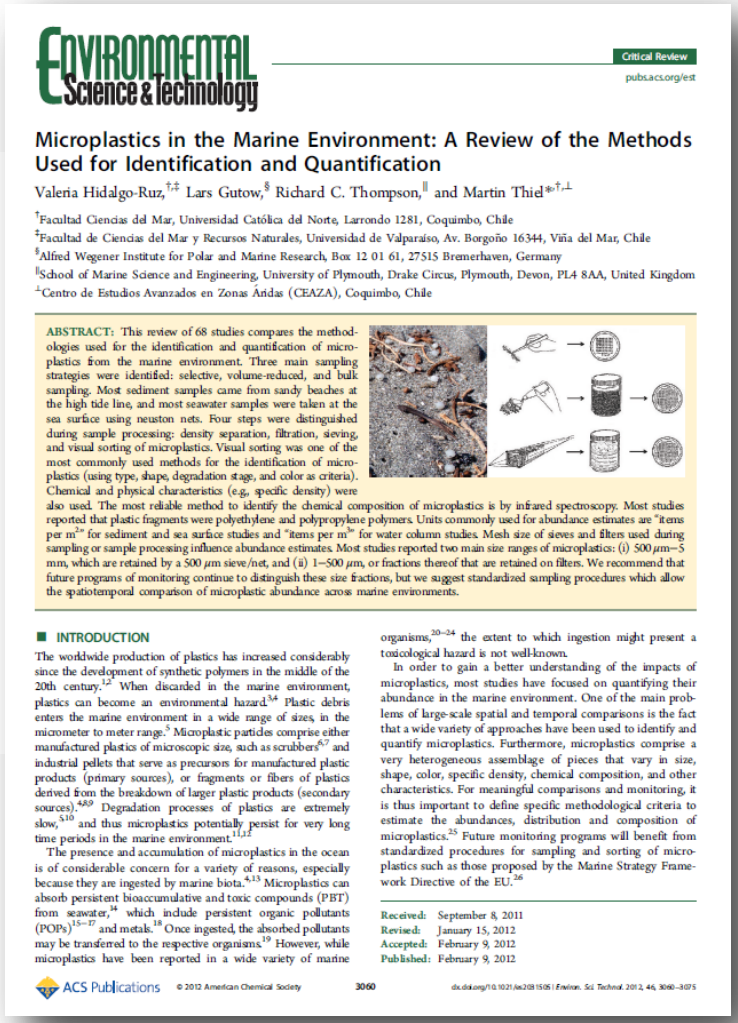
→10:30 am
Isabelle Schultz
(JPI - Oceans Program)
Ecological aspects of microplastics: JPI Oceans aligns research across 16 countries

environment

What was -presumably- intended/expected by JPI-O...

Legislation: the Marine Strategy Framework Directive

The aim of the European Union's ambitious Marine Strategy Framework Directive is to protect more effectively the marine environment across Europe



Environmental Science & Technology — Critical Review — pubs.acs.org/est

Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification

Valeria Hidalgo-Ruz,[†,‡] Lars Gutow,[§] Richard C. Thompson,[∥] and Martin Thiel*[,†,⊥]

[†]Facultad Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile
[‡]Facultad de Ciencias del Mar y Recursos Naturales, Universidad de Valparaíso, Av. Borgoño 16344, Viña del Mar, Chile
[§]Alfred Wegener Institute for Polar and Marine Research, Box 12 01 61, 27515 Bremerhaven, Germany
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ABSTRACT: This review of 68 studies compares the methodologies used for the identification and quantification of microplastics from the marine environment. Three main sampling strategies were identified: selective, volume-reduced, and bulk sampling. Most sediment samples came from sandy beaches at the high tide line, and most seawater samples were taken at the sea surface using neuston nets. Four steps were distinguished during sample processing: density separation, filtration, sieving, and visual sorting of microplastics. Visual sorting was one of the most commonly used methods for the identification of microplastics (using type, shape, degradation stage, and color as criteria). Chemical and physical characteristics (e.g., specific density) were also used. The most reliable method to identify the chemical composition of microplastics is by infrared spectroscopy. Most studies reported that plastic fragments were polyethylene and polypropylene polymers. Units commonly used for abundance estimates are "items per m^{2}" for sediment and sea surface studies and "items per m^{3}" for water column studies. Mesh size of sieves and filters used during sampling or sample processing influence abundance estimates. Most studies reported two main size ranges of microplastics: (i) 500 μm−5 mm, which are retained by a 500 μm sieve/net, and (ii) 1−500 μm, or fractions thereof that are retained on filters. We recommend that future programs of monitoring continue to distinguish these size fractions, but we suggest standardized sampling procedures which allow the spatiotemporal comparison of microplastic abundance across marine environments.

INTRODUCTION

The worldwide production of plastics has increased considerably since the development of synthetic polymers in the middle of the 20th century.[1,2] When discarded in the marine environment, plastics can become an environmental hazard.[3,4] Plastic debris enters the marine environment in a wide range of sizes, in the micrometer to meter range.[5] Microplastic particles comprise either manufactured plastics of microscopic size, such as scrubbers[6,7] and industrial pellets that serve as precursors for manufactured plastic products (primary sources), or fragments or fibers of plastics derived from the breakdown of larger plastic products (secondary sources).[4,8,9] Degradation processes of plastics are extremely slow,[5,10] and thus microplastics potentially persist for very long time periods in the marine environment.[11,12]

The presence and accumulation of microplastics in the ocean is of considerable concern for a variety of reasons, especially because they are ingested by marine biota.[4,13] Microplastics can absorb persistent bioaccumulative and toxic compounds (PBT) from seawater,[14] which include persistent organic pollutants (POPs)[15−17] and metals.[18] Once ingested, the absorbed pollutants may be transferred to the respective organisms.[19] However, while microplastics have been reported in a wide variety of marine organisms,[20−24] the extent to which ingestion might present a toxicological hazard is not well-known.

In order to gain a better understanding of the impacts of microplastics, most studies have focused on quantifying their abundance in the marine environment. One of the main problems of large-scale spatial and temporal comparisons is the fact that a wide variety of approaches have been used to identify and quantify microplastics. Furthermore, microplastics comprise a very heterogeneous assemblage of pieces that vary in size, shape, color, specific density, chemical composition, and other characteristics. For meaningful comparisons and monitoring, it is thus important to define specific methodological criteria to estimate the abundances, distribution and composition of microplastics.[25] Future monitoring programs will benefit from standardized procedures for sampling and sorting of microplastics such as those proposed by the Marine Strategy Framework Directive of the EU.[26]

