Final Report

Defining the BASElines and standards for Microplastics ANalyses in European waters

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Partner 25 - 27: new in kind partners in 2017

Partner 17: Now Havforskningsinstitutt (HI)/Institute of marine Research (IMR).

Partner 21: RapID Particle Systems GmbH (Rap-ID) acquired by Unchained Labs

Partner 27: Now NORCE Norwegian Research Centre AS
Persistent plastic litter amasses. It fragments over time, both before entering and within the marine environment. Together with micro-sized primary plastic litter from consumer products, this leads to an increasing amount of small plastic particles, so called microplastics (MP). The ubiquitous presence and massive accumulation of MP in marine habitats and the uptake of MP by at least 700 marine species biota is now well recognised by scientists and authorities worldwide. However, the impact of plastic particles on aquatic ecosystems is far from understood. A fundamental issue precluding assessment of the environmental risks arising from MP is the lack of standard operating procedures (SOP) for MP sampling and analysis. Consequently, there is a lack of reliable data on concentrations of MP and the composition of polymers within the marine environment. Comparability of data on MP concentrations is currently hampered by the huge variety of different methods, each generating data of very different quality and resolution. Although MPs are recognised as emerging contaminants in the environment, neither sampling, extraction, purification nor identification or quantification approaches are currently standardised, making the increasing numbers of MP studies hardly -if at all-comparable.

The overall goal of the interdisciplinary and international collaborative research project BASEMAN was to overcome these problems through a profound and detailed comparison and evaluation of all approaches from sampling to identification of MP. The collaborative research project BASEMAN combined experienced MP scientists (from different disciplines and countries) in a cutting edge project addressing the JPI Oceans pilot call “Ecological aspects of MP in the marine environment”. BASEMAN was structured in 5 work packages (WPs): Defining baselines for all relevant identification approaches (WP1), Preparation of standardized test samples for inter-lab comparisons (WP2), Inter-lab and inter-method comparisons (WP3), Sampling methodologies for MPs in the marine environment: Standardization, suitability and intercomparison (WP4) and finally a coordination work package Coordination, Integration and Synthesis (WP5).

In WP1 the strength and limitations of different analytical techniques with respect to MP identification, quantification (numbers and masses) were successfully investigated covering different Fourier-transform infrared spectroscopy (FTIR) techniques, Raman-microscopy, pyrolysis-gas chromatography/mass spectrometry (py-GC/MS) and py-GC/MS with Orbitrap. Multi-spectroscopic databases for FTIR and Raman microscopy were generated encompassing “pristine” synthetic polymers, weathered synthetic polymers but also representative natural substances present in environmental matrices. For evaluation of the generated data, dedicated software pipelines were developed. A MP reference kit was produced and used for investigations on MP weathering and spiking of different samples in inter-lab comparison in WP2 and WP3. For inter-lab comparisons, different sample matrices (plankton, sediment and biota) were spiked and provided to the participating BASEMAN partners. Unfortunately, unforeseen problems related to milling, sieving, handling & analyses (size distributions) of the polymer beads and the spiking procedure itself (transfer of the kit to the samples) lead to extreme delays. The inter-lab comparison approach to quantitate the small particle fractions below 100 μm particle size from the named matrices with methods of the current state of the art is considered as failed. However, with considerable further method development (including strict QA/QC criteria), the task is considered feasible. Further, in the scope of WP3 several purification methods were developed and/or optimized for the separation of microplastics from sediments. These included newly developed small-scale sediment separators based on density separation. Different purification methods were successfully investigated of which the use of alkaline digestion, enzymatic digestion, wet oxidation (including Fenton’s reagent) were applied, evaluated and to some extent compared. To reduce contamination risks and allow an easier handling a “purification reactor” was developed. In WP4, sampling methodologies for MP in the marine environment were standardized, evaluated (suitability) and compared. For this task, two joint cruises were conducted (Galway Bay, Ireland and Rias de Vigo, Spain). Both cruises intended to collect environmental samples of benthic sediments and water samples from surface and water column. Samples collected in both cruises were aimed to be processed under the same conditions by the different laboratories involved in order to estimate the associated errors of microplastic counting and identification. Furthermore, marine biota species were suggested that might serve as relevant and appropriate as biomonitoring species with respect to MP in Europe. Three white papers finally were generated: i) Standardization protocols for monitoring microplastics in seawater; ii)
Standardization protocols for monitoring microplastics in sediments; and iii) Harmonized protocol for monitoring microplastics in biota (in collaboration with the JPI Oceans project EPHEMARE).

In general, BASEMAN provided EU authorities with tools and operational measures that can be applied to describe the abundance and distribution of MP in the environment in existing (e.g. MSFD) or future monitoring requirements.
PART A - SCIENTIFIC REPORT

INTRODUCTION

Persistent plastic litter amasses. It fragments over time, both before entering and within the marine environment. Together with micro-sized primary plastic litter from consumer products, this leads to an increasing amount of small plastic particles, so called microplastics (MP). The ubiquitous presence and massive accumulation of MP in marine habitats and the uptake of MP by at least 700 marine species biota is now well recognised by scientists and authorities worldwide. However, the impact of plastic particles on aquatic ecosystems is far from understood. A fundamental issue precluding assessment of the environmental risks arising from MP is the lack of standard operating procedures (SOP) for MP sampling and analysis. Consequently, there is a lack of reliable data on concentrations of MP and the composition of polymers within the marine environment. Comparability of data on MP concentrations is currently hampered by the huge variety of different methods, each generating data of very different quality and resolution. Although MPs are recognised as emerging contaminants in the environment, neither sampling, extraction, purification nor identification or quantification approaches are currently standardised, making the increasing numbers of MP studies hardly-if at all-comparable.

AIMS OF THE PROJECTS

Although microplastics (MP) are recognized as an emerging contaminant in the environment, currently neither sampling, extraction, purification nor identification approaches are standardized, making the increasing numbers of MP studies hardly-if at all-comparable. The overall goal of this interdisciplinary and international collaborative research project was to overcome this problem through a profound and detailed comparison and evaluation of all approaches from sampling to identification of MP. Our collaborative research project BASEMAN combined experienced MP scientists (from different disciplines and countries) in a cutting edge project addressing the JPI Oceans (JPI-O) pilot call “Ecological aspects of MP in the marine environment”. Our project tackled two major topics: i) “The validation and harmonization of analytical methods” which is indispensable for II), the “Identification and quantification of MP”. BASEMAN was structured in 5 work packages (WP): Defining baselines for all relevant identification approaches (WP1), Preparation of standardized test samples for inter-lab comparisons (WP2), Inter-lab and inter-method comparisons (WP3), Sampling methodologies for MPs in the marine environment: Standardization, suitability and intercomparison (WP4), and finally a coordination work package, Coordination, Integration and Synthesis (WP5). In WP1 the objectives were: i) to characterize the effects of artificial/natural weathering on reference MPs; ii) to generate a spectra database (FT-IR, Raman, Pyrolysis-GCMS, HySpex) for pristine and weathered reference particles; iii) to evaluate the strengths and limitations of different analytical techniques without interferences of environmental matrices; and iv) to develop methods with submicron capability in order to define a reasonable (methodological) lower size limit of MP. In the closely related WPs 2 and 3 the objectives were: i) to generate and provide standardized MP-spiked test samples (sediment, plankton, biota) for inter-lab comparisons (WP2 -> WP3); ii) to generate and provide standardised test samples (sediment, plankton, biota) for inter-method comparisons (WP2 -> WP3); iii) to analyse MP-spiked reference samples (sediment, plankton, biota) according to the current analytical workflows in the participating labs with respect to identification, quantification and sizing of MP; iv) to analyse environmental samples (sediment, plankton, biota) (e.g. provided by WP4) according to the current analytical workflows in the participating labs with respect to identification, quantification and sizing of MP; and v) to optimize the extraction and purification of MP from sediment, plankton and biota with respect to matrix disintegration/removal and polymer preservation. WP4 was dedicated to environmental sampling and evaluation of appropriate sample types with the following objectives: i) to develop standardized methods for sampling MPs in the water column and sediments; ii) to identify marine biota species appropriate for MPs monitoring in European regions; iii) to evaluate alternative methods for sampling MPs in the marine environment particularly those deployable from platforms of opportunity; iv) to intercalibrate sampling methods for MPs of varying size; v) to facilitate inter-lab comparisons and analyses of environmental samples of MPs; and vi) to develop validated conversion coefficients and harmonized reporting units for MPs.
In general, BASEMAN aimed to equip EU authorities with tools and operational measures that may be applied to describe the abundance and distribution of MP in the environment. Such tools will permit JPI OCEANS evaluation of member state compliance with existing and future monitoring requirements.

RESULTS AND DISCUSSION

Defining baselines for all relevant identification approaches (WP1)

MP reference kits were developed, produced (e.g. cryomilling, thieving) and distributed by P6 (UBAY) to BASEMAN partners responsible for spiking different environmental samples (plankton, sediment, biota). These samples were used to test the efficiency and recovery of their extraction, purification and analytical methodology. Five polymers (LDPE, PET, PA66, PS, and PVC) which are frequently found in the aquatic environment and cover a large range of possible densities were considered. Three size classes (1 mm, 100 µm and 20 µm) were defined in order to test the limits of the different analysis methods. Each MP kit included 10 particles of 1 mm and 50 particles of each of the defined size categories “100 µm” and “20 µm”. In the case of PVC, only the size classes of 100 and 20 µm were considered, as 1 mm PVC pellets were not available. The aim was to use irregular fragments instead of the widely used spherical particles in order to better depict the environmental heterogeneity of MPs. As irregularly shaped particles are not manufactured, the smaller size ranges had to be produced by grinding. Each of the smaller size classes was produced by a sieving step over 20 µm and 100 µm respectively. As a consequence, the size distribution in the size class 20 µm and 100 µm was broader than expected and that there was an overlap in the size distribution between the 100 µm and the 20 µm sieving fractions. Since the kit fabrication method bears the risk of introducing a certain variance, the exact content of the kits needed to be validated. Possible cross-contamination or loss of particles during the production of the kits and the sample spiking process had also to be investigated. Because the kits were intended to be used for spiking and (see W2 and WP3), they played a central role in the BASEMAN proficiency testing approaches (see WP3). Therefore, the validation of the kits was one of the main objectives. P6 (UBAY) validated the MP reference kits and provided comprehensive information concerning the polymers contained in the kits. For the validation of the kits, 10 kits were randomly selected and transferred to appropriate membrane filters for FTIR analysis according to the same protocol the spiking partners used for spiking of environmental samples (e.g. suspending, mixing, flushing etc.). Larger particles (1 mm) were sorted out manually with tweezers and were analysed with ATR-FTIR spectroscopy. The remaining content was filtered and finally analysed with FPA-based micro-FTIR spectroscopy to assess identity and variance in numbers of particles in the kits. The size of all particles was also measured. Regarding the 1 mm particles, the validation of the kits showed the exact expected number of particles, meaning 10 particles per polymer. Because of the observed broad overlap in size distribution between the 100 and the 20 µm size classes, it was impossible to classify each particle according to one of both size classes. Therefore, the latter were combined and the size of each particle was measured. Results regarding PVC, including all particles, were considered as suitable for the proficiency tests because the standard deviation in the 10 investigated kits was < 20%. The other polymers partly show high variability when considering the whole size range < 1 mm mainly related to smaller particles. In fact, if the results are restricted to specific size ranges and leaving out small particles < 80 µm, a better accuracy with a lower standard deviation can be obtained. For instance, by considering PET particles with a size larger than 80 µm, a standard deviation of only 16% was observed (instead of 42% when all particles < 1 mm were considered).