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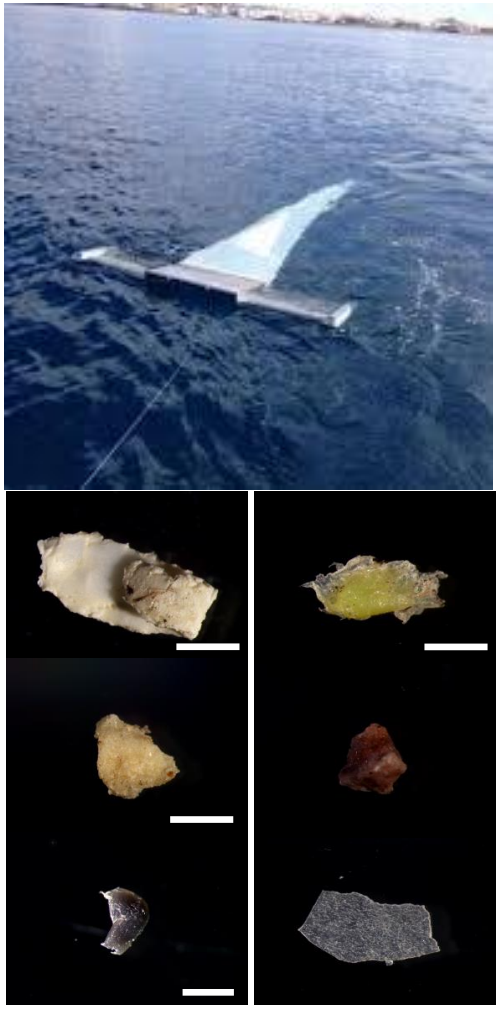
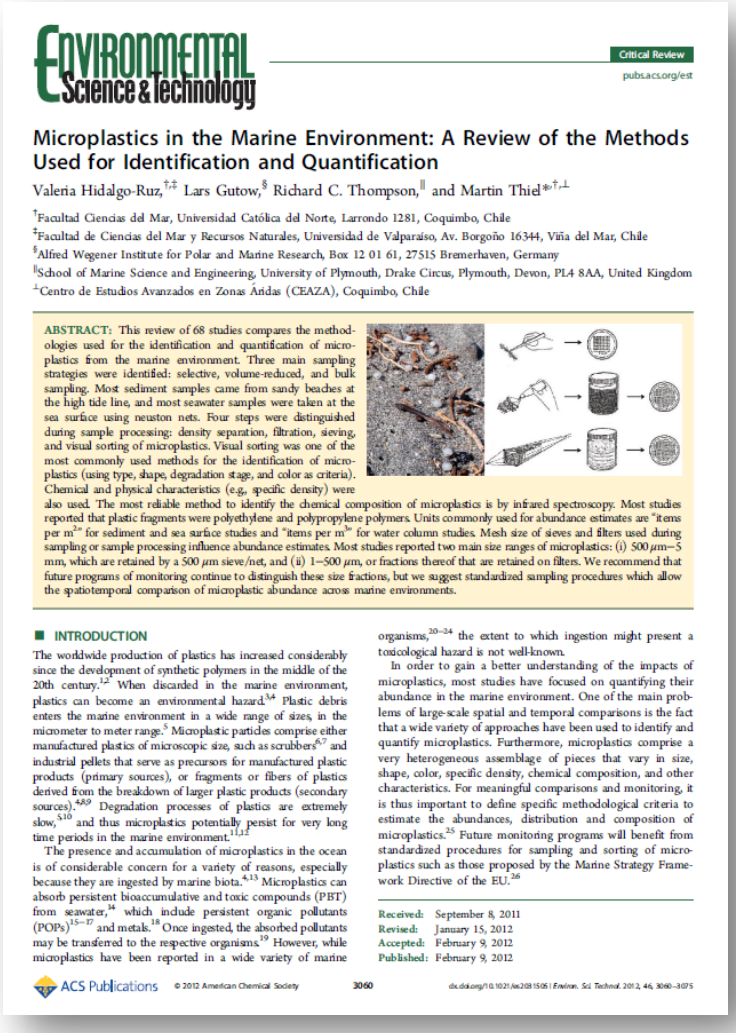
- *"Properties and **quantities of marine litter**...cause harm to the coastal and marine environment" (known as 'Descriptor 10').*
- *This definition **includes microparticles (particularly microplastics)***

What was expected by environmental agencies...





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- Provide **methodological standards** for MP sampling & analysis monitoring
- Enable MSFD MP **monitoring** →Descriptor 10, microplastics

What was expected by environmental agencies...

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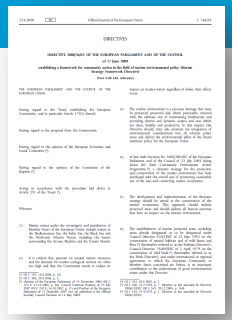
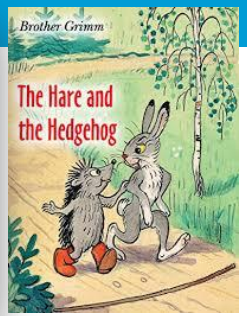
- Focus on **simple, inexpensive methods** (→Monitoring)
- Focus on **large(r) particles**
- **Chemical Identification +/- not necessary**
- Start **ISO process** for methods

What was intended by “science”

- Provide **reliable data on MP** in different environmental compartments
  - **Include +/- all MP-sizes (from 5 mm down to “what is possible”)**
  - **Reliable identification** of polymers
  - **Dynamic improvement of methodologies**

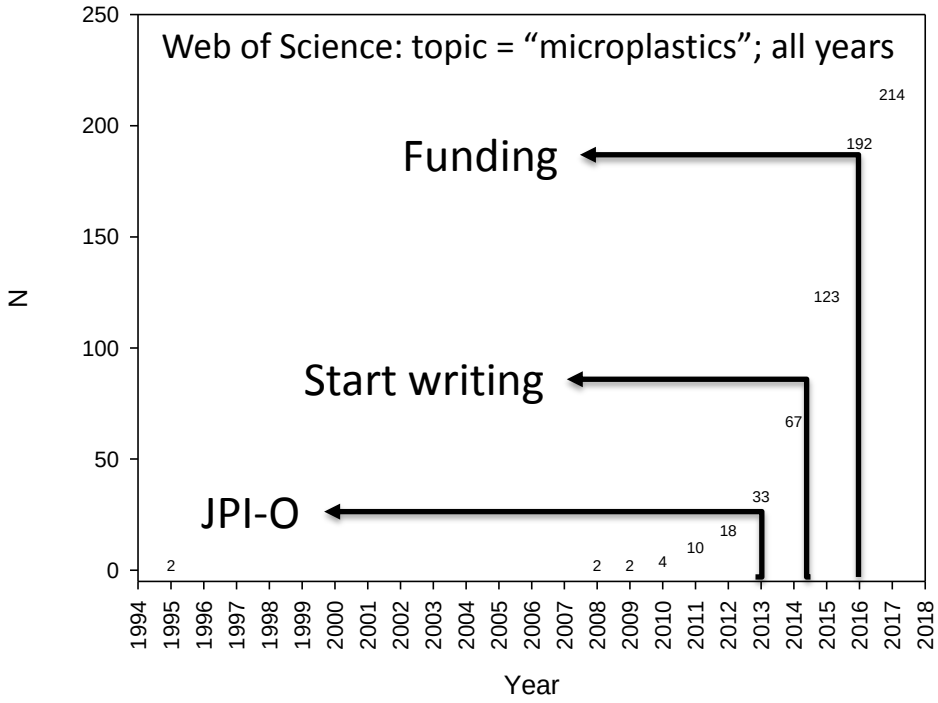
- **Different expections**
- **Different motivations**





Defining the baselines and standards...

Defining the baselines and standards as needed for monitoring for such a dynamic "science"?



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Defining the baselines and standards...for?

Defining the baselines and standards...for?



Defining the BASElines and standards for Microplastics ANalyses in European Waters (BASEMAN)

28 partners from 10 countries (AWI in lead)

- WP 1 **Defining baselines** for all relevant identification approaches
- WP 2 Preparation of **standardized test samples** for inter-lab comparisons
- WP 3 **Inter-lab and inter-method comparisons**
- WP 4 **Sampling methodologies** for MPs in the marine environment: standardization, suitability and intercomparison
- WP 5 Coordination, Integration and Synthesis

- **As proposed in ~2015**

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MICROPLASTICS ANALYSES IN EUROPEAN WATERS

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Highlights and pitfalls of JPI-O BASEMAN

HELMHOLTZ GEMEINSCHAFT

Highlights and pitfalls of JPI-O BASEMAN
Pitfalls...

Development of a **MP reference kit** and definition of methodological baselines

- **To develop and provide a MP reference kit**
  - **9 Polymers** (LDPE, HDPE, PP, PC, PVC, PET, PS, PMMA, PA66)
    - Physico-chemical characterization
    - **3 size fractions**: "→20 µm", "→100 µm", "→1 mm"
      - Grinding/milling & sieving
      - Size distribution
  - **Preparation and provision of "MP kits"** (X Polymers – X numbers- X sizes) for WP2/WP3 - Inter-lab and inter-method comparisons

Preparation of **standardized test samples** for **inter-lab comparisons**

- Preparation of standardized sediment samples, standardized plankton samples and standardized biota samples
  - Sediments: **3 types of sediments** from the wadden sea
  - Biota: soft parts of farmed **blue mussels**, intestines of **farmed salmon** and **wild caught haddock**
  - Plankton: **3 types of plankton** (German Bight) representing different natural polymers (e.g. “silicate” (diatoms), chitin (copepods))
  - “MP kits” (? Polymers ? Numbers ? Sizes) defined by WP1 & WP2
  - 4 replicates (3 contain the “MP kits”, 1 “natural” MP load)

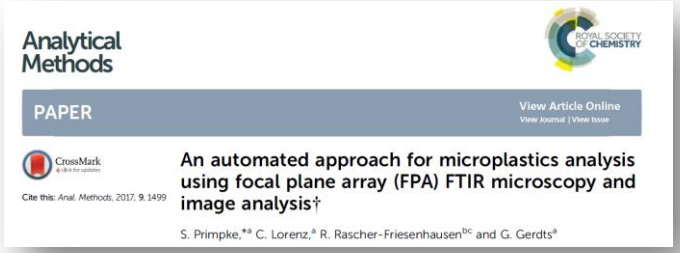
Preparation of **standardized test samples** for **inter-lab comparisons**

- **Problems**
  - Standardized milling, clean environment, storage etc.
  - Transfer of MP-kits to samples (transfer efficiency)
  - ➢ **General QA/QC related problems**
  - ➢ **Outcome ±……**
  - ➢ **However, currently several initiatives ongoing (e.g. QUASIMEME)**
    http://www.quasimeme.org/

Highlights and pitfalls of JPI-O BASEMAN

Highlights...