Detection and identification of MP based on FTIR or Raman. P1 (AWI) and P14 (UDC) generated “in house” FTIR databases (mostly based on ATR-FTIR) by measuring reference material (e.g. pellets, foils, powders) provided by or purchased from different commercial and non-commercial sources (covering “pristine” synthetic polymers (P1 (AWI)), weathered synthetic polymers (P14 (UDC)) and representative natural substances present in environmental matrices (P1 (AWI)). All reference-material used was comprehensively documented and stored in a “reference material collection” maintained by P1 (AWI). In contrast to commercially available databases, all data are “open access” and were provided for the BASEMAN consortium. The FTIR database of P1 (AWI) currently comprises > 350 spectra (in Bruker and JCPD-DX formats) and is permanently extended, the database of P14 (UDC) covers 100 spectra (in Perkin Elmer format). P1 (AWI) generated and provided a Raman database (currently > 230 spectra) as well (based on the “reference material collection” used for the FTIR-database). By using multivariate statistics, the FTIR database was further improved.
Detection and identification of MP based on pyGCMS. P5 (ICBM) introduced the Curie point pyrolysis GCMS (CP-pyGCMS) as a sensitive tool for MP identification and quantification (weight related data) of different polymer types (Fischer and Scholz-Böttcher, 2018; 2019). Recovery tests were provided at µg level and an application to biota (fishes) samples. The CP-pyGCMS method was transferred to a new micro-furnace pyrolysis GCMS system (MF-pyGCMS). Here a higher sample capacity allows improving the sample transfer by using glass fibre filters. In contrast to the published CP-pyGCMS the linearity was enhanced and considerably lower process standard deviations were achieved. The improved method was demonstrated analysing different sea salts and “fleur de sel” samples from the Atlantic Ocean and the Mediterranean Sea (publication in prep). This study emphasizes the sensitivity of the method and enables a comparison of the qualitative and quantitative MP loads of natural salts at different locations of the study areas. Furthermore MF-pyGCMS and micro-attenuated total reflection Fourier transform infrared spectroscopy (µ-ATR-FTIR) were compared as complementary identification tools for larger weathered MP particles and fibres isolated from river sediments (Käppler et al., 2018). Concerning small MP particles (< 500 µm) FTIR-imaging and MF-pyGCMS are currently compared (publication in prep). P17 (NIFES) furthermore performed a method pilot test identifying py-GC/MS Orbitrap as a potential option for lowering plastic identification detection limits, increasing quantification accuracy and at the same time screening for unknown polymer contaminations, leading to an application note with Thermo Fisher (https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-10643-gc-ms-microplastics-biological-matrix-an10643-en.pdf; publication in prep).

Artificial/natural weathering on synthetic polymers. A weathering pilot study was deployed and analytical methodologies implemented by P14 (UDC). For weathering, the different polymer particles as provided by P6 (UBAY) were used (in total 9 polymers). The MPs were subjected over 10 weeks to different treatments: simulated marine conditions, dry conditions and seawater in darkness. Their characterization was performed by FTIR, NMR (1H and 13C) and SEM (scanning electron microscopy). A collection of FTIR spectra was obtained for each of the “pristine” polymers and for each stage of the weathering study (and each polymer). They are still under comparison to ascertain differences between the polymers and, eventually, different weathering behaviours (trends). Weathered polymers have been distributed to other BASEMAN’s partners upon request. The interpretation of the NMR (nuclear magnetic resonance) spectra obtained so far is still under way. SEM microscopy (Scanning electron microscopy) was employed to characterize visually the evolution of the polymers; in essence, a mechanical evolution (publication in prep).
Standardized test samples and inter-lab comparisons (WP2 & WP3)

Preparation of standardized test samples

Sediment. Three natural reference standard types (overall 200 kg) were sampled, aliquoted in 1 kg portions and characterized in terms of grain size distribution and bulk elements (CNHS) by P5 (ICBM). The “sandy” and “silty muddy” sediments were taken from the coastline of the East Frisian Wadden Sea. A third almost inorganic fine-grained sand < 200 μm originates from a Pleistocene sand bit and is supposed to be MP free. Samples were stored at -20°C. Spiking with the MP-kit provided by P6 (UBAY) was performed under particular conditions and in a distinct way to assure that no leftovers of any particles in the kit vial/lit occurred (under constant optical control (microscope)). The developed spiking protocol was provided for P1 (AWI) and P17 (NIFES) responsible for plankton and biota samples respectively. Each spiking kit vial was numbered, and preserved sample related to be traced back if necessary. Finally MP spiked samples (in triplicate) were mailed frozen to the BASEMAN partners participating in the inter-lab proficiency test.

Plankton. During three research cruises in the German Bight, natural plankton samples were taken by P1 (AWI) to provide material of different complexity (and different natural polymers or abiotic compounds, e.g. chitin, silica). Samples were taken in spring (diatom dominated plankton) and summer (dinoflagellates and zooplankton) near the island of Helgoland. A third sample was taken in summer in the Elbe estuary to include a sample type with high content of detritus (including resuspended material). All samples were taken with either phytoplankton (100 or 20 μm) or zooplankton (300 μm) nets. The samples were collected in 100 L barrels and dispensed in 1 L containers (700 ml sample volume) either directly on board of the research vessel or in the lab. From each sample set, three subsamples were analysed with respect to plankton composition (Utermöhl), particle numbers (FlowCam) and wet/dry weight of particulate matter. All containers were stored frozen (-20°C) until spiking with the MP reference kit (P6 (UBAY)). Spiking of the samples was performed in accordance to the protocol developed by P5 (ICBM). Each spiking kit vial was numbered, and preserved sample related to be traced back if necessary. Finally MP spiked samples (in triplicate) were mailed frozen to the BASEMAN partners participating in the inter-lab proficiency test.

Biota. P17 (NIFES) prepared biota samples of three seafood species. Nine kg soft parts of farmed blue mussels and 9 kg intestines of farmed salmon were minced in total. 9 kg intestines of wild caught haddock were cut by scissors to 3-4 cm large parts (Haddock intestines can be very stringy and cannot be minced and contain sometimes residues of for instance sea urchins etc.). All samples were partitioned into 100 g portions (in glass jars). Manual remixing preceded all partitioning steps. Spiking of the samples were performed in accordance to the protocol developed by P5 (ICBM) with slight modifications. Each spiking kit (P6 (UBAY)) vial was numbered, and preserved sample related to be traced back if necessary. Finally MP spiked samples (in triplicate) were mailed frozen to the BASEMAN partners participating in the inter-lab proficiency test.

Inter-lab comparison (proficiency test)

Although in 2017 the MP reference kit was finally provided (and evaluated) to all partners responsible for the preparation of standardized test samples, it has to be stated that general problems (requirements) related to QA/QC were underrated with respect to the generation of the MP reference kit or MP-spiked samples for the Inter-lab comparison approach (proficiency test). Especially unforeseen problems related to milling, sieving, handling & analyses (size distributions) of the polymer beads and the spiking procedure itself (transfer of the kit to the ring trial samples) lead to extreme delays. As already pointed out, only the large particles (1 mm) showed the exact expected number of particles in the validation of the kits, meaning 10 particles per polymer. Smaller particles overlap in size distribution between the 100 and the 20 μm size classes and it was impossible to classify each particle according to one of both size classes. 12 laboratories have delivered results per March 2019, namely P1 (AWI), P4 (GEOMAR), P5 (ICBM), P6 (UBAY), P17 (NIFES), P2 (NIVA), P3 (NILU), P9 (LOV), P7 (IMMM), P14 (UDC), P10 (IAMP-CNR) and P12 (FCT NOVA) having extracted and analysed the different samples according to their methods. Plastic content was analysed with either FTIR (ATR-FTIR, FTIR-microscopy/imaging or py-GC/MS by several partners.
Small particles (20 - 100 μm). Three laboratories provided results so far. Recovery rates varied widely, the consortium deemed current methods as not quantitative at the current time point. Main introductions of errors were identified: i) the number of 100 μm particles added to the kit varied per polymer between 32 and 183 according to a validation test carried out on 10 kits by partner P6 (UBAY); ii) transfer from the glass vials to the sample was not quantitative. Due to the shape of the glass vial and electrostatic forces, an unpredictable number of particles was not transferred to the sample, even upon repeated washing steps with water and ethanol; and iii) particles lost during extraction procedure, which necessitates repeated treatments by using bases, detergents, oxidative, acids and/or enzyme containing solutions. The challenge of preparing reference material and quantitative extraction for particles below 100 μm may currently be larger than the challenge of their endpoint analysis. The inter-lab comparison approach (proficiency test) to quantitate the small particle fractions below 100 μm particle size from the named matrices with methods of the current state of the art is considered as failed. However, with considerable further method development, the task is considered feasible.

Large particles (1 mm). The kits contained the appropriate number of particles with satisfying deviation. In a test of 10 kits with 40 particles each, only one particle was missing. All added polymer types, polystyrene (PS), polyethylene (PE), nylon (PA66) and polyethylene-terephthalate (PET), were chemically identified by either FTIR or py-GC/MS by 11 partners, and counted by 12 partners. Samples with and without added 10 plastic particles per polymer type, were clearly distinguished by all partners in a blind test. One laboratory also found one particle of PP in a spiked sample of plankton in the Elbe estuary, even though PP has not been added. Those laboratories who had run procedural controls did not find plastic fragments in those. Fibres were reported, but considered as contamination. In conclusion, laboratory contamination with plastic particles (fragments or beads, not fibres) in the size range of several hundred micrometres is low under the applied precautious conditions, but also natural occurrence is very low; in this case, zero in haddock and salmon intestines, heated and unheated silty-muddy sediment; 4 particles in 900 g of blue mussel soft parts, 2 particles in 8 litres of each plankton draw, 2 particles in 5 kg sandy-silty sediment. For blue mussels, 9 laboratories provided results, recoveries ranged between 95 and 21 %. For salmon intestines, 7 laboratories provided results, recoveries ranged between 88 and 56 %. One failed, one provided only results for one of three parallels. For haddock intestines, 7 laboratories participated. 6 laboratories provided results for at least two of the three parallels, recoveries ranged between 91 and 33 %. One provided only results for one of three parallels. For gold-standard matching protocols, PE and PA66 had the tendency to be recovered worst, while PS and PET were recovered best. For sediment, one laboratory had only analysed PE. For all three sediment types, 5 laboratories provided complete results. For heated sediment, recoveries ranged between 93 and 59 %. The laboratory with the lowest recovery rate had additionally a number of plastic particles in the blank sample that corresponds to 25% of the number of added particles in the recovery test samples. For silty-muddy sediment, recoveries ranged between 88 and 38 %. For sandy-silty sediment, recoveries ranging between 93 and 21 %. For both plankton samples, 8 laboratories provided results, all recoveries ranging between 80 and 99%.

Inter-method comparisons (WP3)