Development of methodological baselines



FTIR Imaging

- **MPApp**
- Pipeline is open source (Python-code and "curated" database)
- Automated identification, counting and sizing of MP (Numbers, sizes → toxicological studies)
- Size limit: ~11 µm

→01:55 pm
Sebastian Primpke
(Alfred Wegener Institute)
Harmonized Analysis of Microplastics by FTIR Spectroscopy and Imaging

Development of methodological baselines

FTIR Imaging

- **“Curated” database**
- Usage of multivariate statistics
- “Conservative” affiliations to clusters (not single entries)
- Permanently expanded
- Available upon request

Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-018-1156-x

RESEARCH PAPER

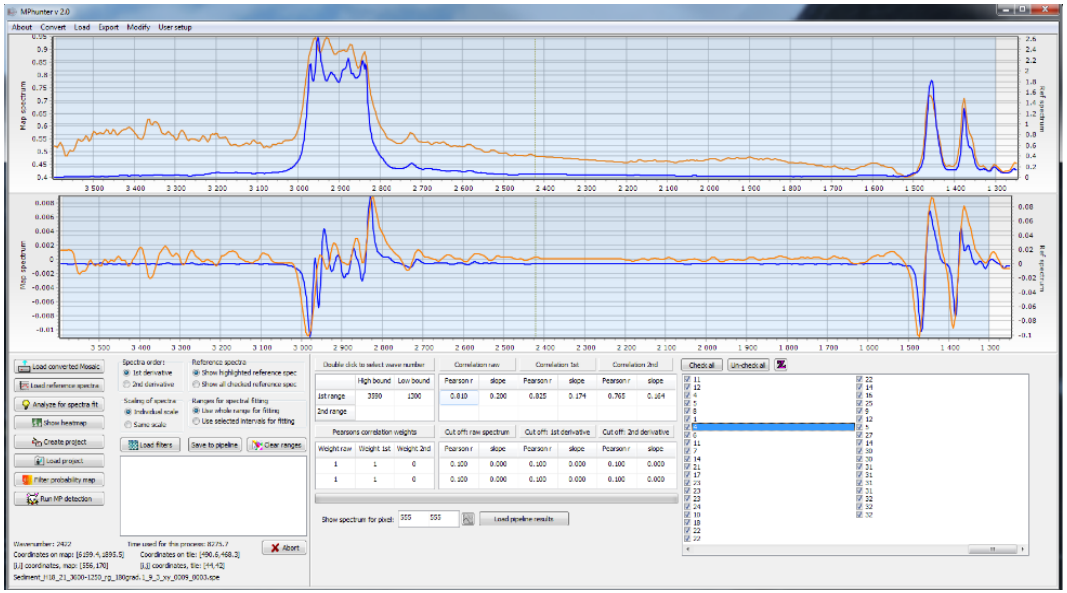
Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy

Sebastian Primpke[1] · Marisa Wirth[1,2] · Claudia Lorenz[1] · Gunnar Gerdts[1]

Received: 22 February 2018 / Revised: 20 April 2018 / Accepted: 18 May 2018

→01:55 pm
Sebastian Primpke

Development of methodological baselines

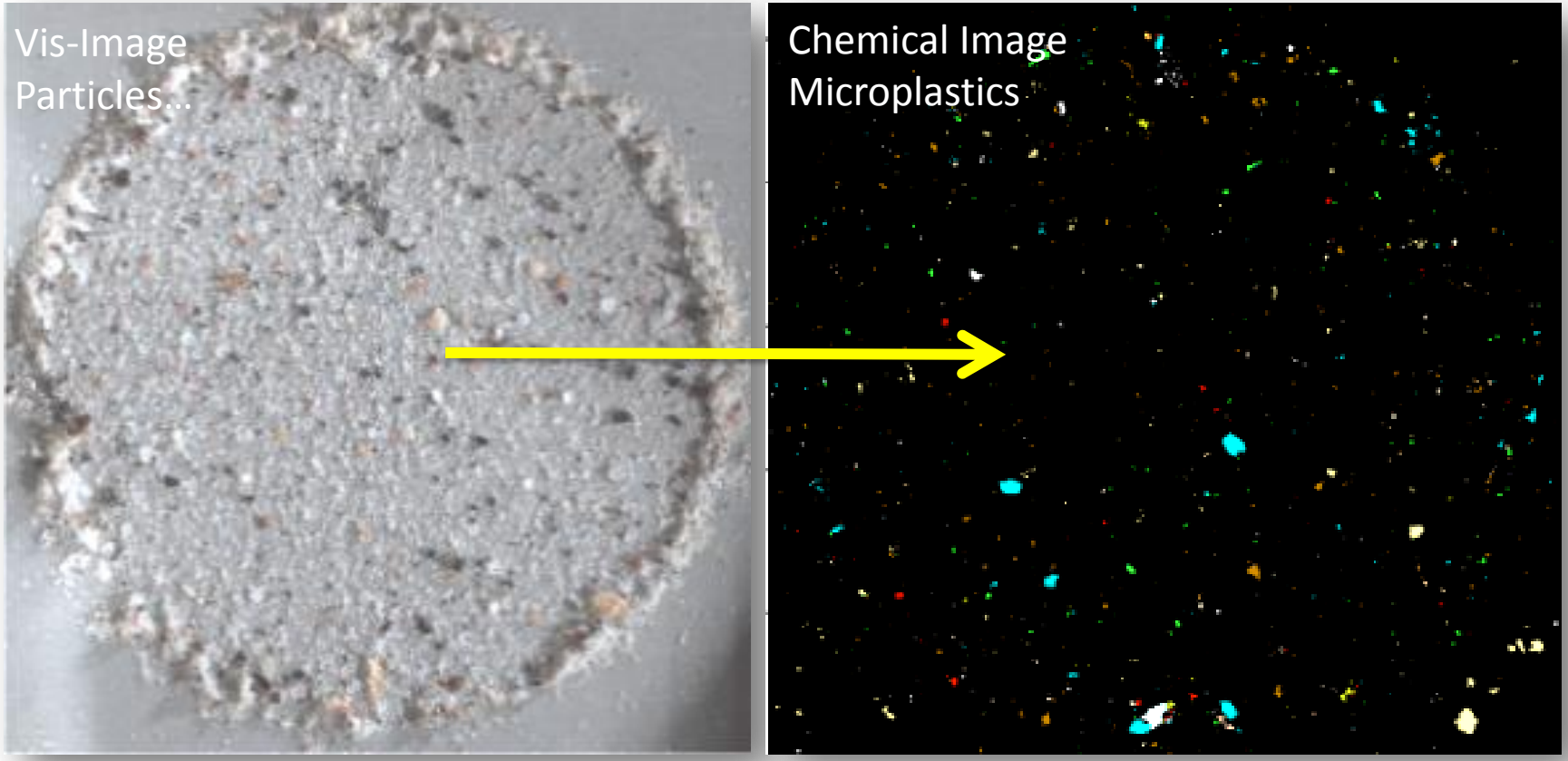


FTIR Imaging

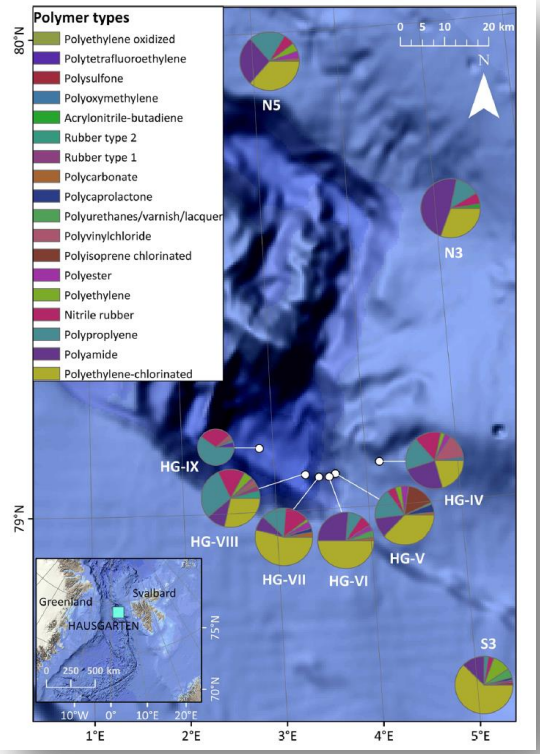
- **MPHunter**
- Delphi-based GUI environment
- Initial database comparison
- ~„Imaging“
- Import functions
  - Bruker
  - Agilent
  - (Thermo)
  - (Perkin-Elmer)

→01:55 pm
Sebastian Primpke

Development of methodological baselines
“Suspicious particles” become microplastics...