Extraction approaches

P5 (ICBM) determined the basic MP load of sediments from the German Wadden Sea applying a newly developed (by P5 (ICBM)) miniaturized MP sediment separation unit (publication in prep). Triplicates of kilogram aliquots from sandy and muddy sediments were pre-concentrated and consecutively analysed by MF-pyGCMS. The sandy sediments contained 2 to 3 different polymers, with PS being present in all three sediments. Additionally PMMA, PET and PE were present. The absolute contents (sum of all MP types) ranging were the ppm-range with MP-levels of 19 µg ± 16 µg MP kg⁻¹ wet sediment. The high standard deviation reflects the natural inhomogeneity of MP particle distribution in the sample. The muddy sediments contained a higher number of different polymers (up to 5/sample) with PET, PS and PE being found in all three samples. The absolute MP contents were between 55 µg ± 9 µg MP kg⁻¹ wet sediment. Here the particle distribution seems to be more homogeneous. P15 (IVL) tested the degradation of the sediment organic matrix using TRIS (pH 8) with pancreatic enzymes (Creon®, Mylan), treatment with a saline solution of SDS (0.75%) and no pre-treatment at all. The choice of methods has been focused on efficiency and minimal impact on the synthetic polymers.
Subsequent to pre-treatment, MP were recovered from sediments by density separation. **P15 (IVL)** performed density separation in newly developed separation towers (**P15 (IVL)**) as a smaller modification of the MPSS (Imhof et al., 2012). To each tower ~4 l of saturated saline solution and ~400-600 g wet weight sediment was added and mixed slowly for approximately 3-5 hours. When the rotor is stopped, the particles are allowed to settle or surface overnight leaving the saltwater clear. In order to afford running many replicates while at the same time avoiding creating new pollution problems with toxic chemicals, **P15 (IVL)** used saturated NaCl rather than NaI, Na2WO3 or ZnCl2 for density separation. Due to its lower density (1.2 g cm−3) NaCl will not be optimal for retrieving all types of plastic, e.g. PVC, PET and PS or rubber. For these materials, smaller systems with saturated NaI were used which has a density of 1.8 g cm−3. Filtration was performed in direct connection with the density separation apparatus by adding a tower with sequential filter on top (filter sequence 300, 100, 20 µm). The remaining liquid fraction was collected and stored for further filtration onto filters with smaller pore sizes. All filtered samples were analysed under a stereomicroscope and microparticles were characterized into appropriate classes and quantified. Subsequent verification of suspected plastics was verified using FTIR. A series of recovery experiments have been performed in order to test the efficiency. The best recovery (70-100 %) of MP from spiked sediments was obtained when pre-treating thawed sediment with SDS in a saturated NaCl solution. A volume corresponding to the water content of the sediment was added to a final SDS concentration of 0.75 v/v %. The sediment was vigorously shaken and kept at 20 °C on 200 rpm for one hour, being turned once after 30 minutes, before being added to the density separator filled with saturated, NaCl. Added fragments of PET were not recovered in the density separation since they have a higher density (1.37 - 1.46 g cm−3) than NaCl (1.2 g cm−3). All recovered plastic fragments were subsequently analysed with FT-IR to determine potential weathering during treatment (publication in prep). **P1 (AWI)** successfully determined the MP load of sediments from the North Sea (from the Frisian Coast to the English Channel) and deep sea sediments from the Fram Strait by using the MPSS and ZnCl2 (1.7 g cm−3) as density separation fluid (Lorenz et al., 2019, Bergmann et al., 2017). **P1 (AWI)** designed, constructed and patented a new sediment separator (Patents DE 10 2016 008 966 A1 and EP 3 272 421 A1). The new separator follows the general principle of the MPSS but has the following advantages: i) reduced amount of separation fluid; ii) complete straight line from the sample chamber to the upper inspection glass; iii) mixing by compressed air; and iv) possibility to run more than one sample (currently 4). The separator is currently being evaluated (publication in prep). **P4 (GEOMAR)** extended and optimized an already existing in house method for the extraction and quantification of MP in marine sediment with respect to the 9 most abundant polymer types in the marine realm (PP, LDPE, HDPE, PS, PA, PMMA, PC, PET, PVC), covering: i) digestion of organic material in the sample; ii) density separation (poly-tungstenate solution) and sieving into different particle size classes; iii) transfer of particles for centrifugation; iv) vacuum filtration of supernatant onto glass fibre filter; and v) microscopic quantification and identification of particles on the filter by Raman spectroscopy (publication in prep). **P6 (UBAY)** tested a new device for density separation. Sediment samples generally require a density separation due to the co-occurrence of mineral particles in samples. Carrying out a density separation of sediments represents different challenges depending on the sample volume and size of particles targeted. The use of the MPSS (Imhof et al., 2012) is useful for high volume samples when larger (>1mm) and average size particles (>100µm) are targeted. Classic separation funnels are used when very low amounts of sediments are treated. In the framework of the project, a new separation device was developed in order to offer more adaptability. It can be used with variable size samples and is intended to target different sizes. It is made of glass with a stainless steel holder and an automated motorized stirrer (publication in prep).

**Purification approaches**

**P1 (AWI)** applied Fenton’s reagent for purification of deep-sea sediment samples. The Fenton reaction is an oxidative process based on the initiation and catalysis of H2O2 by a ferrous ion (Fe2+). This leads to the formation of hydroxyl as well as hydroperoxyl radicals (Fe3+ + H2O2 → Fe2+ + ·OH + OH−). Nine samples originating from the Long-Term Ecological Research (LTER) observatory HAUSGARTEN west off Svalbard (Norway) at ca 79°N in the Fram Strait were analysed (from depths between 2342 m and 5570 m). Sediment samples were taken by multiple corer during the expedition ARK 29.2 of the research icebreaker RV Polarstern in summer 2015. The concentrations of MP for the different stations ranged between 42 and 6595 MP kg−1 dry weight. Overall, 18 different polymers were detected by FTIR-Imaging and subsequent image analysis (Bergmann et al., 2017). **P6 (UBAY)** also tested the use of hydroperoxide with ferrous irons up to a temperature of 30°C (Fenton’s reagent) in addition to the enzymatic digestion developed by Löder et al. (2017). It was
proven to be very successful concerning the removal of organic material and not to be harmful for the integrity of the MP contained in the BASEMAN kits. High volume samples require often a subsampling as the amount of natural organic particles after treatment is still too high for a proper FTIR (FTIR-imaging) measurement (publication in prep). P1 (AWI) applied the newly developed and patented “purification reactor” (Patents DE 2016 123 324 B3 and EP 3 329 994 A1) in combination with the published enzymatic protocol (Löder et al., 2017) for determination of the MP load in plankton and sediment samples from the North Sea (from the Frisian Coast to the English Channel). The automated FTIR Imaging approach yielded concentrations between 0 and 245 MP m⁻³ in seawater. MP concentrations in sediments were far higher (3 - 1200 MP kg⁻¹ dry weight). On average 98% of MP particles were < 100 µm in sediments and 86% in surface waters. A total of 17 different synthetic polymers were detected (Lorenz et al., 2019). P1 (AWI) analysed arctic ice cores for the presence of MP. Due to the low organic content of the sample (TOC data) and only few detectable particles in total (analysed by FlowCam), wet oxidation by H₂O₂ was directly performed on the final analysis filters (Anodisc). The highest MP particle concentration (1.2 ± 1.4 × 10⁶ MP m⁻³) was detected in an ice core taken in the pack ice of Fram Strait. The MP concentrations of all sea ice cores were highly variable with the second highest MP concentration found in the land-fast ice of the Fram Strait (4.1 ± 2.0 × 10⁵ MP m⁻³). Most of the MP particles identified in the sea ice cores were smaller than 50 µm. On average 67% of the particles were within the currently smallest detectable size class of 11 µm. In total, 17 different polymer types were identified (Peeken et al., 2018). P15 (IVL) analysed MP in biota involving: i) degradation of the tissue matrix; ii) filtration of extracted MP onto appropriate filters; and iii) quantification and characterization of MP particles. When gut content of for example fish is part of the sample, removal of mineral grains by density separation may be necessary prior to filtration. P15 (IVL) tested and evaluated three different methods for tissue degradation encompassing incubations with: a) KOH (1 M) and agitation in room temperature; b) addition of SDS (2.5%) followed by addition of a set of enzymes (laundry detergents Biozym F and SE, Spinnrad®) and agitation at 37 °C; and c) addition of pharmaceutical pancreatic enzymes (Creon®, Mylan) with Trizma hydrochloride solution (1 M, pH 8) and subsequent agitation at 37 °C. The focus has been to develop a time- and cost effective method providing a filter cover low enough to enable identification of the anthropogenic particles and causing minimal damage to the plastic polymers. The most efficient, least time consuming and least harsh on the plastic polymers (verified by FT-IR partly as carbonyl index) was the Creon/Trizma treatment. This method is currently used as standard procedure for retrieving MP from marine biota in our laboratory and has been successfully used for amphipods, gastropods and bivalves (publication in prep). P7 (IMMM) and P9 (CNRS-LOV) compared two purification protocols for biota (tissues) leading to different rates of MP recovery. In both cases, identification was identically performed on MP > 1 mm using an FTIR microscope in reflection mode with manual and visual inspection of the filtering membranes. Comparing the two protocols, the largest difference was observed for heavy synthetic polymers (e.g. PET) recovery, which was very low using P7 (IMMM) protocol. The P9 (CNRS-LOV) protocol was more complicated compared to that developed by P7 (IMMM), as several chemicals and steps were added (ultrasonication, adding of Tween, washing step with ethanol) but allowed on average to recover 3 times more MP. However, even with this protocol, MP recovery from salmon samples was still difficult and significantly lower than for mussels and haddock. However, the same protocol allowed very good recoveries with respect to plankton samples (publication in prep).

**Sampling methodologies for MPs in the marine environment: Standardization, suitability and intercomparison (WP4)**

The aim was to develop harmonised/standardised methods for sampling MPs in the water surface, water column, benthic and intertidal sediments and to suggest (define) marine biota species that might serve as relevant and appropriate biomonitoring species with respect to MP in Europe. One of the first activities of this WP was the organisation of a sampling cruise (BASEXEPIMIPS 201704) on board of RV Ramon Margalef between 17-24th of April 2017. This cruise coordinated by P13 (IEO), allowed several project partners (to embark on a sampling opportunity to collect sediment and surface water samples using different sampling tools (e.g. van Veen grab, box corer, bongo and manta nets). Outstanding were the water surface samples (vertical profiles using a bongo net and comparison between bongo and the manta nets with different mouth apertures). P13 (IEO), P8 (GMIT), P7 (IMMM), P24 (IPMA), P1 (AWI), P23 (SAHFOS), P22 (OGS), P10 (CNR-IAMAC) and P5 (ICBM) have been involved in the cruise or analysed samples. Due to the only recently finished standardized protocols, most of the analyses are unfortunately still ongoing. More details about this cruise can be found in the following link: https://doi.org/10.13140/RG.2.2.27319.32162. P8 (GMIT) collected further sediment samples in Galway Bay for comparing sampling and separation methods. It turned out, that all
commonly used benthic grabs may be used for sampling MP in subtidal sediments and that sodium chloride (as extraction solution) might be suggested as a pragmatic option for large scale monitoring purposes. Preliminary results show the sediment station sampled in Galway Bay had an average 104 MP kg$^{-1}$ of dry sediment (Pagter et al., 2018). P15 (IVL) was involved in developing robust methods for sampling of MP in seawater (both water column and sea surface), sediments and biota. Evaluation of sampling methodologies has been carried out for seawater, sediments and biota in temperate and Arctic waters within the framework of other research projects and partly supported by BASEMAN funding. P15 (IVL) e.g. tested sampling of MP using a Manta trawl, in situ pumping through a series of filters (normally 300 and 100 or 20 µm), and by collecting a larger amount of sea water and filtering it in the laboratory. The aim of that approach was to clarify whether the net of a Manta trawl can be cleaned sufficiently between samples to accurately quantify MP and fibres (cross-contamination). Pumping allows passing large amounts of water through the filters. This is highly useful in areas where contamination is expected to be low as in the Arctic. Both phyto- and zoo plankton blooms complicate sampling by clogging filters. Collecting water and filtering it in the laboratory has resulted in higher counts of fibres than obtained through in situ pumping from the same water body. This indicates that the irregular shape of fibres may allow them to escape even through very small filter sizes aided by the pressure created by the pump itself and the extensive amounts of water passed through the filters during field pumping. In conclusion, our experience is that the method of choice depends on the question asked and the conditions in the recipient sampled. All the applied methods are useful (publication in prep).

The sampling cruise BASEXEPEMIPS 201704 and the dedicated WP4 workshop were important milestones to collect environmental samples and to have a forum debate where project partners have organised into work groups in order to achieve standardised protocols with respect to: i) floating MP in surface waters and in the water column (P13 (IEO)); ii) monitoring MP in sediments (P8 (GMIT)); and iii) selecting and using biota to monitor MP (P12 (NOVA) and P8 (GMIT)). Finally P8 (GMIT), P13 (IEO) and other WP4 partners (and partners from the JPI Oceans project EPHEMARE (biota)) published the white papers: i) Standardization protocols for monitoring microplastics in seawater (https://doi.org/10.13140/RG.2.2.14181.45282); ii) Standardization protocols for monitoring microplastics in sediments (https://doi.org/10.13140/RG.2.2.36256.89601/1); and iii) Harmonized protocol for monitoring microplastics in biota (https://doi.org/10.13140/RG.2.2.28588.72321/1). All three white papers are uploaded to the platform ResearchGate (https://www.researchgate.net/project/BASEMAN-JPI-OCEANS-Defining-the-baselines-and-standards-for-microplastics-analyses-in-European-waters-Project-Coordinator-Dr-Gunnar-Gerdts-Alfred-Wegener-Institute-Helmholtz-Centre-for-Polar-and-Mar) and are attached as a supplement to the final report.