Development of methodological baselines
Case study 1: Arctic deep sea sediments



Environmental Science & Technology
Article
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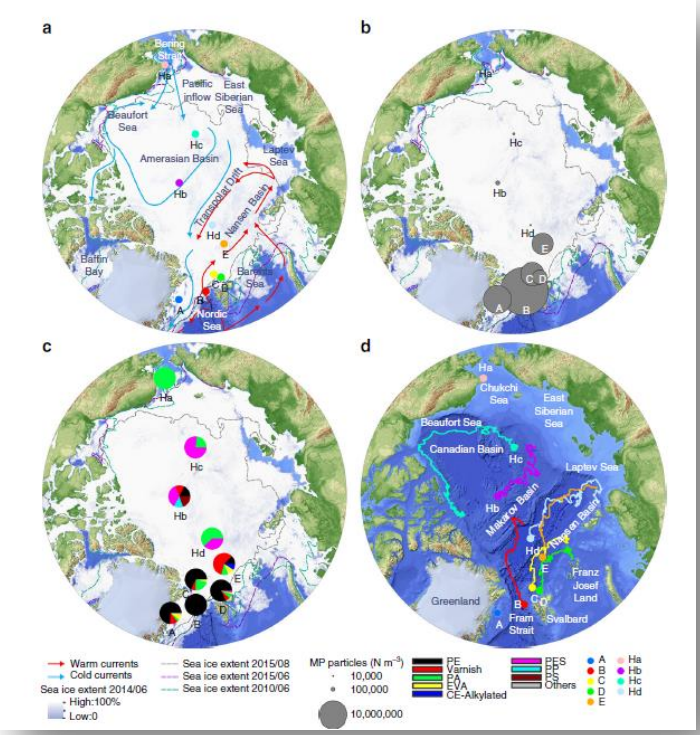
High Quantities of Microplastic in Arctic Deep-Sea Sediments from the HAUSGARTEN Observatory

Melanie Bergmann,[*,†,∥] Vanessa Wirzberger,[‡,§,∥] Thomas Krumpen,[⊥] Claudia Lorenz,[‡] Sebastian Primpke,[‡] Mine B. Tekman,[†] and Gunnar Gerdts[‡]

- $10^0 – 10^3$ (42 – 6595) MP kg^{-1}
- 18 different polymers were detected
- ~80% of the MP were ≤25 µm

Development of methodological baselines
Case study 2: Arctic sea ice




nature COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-018-03825-5 OPEN

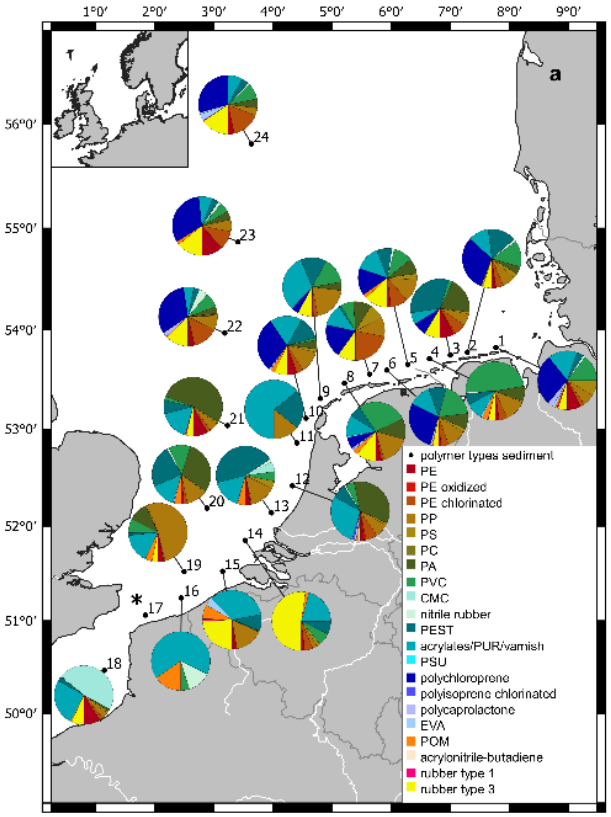
Arctic sea ice is an important temporal sink and means of transport for microplastic

Ilka Peeken[1], Sebastian Primpke[1], Birte Beyer[1], Julia Gütermann[1], Christian Katlein[1], Thomas Krumpen[1], Melanie Bergmann[1], Laura Hehemann[1] & Gunnar Gerdts[1]

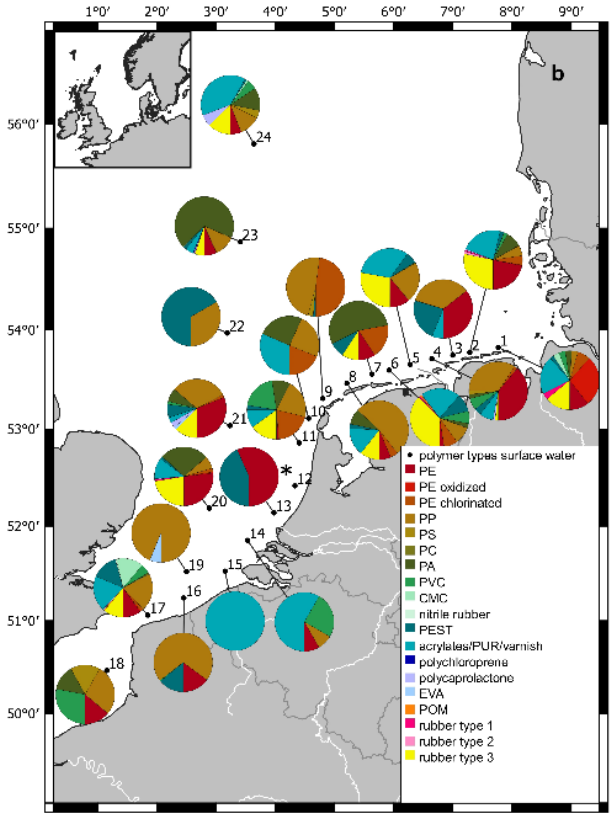
- Max. 10^7 MP m^{-3}
- 17 different polymers were detected
- 67% of the MP were ~11 µm

Development of methodological baselines
Case study 3: North Sea surface waters & sediments

Sediment



Water



Numbers & Identities

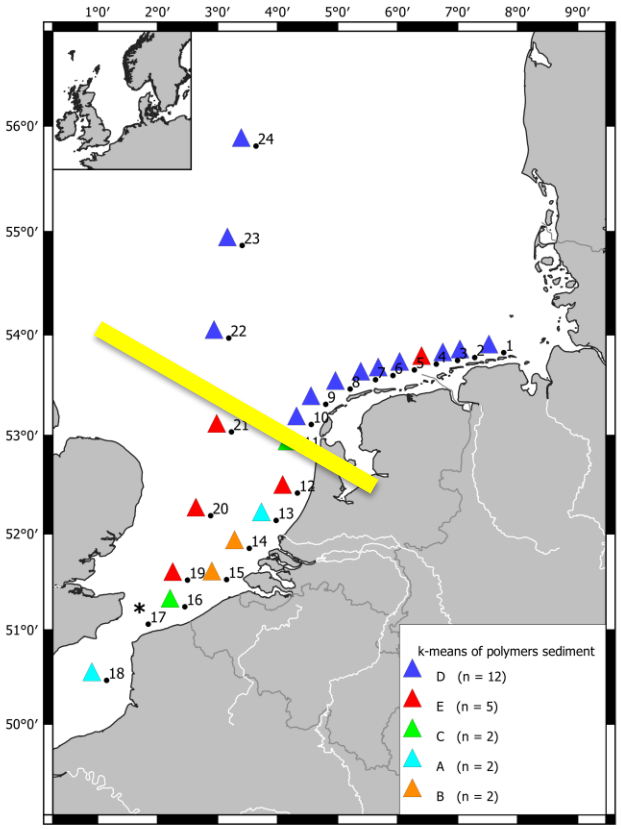
Water
- 0.06 - 245 MP m^{-3}
- 17 polymers detected

Sediment
- 3 - 1200 MP kg^{-1}
- 19 polymers detected

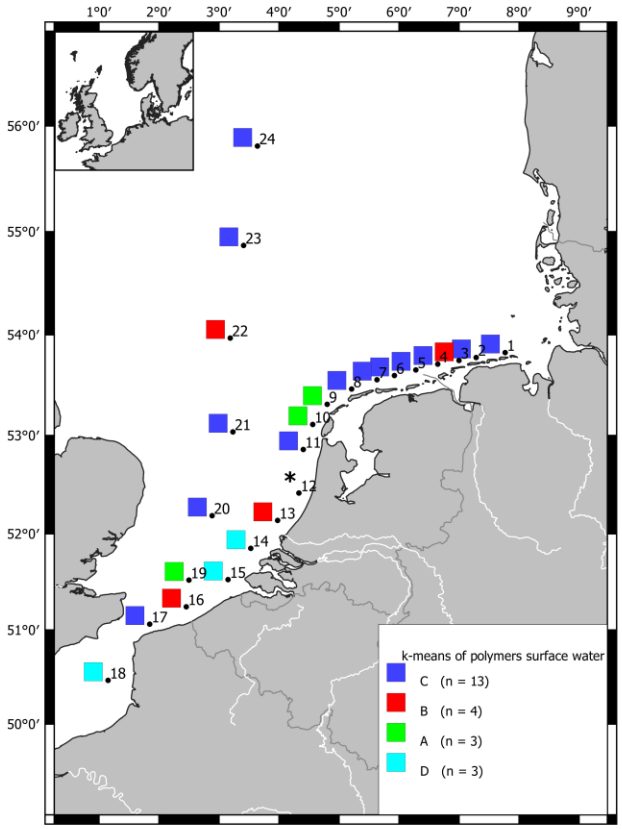
Lorenz et al. (2019)

Development of methodological baselines
Case study 3: North Sea surface waters & sediments

Sediment



Water



Patterns

- Multivariate statistics
- Kmeans/SIMPROF

Lorenz et al. (2019)

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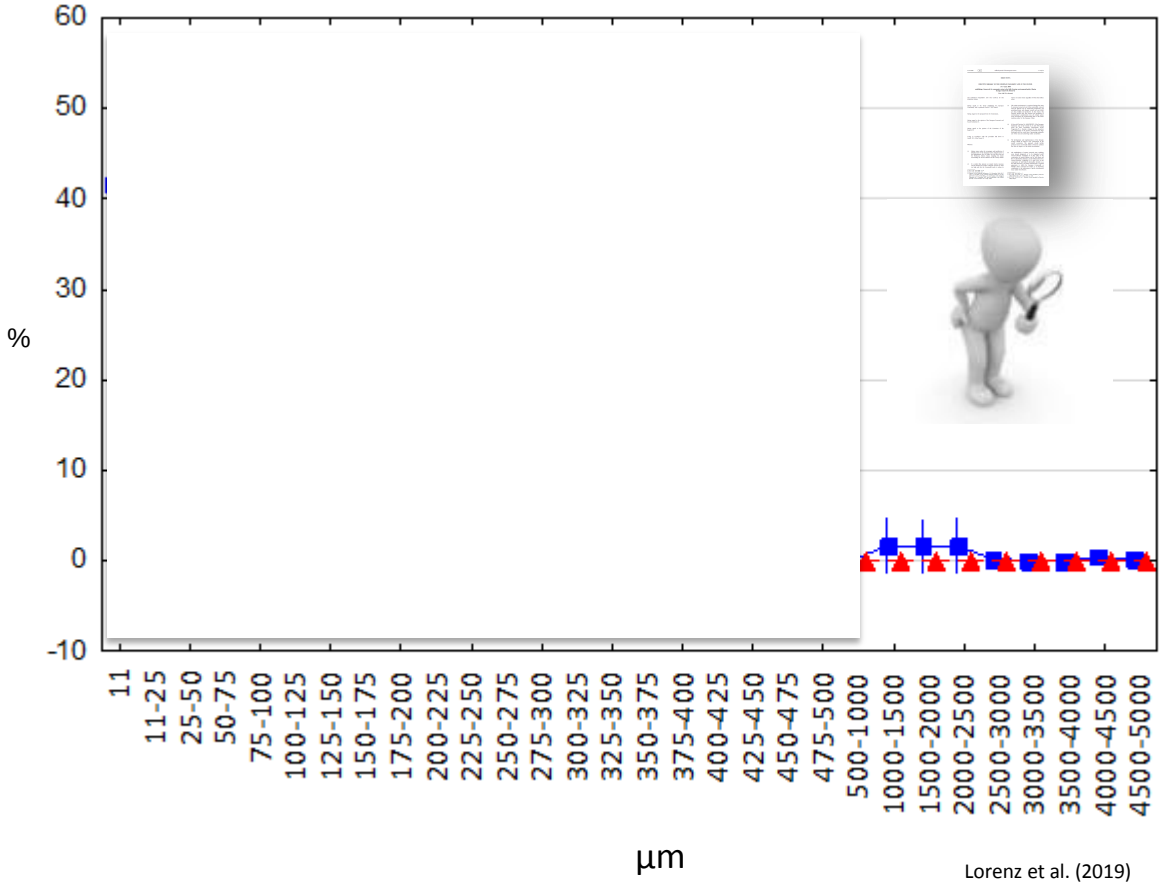
Case study 3: North Sea surface waters & sediments

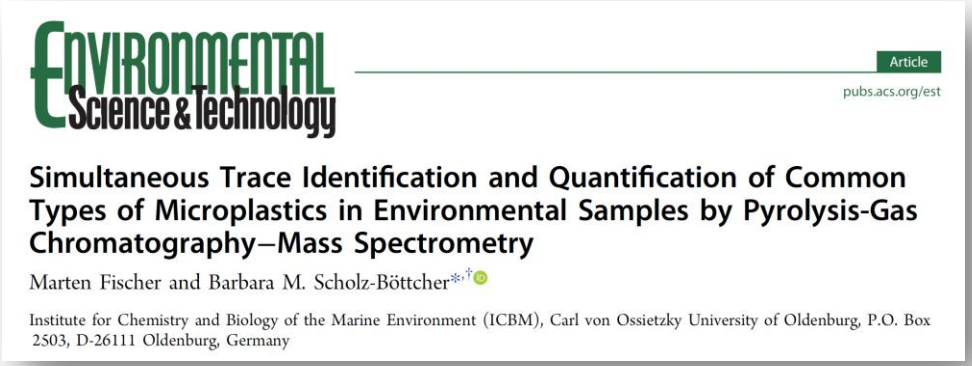


Lorenz et al. (2019)

Sizes

MSFD: *"Properties and **quantities of marine litter…**cause harm to the coastal and marine environment" (known as 'Descriptor 10')?????????*

Development of methodological baselines
Its not all about numbers...



ENVIRONMENTAL Science & Technology

Article

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Simultaneous Trace Identification and Quantification of Common Types of Microplastics in Environmental Samples by Pyrolysis-Gas Chromatography–Mass Spectrometry

Marten Fischer and Barbara M. Scholz-Böttcher*,†

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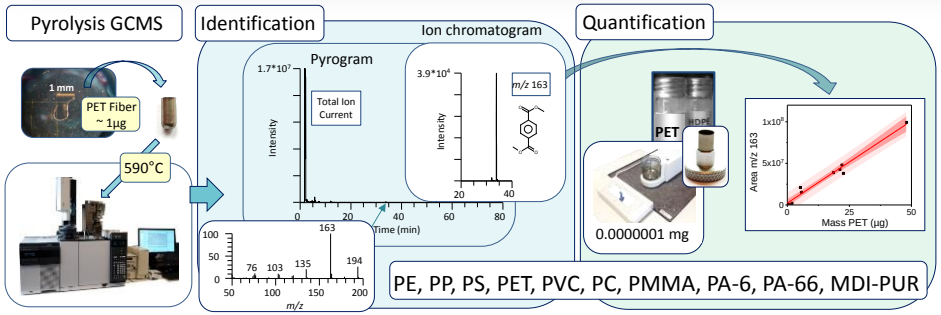
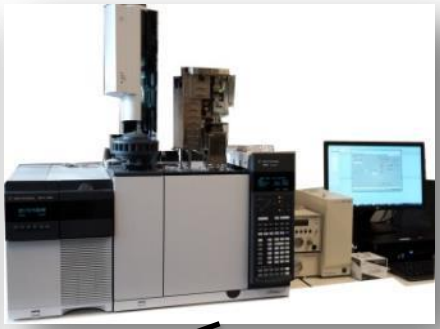
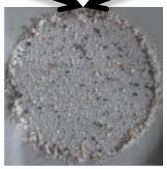


PyGCMS

- **Mass related MP-quantification** on a trace level (µg and below) (**Mass → Budgets**)
- **Simultaneous identification and quantification of 10 common plastic types** in complex environmental samples

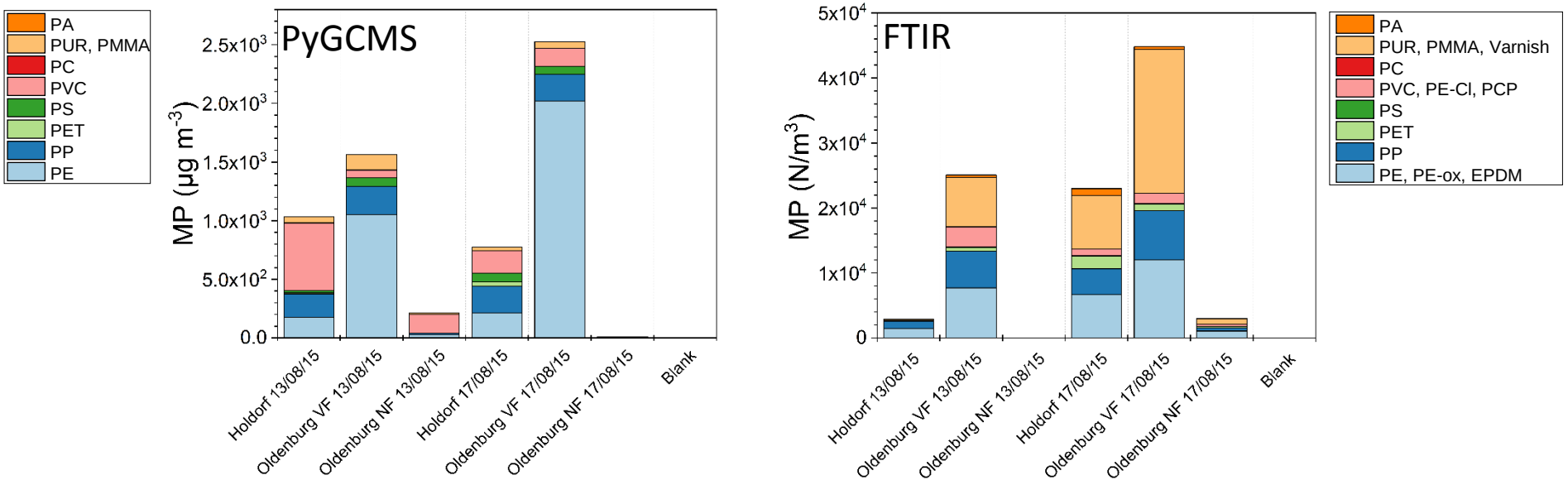
Development of methodological baselines
Comparison of methods...





FTIR Imaging & PyGCMS

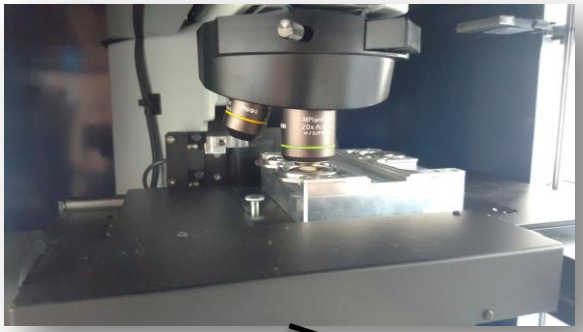
Development of methodological baselines
Comparison of methods... FTIR (Imaging) & PyGCMS

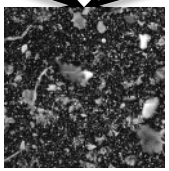


Primpke, Fischer et al., (in prep)

- Samples: Treated waste water
- **Similar qualitative compositions**!
- **Quantitative results differ** since larger particles dominate the pyrolysis signal

Development of methodological baselines
Comparison of methods...





FTIR Imaging & Raman-microscopy

Development of methodological baselines
Comparison of methods... FTIR (Imaging) & Raman-microscopy

Environmental Science & Technology

Article

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Comparison of Raman and Fourier Transform Infrared Spectroscopy for the Quantification of Microplastics in the Aquatic Environment

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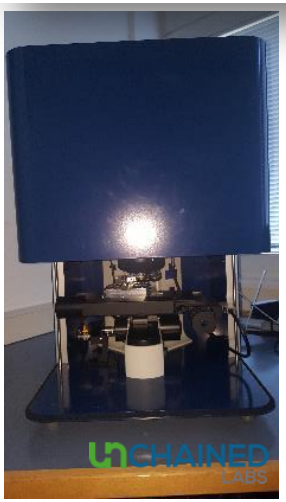
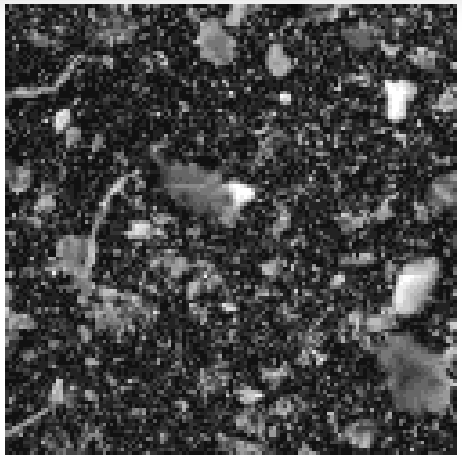




„Hand-sorted“ MP

- Alternative to ATR-FTIR
  - Automatic detection & identification
  - No compression (destruction)

MP 10–500 µm

- More diverse polymer composition
- Higher numbers (more accurate?)
  - Raman: 38–2621 MP m^{-3}
  - FTIR-Imaging: 22–228 MP m^{-3}
- Measuring time
  - Raman: 43 hours!
  - FTIR-Imaging: 8 hours (meanwhile 4 hours)

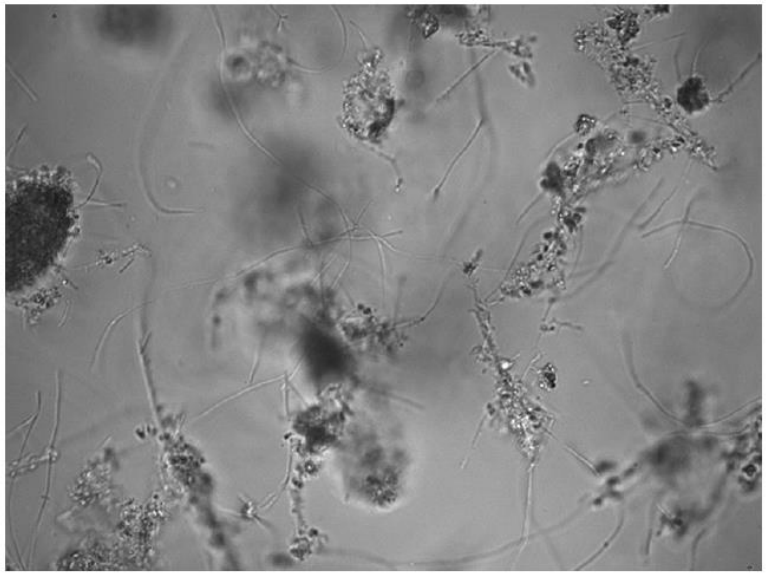
Development of methodological baselines
Fibers not included...(so far)

FTIR Imaging

- Not included in the image analysis so far
- Neural networks etc. not successfull

Development of methodological baselines Fibers?...

OPUS College of Engineering
MARQUETTE UNIVERSITY



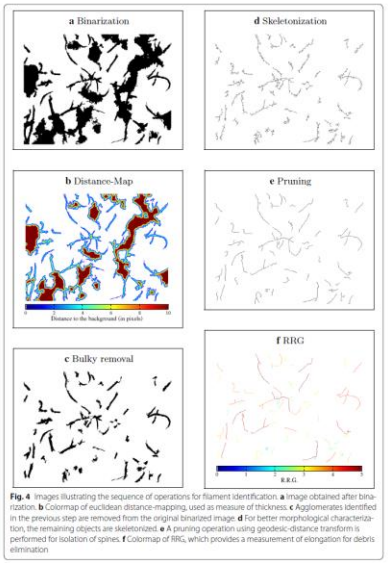


Fig. 4 Images illustrating the sequence of operations for filament identification. **a** Image obtained after binarization. **b** Colormap of euclidean distance-mapping, used as measure of thickness. **c** Agglomerates identified in the previous step are removed from the original binarized image. **d** For better morphological characterization, the remaining objects are skeletonized. **e** A pruning operation using geodesic-distance transform is performed for isolation of spines. **f** Colormap of RRG, which provides a measurement of elongation for debris elimination

FTIR Imaging including fibers

- Initial MATLAB script
- Transposed to Python code
- Fully integrated in Python-pipeline (MPApp)

Dias et al. BioMed Eng OnLine (2016) 15:64
DOI 10.1186/s12938-016-0197-7

BioMedical Engineering OnLine

RESEARCH Open Access

Image processing for identification and quantification of filamentous bacteria in in situ acquired images

Philipe A. Dias[1,2*], Thiemo Dunkel[3], Diego A. S. Fajado[2], Erika de León Gallegos[3], Martin Denecke[3], Philipp Wiedemann[4], Fabio K. Schneider[1] and Hajo Suhr[2]

Journal Name

ARTICLE

Automated Identification and Quantification of Microfibres and Microplastics

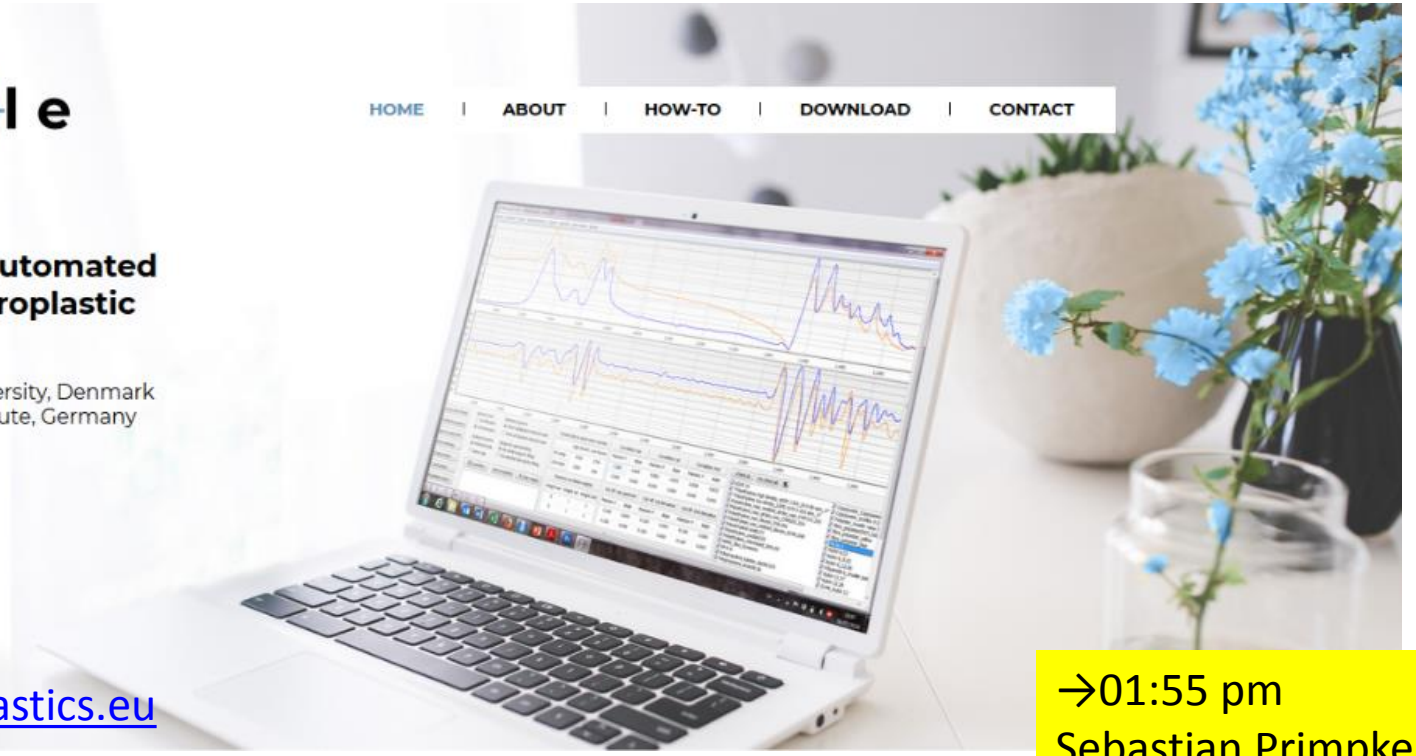
S. Primpke,[a,*] P. A.Dias [b] and G. Gerdts[a]

Esm2Received 00th January 20xx,
Accepted 00th January 20xx

→01:55 pm Sebastian Primpke

Development of methodological baselines
“The need for speed” and “usability” (→monitoring)

- MPApp & MPHunter now in a common GUI environment



www.simple-plastics.eu

→01:55 pm
Sebastian Primpke

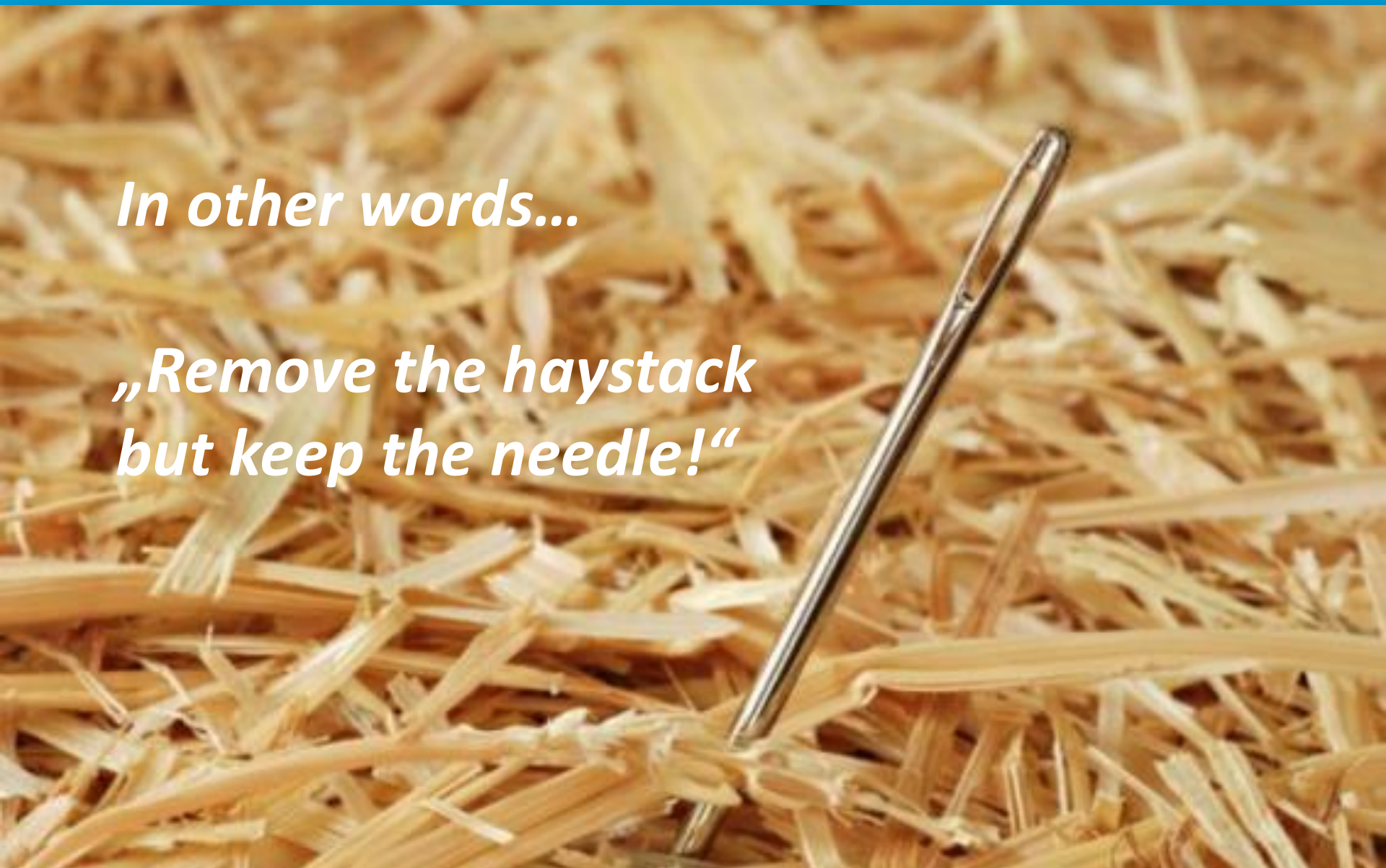
Inter-lab and inter-method comparisons

Inter-method comparison of **extraction approaches**

- Objective: To **optimize the extraction of MP** from sediment

Inter-method comparison of **purification approa**ches

- Objective: To **optimize the purification of MP** from sediment, plankton and biota in respect to matrix disintegration/removal and polymer preservation



In other words…

„Remove the haystack but keep the needle!“

Inter-lab and inter-method comparisons
"The sediment haystack"

Microplastics Sediment Separator (MPSS)

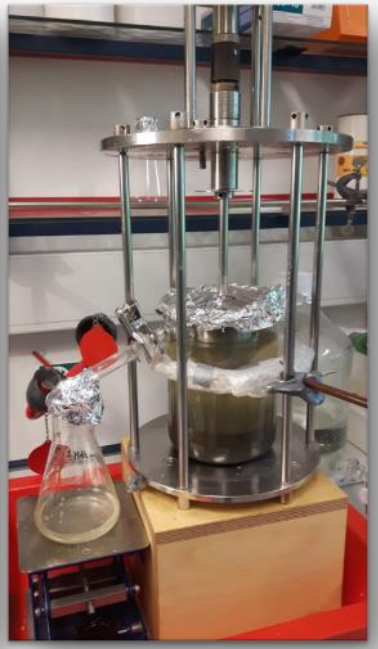
- Based on density separation
- 1 - 3 kg sediment-sample
- High recovery rate
- Commercially available (and published)
- Improvement necessary!
  - ~30 L prefiltered $ZnCl_2$ solution
  - 1 sample in ~24 hours
  - Mixing by stirring (milling..)
  - Geometry
  - Intransparent
  - Expensive!

Inter-lab and inter-method comparisons
"The sediment haystack"

IVL solution

Cheaper

ICBM solution



Smaller

AWI solution

Smarter..;-)

Inter-lab and inter-method comparisons
"The sediment haystack"

AWI Sediment Separator

- 1 kg sediment-sample
- **~9 L** prefiltered $ZnCl_2$ solution (filling from below through 10 µm filter)
- **4 samples** in ~24 hours ("upscalable")
- **Mixing by aeration**
- **Geometry** (straight line)
- **Transparent**
- Currently being evaluated

Inter-lab and inter-method comparisons
"The natural polymer haystack"

Environmental Science & Technology

Article

Cite This: *Environ. Sci. Technol.* 2017, 51, 14283−14292

pubs.acs.org/est

Enzymatic Purification of Microplastics in Environmental Samples

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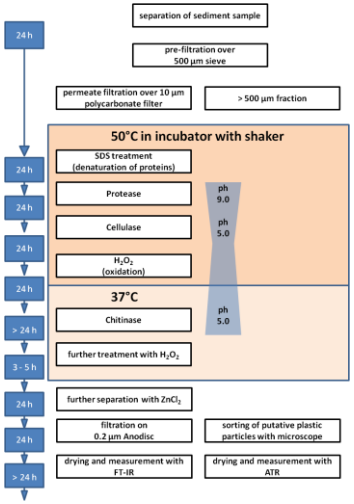


Enzymatic maceration

- Sequential usage of inexpensive technical enzymes and chemicals (Proteinase, cellulase and chitinase; SDS) → MSFD
- **Degradation of organic residues**
- **No degradation of synthetic polymers**
- Improvement necessary!
  - **Time consuming**
  - Risk of contamination (several manual steps…)

Inter-lab and inter-method comparisons
"The natural polymer haystack"

However...

AWI MP reactor

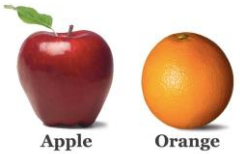
- Very **simple design** (stainless steel tube)
- Sample stays permanently in the reactor
- **Prevention of contamination** (10 µm stainless steel meshes (top/bottom)
- **Simple usage** (fill/drain of reagents by vacuum/pressure)
- "**Upscalable**" (several samples)

Lessons learned (?)

- Analytical approaches?
  - **+/-**
    - Numbers & identities: µFTIR, Raman
    - Masses: PyGCMS, TED-GCMS
- Extraction & Purification?
  - **Yes!**
    - Still time & labour consuming
    - Chemical and physical treatments: Keep the needle but remove the haystack
- Sampling?
  - **+/-**
- QA/QC?
  - **Yes**

Future challenges

- Environmental surveillance versus basic science
- Static standards (ISO) versus dynamic improvements



- Cross-ecosystems analyses

- *In situ* MP-conformation
- Single MPs or aggregates?

JPI OCEANS

BASEMAN
MICROPLASTICS ANALYSES IN EUROPEAN WATERS

ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG

Thank you for your attention

AALBORG UNIVERSITET

uniResearch

MSI

LOV

rapid Particle Systems

OGS

AWI

IVL Swedish Environmental Research Institute

GMIT

IAMC

Università di Siena 1240

SAHFOS

UNIVERSIDADE DA CORUÑA

ipma instituto português do mar e da atmosfera

UNIVERSITÄT BAYREUTH

NIFES NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH

Université du Maine

NILU Norsk institutt for luftforskning Norwegian Institute for Air Research

GEOMAR

UNIVERSIDADE NOVA DE LISBOA

ICBM

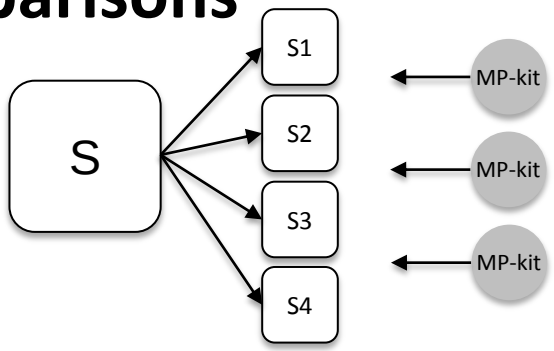
ULg UNIVERSITÉ de Liège

TU WIEN TECHNISCHE UNIVERSITÄT WIEN Vienna University of Technology

HELMHOLTZ GEMEINSCHAFT

NIVA Norsk institutt for vannforskning

Preparation of **standardized test samples** for **inter-lab comparisons**



- Thawing
- Addition of “MP kits”
  - “Common” transfer protocol (after 1st experiences...)
  - Usage of antistatic device (after 1st experiences...)
  - Recording tags of sub-samples and vials