**IMPACT - OUTCOME**

In the scope of the project, several project partners were involved to contribute to European and international regulations, policies and management practices. Contributions were performed for example to the GESAMP Report 93: SOURCES, FATE AND EFFECTS OF MICROPLASTICS IN THE MARINE ENVIRONMENT: PART 2 OF A GLOBAL ASSESSMENT (P2 (NIVA),P8 (GMIT), P12(FCT-NOVA)) and the national report of PlastikNet “Mikroplastik-Analytik: Probennahme, Probenaufbereitung und Detektionsverfahren” P1 (AWI), (https://bmbf-plastik.de/index.php/de/publikation/diskussionspapier-mikroplastik-analytik).

Experts from the consortium (P1 (AWI), P2 (NIVA)) were invited to contribute to regulations required by the California Senate Bill 1422 for standard operation protocols for microplastic sampling and analysis in California, USA, until 1st July 2021.

The following innovations were developed during the project:

unkritisch und beantwortet, Erteilungsbeschluss liegt vor, Patentschrift wird demnächst ausgegeben. Patentanmeldung (EP EP 3 272 421 A1) in Europa (erweiterter Recherchenbericht liegt vor: negativ (keine Neuheit), aber entkräfbar, Erwiderung zusammen mit Prüfungsantrag) (P1 (AWI)); A Freeware software tool “Systematic Identification of MicroPlastics in the Environment (siMPle)” for the analysis of FTIR and Raman spectra from single and imaging measurements (P1 (AWI), P26 (AAU)) was provided and is available via the website https://simple-plastics.eu/.

New cooperations and collaborations were developed during the project time as members of the consortium are currently part of the steering committee for the Microplastic Interest Group from the Society of Environmental Toxicology and Chemistry (SETAC) (P1 (AWI), P2 (NIVA)) and invited experts on microplastics sampling and analysis (P1 (AWI), P2 (NIVA), P3 (NILU), P5 (ICBM), P15 (IVL), P17 (NIFES)) for the Arctic Monitoring and Assessment Programme (AMAP), a Working Group of the Arctic Council.

The project contributed to the exhibition Ocean Plastics Lab (P1 (AWI)) with materials and pictures (https://oceanplasticslab.net/de/). An intensive course on MP at the Ocean Outlook conference, Woods Hole, MA, USA was performed by P17(NIFES) and P27(NORCE) in 2018. P26 (AAU) in Aalborg performed a PhD workshop on the analysis of microplastics with contributions from P1 (AWI). Another PhD course will be held in 2019 with contribution of P1 (AWI), P8 (GMIT) and P26 (AAU) again in Aalborg.

The project will be followed by future projects by an application for the new JPI Oceans call “Sources, distribution & impact of microplastics in the marine environment” by the project “Fluxes and fate of microplastics in northern European waters (FACTS)” and by an application for the Horizon 2020 call CE-SC5-30-2020.

CONCLUSION

The project has been implemented to the best possibilities of the participating laboratories. The research topic is comparably new and still highly experimental. During BASEMAN, connecting Europe’s foremost laboratories with respect to MP research the marine environment, it became clear that chemical identification and quantification of larger MP can be achieved by most laboratories, while identification, quantification and sizing of small MP (< 500 µm) is not yet achieved as a standard with sufficient measurement uncertainty. One necessary step towards that enterprise has simply to be the general acceptance of European public authorities responsible for environmental surveillance in the marine realm (e.g. environmental agencies) that more dedicated efforts but also resources are needed to facilitate the analyses of small MP. This is of utmost importance, as: i) it becomes more and more evident that the majority of MP particles (with respect to numbers) in the environment is small (also findings of BASEMAN); and ii) long term toxicity of small MP has been demonstrated in over 100 publications by now (size matters!). However, it is currently widely unknown what the concentrations and compositions of such small MP are in the marine environment. Therefore, no risk assessment can be performed without quantitative data on small MP from all relevant matrices. Such a risk assessment is necessary to decide upon meaningful measures to counteract the increasing plastic pollution we are facing. Furthermore, for a deeper understanding of MP sources, transport paths of MP and MP sinks in and across ecosystems, harmonization is needed between marine, freshwater, terrestrial and possibly atmospheric research or monitoring approaches. Currently most research on MP in the freshwater, terrestrial -and recently atmospheric- environment, was and is focused on small MP, whereas in the marine environment, the focus is still on particles > 300 µm (which are even not always subsequently chemically identified/verified as synthetic polymers). In the framework of BASEMAN, we were able to show, that all analytical approaches and tools for identification, quantification and sizing of even small MP particles (current lower thresholds: ~10 µm (FTIR), 1 µm (Raman)) are at hand, are constantly improving and cannot be seen as a general bottleneck any more (if more labs get access to the necessary analytical equipment). However, for accelerating the whole pipeline from sampling to analysis (for instance for monitoring approaches), time-efficient and effective approaches for removal of the referring environmental matrix (where MP is embedded) still have to be developed (or improved) for processing large sample numbers. Finally, it will be of utmost importance that appropriate QA/QC measures (and designs) are incorporated in future approaches on a mandatory basis.
REFERENCES


LIST OF SCIENTIFIC QUALIFICATIONS (PHD, MSC AND BSC THESES)

2016 - 2018/19 (exemplarily)
(Hosting BASEMAN partners in bold characters)


LIST OF PUBLICATIONS

Peer reviewed (ISI) with funding remark (BASEMAN)
(Responsible (first/corresponding author) BASEMAN partners in bold characters)


others
2016 - 2018/19 (exemplarily)
(e.g. joint activities with respect to BASEMAN, affiliation not further specified)


João P.G.L. Frias, Elena Pagter, Róisín Nash, Ian O’Connor, Olga Carretero, Ana Filgueiras, Jesus Gago, Joana Antunes, Filipa Bessa, Paula Sobral, Alenka Goruppi, Valentina Tirelli, Maria Luiza Pedrotti, Giuseppe Suaria, Stefano Aliani, Clara Lopes, Joana Raimundo, Miguel Caetano, Luca Palazzo, Giuseppe Andrea de Lucia, Andrea Camedda, Soledad


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**LIST OF CONFERENCE PRESENTATIONS**

**2016-2018/2019 (exemplarily) with funding remark (BASEMAN)**

(Responsible (first/corresponding author) BASEMAN partners in bold characters)


Bjorøy, Ø., Oveland, E., Kögel, T. (2018). Quasimeme Microplastics analysis workshop, Netherlands; Combining pyrolysis with GC-Orbitrap for the analysis of microplastics *(P17 (NIFES))*


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Kögel, T. (2017). BASEMAN meeting Lisboa, Portugal; WP2 Biota preparation (P17 (NIFES))


Kögel, T. (2017). Oslofjordkonferansen NJFF, Oslo, Norway, Uønskede stoffer i sjømat, en studie fra Oslofjorden og mikroplast (P17 (NIFES))

Kögel, T. (2017). Technician Forum, Institute of biomedicine, University of Bergen, Norway; Påvirkes Norsk sjømat av mikroplast? (P17 (NIFES))


Kögel, T. and Måge, A. (2017). The Nordic Council of Ministers, Oslo, Norway; Påvirkes sjømaten av mikroplast? (P17 (NIFES))

Kögel, T., Bjorøy, Ø., Bank, M. and Monica Sanden (2018). Arctic Frontiers, Tromsø, Norway; Effects of micro- and nanoplastics on physiology of biota: A review (P17 (NIFES))


Lusher, A.L., (2018). What are the environmental challenges with the use and disposal of plastics? Norwegian Academy of Technical Sciences Seminar 15 February, Trondheim, Norway (P2 (NIVA))


Olsen, M. (2018). From waste to pollution- challenges, sources, solutions. Workshop on plastic waste and marine pollution, Shanghai, China (IVL)

Olsen, M. (2018). From waste to pollution, Miljøringen, Sanderfjord, Norway (P15 (IVL))


LIST OF NON-SCIENTIFIC, POPULARIZED PUBLICATIONS
2016-2018/2019
-

LIST OF WORKSHOPS, STAKEHOLDER MEETINGS (ETC)
2016-2018/2019 (exemplarily)
(e.g. joint activities with respect to BASEMAN, partner affiliation not further specified or listed)

Intensive course on micro-plastics at the Ocean Outlook conference, Woods Hole, MA, USA; 2018 (Marte Haave, Scott Gallager, Mark Hahn, Tanja Kögel and Bjørn Einar Grøsvik)


GESAMP workshops (Sep 2017, March 2018, June 2018) (Amy Lusher, Martin Hassellöv)

LIST OF PUBLIC ACTIVITIES - FLYERS, BROCHURE, VIDEO/FILM
2016-2018/2019 (exemplarily)
(diverse contributions with respect to BASEMAN or generally to “microplastics”; partner affiliations not further specified or listed)

GEOMAR News 02/2018, Dominiert Plastik den Ozean der Zukunft?,

Panel discussion and press interviews at the Opening of the KDM Ocean Plastic Lab, 22 October 2018, Berlin

TV documentary on pollution from polymer fibers of clothing, NDR, Markt im Dritten, March 2018
https://www.zdf.de/gesellschaft/sonntags/plastik-freund-oder-feind-104.html
http://www.nwzonline.de/oldenburg/wirtschaft/im-stadthafen-den-ozean-erleben_a_31,0,2175252962.html
http://future.arte.tv/de/plastik-unseren-meeren; https://plastikmeere.arte.tv/#/chapter/1-4

ZDF Internet-Channel „Jäger und Sammler“, https://www.youtube.com/watch?v=KylRxCprbY

WDR, „Könnes kämpft“, https://www.youtube.com/watch?v=MAIxawUxtpE
Arte “Was ist Mikroplastik?”; https://www.arte.tv/de/videos/075351-000-A/was-ist-mikroplastik/

“Immer Meer Plastik - durch Mikroplastik in Kosmetikartikeln”; Jäger & Sammler
https://www.youtube.com/watch?v=KvlRxHCrpbY

Interview for the radio show “Os dias do Futuro” RTP/ Antena1 with the Theme “Ocean without plastics/ Um oceano sem plásticos”, 17 June 2017; https://www.rtp.pt/play/p383/e294377/os-dias-do-futuro


Camera interview with Terje Alming for museum of fishery, Bergen, about farming (Jan-Erik Lock), wild fish (Arne Duinker) and microplastic (Tanja Kögel)

Background info and video interview "Forskerne jobber på spreng for å finne svar: Kan mikroplast gi oss hormonforstyrrelser?"

https://www.aftenposten.no/norge/j/02bmJ/Forskerne-jobber-pa-spreng-for-a-finne-svar-Kan-mikroplast-gi-oss-hormonforstyrrelser

Interview "– Vet ikke hvor skadelig mikroplast er for mennesker" https://www.nrk.no/norge/-vet-ikke-hvor-skadelig-mikroplast-er-for-mennesker-1.13371091

Background info and NRK radio interview “Nyhetslunsj” "Plast i sjømat" 10.2.2017
https://radio.nrk.no/serie/nyhetslunsj/NPUB44003017/10-02-2017#t=37m43s

LIST OF SOCIAL MEDIA ACTIVITIES
2016-2018/2019
As a result of the 2015 call for research proposals 'Microplastics in the marine environment', four projects (BASEMAN, EPHEMARE, PLASTOX and WEATHER-MIC) were funded under the framework of JPI Oceans by the following ministries and funding agencies: