ANDROMEDA]website address: https://www.andromedaproject.net

FINAL REPORT

ANALYSIS TECHNIQUES FOR QUANTIFYING NANO- AND MICROPLASTIC PARTICLES AND THEIR DEGRADATION IN THE MARINE ENVIRONMENT

Andromeda

Period covered: 05 May 2020– 30 September 2023

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PUBLISHABLE SUMMARY

Plastic polymers coming from both industrial and domestic products have also been defined as plastic particles spanning a large spectrum of size from macro- to microplastics (MP: 1 µm - 5 mm) and nanoplastics (NP: 1 < µm), shapes, and chemical composition. These polymers are made up of a series of monomers and additives and have been found in soil, water, air, sediment, fish, and snow. Although knowledge in this domain is still very limited, there is small evidence of the impact of MP and NP consumption on aquatic organisms and on human health, e.g. by affecting cardiovascular mortality in adult men. A proper study of the fate and impacts of microplastics and nanoplastics requires, on the one hand, appropriate measurements of MPs and NPs and, on the other hand, information on the fragmentation and degradation of plastic particles and their ability to release toxic additives (Figure 1).

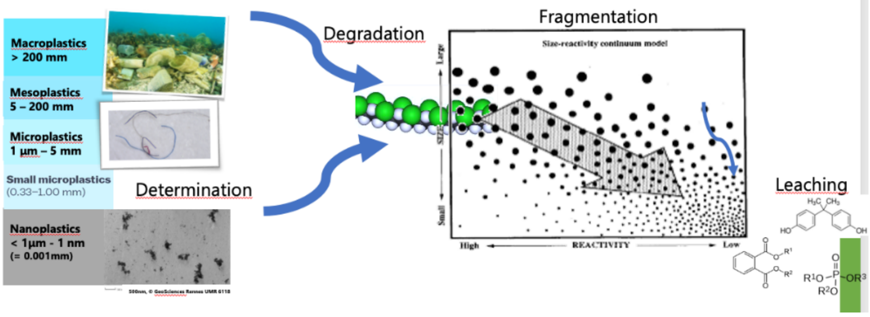

Figure 1: Andromeda Scientific challenges linked to the study of plastics.

Andromeda focuses on (1) cost-effective analysis of MP by in situ-methods and low-cost laboratory analysis, (2) comparison and cross-validation of different analytical methods for MPs including the analysis of tire wear particles (TWPs), fibers and paint flakes, (3) development and optimization of advanced techniques to measure and quantify small and challenging types of MP and NP particles, (4) investigation on the degradation of plastic into micro- and NP particles and (5) release of organic additives and finally (6) on the dissemination of project results and developed protocols to a range of audiences, including public authorities, the private sector, academia and the general public (Figure 2).

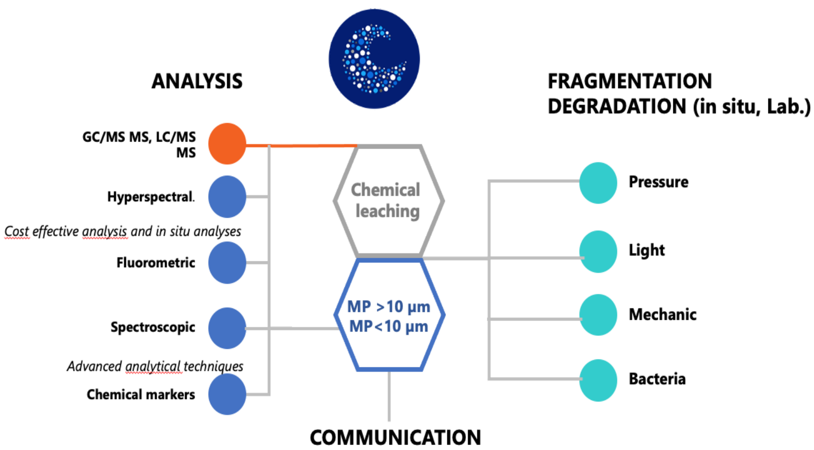

Figure 2: Andromeda general Research plan

The Andromeda consortium represented an international team with 15 partners from 11 countries that have significant experience in international research cooperation. The project, which is organized into 5 work packages (WP), strengthened existing collaborations and enabled us to exchange and collaborate on sampling, analysis, and incubation methods for plastics, as well as on the dissemination of information. Moreover, project partners included 3 in-kind partners from Wageningen University, The Netherlands and from McGill University and Merinov, Canada, who actively contributed to the ANDROMEDA project tasks.

The Andromeda goals are specifically targeted through its WP structure.

- WP1 focused on general management. The collective management team, comprising the coordinator and WP leaders, met regularly by videoconference, and met 3 times with the advisory board. This work package has enabled exchanges of information between the various groups, the organization of at-sea campaigns and joint laboratory work, publications, addressing policy and the scheduling of meetings.

- WP2 deals with development of new cost-effective sampling techniques and in situ analyses and laboratory techniques for MP determination. Indeed, sampling devices were developed as well as new analyses techniques using automated image analysis, fluorescence microscopy and machine learning as well as a smart phone application. These techniques were elaborated, tested in situ and used in different marine environments.
- WP3 deals with the development of a toolbox complementary, analytical instrumental methods and workflows to consistently and reliably detect, identify, quantify (mass/number) and characterize MP and NP in environmental samples. In addition, the WP3 project team provided Reference Materials (RMs) in the 1-100 µm-size range for use in the various Andromeda experiments, including those with properties specifically needed to address the research questions and tasks of WP3.

- WP4 is concerned with the study of the degradation of plastics in the marine environment under various external forcings, and their ability to release dissolved additives, which have also been identified as toxic to living organisms. Material has been degraded in situ in the Mediterranean and Baltic Seas, or in the laboratory under different conditions of light pressure, and subsequently studied in the laboratory.

- WP5 deals with the communication of the Andromeda program. The WP5 team developed classic communication tools to ensure visibility of the project and its research (twitter account, website) and developed in close collaboration with researchers from other WPs information-rich documents for the general public on plastics and the use of the WP2 smartphone application, thereby contributing to developing citizen science around plastic pollution in the marine environment. A project brochure has been developed and together with all other developed information material is available for download via the project website. The team also developed, tested and implemented two online stakeholder workshops to support and contribute to WP2 work on the cost-effectiveness of microplastics analysis methods for seawater samples.

WORK PACKAGE 1: PROJECT COORDINATION

WP1 deals with project management and was punctuated by (1) regular quarterly meetings of the Andromeda steering committee, comprising the WP leaders and the coordinator, and (2) meetings of the advisory board, comprising the steering committee and experts, external to the project (Figure 3). At the same time, regular meetings were held for each WP, depending on the objectives and ongoing projects, that required collective dialogue between the various participants. The general project has been presented pro-parte or in its entirety at several national and international meetings (the 12th Kobe University congress, Brussels, 2022; EU missions, restore our ocean and waters by 2030, Brussels, 2023; ASLO Palma, 2023). The project has been presented to the Maltese ministry of education in La Valette thanks to our Malta partner (A. Deidun) during the Malta meeting (May 2022).

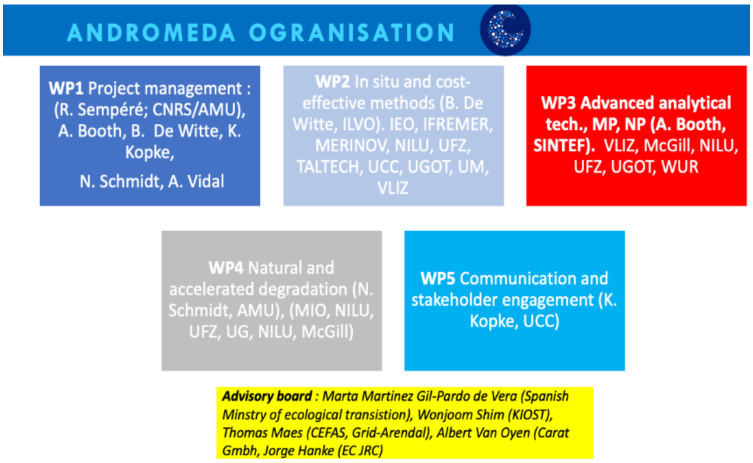

Figure 3: Andromeda general organization

Task 1.1 Project coordination

A virtual kick-off meeting was held in May 2020 with all project members, followed by another general consortium meeting in September 2020. Mailing lists were established right at the beginning of the project to ensure easy communication within WPs as well as between all project members. The general consortium meeting was realized in January 2022 (online) followed by two in-person meetings in Malta (June 2022) and in Galway in September 2023. The first general meeting was held virtually due to the current pandemic.

Task 1.2 Risk management

A risk assessment was made within the first six months of the project. Environmental and personal risks, such as the release of MPs into the environment or the exposure of laboratory staff to UV irradiation during MP degradation studies, were conscientiously evaluated. The levels of risks were found to be acceptable. Furthermore, all project partners concerned by these experiments already have considerable experience working with micro- and nanoplastics and UV irradiation and are familiar with the safety precautions that need to be taken.

Task 1.3 Initial protocol harmonization

Two tables summarizing the analysis methods and the artificial MP degradation methods available within the project were established and can be visualized under https://www.andromedaproject.net/research-methodologies. The tables include information on strengths and weaknesses of each method, personnel and equipment cost indexes (low/medium/high), the links of these methods to Andromeda workpackages as well as a reference person within the consortium.

Task 1.4 Material selection

Pristine reference materials used in degradation studies and as test materials for MP analysis were selected in a common effort and provided by CARAT GmBH (Germany). Different size ranges were selected (e.g. 100-300 µm, 300-500 µm, etc.) as well as various polymer types (LDPE, HDPE, PVC, PET, PP, PS, PTFE, PUR), including a post-consumer HDPE material and plastics with high/low additive content. Furthermore, car tires for the production of tire wear particles (TWP) were selected and provided through the University of Gothenburg and the Helmholtz-Centre for Environmental Research (UFZ).

Work Package 2: In-situ and cost-effective methods

Introduction

Microplastics occur in a wide range of sizes, shapes, colours or polymer types. This hampers the application of one method to measure all microplastics. Methods should be fit for purpose, and for monitoring purposes, large scale research projects or citizen science, measuring microplastics of a few micrometres is generally not necessary. Therefore, the Andromeda work package 2 focused on the optimisation of different sampling and analysis methods to analyse microplastics >50, >100 or >300 µm in a cost-effective way.

Cost-effective MP analysis can be mediated by efficient sampling. Within the first task of WP2, MP samplers have been optimized for both water and air matrices. Next, in-situ methods also allow cost-effective assessment of microplastic occurrence. An ANDROMEDA smart phone app was developed, which incorporates AI through a Machine Learning algorithm for a more engaging citizen science experience. Third, in-laboratory, cost-effective techniques may be implemented, based on the use of low-investment equipment or high degree of automatization. Different methods based on hyperspectral imaging, chemical markers and fluorochromes were developed or optimized to apply in the laboratory. Cost-effectiveness of frequently applied methods was assessed and cross-validation exercises were conducted.

Results and discussion

Cost-effective sampling techniques

An optimized MP sampling device for water, which can be attached to a pumping system or ferrybox, has been developed and tested (Figure 4). The device/method allows the sampling of MP with pre-defined particle sizes. Sieves of 300, 100 and 50 µm were used within the project. The tests included sampling with the devices attached to a ferrybox on a commercial ferry and ferryboxes or pumping systems on board research vessels. The sampling protocol was developed. The efficiency of the method in different environments (Baltic Sea, Atlantic Ocean, Mediterranean Sea) and seasons were tested. Comparisons with sampling methods using nets (e.g. Manta) and parallel sampling with three devices were conducted. Differences between methods were explained although a high degree of similarity of the results in the distribution patterns of MPs is shown.

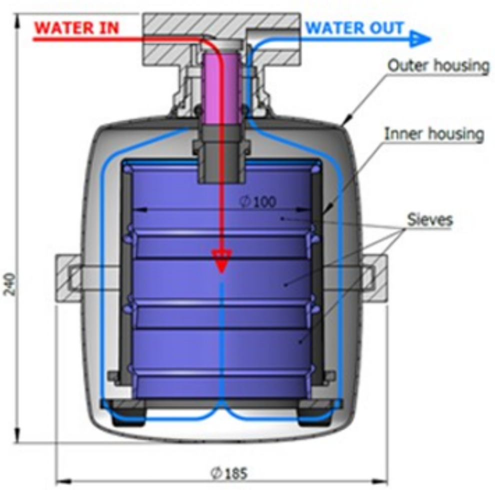

Figure 4: Schematic representation of the MP sampling device for water samples (TalTech).

Two MP sampling devices for air have also been developed and tested. An active air sampler and a deposition sampler were placed at the meteorological station in Marseille, France for feasibility testing. We sampled in a period of three months for periods of 14 days per sample. To further evaluate the application of the sampling devices on board a vessel, an active air sampler was used during the second intercalibration cruise in 2022 in Vigo (Spain). Both samplers consist entirely of metal, while the active sampler consists of a filterholder containing a stainless-steel filter (5 µm pore size), connected to a pump. A flowmeter allows to monitor the amount of air that has been filtered through the filter. A second filterholder, attached in parallel but without an active air flow allows to conduct field blanks. The set-up permits to sample suspended microplastic particles in an easy way as long as electricity is available, and under conditions minimizing contamination due to the use of metal in the conception of the filterholders. The passive sampler consists of a metal bucket collecting dry and wet deposition during 14-day periods. This represents a very cost-effective sampling method, easily replicable in different countries.

In-situ analysis

An Andromeda smartphone app was developed which allows in-situ microplastic monitoring on beaches. The app was launched in May 2023 and is available for free download on both the ANDROID and iOS platforms, being applicable to any beach along the European coastline. A dedicated factsheet (ANROMEDA App Factsheet ENGLISH (squarespace.com)) has been delivered/produced within the project on the development of the same app and has been translated into numerous languages. The app has been extensively used already in Malta, with the geographical location of each report being plotted on a European-scale map by tapping into the mobile device's GPS. The user ideally registers for an account once the app has been downloaded so that he/she can view the history of all the reports they have submitted. The app contains a link to the YouTube video (https://youtu.be/1kBfm1qhTEI) which demonstrates the simple procedure one has to perform in order to extract microplastics from sand, as well as tips on how to take good-quality photos of the same microplastics on the provided background/template for optimal algorithm functionality. Upon being received at the University of Malta, the submitted photo is subjected to the Machine Learning algorithm and the extracted attributes (number, colour, dimensions, surface roughness) are stored in the database and are relayed back to the user as feedback.

For optimal operation of the app, a simple beach microplastic extraction toolkit, consisting of two sieves (0.5 mm and 1.0 mm), a shovel, a ruler, a 0.5 m quadrat and a custom-designed, QR code-loaded, white paper template for photos should be provided on the beaches where the ANDROMEDA campaign is being promoted. The use of such toolkits will also enable the standardization of the data being collected so that microplastic data being collected on different beaches can be compared. Additional relevant recommendations include: a.) the use of seaside panels/banners on beaches where the ANDROMEDA smart phone app is being conducted so as to attract the attention of passers-by; b.) the engagement of beach supervisors where these are available (e.g., on Blue Flag beaches) so that they can assist in the management of the microplastic beach extraction toolkits (handing out and retrieval) and c.) the recovery of the extracted microplastics into glass jars, as opposed to them being discarded back onto the beach, so as to contribute to environmental targets as well.

Cost-effective analysis techniques

Use of hyperspectral imaging can allow identification of microplastics down to 100 µm routinely. An evaluation platform for a novel snapscan type of hyperspectral sensors was build, covering the short-wave infrared wavelength (1100-1700 nm). The setup could be either designed in a simple benchtop standalone system with lens and illumination and stage control, or more advanced integrated in a microscope setup. Prior to classification, a preprocessing method was applied to prepare the data, which used the 1st derivative in combination with Savitzky-Golay smoothing and length normalization. A classifier for microplastic detection was developed, consisting of a Neural Network with an input layer of 102 neurons and three hidden layers with 200, 100 and 50 neurons. The reLu function served as the activation function for the hidden layers and a soft-max activation function was used to produce a probability distribution in the output layer which consisted of a number of neurons equivalent to the number of polymer classes. Furthermore, 50 epochs were used for the final classifier and Synthetic Minority Oversampling Technique (SMOTE) was applied to balance the data. In addition, a particle binning of 10 was applied to the samples.

Validation showed that the classification model was able to separate large pristine plastic particles. However, the classifier shows a noticeable drop in accuracy when it comes to weathered particles. Therefore, a good training dataset is important, also for future research and both, pristine and weathered particles, must be included into the training. Furthermore, the wavelength needs further investigation. In this project, a wavelength range of 1100-1700 nm was used, excluding the 1700-2500 nm range. Although this shorter wavelength range provides less information, it offers the opportunity to use the more cost-effective SWIR cameras compared to more expensive NIR cameras.

The fluorescent staining of MPs using the hydrophobic dye NR, frequently used in histology, enables the rapid screening of environmental samples for the presence of MPs using fluorescence microscopy. The automation of inexpensive fluorescence staining methodologies as an alternative to often used, spectroscopy-based methodologies has aroused as a promising alternative for the development of cost-effective screening methods for MPs analysis. Within Andromeda, a multi-filter (UV, blue, green) approach was optimized (Meyers et al., 2022, 2024b in prep., 2024c in prep.) (Figure 5). A two-step semi-automated classification method was created which combines (RGB)-colour quantification based on the automated image analysis of NR-stained particles filters photographed with a fluorescence microscope (FM) with a supervised machine learning (ML) classification tree (CT) and random forest (RF) model, developed in an open-source environment, using the software ImageJ and Rstudio. Two models were developed: the first one, the Plastic Detection Model (PDM) can predict with high accuracy whether particles are of plastic origin or of non-plastic origin based on their fluorescent colouration (95.8% correctly classified particles), and the second one, the Polymer Identification Model (PIM), can identify plastic polymer types (88.1% correctly classified particles). Sample processing protocols were optimised for the extraction of MPs from different marine matrices. These protocols ensure the efficient use of the analysis protocol mentioned earlier. The protocols were validated, and special attention was given to the analytical QA/QC associated with the validation of these protocols. Developed sample processing protocols were successfully used to extract MPs from various matrices at and near dredge disposal site Zeebrugge Oost, as well as at reference areas.

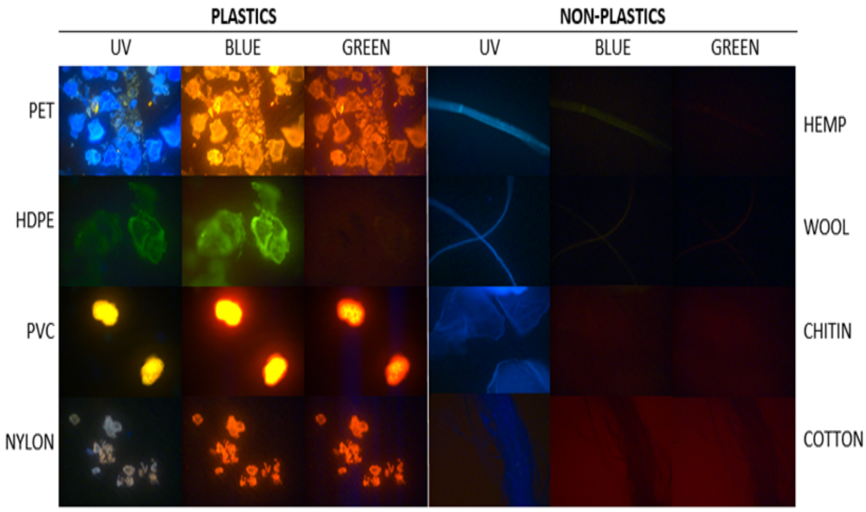

Figure 5: Microplastic and non-plastics particles, stained with Nile-Red and visualised through 3 different filters (ILVO-VLIZ).

Suitable chemical markers to assess the exposure of biota to microplastics, including tire wear particles (TWPs), are currently still missing. TWP are characterized by a different set of additives than plastic polymers, requiring two separate approaches for the determination of the presence of MPs in biota. A three-tiered procedure was carried out with 1) identifying relevant and specific chemical markers for TWPs and polymers; 2) exposure experiments to characterize the uptake potential of the pre-identified marker candidates and 3) marker analysis in field samples with known MP exposure. To cover the 1st step, organic extracts of virgin and weathered TWPs from a variety of tires as well as the ANDROMEDA plastics were analysed for potential marker candidates. Benzothiazoles, guanidines, 6PPD and other PPDs were selected as TWP-specific markers while UV 326 -329 as well as Irgafos were found to be prevalent in most plastic polymers containing additives. Zn was included as a potential marker for TWP exposure. In step 2, *ad hoc* TWP and high-density polyethylene (HDPE) exposure experiments were performed using blue mussels (*Mytilus edulis*). Wild mussels were collected, cleaned and left to depurate. Three sub-groups (n=8 each) were established: controls, low particle concentrations (1.0 g in 10 L seawater) and high particle concentrations (10 g in 10 L seawater). Samples were taken for chemical analysis after 1, 3, 7 and 14 days. After t_3 (7 days) the mussels were moved to fresh tanks for depuration. In samples from TWP exposure, a total of 20 target and 17 suspect tire-related compounds were detected by combining UPLC-HRMS and orbitrap GC/MS. These include vulcanization accelerators (such as guanidines and thiazoles), and antioxidants (such as p-phenylendiamine compounds). Diphenylguanidine was most abundant, reaching concentrations of 600 µg/g. Among the antioxidants, 6-PPD, other PPDs and related transformation products were detected, even 1 week after exposure time. Also, BENPAT compounds were still present in the mussel soft tissue after the depuration time. The Zn content of mussels showed no notable differences compared to control samples. Biologically accumulated organic chemicals were found to accurately reflect TWP exposure in biological samples exposed under controlled conditions, while Zn levels were not indicative of TWP uptake.

For step 3, plasma samples of Northern fulmar with known amount of ingested plastic particles were analysed for UV-compounds as well as crab- and blue mussel samples from known TWP exposure sites. The presence of the selected markers could be shown for all selected species. Reference samples were significantly lower than the samples from polluted sites. Our results indicate that blue mussels and crabs can be used as bioindicators for the exposure to MP and TWP, whilst plasma of Northern Fulmar is not resulting in conclusive information on MP exposure. Further, the selected markers proved to be useful indicators of recent exposure to MPs and TWPs.

Cross-validation and cost-effectiveness evaluation

Two cross-validation exercises were conducted: in 2021, a campaign took place in the Bay of Marseille (France) focused on sediment samples, and in 2022 in Vigo (Spain), focused on water samples. During the campaigns, two types of samples were taken: control samples, being a Negative Control (NC) and a Positive Control (PC) for inter-comparison; and in-situ samples to study contamination. For the sediment sample cross-validation exercise, a NC was made from decontaminated sediments, collected on site, while the PC corresponds to NC spiked with known concentrations of artificial polymers. In-situ samples were taken at different stations and depths. Each partner used its own extraction and identification techniques to identify microplastics and no a priori selection of techniques was done. During the inter-comparison exercise, recovery rates varied between 47% and 113%. However, none of the partners achieved the exact expected concentration of MPs. The choice of extraction technique has a major impact on the recovery rate. The extraction and identification of MPs are highly improved with addition of a digestion step (using H_2O_2). The use of high-density solutions such as $ZnCl_2$ or NaI proves to be more effective compared to extraction with NaCl. With regard to MP identification and characterization, reference techniques such as infrared spectroscopy (FTIR) are the most reliable, but the identification using fluorochrome method remains a cost-effective and efficient solution, especially on fragments larger than 300 µm (Gerigny et al., 2024 in prep.).

Environmental contamination results show considerable variability. Although the average environmental signal remains similar between the different identification techniques, the reliability varies, particularly in terms of false-positive and false-negative results. Contamination of sediment by MPs is more significant in the Calanques de Marseille (Marine Protected Area – National Park of Calanques) than in the Bay of Marseille, an area considered to be highly anthropized (Gerigny et al., 2024 in prep.).

To investigate the cost-effectiveness of commonly used analysis techniques for MPs in seawater, an online survey was performed. A detailed hypothetical scenario was developed. In the scenario, we considered a situation in which five seawater samples were acquired with a manta net targeting > 300 µm MPs, on board of a research vessel in the North Sea. Details of the respondents' MPs analysis workflow were investigated, i.e. of the sample acquisition, sample processing (preparing the sample for MP analysis) and sample analysis step (analysis of the MP content). Equipment and labour costs were calculated for 6 different analysis technique categories: 1. (fluorescence) (stereo)microscopy ((F)(S)M)), comprising all purely microscopy-based techniques; 2. (stereo)microscopy combined with ATR-FTIR ((S)M+ATR-FTIR); 3. (stereo)microscopy combined with FTIR microspectroscopy ((S)M + µ-FTIR); 4. fluorescence (stereo)microscopy combined with FTIR microspectroscopy F(S)M + µ-FTIR; 5. (stereo)microscopy combined with Raman microspectroscopy ((S)M + µ-Raman); and 6. all GC-MS-based techniques (GMBT). This led to a predictive tool, which demonstrated variations in cost-effectiveness according to the country of employment (Meyers et al., 2024a, in prep.)

Conclusion and prospective

No single method currently allows measuring microplastics covering all sizes, shapes, colours and polymer types. This stresses the importance for selecting a method fit for purpose. The Andromeda work package 2 focused on the optimisation of different sampling and analysis methods to analyse microplastics >50, >100 or >300 µm in a cost-effective way. A central key to obtain cost-effective methods is automation at each step of the workflow. Within the project, MP sampling devices for unmanned water and air sampling were developed. Sampling methods have been proven to be efficient and cost-effective for different (including ecologically relevant) size classes. Analysis can also be automated by the use of automated imaging techniques. The use of image recognition algorithms in hyperspectral VIS/NIR imaging or Nile red-based methods allow laboratory analyses of microplastic samples from various matrices in a cost-effective way, even combining microplastic analysis with polymer identification. This allows a higher throughput of samples or may allow pre-screening of samples in order to reduce the workload on high-end techniques such as µFTIR or µRaman. Within Nile-Red applications, the use of a multi-filter approach was found to be a key element to identify pristine as well as weathered plastics with high accuracy. For the long-term success and legacy of the ANDROMEDA smart phone app, there is a need for regular demonstration exercises on target beaches with different communities of potential users, including schoolchildren, ramblers, eNGOs, bathers, etc. A continuous media presence is also essential, so as to generate enough awareness about the existence of the app and high-profile outreach events still need to be conducted in future months and years, despite the formal termination of the project. The University of Malta will keep hosting indefinitely (way beyond the lifetime of the project) the database of reports coming in through the app and the composition of extracted beach microplastics will be continuously analysed. The online portal which will display the results extracted from the submitted microplastic photos will be hosted indefinitely at this URL: https://ocean.mt/2023/03/02/andromeda/.

Use of chemical markers to measure plastic exposure in biota indicates the high potential of this method and offers excellent opportunities for plastics which are difficult to measure with spectroscopy-based techniques such as TWP. However, the direct link to the exposed/ taken up amount and kinetics of plastic and TWP is still under consideration. Attention must be given to choosing chemicals specific to plastic contamination, avoiding those used in other applications and already posing a global environmental pollution issue as well (e.g., phthalates or chlorinated paraffins, PBDEs). The Andromeda WP2 proves that methods based on chemical markers, hyperspectral imaging or Nile-Red based techniques, as optimized within Andromeda, all have specific characteristics and selection of the best available technique depends on the type of matrix and the research or monitoring purpose. The availability of a portfolio with different techniques for microplastic analysis is of high value. However, cross-validation exercises on sediment and water samples showed that variability in MP analysis results is still high, also for spectroscopy-based methods, and that it is important for future microplastic analysis to harmonise required quality criteria rather than putting forward one single analysis method. The developed cost-effectiveness tool which takes into account equipment cost, man hour cost as well as method quality, can be a large aid to researchers and policy in selecting the best available and fit-for-purpose method.

DELIVERABLES AND MILESTONES

DELIVERABLES (WHERE APPLICABLE)

Deliverable	**Partner responsible**	**Date of submission**	**Comment**
2.1 Automated sampling device for ferrybox	Taltech	14/09/2021	Ferrybox system delivered and employed during common cruises.
2.2 Smartphone app	UM	16/05/2023	Official launch at the Esplora interactive Science Museum (Malta)

2.3 Smartphone app online platform	UM	16/05/2023	Official launch at the Esplora interactive Science Museum (Malta)
2.4 Hyperspectral imaging protocol	GU	18/12/2023	Protocol will be published in the Andromeda protocol bundle.
2.5 Chemical marker protocol	NILU/UFZ	30/09/2023	Protocols published on project website. Scientific publications including protocols are in preparation.
2.6 Fluorometric method protocols	ILVO	01/07/2022	Protocols developed for matrices water, sediment and marine biota. Protocols will be published in the Andromeda protocol bundle.
2.7 Intercomparison paper	IFREMER	30/09/2023	Paper in preparation, first draft prepared by project end.

MILESTONES (WHERE APPLICABLE)

Milestone	**Partner responsible**	**Progress**	**Comment**
2.1 Sample collection (by developed samplers)	Taltech/NILU	Completed	Samples were collected within cruises at Baltic Sea, North Sea, Gulf of Biscaye and Med. Sea
2.2 Launch smartphone app	UM	Completed	Official launch at the Esplora interactive Science Museum (Malta)
2.3 Air sampling method	NILU	Completed	
2.4 Hyperspectral imaging method	GU	Completed	
2.5 Chemical marker comparison	NILU	Completed	
2.6 Chemical marker method	NILU	Completed	Method protocols taken up in Deliverable 5.6.
2.7 Dye and pigment selection	ILVO	Completed	Different dyes were tested and final methods were developed using Nile Red.
2.8 Fluorometric method	ILVO	Completed	Method protocols taken up in Deliverable 5.6.
2.9 Sampling cruise with WP2 partners	IFREMER/IEO	Completed	Sampling cruise 1 in mediterranean, sampling cruise 2 in Gulf of Biscaye

2.10 Fluorometric method for air samples	NILU	Completed	
2.11 Cross-validation exercise	ILVO	Completed	Publication submitted

Dissemination activities (including a list of scientific publications) (Max. 3 pages)

Publications:

- Foscari *et al.* Tire wear particles in coastal areas: suitable chemical indicators in blue mussels (*Mytilus Edulis*), in prep.
- Gerigny, O., Blanco, G., Lips, U., Buhhalko, N., Chouteau, L., Georges, E., Meyers, N., Vanavermaete, D., Galgani, F., Ourgaud, M., Papillon, L., Sempere, R., De Witte, B. 2024. Comparative analysis of microplastics detection methods in marine sediment. In prep.
- Gestalt et al *et al.* Identification and usefulness of chemical indicators for tire wear particle exposure to marine organisms in the Norwegian coast, in prep.
- Hägg, F., Herzke, D., Nikiforov, V. A., Booth, A. M., Sperre, K. H., Sørensen, L., Creese, M. E. & Halsband, C. 2023. Ingestion of car tire crumb rubber and uptake of associated chemicals by lumpfish (*Cyclopterus lumpus*). *Frontiers in Environmental Science,* 11. doi: 10.3389/fenvs.2023.1219248
- Meyers, N.; Catarino, A.I.; Declercq, A.M.; Brenan, A.; Devriese, L.; Vandegehuchte, M.; De Witte, B.; Janssen, C. and Everaert, G. (2022). Microplastic detection and identification by Nile red staining: Towards a semi-automated, cost-and time-effective technique. Science of the Total Environment, 823, p.153441. Doi: 10.1016/j.scitotenv.2022.153441
- Meyers, N.; Kopke, K.; Buhhalko, N.; Mattsson, K.; Janssen, C.; Everaert, G.; De Witte, B. 2024a. Value for money: a cost-effectiveness analysis of microplastic analytics for seawater. Microplastics and Nanoplastics. accepted.
- Meyers, N.; De Witte, B.; Schmidt, N.; Herzke, D.; Fuda, J.; Vanavermaete, D; Janssen, C.; Everaert, G. 2024b. From microplastics to pixels: Testing the robustness of two machine learning approaches for automated, Nile red-based marine microplastic identification. In prep.
- Meyers, N.; Everaert, G.; Hostens, K.; Schmidt, N.; Herzke, D.; Fuda, J.; Janssen, C.; De Witte, B. 2024c. Towards reliable data: A validated, machine learning-based approach for microplastics analysis in marine organisms by Nile red staining. In prep.

Press releases

- Belgen forceren doorbraak in opsporing microplastics in zee: "Veel efficiënter in kaart te brengen" (English: Belgians force a breakthrough in detecting microplastics in the sea: "Much more efficient to map"). https://www.vrt.be/vrtnws/nl/2022/02/15/belgische-wetenschappers-forceren-doorbraak-in-onderzoek-naar-mi/ (in Dutch).

Work package 3: Advanced analytical techniques

The main aim of WP3 was to develop a toolbox of coherent, complementary, analytical instrumental methods and workflows to consistently and reliably detect, identify, quantify (mass/number) and characterise MP and NP in environmental samples. To achieve this, WP3 had the following objectives:

- Establish robust and validated instrumental methods, associated sample preparation protocols and analytical workflows for identification and size characterization of MP down to 10 µm filter pore size.
- Push the limits of currently mature methods to approach characterization of MP with a 1 µm filter pore size.

- Conduct exploratory work on analysis of NP of polymer origin to at least down to 0.2 µm with selective and non-selective methods.
- Develop specific combinations of analytical tools for the determination of TWPs, synthetic fibres, and paint flakes.

Task 3.1. MP ≥10 µm in environmental samples

Introduction: Spectroscopic techniques such as µ-FTIR and µ-Raman can easily be used to identify microplastic particles down to 10 µm. In automated applications where large numbers of spectra are required within limited time often the spectra quality limits the efficiency. To identify a particle with FTIR or Raman a good enough spectrum is required, in principle a spectrum without any artefacts or instrumental noise. To produce high quality spectra, many parameters can be optimised such as optimising laser wavelengths and energy (Raman), spectral resolution, confocal optics, focus and number of scans. However, it is not practical to improve all parameters since it takes time and is especially not suitable for environmental samples where there are many particles and many uninteresting ones. Moreover, a simple and quick batch processing will not be a solution. This task focused on developing a deep-learning method for removal of instrumental noise and unwanted spectral artefacts in FTIR and Raman spectra for automated analysis of environmental samples.

Results: The autoencoder was first used for noise reduction, the neural net was trained on spectra with added noise to be able to reconstruct spectra with high levels of noise. Then, the neural net was trained on spectra with added spectral distortions together with noise. For Raman spectra where limitations arise from low signal to noise ratio, fluorescence and cosmic rays the neural net were trained on these disturbances.

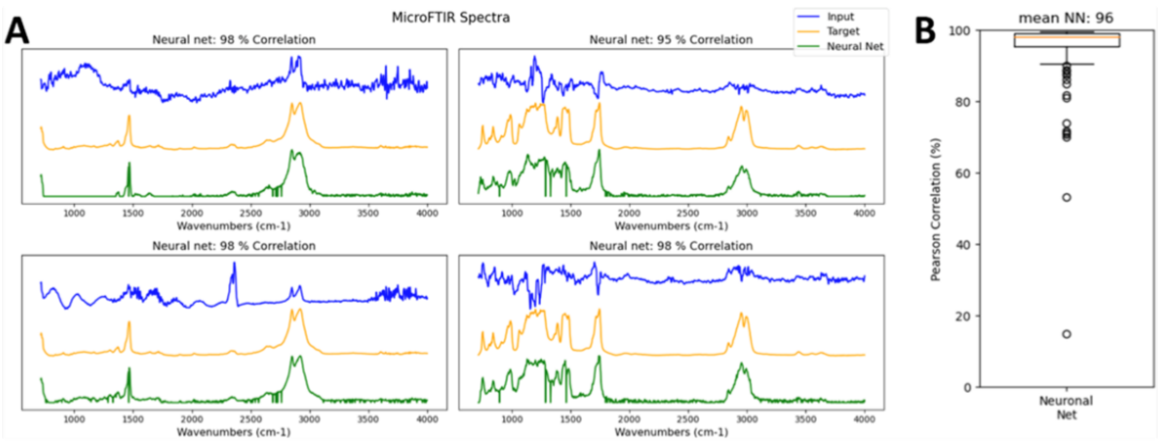

Figure 6: Restoration of FTIR of complex spectral distortions including Mie scattering. A) Application to real µ-FTIR spectra of cryomilled particles and B) the corresponding distribution.

Discussion & conclusions: There is a high potential of using autoencoding networks to reconstruct FTIR and Raman spectra. Neural nets can recognize and restore any kind of spectral artefacts, it is done in one step and within a short time. However, it is limited in identifying particle types as it will reconstruct spectra depending on what is available in the library, i.e. if a spectra is not in the library, it will match it to another in the library. Moreover, when applying the neural net to new spectra it has to be retrained. There is a high potential of using autoencoding networks for reconstructing FTIR and Raman spectra which is of high relevance for environmental samples especially when targeting specific particles such as within monitoring approaches.

Additional activities: Work was also conducted on method development for hyperspectral imaging, a cost-effective method for microplastic identification. In addition to the experimental work conducted within Task 3.1, knowledge generated in Andromeda related to polymer identification, quality assurance and control (QA/QC), and data reporting in microplastic analysis and monitoring was used in the preparation of a manuscript looking at methods for monitoring microplastic pollution in the Arctic. Finally, Task 3.1 contributed to a small study looking into the current state of microplastic research data in terms of trends in the availability and sources of open data.

Publications:

- Brandt, J., Mattsson, K., Hassellöv, M. (2021) Deep Learning for Reconstructing Low-Quality FTIR and Raman Spectra: A Case Study in Microplastic Analyses. Analytical chemistry 93(49), 16360-16368 [doi: 10.1021/acs.analchem.1c02618].
- Schwarte, J., Brandt, J., Mattsson, K., Hassellöv, M.. Development of Monitoring Techniques for Identification of Microplastic Particles in Environmental Samples Using Hyperspectral Imaging. *Manuscript in preparation*.
- Primpke, S., Gerdts, G., Strand, J., Scholz-Böttcher, B., Aliani, S., Patankar, S., Lusher, A., Booth, A.M., Gomiero, A., Kögel, T., Galgani, F., Provencher, J., Vorkamp, K. (2022). Monitoring of microplastic pollution in the Arctic: Recent developments in polymer identification, quality assurance and control (QA/QC), and data reporting. Arctic Science 9(1) 176-197 [doi: 10.1139/AS-2022-0006].
- Jenkins, T., Persaud, B., Cowger, W., Szigeti, K., Roche, D.G., Clary, E., Slowinski, S., Lei, B., Abeynayaka, A., Nyadjro, E.S., Maes, T., Thornton Hampton, L., Bergmann, M., Aherne J., Mason, S.A., Honek, J.F., Rezanezhad, F., Lusher, A.L., Booth, A.M., Smith, R.D.L., Van Cappellen, P. (2022). Current State of Microplastic Pollution Research Data: Trends in Availability and Sources of Open Data. Frontiers in Environmental Science 10, 912107 [doi: 10.3389/fenvs.2022.912107].

Task 3.2. Robust techniques for MP 10-1 µm

Introduction: Particles in the range 1-10 µm are challenging due to their small size and because particle number increases with decreasing size. Automated identification is crucial and in µ-FTIR and Raman microscopy the particles are, in general, identified on membranes. This task focused on developing membranes to improve identification of small MPs in environmental samples.

Results: Polycarbonate membranes were coated with 100 nm platinum on both sides using an e-beam evaporator. The coated membranes were tested for different types of environmental samples such as seawater and tap water. The membranes could handle high pressure, filtrate large volumes, had a smooth and mirror looking appearance, could handle treatment for removing natural organic matter, no disturbing background signal and no fluorescence.

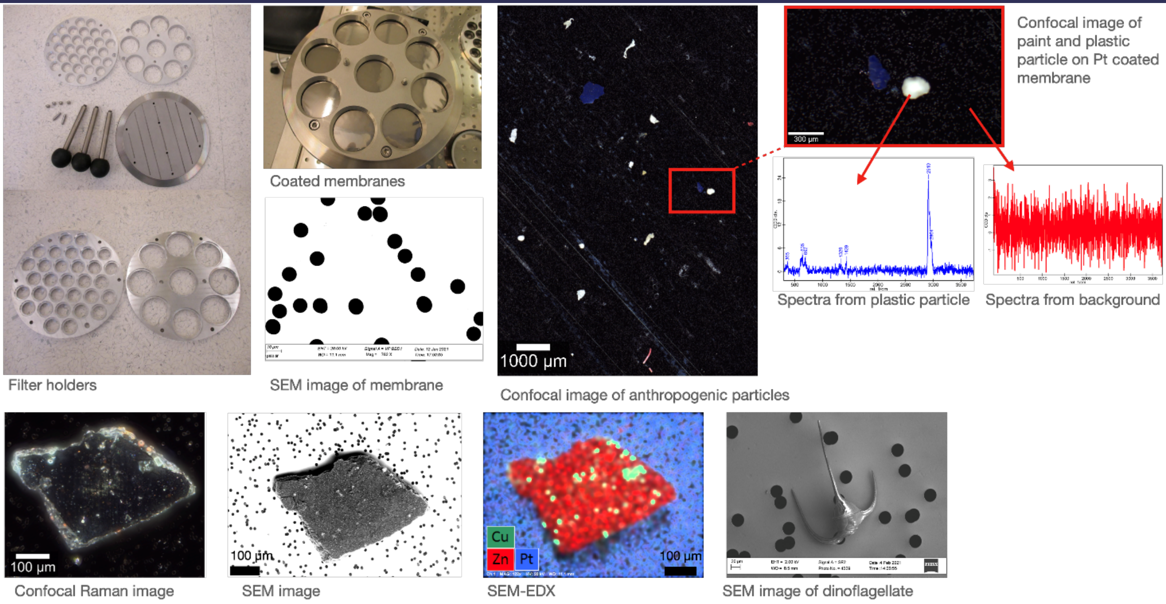

Figure 7: Metal coated membranes.

Discussion & conclusion: Filtrating the samples in a cascade filtration set-up with a 10 µm and a 1 µm membrane makes the analysis easier since the particle size distribution on the 1 µm membrane will be limited. The membranes are first imaged in BF or DF, then a threshold is applied to determine what particles to analyse. Finally, the particles are analysed with Raman spectroscopy and the spectra are evaluated. The major challenge is the amount of non-plastic particles and to apply a good threshold with no interference from the membrane holes. By pre-treating the sample before analysis in combination with imaging in BF there is no problem to identify particles in the size range of 1 to 10 µm. However, if many different coloured particles are present, two analyses can be required, one for the brighter particles and one for the dark ones. Platinum coated membranes in many pore sizes that can handle pre-treatment and filter large volumes at high pressure is key for analysing MPs in the range 1-10 µm with Raman microscopy.

Publications:

- Mattsson, K., Hagberg, M., Hassellöv, M. Platinum vaporization-deposition coated polycarbonate membranes for comprehensive, multimodal, and correlative microscopic analysis of micro-and nanoplastics and other environmental particles. Talanta, Volume 269, 125435, [https://doi.org/10.1016/j.talanta.2023.125435.]

Task 3.3. Direct identification of NPs

Introduction: For analysis of plastic NPs normal µ-FTIR nor µ-Raman can be used due to their detection limit. However, combining techniques can improve the analysis and lower the detection limit. This task focuses on exploring the use of SEM-Raman for identification of nanosized plastic particles from the environment.

Results: Tap water and seawater from different locations were filtered through a cascade filtration setup with 100, 30, 10, 1 and 0.4 µm membranes. The smallest membrane, 0.4 µm was analysed using SEM-Raman. First, the membranes were imaged with SEM to locate the particles of interest. Then, while still in the SEM, Raman was used on the particles to obtain a spectrum which was compared to our in-house library, where a HQI value of ≥80% was considered a match.

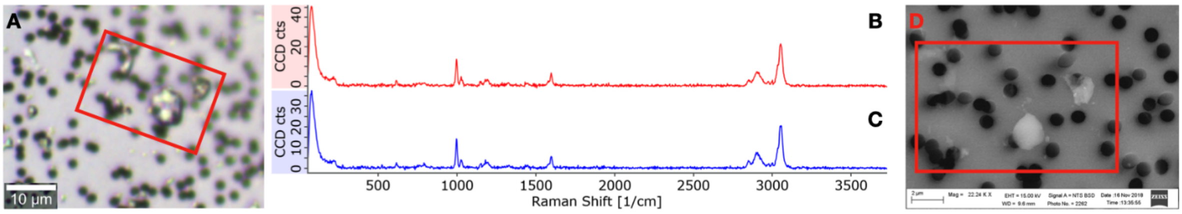

Figure 8: Principle of detection of nanoparticles; A) Confocal image, B) obtained Raman spectra C) reference spectra and D) SEM image of analysed particle.

Discussion & conclusion: Combining techniques can be challenging since each technique requires optimization of specific parameters. The membranes developed in task 3.2 improve identification since the membranes have good properties in both SEM and Raman. In general, there are more particles with decreasing size. This is also true for natural organic particles and minerals. Therefore, it is crucial to have a treatment method for reduction of disturbing, non-interesting particles to speed up the identification and the potential interference. SEM-Raman is a promising technique for identifying plastics NPs.

Publications:

- Karin Mattsson and Martin Hassellöv. Novel inline cascade filtration method to reveal distribution of small microplastics in drinking waters. Manuscript in preparation.
- Karin Mattsson and Martin Hassellöv. Distribution of small microplastics in Skagerrak. Manuscript in preparation.

Task 3.4. Hyphenated techniques for nanoplastics

Introduction: The aim of this task was to develop a hyphenated fractionation approach to obtain continuous nanoplastic (NP) size distributions followed by identification and quantification. A continuous fractionation technique building on field flow fractionation (FFF), in this case asymmetric flow FFF (AF4) fitted with a multi-angle light scattering detector (MALS), was studied and potential limitations and bias identified. Methods were then developed for the extraction and sample transfer of the different NP size fractions (aqueous dispersions) collected by FFF. Finally, pyrolysis gas chromatography mass spectrometry (pyGC-MS) analysis approaches were developed and optimised for the mass-based quantification of NP by polymer type. Test materials included three polystyrene (PS) NP materials of different sizes (PSL60, PS2, PS3), which were analysed as mono-modal samples (i.e., individually) and as a tri-modal mixture to allow particle size separation and recovery to be assessed.

Results: Analysis of the mono-modal samples by AF4-MALS revealed retention times (RTs) of 20-26 min for PSL60, 24-34 min for PS2, and 33-46 min for PS3 (Fig. 3.4). Analysis of the 3 PS NPs as a mixture indicated all particle types could be separated and isolated as individual fractions, with only a minor overlap in RTs. Subsequent sample processing and analysis by pyGC-MS was compared by two labs, with determined concentrations of 33.8%, 43.2%, and 46.4% (lab 1) and 314.1%, 39.4%, and 31.7% (lab 2) for PS2, PS3, and PSL60, respectively. These values account for the determined losses during the sample work-up procedure and during the fractionation with AF4, as well as the incomplete collection of each fraction. For the tri-modal sample, recoveries were 16.7%, 126.4%, and 48.1% (lab 1) and 28.7%, 208.8%, and 78.8% (lab 2) for PS2, PS3, and PSL60, respectively. The results from the tri-modal analysis indicate that there are further complications when the particles are present in a mixture.

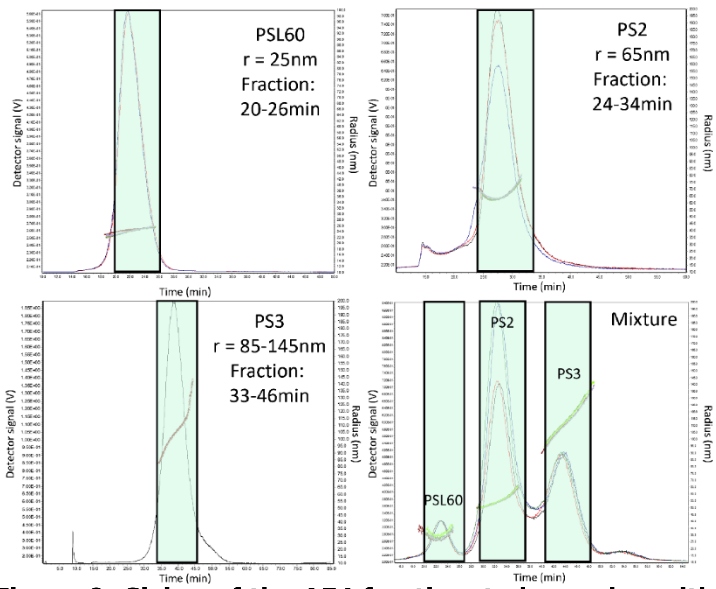

Figure 9: Sizing of the AF4 fractionated samples with multi-angle light scattering (MALS) and the time windows (green boxes) of fraction collection. The time is shown as FFF retention time. The top left, top right, and bottom left figures show fractionation of individual/mono-modal samples (PSL60, PS2, PS3) including the radii of gyration (Rg) while the bottom right figure shows fractionation of a mixture of three PS NPs. Reproduced from Huber et al., 2023.

Discussion and conclusion: For most mono-modal PS samples, >50% of the NPs were not recovered. This difference is not explained by the losses in the extraction and sample processing steps determined in the recovery experiments, suggesting other losses are occurring. One contributing factor may be the incomplete elution of the sample NPs during the AF4 fractionation process, which may derive from particles eluting outside of the defined time windows. As the extraction procedure is indiscriminate toward the different particles, the tri-modal data suggest that mixtures impact the separation capacity of the AF4 approach. Although pyGC-MS proved highly suitable for the identification of PS NPs in fractionated samples, only semi-quantification by mass could be achieved due to the variability in recovery. While the concentration of samples was achieved by solvent extraction and evaporation in the current study, an alternative approach could be to concentrate particles from the collected fractions on filters with small pore sizes (e.g., Anodisc™). The analytical combination of FFF and pyGC–MS seems a promising approach for the identification and semi-quantitation of polymers in heterogeneous samples, but there is a need for further method development and optimization that specifically focuses on improved and reproducible fractionation, extraction, and quantification. PyGC-MS is a suitable approach for mass-based NP quantification, but it is important to highlight that the technique provides no direct information on particle size and shape.

Publications:

- Huber, M. J., Ivleva, N.P., Booth, A. M., Beer, I., Bianchi, I., Drexel, R., Geiss, O., Mehn, D., Meier, F., Molska, A., Parot, J., Sørensen, L., Vella, G., Prina-Mello, A., Vogel, R., Caputo, F. (2023). Physicochemical characterization and quantification of nanoplastics: Applicability, limitations and complementarity of batch and fractionation methods. Analytical and Bioanalytical Chemistry 415, 3007–3031 [doi: 10.1007/s00216-023-04689-5].

Task 3.5. Optimised analysis of microfibers

Introduction: Microfibres often represent a significant proportion of the total microplastic particles present in an environmental sample. However, their robust quantification can be difficult to achieve as many fibres do not lie flat in a 2-D plane on the filters used in µFTIR analysis of microplastic. This can lead to individual fibres being quantified as multiple particles and leading to overestimation of particle numbers. The aim of this task was to evaluate the suitability of calcium fluoride (CaF_2) and barium fluoride (BaF_2) cover slides that 'flatten' microfibers into a single focal plane. These were sourced and evaluated for their cost, effectiveness and re-use on an laser direct infrared (LDIR) chemical imaging system and on a µFTIR system. Testing was conducted with a suite of white PET, orange acrylonitrile (polyacrylic), blue acrylonitrile and white nylon fibres produced from commercial textile yarns.

Results: Pre-testing with the LDIR system indicated that this technique was able to image and identify all fibres as single particles without a cover slide being used (Figure 10a). Analysis of the fibre samples by µFTIR with the CaF_2 slides was unsuccessful and resulted in poor imaging overall. In contrast, the BaF_2 cover slides (25 mm diameter, 1 mm thickness) allowed good imaging by µFTIR, although a slight 'haze' was evident on the resulting images. However, all fibres were identified as single particles. Where overlap of particles occurred, there were still issues with maintaining single fibres in the focal plane along their entire length. In many cases, the baseline of the spectra had a 'wavy signal', which may result from the presence of the BaF_2 cover slide.

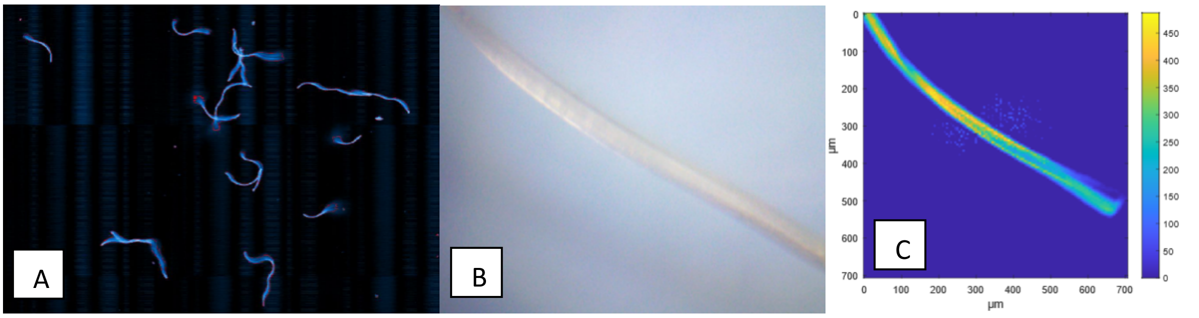

Figure 10: (A) LDIR image of white PET fibres without cover slide, showing good identification of each fibre as a single particle. (B) µFTIR optical microscopy image of an acrylonitrile fibre when using the BaF_2 cover slide. (C) FTIR image of same acrylonitrile fibre using the BaF_2 cover slide.

Discussion & conclusion: Although we did not use any plate or cover the fibres in the LDIR analyses, they could be easily detected as single particles, suggesting LDIR has advantages over μFTIR for the analysis of microplastic fibres. Despite their narrow diameters (ca. 20-30 μm), the LDIR instrument was generally able to identify most fibres as the correct polymer type, but there is room for improvement, concerning the S/N ratio and resulting lower hit quality. In the case of the μFTIR analysis, the BaF_2 cover slide did reduce the occurrence of fibres being identified as multiple particles. However, there are still issues maintaining entire fibres in the focal plane when they overlap with each other and the cover slide reduces the quality of the FTIR spectra slightly. LDIR analyses of fibres, which has rarely been reported in the literature, seems to be able to cope better with fibres that do not entirely lie within a single focal plane and cover slides were not necessary. In the case of μFTIR, the cover slides help flatten the fibres and maintain them in the focal plane. However, this comes with a reduction in the optical microscope imaging quality and greater background signal in the FTIR spectra. For laboratories using μFTIR for microplastic analysis, a BaF_2 slide may offer advantages for samples with a high proportion of fibres.

Task 3.6. Tyre wear particle characterization

Introduction: This task focused on understanding the transformation of tyre wear particles (TWPs) in marine systems, considering their size, composition, and density changes. It links to chemical marker assessment (WP2) and simulated degradation studies (WP4). The goal was to identify stable properties and analytical parameters for TWPs, evaluating existing detection techniques and adapting them for marine environments. Mass spectrometry and elemental analysis were used for bulk and single particle analysis.

Results: Various cryo-milled tire tread (CMTT) mixtures were produced from different tyre materials. A size distribution of ~200 μm was determined for all materials and the morphological heterogeneity was assessed by scanning electron microscope (SEM). Over 20 tyre-related chemicals were identified by high resolution LC-MS after developing a suitable solvent extraction method. The chemicals consisted of several additives known to be used in tyre production, including vulcanisation accelerators (e.g., thiazoles and phenylguanidines) and antioxidants (e.g., phenylenediamines). Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) was tested for identifying TWP elemental composition and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-ToF-MS) for polymer analysis. Finally, pyGC-MS was used to measure TWP in air samples. To study the usefulness of TWP related additives as markers of TWP ingestion by marine organisms, GC/Orbitrap HRMS was used to identify 7 indicator chemicals in fish and mussels exposed to TWP under laboratory conditions.

Discussion & conclusion: The analysis of the organic extracts was an efficient method for detecting tire material in environmental samples, as the additive reservoir in the TWPs overcomes matrix effects and additives remain measurable even after long ageing processes. In contrast, MALDI-ToF-MS was not suitable for as the laser energy completely fragmented the polystyrene and no characteristic decomposition products remained. Use of pyGC-MS for identifying rubber chemical markers was successful, suggesting it is one of the few analytical technologies currently available that is able to reliably detect and quantify TWP. The usefulness of indicator chemicals for ingested TWP by marine organisms was shown in the laboratory exposure studies and further tested on field samples (crabs, mussels) collected at TWP contaminated sites. The relatively slow excretion/metabolization of selected indicator chemicals over several days allows for the method to be applied to marine species such as blue mussels, and promotes their use as a bioindicator for TWP emissions and exposure. The widely available instrumentation needed (GC-or LC/MS/MS) for the detection of the identified indicator chemicals and the wide distribution of blue mussels along European coastlines would permit their use as bioindicators of TWP pollution in the marine environment. LA-ICP-MS analysis of TWPs was unsuccessful owing to the variability of the Zn peak and the high background interference from natural carbon sources. The transformation of TWPs in the environment, including changes in size, composition, and density, must be taken into account when analysing TWPs in marine systems. For this purpose, some of the techniques applied (LC-HRMS, pyGC-MS, SEM) turned out to be very efficient compared to others (LA-ICP-MS, MALDI-ToF-MS) for the analysis of TWPs.

Additional activities: In addition, work was conducted on the sources, fate and distribution of tyre and road wear particles (TRWPs) from city to sea. TRWP in surface sediments were compared to TRWP from a road simulator, where different brands of summer, winter and studded tyres were used. The particles abundance and physicochemical properties were characterised. Finally, the ingestion of TWPs and the uptake of tyre-related chemicals by a representative marine fish (lumpfish, *Cyclopterus lumpus*) was investigated.

Publications:

- Foscari, A., Schmidt, N., Seiwert, B., Herzke, D., Sempéré, R., Reemtsma, T. (2023). Leaching of chemicals and DOC from tire particles under simulated marine conditions. Frontiers in Environmental Science, 11, 1206449. [doi: 10.3389/fenvs.2023.1206449].
- Mattsson, K., Aristéia de Lima, J., Wilkinson, T., Järlskog, I., Ekstrand, E., Andersson Sköld, Y., Gustafsson, M., Hassellöv, M. Tyre and road wear particles from source to sea. Microplastics and Nanoplastics 3, 14 (2023). [doi: 10.1186/s43591-023-00060-8].
- Hägg, F., Herzke, D., Nikoforov, V.A., Booth, A.M., Sperre, K.H., Sørensen, L., Creese, M.E., Halsband, C. (2023). Ingestion of car tire crumb rubber and uptake of associated chemicals by lumpfish (*Cyclopterus lumpus*). Frontiers in Environmental Science 11, 121924 [doi: 10.3389/fenvs.2023.1219248]
- Wilkinson, T., Järlskog, I., Aristéia de Lima, J., Gustafsson, M., Mattsson, K., Andersson-Sköld, Y., Hassellöv, M. Shades of grey - Tire characteristics and road surface influence tire & road wear particle (TRWP) abundance and physicochemical properties. Frontiers in Environmental Science 11, 1258922. [doi: 10.3389/fenvs.2023.1258922]

Task 3.7. Paint particle characterization

Introduction: Paint particles are commonly found in marine environmental samples. Paint particles are made of polymers but also contain binders, pigments, fillers and extenders, solvents and additives, making the identification process more challenging since not commonly microplastic tools such as FTIR or Raman can be used. This task focuses on development of methods for detection and characterization of paint microplastics from the marine environment.

Results: Manta trawl (>300 µm), water samples from 0.5 m depth (>10 µm) and collection of filtrate through 10 µm membranes (0.1-10 µm) were samples at six marinas around the Swedish coast. Particles from the trawl samples and water samples >10 µm were visually identified under a light microscope (Zeiss AxioImager M2), photographed and classified into morphological classes (colour, size, shape etc). These membranes were also analysed with SEM-EDX using an automated method (SmartPI) for analysis of the particle's elemental contrast of their copper content. The particles containing copper were then fully analysed for their elemental composition and morphological parameters (size and shape). The filtrate was analysed with spICPMS (0.1-10 µm) and copper-rich particles were detected and their number concentration and size were quantified.

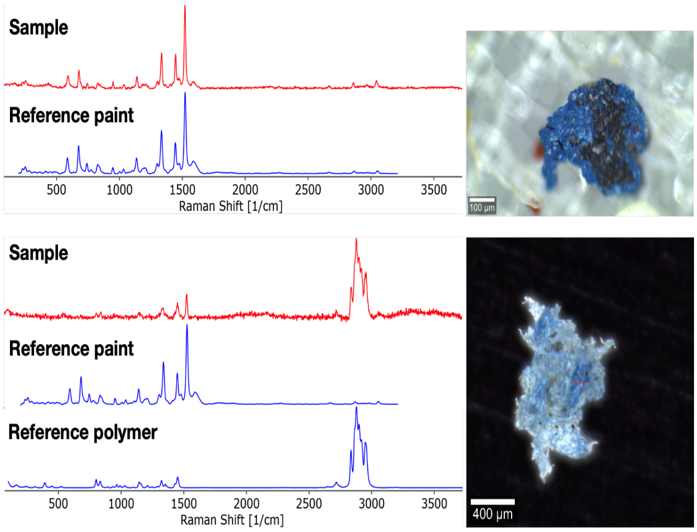

Figure 11: Example of blue paint particle (top) and blue plastic particle (bottom), with spectra acquired using Raman microscopy (red) compared to spectra of reference paint and polypropylene.

Discussion & conclusion: Using colour as the main criteria increases the risk of false positives for paint particles without strong colour (e.g., bright blue or red), while coloured plastics can be mistaken and identified as paint particles. Size, shape and textual characteristics corresponding to paint flakes may be used to strengthen the identification. Using metal content as the main criteria offers an advantage in identifying antifouling paint particles, however, non-paint particles may be mistakenly categorised as paint particles if they contain metals. Using combinations of metals (e.g., copper-zinc) as a fingerprint for identification of antifouling paint particles is promising. For smaller sized particles spICMS can be used, however, the information is limited to one element. Combining techniques such as visual (LM) and chemical identification (SEM-EDX) offers a way forward.

Publications:

- Gondikas, A., Mattsson, K., & Hassellöv, M. (2023). Methods for the detection and characterization of boat paint microplastics in the marine environment. Frontiers in Environmental Chemistry, 4, 1090704 [doi: 10.3389/fenvc.2023.1090704].

DELIVERABLES AND MILESTONES

DELIVERABLES

Deliverable	Partner responsible	Date of submission	Comment
3.1. Scientific paper(s): Manuscript describing fully automated and validated workflows developed within the ANDROMEDA project for the characterization and quantification of polymer MP down to 10 µm (≥10 µm) in environmental samples.	University of Gothenburg	2022	During the project it became clear that such a manuscript was not really suitable for peer-review publication due to lack of novelty at this point in the research field. A total of 4 alternative manuscripts on related topics were prepared and published instead.
3.2. Guidance white paper: A white paper will be produced with guidelines for science advice to regional seas conventions (HELCOM, OSPAR, and Barcelona Convention) and national authorities on the spectroscopic identification of MPs in environmental monitoring.	University of Gothenburg	Multiple documents submitted over 2021-2023	This task involved activity from multiple Andromeda partners. Direct input has been provided to documents produced by OSPAR (MP indicator for sediments group, ICGML), ICES (WGML), AMAP (LMEG), EU MSFD (TGML). A detailed summary of the documents is provided in the dissemination section.
3.3. Scientific paper(s): Manuscript describing the robust approach(es) developed within the ANDROMEDA project for the characterization and quantification of polymer MP in the range 1-10 µm in environmental samples.	University of Gothenburg	2024	Manuscript is published
3.4. Scientific paper: Manuscript describing the development and evaluation of SEM-Raman characterization and quantification of NP	University of Gothenburg	2024	Two manuscripts is in preparation (UGot)

particles in simple aqueous environmental matrices.			
3.5. Scientific paper: Manuscript evaluating the viability of a stepwise, hyphenated fractionation multi-technique quantification approach for a comprehensive determination of size distribution and quantification of NP particles in simple aqueous and possibly complex sediment and biological environmental matrices.	SINTEF Ocean	2023	Manuscript published
3.6. µFTIR filter cover report: A short report will be prepared describing the production of the final filter cover slide and its validation for use in the analysis of microfibers by µFTIR.	SINTEF Ocean	2023	An internal report prepared and submitted to the project team.
3.7. Scientific paper(s): Manuscript describing the extraction and chemical analysis approach(es) developed within ANDROMEDA for the characterization and quantification of pristine and degraded TWPs in environment samples.	UfZ	2023	Four manuscripts published
3.8. Scientific paper(s): Manuscript describing the extraction and analysis approach(es) developed within ANDROMEDA for the characterization and quantification of paint flakes in environment samples.	University of Gothenburg	2023	Manuscript published
3.9. Scientific paper: Joint draft paper on 'Mastering the complexity of MPs down to the micrometre size range' based on the work conducted in Task 3.8.	SINTEF Ocean	Not completed	Due to time and budget constraints it was not possible to complete this activity. See also related milestones 3.10 and 3.11.

MILESTONES

Milestone	Responsible	Progress	Comment
M3.1. Optimized metal coated membrane filter	University of Gothenburg	Completed	Manuscript published
M3.2. SOP for small MPs	University of Gothenburg	Completed	SOP delivered to project coordinator for inclusion in SOP compendium
M3.3. NP isolation approach	SINTEF Ocean	Completed	Manuscript published
M3.4. NP characterization and quantification	University of Gothenburg	Completed	Method has been defined for SEM-Raman. Manuscript in preparation.
M3.5. Characterization of reference materials	SINTEF Ocean	Completed	Done for most instruments used in ANDROMEDA. Unable to produce an open-source spectra library due to time and cost constraints. Other open-source spectral libraries have become available since the project started.
M3.6. Optimized filter cover for µFTIR	SINTEF Ocean	Completed	Report finalised
M3.7. Analysis techniques for TWPs	UfZ	Completed	Proposed work on TWPs in WP3 became transferred to WP2 and WP4. More details are presented for WP2 and WP4.
M3.8. Paint particle spectral database	University of Gothenburg	Completed	Manuscript published. Unable to produce an open-source spectra library due to time and cost constraints.
M3.9. Stakeholder dialogue	University of Gothenburg	Completed	Dialogue with relevant stakeholders was maintained throughout project period.
M3.10. Analytical data collection	SINTEF Ocean	Not completed	Due to time/budget constraints it was not possible to complete this activity.
M3.11. Function fitting and calibration	SINTEF Ocean	Not completed	Due to time/budget constraints it was not possible to complete this activity.

WORK PACKAGE 4: NATURAL AND ACCELERATED DEGRADATION

After being released into the marine environment, macroplastic waste is subjected to photo- and biodegradation, in addition to physical aging processes, giving rise to plastic debris at the micro-and nanoscale. As these degradation processes are very slow, however, the flux of micro- and nanoscale particulates from terrestrial environments is thought to be the major source of marine pollution. Plastic degradation is also considered a significant source of dissolved organic carbon (DOC) release. This plastic-sourced DOC includes oligomers at different oxidation levels, as well as plastic additives such as phthalates (PAEs), organophosphate esters (OPEs), and bisphenols (BPs) and a myriad of additional molecules. Modelling exercises have shown that only 1% of the estimated plastic waste actually floats on the ocean surface, suggesting that most of the plastic load is distributed within the ocean and most likely on the sediment surface (especially for the denser particles). However, there is insufficient knowledge about the processes affecting plastic distributions and degradation and how this might influence the release of additives under varying environmental conditions. The various degradation and additive release processes on different types of materials are addressed in this WP4 both under experimental conditions and in the deep-sea environment.

Task 4.1. Fragmentation and degradation processes (quantifying degradation)

Introduction:

This task served as a foundation for identifying and quantifying degradation mechanisms and generated products from other tasks, while also linking to methods developed in WP3. The fragmentation and degradation of plastic polymers and tire material was assessed by using a range of analytical techniques, allowing for measurement and quantification across various size scales.

Result and discussion:

The influence of degradation processes on microplastic polymers (MPs) and tire wear particles (TWPs) was assessed by different point of view across the work packages (WPs). To investigate morphological changes of the reference material due to the ageing, microscopy techniques (such as light microscopy (LM) and scanning electron microscope (SEM)) and particle sizing techniques (such as laser obscuration) resulted suitable for the purpose. As an examples, among the plastic polymers tested (such as polystyrene (PS), polyethylene (PE) and polyvinylchloride (PVC)), only PS showed visible changes in size and shape after 12 weeks of photo-degradation by a yellowing and crack formation on the particle’s surface, leading to a 70% increase smallest size class (< 300 µm) compared to the starter material

(500-700 µm). Regarding TWPs, SEM imaging allowed to identify biofilm formation on the particles due to the degradation processes tested. MPs chemical changes were tested by Fourier transform infrared microscopy (µFTIR), revealing no changes in the spectra of the MPs tested (PS, PE, PVC). Regarding TWPs, chemical changes were assessed by LC-MS, which organic composition resulted highly affected by the degradation processes applied, such us photo-degradation and hyperbaric pressure conditions (see more details in T.4.4 and T4.5 reports). TWPs detection was tested by ICP-MS, which highlighted that by increasing the complexity of the matrix, the detection of the characteristic chemical markers (such as S and Zn) might not be relevant (see more details in T2.4 and T3.6 report). The total amount of dissolved organic carbon (DOC) revealed how much the degradation process influenced the leaching of organic compounds, highlighting the high amount of chemicals MPs and TWPs related which still unknown.

Conclusion:

The results obtained highlighted the importance of considering different analytical techniques in order to identify and quantify the effects degradation mechanisms on different types of materials/particles tested, since morphological changes might not reflect changes on the chemical composition (e.g., PS) and vice-versa.

Milestone 1.1
Deliverables: The review paper manuscript ("The fate and impact of plastic in the marine environment – highlighting the implications of UV degradation"), submitted to the Journal of Hazardous Materials.

Task 4.2. Natural plastic degradation

Task 4.2 aimed to produce naturally weathered/degraded MPs through a deployment of MP reference materials in coastal surface waters and in the deep sea. The deployment in surface waters (< 1 m depth) was carried out in the Norwegian Sea (Tromsø, Norway; N 69.64, E 18.95), while the deployment in the deep sea (2380 m depth) was conducted in the Mediterranean Sea (off the French coast, N 42.48, E 06.02). For this, pristine reference materials were placed in stainless-steel tubes with a mesh size of 40 µm, which in turn were placed in stainless-steel cages to sustain the physical strains present in the marine environment, such as waves and currents or high hydrostatic pressure (Figure 12). The cage deployed in surface waters was regularly cleaned mechanically, to remove algal and bivalve growth and ensure a certain degree of UV exposure and water movement within the exposure device. The MPs were exposed to the natural environment for 12 months, from spring 2021 to spring 2022. After retrieval, the MP particles were distributed to partners from WP2 and simultaneously characterized concerning their additive content within WP4.

The deployment and retrieval of the MPs was disseminated in the ANDROMEDA Newsletter Issues No. 1 and No. 2, respectively. The characterization of the extractable additives from virgin vs. naturally weathered MPs was performed as part of a master thesis at NILU. The results of these analyses will be integrated into a manuscript on chemical markers within WP2 (deliverable D2.5). Finally, the naturally weathered MPs were used within WP2 for a comparison with virgin MPs for the optimization of protocols for the analysis of MPs by fluorometric methods. The outcomes are summarised in a scientific paper currently in preparation (deliverable D2.6).

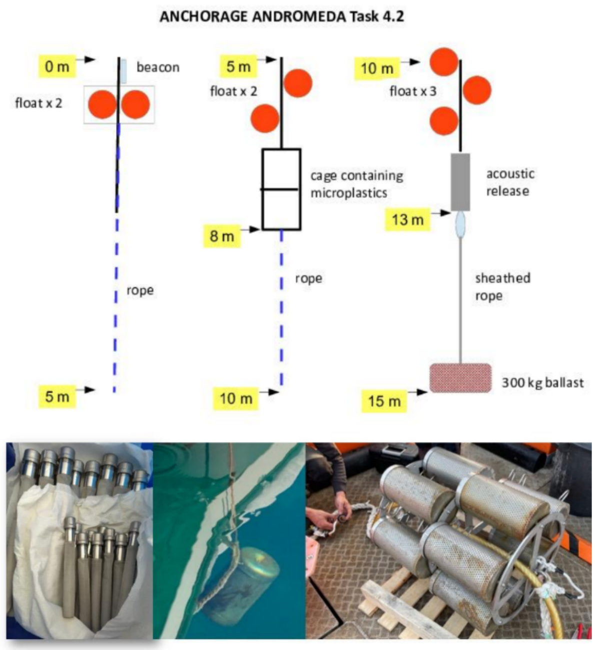

Figure 12: (Above) Schematic plan of the deep-sea anchorage deployed at 2400 m depth. (Below) Pictures of the stainless-steel tubes containing MP samples (left), the stainless-steel cage deployed in Norwegian surface waters (middle) and the cages deployed in the Mediterranean deep-sea (right).

Milestone 4.2

The milestone 4.2, i.e. the completion of the *in situ* natural degradation of pristine plastics in marine surface waters and deep seawater and distribution to WP2 partners, was reached in month 21, i.e. on time (target value in the project proposal: month 24).

Publications:

- Sarno, A., Olafsen, K., Kubowicz, S., Karimov, F., Sait, S.T.L., Sørensen, L., Booth, A.M. Accelerated Hydrolysis Method for Producing Partially Degraded Polyester Microplastic Fiber Reference Materials. Environmental Science & Technology Letters, 2020. https://dx.doi.org/10.1021/acs.estlett.0c01002?ref=pdf
- Foscari, A., Schmidt, N., Seiwert, B., Herzke, D., Sempéré, R., Reemtsma, T. Leaching of chemicals and DOC from tire particles under simulated marine conditions. Frontiers in Environmental Science, 2023. doi: 10.3389/fenvs.2023.1206449
- Hernandez, L.M., Howarth-Forster, L., Sørensen, L., Booth, A.M., Vidal, A., Tufenkji, N., Sempéré, R., Schmidt, N. The fate and impact of plastic in the marine environment – highlighting the implications of UV degradation. *Submitted.*
- Schmidt N., Foscari, A., Garel, M., Tamburini, C., Seiwert, B., Herzke, D., Reemtsma, T., Sempéré, R. Tire particles in deep-sea conditions: interactions between hydrostatic pressure, bacterial growth and chemical leaching. *In prep*.
- Fauvelle, V., Regis, J., Schmidt, N., Vu, T.K., Sempéré, R., Grenz, C., Maeght, J.L., Verdoux, P., Pringault, O., Dolla, A., Milliton, C., Cuny, P., Rigaud, S. First evidence of additive fluxes from sediment buried plastic debris to the water column. *In prep.*
- Schmidt, N., Foscari, A., Vidal, A., Denonville, C., Herzke, D., Booth, A.M., Sempéré, R., Sørensen, L. UV-degradation and release of additives from HDPE, PS and PVC microplastic under environmentally realistic conditions. *In prep.*

DELIVERABLES (WHERE APPLICABLE)

Deliverable	**Partner responsible**	**Date of submission**	**Comment**
D4.2 Degraded MP reference materials	NILU		

MILESTONES

Milestone	**Partner responsible**	**Progress**	**Comment**
M4.2 Natural plastic degradation	NILU	Completed	

Task 4.3. Optimization of accelerated degradation protocols

The aim of the task was to define and optimize accelerated degradation protocols that allow the study of plastic degradation over short timescales, but which generate environmentally relevant degradation products, particularly for use across the other tasks of the project. While several options were outlined prior to project initiation, two pathways for degradation were deemed most promising to allow surface degradation and fragmentation in a timeframe allowing study within the course of months (rather than several years); 1) UV-degradation in seawater and 2) alkaline hydrolysis. The extent of physical degradation was determined by change in size distribution (light microscopy) and by observation of surface degradation (SEM), whereas chemical degradation was quantified by measuring the production of polymer degradation products.

UV-degradation in seawater in a UV solar simulator chamber allowed simulation of weathering at the water surface under European mean UV irradiance with an acceleration ratio of nearly 10:1 (24 hours exposure corresponding to ~9.5 days in the environment) (Sait et al., 2021). In addition to severe fragmentation for certain polymers (e.g. PET, PA), surface cracking or hole formation was observed, as well as leaching of both additives and monomers/polymer degradation products (Sarno et al., 2021; Sørensen et al., 2021). Alkaline hydrolysis provided linearly increasing degrees of degraded PET over just a few hours, with full decomposition into molecular fragments occurring after 3 h. The formation of degradation products (ethylene glycol and terephthalic acid) as well as the lack of surface chemistry changes (FTIR) was comparable between the hydrolysis and UV exposures, suggesting that partial hydrolysis may be a rapid and simple way of producing degraded PET reference materials (Sarno et al., 2021).

Task 4.4. Comprehensive UV-degradation studies

In the environment, most microplastic (MP) particles break down very slowly through a combination of photodegradation, oxidation and mechanical abrasion, with the major degradation step being UV-initiated oxidation (Andrady, 2015; Booth et al., 2018). However, to date there is very limited data on the rates at which different polymers degrade and fragment under environmental conditions (Andrady, 2015; Booth et al., 2018). The objective of this study was to investigate the effects of UV exposure on different polymer MP in the marine environment. Cryomilled soft polyvinylchloride (PVC), polystyrene (PS) and UV-stabilized high-density polyethylene (HDPE), size range 250-500 µm, were placed in quartz glass tubes with sterile filtered natural seawater (1 g MP/L). UV exposure were performed using an Atlas Suntest CPS+ instrument fitted with a Xenon lamp and daylight filter, with sampling after 0, 1, 2, 4, 8 and 12 weeks (corresponding to 0, 5, 10, 20, 40 and 60 weeks of exposure under European mean UV irradiance) (Gewert et al., 2018; Sait et al., 2021). At each time point, particles and leachates were separated by filtration (5 µm). Comprehensive analysis of particles[a] and leachates[b] was performed to investigate changes in MP size distribution[a] as an indication of fragmentation (G3 Morphologi, light microscopy), changes in surface morphology[a] (SEM) or surface chemistry[a] (FTIR), the formation and release of NPs[b] (NTA), as well as the loss of additives from the particles[a] and the leaching of additives[b] into seawater.

Surface cracking was observed in several of the particles as well as small changes in particle size distribution. However, no NPs could be observed in the leachates. While the polymer surface chemistry was unchanged, loss and leaching of additives was observed for all particle types. In line with concurrent research, we therefore suggest that MP chemicals are a particular concern, also under UV-exposure conditions. A manuscript is being prepared and will be submitted to a scientific journal in spring 2024.

Deliverables: Articles: study examining the effects of UV exposure on TWP compared to dark conditions. [Frontiers | Leaching of chemicals and DOC from tire particles under simulated marine conditions (frontiersin.org)]

Task 4.5. Deep seawater MP degradation under laboratory conditions

Environmental factors such as temperature, salinity and UV irradiation can influence the leaching of chemicals from anthropogenic particles, such as microplastics and tire particles. The deep sea is considered as a final sink for most anthropogenic particles, but very little is known on the effects of increased hydrostatic pressure on the leaching of chemicals. To tackle this question, we collected seawater at the surface (-1 m) and in the deep sea (-2000 m), filtered it (0.7 µm GF/F), divided it into several replicates and added tire particles at a concentration of 1 g/L. Next, half of the samples were poisoned with $HgCl_2$ in order to be able to compare biotic and abiotic conditions. Then, the samples containing deep-sea water were submitted to a pressure of 20 MPa using hyperbaric pressure equipment, while those containing surface seawater were kept at atmospheric pressure. All samples were kept in the dark at 13 degrees (*in situ* temperature during seawater collection) to avoid influences of differing light and temperature conditions. Samples were sacrificed after 6 h, 24 h, 7 days and 14 days. The leachates were analyzed for 20 organic chemicals, dissolved organic carbon (DOC) and inorganic nutrient concentrations (NO^-_3, PO^{3-}_4, $Si(OH)_4$) and prokaryotic abundance.

We observed an increase in DOC concentrations over time, in accordance with the increased concentration of organic target chemicals in the leachates. Generally, chemical concentrations were higher in deep-sea condition samples (Figure 13), potentially due to the effect of high hydrostatic pressure on the behavior and spatial distribution of tire particles (sinking and agglomeration). For some compounds, such as 6-PPD quinone, a transformation product of 6-PPD, higher concentrations were observed in biotic conditions, indicating the occurrence of biotransformation processes. Samples containing tire particles exhibited a higher prokaryotic abundance compared to control samples, especially in surface water samples, suggesting that natural marine bacterial assemblages are able to use tire particles and/or leached chemicals as an energy source for growth.

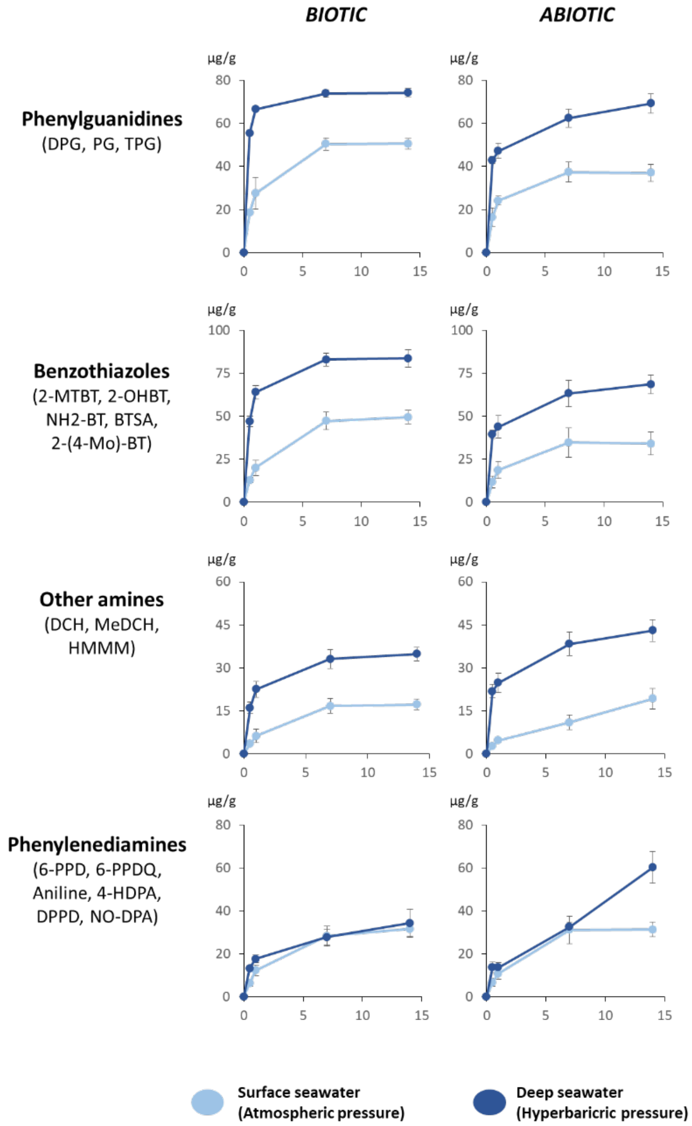

Figure 13: Graphs reporting the summed concentrations of the quantified compounds divided into the following classes: Phenylguanidines (DPG, PG, TPG), Benzothiazoles (2-MTBT, 2-OHBT, NH2-BT, BTSA, 2-(4-Mo)-BT), Phenylenediamines (6-PPD, 6-PPDQ, Aniline, 4-HDPA, DPPD, NO-DPA), and other Amines (DCH, MeDCH, HMMM). Concentrations found in surface (atmospheric pressure) and deep (hyperbaric pressure) seawater conditions are reported in light blue and dark blue, respectively. x-axis represents exposure time in days.

DPG: Diphenylguanidine; PG: Phenylguanidine; TPG: Triphenylguanidine; 2-MTBT: 2-Methylthiobenzothiazole; 2-OHBT: 2-hydroxybenzothiazole; NH2-BT: 2-aminobenzothiazole; BTSA: Benzothiazole-2-sulfonic acid; 2-(4-Mo)-BT: 2-(4-morpholinyl)benzothiazole; 6-PPD: N-(1,3-dimethylbutyl)-N-phenyl1,4-phenylenediamine; 6-PPDQ: 6-PPD quinone; 4-HDPA: 4-Hydroxydiphenylamine; DPPD: N,N'-Diphenyl-p-phenylendiamine; NO-DPA: N-Nitrosodiphenylamine; MeDCH: N-Methyldicyclohexylamine; HMMM: Hexa(methoxymethyl)melamine.

The outcomes are summarized in a scientific paper currently in preparation (deliverable D4.5).

Milestone 4.6

The milestone 4.6, i.e. the completion of the investigations into the role of hyperbaric conditions on MP degradation and release of additive chemicals was reached before the deadline of month 30. All the results have been compiled and a scientific paper (deliverable D4.5) is in preparation.

DELIVERABLES (WHERE APPLICABLE)

Deliverable	Partner responsible	Date of submission	Comment
D4.5 Scientific paper	AMU-MIO		In preparation. Expected to be submitted in spring 2024

MILESTONES (WHERE APPLICABLE)

Milestone	Partner responsible	Progress	Comment
M4.6 Hyperbaric degradation studies	AMU-MIO	Completed	

Task 4.6. Kinetic of MP degradation in marine sediments

Intro: This task consists in a 22-months *ex situ* study of the plastic additives release from plastic debris (recycled low-density polyethylene, 200-500 µm) buried in a sediment sampled from a marine lagoon (salinity 32). This experiment was completed on M28, thus validating the milestone M4.7 "Completion of the investigations into the role of MP microbial degradation in marine sediments".

Results: we showed that the presence of plastic in the sediment could be accompanied by a release of additives i) into the interstitial waters, then ii) into the water column. Of the 16 substances analysed (9 organophosphate esters and 7 phthalates), tri-n-butyl phosphate (TnBP) was measured in the various compartments of the reconstructed sedimentary system. Its dynamics in the interstitial waters varied over time, with a rapid appearance at the start of exposure (1 week) and a slower disappearance over several months (Figure 14).

Discussion: The TnBP measurements in the interstitial and supernatant waters was directly linked to the release from plastic debris, since controls (sediment not spiked with reference recycled low-density polyethylene) did not show TnBP presence in any compartment of the reconstructed sediment system. The disappearance phenomenon can be attributed to i) bio/geochemical degradation or ii) transfer to other compartments (i.e. particulate sediment). Notably, this disappearance is greatest in the most superficial layer of the sediment (oxic zone of the sediment). The higher TnBP concentrations measured in the supernatant water column of the plastic-enriched sediments, compared with the control incubations, suggest that this decrease is partly controlled by the transfer of TnBP to the water column. In the deeper interstitial waters (suboxic and anoxic/sulphidic zones, respectively), despite their decreases, TnBP concentrations remained significantly higher than those measured in the control experiments at the end of the experiment, probably indicating less favourable TnBP degradation and/or export kinetics under these conditions. However, the concentration gradients indicate the maintenance of a diffusive flow from the deep layers to the superficial layers of the sedimentary column and to the water column, which is maintained even 2 years after burial of the plastic debris.

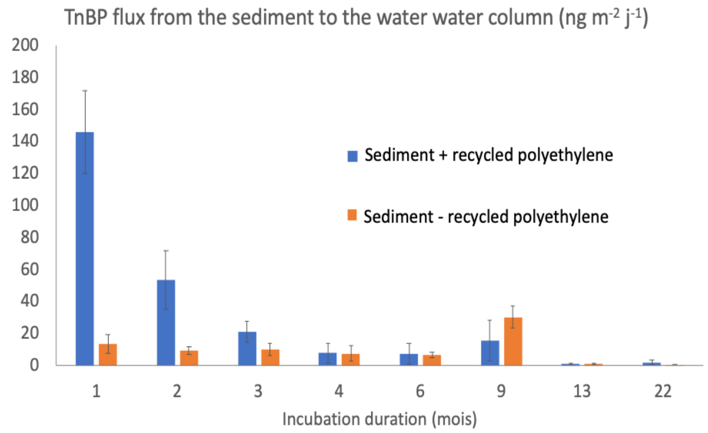

Figure 14: plastic additive flux from plastic-rich sediment to the water column.

<u>Conclusions</u>: The main result of this experiment was to show that, while sediments were known to be a sink for plastics in the marine environment, they could also be sources of additives for the water column. The main conclusions are the object of a dedicated article in currently being finalized (V. Fauvelle, J. Regis, N. Schmidt, T.K. Vu, R. Sempéré, C. Grenz, J.-L. Maeght, P. Verdoux, O. Pringault, A. Dolla, C. Milliton, P. Cuny, S. Rigaud. First evidence of additive fluxes from sediment buried plastic debris to the water column). This article will partly comply the deliverable D4.5 "a manuscript comparing MP degradation through different mechanisms (UV, biological) under different pressures (hyperbaric studies) within characteristic marine environments: Arctic/Mediterranean, surface/deep seawater, seawater/marine sediment."

WORK PACKAGE 5: COMMUNICATION & STAKEHOLDER ENGAGEMENT

WP5 was responsible for communicating and disseminating project activities and outputs and stakeholder engagement. WP5 collaborated with all project partners to ensure project results and outputs are communicated in a scientifically robust manner using the most appropriate means to reach intended audiences and directly engage key stakeholders. Targeted dissemination approaches were used to ensure project information is circulated to and assimilated by project audiences across disciplines and sectors. WP5 engaged with and utilized resources and opportunities provided by JPI Ocean to amplify WP5 efforts and to ensure relevant activities adhered to JPI Oceans best practices guidelines. WP5 had the following specific objectives:

- To use a variety of tools to communicate and disseminate project activities, findings, and results.
- To raise awareness of degradation and fragmentation pathways of MPs originating from defined consumer products and to share this information in close collaboration with the project partners, ensuring project communication is scientifically correct.
- To develop and use project specific targeted dissemination approaches to ensure project information is distributed to, and understood by, both experts and non-experts, linking and building upon relevant on-going activities, research, and projects.
- To engage key stakeholders throughout the project, using a collaborative approach that fosters dialogue between project partners and key stakeholders.

Task 5.1 Dissemination Plan:

A project dissemination plan (D5.1) was developed in month 3 to identify the communication and dissemination objectives for project outputs, activities, and results and to allow for targeted communication and dissemination to European and national bodies dealing with project relevant policy and legal instruments, relevant intergovernmental organization, technical group marine litter and Regional Sea Conventions, project relevant industries and to raise awareness in the public domain. The dissemination plan also provides guidelines and protocols for project dissemination and communication in line with JPI Oceans best practice and communication support and was an iterative document which was updated throughout project duration. The plan includes a communication and dissemination log that documents all partner efforts relevant to WP5 and how they were supported and amplified via WP5 activities. Task 5.1 also included the development of the project logo (M5.1), which was developed in month 1 in collaboration with project partners to ensure project partners feel the logo adequately represents the project and themselves. The final logo was made available in different formats, colour, and greyscale.

Task 5.2 Project website and Social Media:

The ANDROMEDA project website was deployed in month 2 (M5.2/D5.2) to ensure the project's online visibility, and to provide easily accessible information to a range of audiences in conjunction with the ANDROMEDA Twitter account. The project website comprises of several static pages (detailing project aims, consortium details, funding acknowledgements etc.), and a News page that was updated regularly based on partner activities and outputs. Additional pages were created as the project progressed such as the Multimedia Page and Publications Page. The project website contained by the end of project duration over 24 links to downloadable project multimedia items, and over 70 news posts. In addition to the project website, WP5 used social media (Twitter) to facilitate complimentary information exchange with relevant audiences and to connect with relevant activities to support fast and effective correspondence for project outreach in relation to events, activities, and updates. The ANDROMEDA twitter had over 670 Followers by the end of the project duration.

Task 5.3 Stakeholder Engagement:

ANDROMEDA stakeholder engagement was undertaken through the use of tools outlined in Task 5.2 and 5.4, in addition to partner direct contact at events, meetings, via phone and e-mail. MaREI UCC developed, tested and implemented two online stakeholder workshops (M5.3) to support work undertaken on the cost-effectiveness of microplastics analysis methods for seawater samples. The first workshops was implemented on the 1st of February 2023 and the second workshop on the 7th of March 2023. Both workshops made recommendations for policy makers and decision makers, researchers and other stakeholders and the results of these stakeholder events are available in two online reports with a further peer reviewed publication being submitted (D5.6). The final in-person partner meeting (M5.4) of the ANDROMEDA project consortium was held on the 13th of September 2023 in Oranmore, Co. Galway, Ireland. This meeting was held back to back with the JPI Oceans microplastics project meeting at the Marine Institute in the same location, where ANDROMEDA delivered 8 oral presentations, 8 poster presentations, and chaired two sessions.

Task 5.4: Project fact sheets and E – newsletters:

Multiple project fact sheets (D5.3) were developed throughout the project lifecycle to cater to different target audiences and to present different aspects of the project, which are available for download from the project website. The project brochure was developed early in project duration, followed by the project pull-up banner to support partner efforts at events, conferences and meetings. Two ANDROMEDA flyers were developed to support the ANDROMEDA stakeholder workshop implementation prior to the events. The ANDROMEDA Microplastics Factsheet and the ANDROMEDA smartphone App Factsheet were developed to target younger audiences and to support public outreach undertaken as part of Task 5.5 in partner countries. Both of these factsheets are available for download from the project website in eight languages. Additional poster templates were created for ANDROMEDA partners to use at events such as ALSO ASM 2023 and the JPI Oceans end-term meeting. Three project newsletters (D5.4) were delivered throughout the project and were distributed widely. The yearly project newsletter summarized project information such as WP specific activities, conferences attended, publications and project news items. The newsletters highlight the projects progress for each year and are available for download from the project website.

Task 5.5 School Outreach

ANDROMEDA partners reached out to younger audiences including schools in partner countries to raise awareness about Microplastics, the ANDROMEDA project and to promote the developed citizen science smartphone app (Task 2.2). Outreach activities commenced in Spain and Malta together with beach clean sampling activities across eight test sites (M5.5). These events utilized the factsheets developed under Task 5.3 in their national languages. University of Malta have also partnered with Plastic Pirates Go Europe to expand the project's school outreach programme, which will facilitate direct engagement with younger audiences in Europe to encourage uptake and engagement with the ANDROMEDA smartphone application and Europe-wide citizen science campaign.

Task 5.6 Protocols for Microplastic Analysis

Task 5.6 supported the development and collation of protocols that describe the analyses of microplastics particles. The ANDROMEDA portfolio of protocols aims to facilitate the production of comparable data outside of the project and beyond the lifetime of the project by other research groups. This work was undertaken in conjunction with the ANDROMEDA's technical WPs, and the portfolio will be formally launched and disseminated in early 2024, with linked social media campaign. Eight ANDROMEDA protocols for Microplastic Analysis been finalized, collated and edited into: De Witte, B., Power, O-P., Fitzgerald, E. and Kopke, K. (2023). ANDROMEDA Portfolio of Microplastics Analyses Protocols. ANDROMEDA Deliverable 5.5. JPI Oceans ANDROMEDA project.

DELIVERABLES

Deliverable	Partner responsible	Date of submission	Comment
D5.1 Dissemination Plan	MaREI UCC	Month 3 – First version Month 36 – Final version	Complete
D5.2 Project website and social media	MaREI UCC	Month 2	Complete
D5.3 Project Factsheets	MaREI UCC	Month 36	Complete
D5.4 E-news letters	MaREI UCC	Month 36	Complete
D5.5 MP analyses protocols	ILVO & MaREI UCC	Month 36	Complete
D5.6 Scientific Journal Publication	VLIZ, ILVO, MaREI UCC, Tallinn University of Technology, University of Gothenburg	Meyers et al., (submitted)., Value for money: a cost-effectiveness analysis of microplastic analytics.	In Progress

MILESTONES (WHERE APPLICABLE)

Milestone	Partner responsible	Progress	Comment
M5.1 Project logo	MaREI UCC	Complete	Complete
M5.2 Project website launch	MaREI UCC	Complete	Complete
M5.3 Project Stakeholder Meeting	MaREI UCC	Complete	Complete
M5.4 Final project meeting with stakeholders	Université d'Aix-Marseille & MaREI UCC	Complete	Complete

M5.5 School Outreach Campaign	University of Malta & MaREI UCC	Complete	Complete

DISSEMINATION ACTIVITIES (INCLUDING A LIST OF SCIENTIFIC PUBLICATIONS)

List of scientific publications

To date, the Andromeda project has produced 30 published peer-reviewed manuscripts. A full overview of the manuscripts can be found here: https://www.andromedaproject.net/publications

In addition, multiple Andromeda partners have directly contributed to key documents produced by OSPAR (MP indicator for sediments group, ICGML), International Council for the Exploration of the Seas - Working Group on Marine Litter (ICES WGML), Arctic Monitoring and Assessment Programme Litter and Microplastic Expert Group (AMAP LMEG), EU Marine Strategy Framework Directive Technical Group on Marine Litter (MSFD TGML):

- OSPAR (MP indicator for sediments group, ICGML): Guidelines for the monitoring of microlitter (including microplastics) in seafloor sediments for the OSPAR Maritime Area. OSPAR Report from ICGML. Working draft November 2023.
- ICES (WGML): ICES. 2022. ICES manual for seafloor litter data collection and reporting from demersal trawl samples. ICES Techniques in Marine Environmental Sciences Vol. 67. 16 pp. doi: https://doi.org/10.17895/ices.pub.21435771
- AMAP: AMAP, 2021. AMAP Litter and Microplastics Monitoring Guidelines. Version 1.0. Arctic Monitoring and Assessment Programme (AMAP), Tromsø, Norway, 257pp.
- EU MSFD: Guidance on the Monitoring of Marine Litter in European Seas An update to improve the harmonised monitoring of marine litter under the Marine Strategy Framework Directive, EUR 31539 EN, Publications Office of the European Union, Luxembourg, 2023, ISBN 978-92-68-04093-5, doi:https://doi.org/10.2760/59137, JRC133594.

Press, Radio & Events Targeting Public Audiences

- Press, Norwegian, December 2020: Andy Booth & Lisbeth Sørensen interview for Gemini.no entitled 'Skal finne usynlig nanoplast i havet' where they discussed the impacts of microplastics & ANDROMEDA. https://gemini.no/2020/12/skal-finne-usynlig-nanoplast-i-havet/
- Press, Norwgian, April 2021: Andy Booth in Teknisk Ukeblad article entitled 'Eksperten svarer: 5 spørsmål om nanoplast i havet' (The expert answers: 5 questions about nanoplastics) https://www.tu.no/artikler/eksperten-svarer-5-sporsmal-om-nanoplast-i-havet/509161
- Norwegian Environment Agency Seminar, April 2021: https://www.andromedaproject.net/news/norwegian-environment-agency-seminar with Andy Booth
- Radio Interview, Spanish, July 2021 with P. Otero https://www.ivoox.com/espanoles-mar-la-patrona-del-audios-mp3_rf_73011949_1.html
- Press, Spanish, August 2021 with Pablo Ortero, Jesus Gago and Patricia Quintas https://www.revistaalimentaria.es/consumidora/actualidad/nos-preocupa-la-basura-marina
- Press, Flemish (Dutch), February 2022: Nelle Meyer's in VRT News https://www.vrt.be/vrtnws/nl/2022/02/15/belgische-wetenschappers-forceren-doorbraak-in-onderzoek-naar-mi/
- Press, Spanish, March 2022: Jesus Gago in article 'Más basura, ino gracias!' https://www.farodevigo.es/faroeduca/2022/03/22/basura-gracias-64114090.html
- Press, Norwegian, April 2022: Andy Booth in forskning.no entitled 'Tørketrommelen slipper ut masse mikroplast' (The Dryer Releases a Lot of Microplastics) https://www.forskning.no/forurensning-teknologi/torketrommelen-slipper-ut-masse-mikroplast/2011215

- Press, Spanish, May 2022: Jesús Gago in Cerna Magazine https://adega.gal/web/media/documentos/contaminacion_plastica.pdf
- Press June 2022: Newspoint article to promote the launch for the citizen science smartphone app in Malta. https://www.um.edu.mt/newspoint/news/2022/05/coupling-citizen-science-with-artificial-intelligence-the-and-romeda-innovative-microplastic-recording-tool?utm_source=update&utm_campaign=update&utm_medium=email&utm_content=other
- Podcast, September 2022: Andy Booth at Finding Genius Podcast https://open.spotify.com/episode/4LcE7AHHKhrOQVupJsYN95
- Public Science Event, September 2022: National Science in the City Event with Alan Deidun showcased the work of in Fort St Elmo in Vallett.
- Press, Spanish, February 2023: about the ANDROMEDA Microplastics sampling campaign along the coast of Vigo. With IEO, CSIC & TalTech Link: bit.ly/3Z5YrlY
- Radio, Spanish, February 2023 with Pedro Pardo: https://www.rtve.es/play/audios/espanoles-en-la-mar/espanoles-mar-pedro-pardo-vuelve-encabezar-direccion-apecac-28-02-23/6823542/
- Radio, February 2023 with Lucía Viñas: https://www.rtve.es/play/audios/galicia-informativos/cronica-galicia-21-02-23-escuchar-ahora/6816431/
- Public Event, March 2023: Alan Deidun, Malta National Aquarium, 200 students. https://www.andromedaproject.net/news/malta-national-aquarium-event
- Public Event, May 2023: launch of the ANDROMEDA citizen science microplastic campaign and smartphone app. Multiple links: https://www.youtube.com/watch?v=vD2lPRCX-DA, https://westmed-initiative.ec.europa.eu/change-starts-with-you-citizen-science-campaign-on-plastic-in-the-ocean/, https://www.um.edu.mt/newspoint/news/features/2023/05/become-researcher-whilst-on-beach, https://www.gov.mt/en/Government/DOI/Press%20Releases/Pages/2023/05/16/PR230726.aspx
- Public Event & Teacher Training, May 2023: IEO partners with local Losada School and 30 international students from across Hungary, Greece, and Belgium. Gustavo Blanco Heras provided training to schoolteachers & demonstration Smartphone App https://twitter.com/Andromeda_EU/status/1661714985750495233?s=20
- Press, June 2023: Andy Booth in Tackling marine debris and microplastics pollution: tough challenges ahead. Booth, Andy; Rajwi, Tiki. The Hindu [Newspaper] 2023-06-03. https://www.thehindu.com/news/national/kerala/tackling-marine-debris-and-microplastics-pollution-tough-challenges-ahead/article66928073.ece
- Public event, July 2023: Umalta at sea-side event with Girl Guides from Belgium https://www.andromedaproject.net/news/ta-fra-ben-seaside-event
- Press, July 2023: The ANDROMEDA project featured in a Maltese national research magazine called THINK (page 6). https://thinkmagazine.mt/3d-flip-book/think-41-equity/

Non-scientific and non-peer reviewed publications (popularised publications)

- ANDROMEDA Brochure, ANDROMEDA Project Pull Up Banner, ANDROMEDA Microplastics Factsheet & ANDROMEDA Smartphone App Factsheet (both available in Dutch, English, Estonian, French, German, Maltese, Norwegian, and Spanish) and ANDROMEDA Newsletters Issues 1, 2 and 3: https://www.andromedaproject.net/multimedia
- Kopke K., Meyers N., Dozier A., Fitzgerald E., Power O-P., Agnew S., Everaert G., De Witte B., (2023). Scientist Perspectives on the Cost-Effectiveness of Microplastic Analysis Methods for Seawater Samples: ANDROMEDA Workshop 1 Event Summary & Participant Recommendations on Cost-effectiveness. JPI Oceans project. DOI: 10.13140/RG.2.2.17205.86241
- Kopke K., Meyers N., Dozier A., Fitzgerald E., Power O-P., Agnew S., Sempéré R., (2023). Policy & Decision Makers Perspectives on the Cost-Effectiveness of Microplastic Analysis Methods for Seawater Samples: ANDROMEDA Workshop 2 Event Summary & Participant Recommendations on Cost-effectiveness. JPI Oceans project. DOI: 10.13140/RG.2.2.27272.19204

On-line Engagement and Support are documented in D5.1 communication and dissemination log, which is 81 pages long in A4 landscape

- Website: https://www.andromedaproject.net/ description in T5.2 &
- Social Media: https://twitter.com/andromeda_EU description in T5.2

IMPACT

In line with the 2019-JPI-microplastic call, Andromeda contributed to most of the expected outcomes and impacts and reached out to different target groups' categories (see WP description). All these outcomes have been reached at medium time (2-3 years after the beginning of the project).

Optimised sampling methods have been developed as part of WP2 and which enable validated sampling that can be compared with conventional MP techniques in water and atmosphere. Indeed, an optimised MP sampling device for water, which can be attached to a pumping system or ferrybox, has been developed, tested, compared, and used in different environments (Baltic Sea, Atlantic Ocean, Mediterranean Sea). Similar work was carried out for two MP sampling devices. An Andromeda smartphone allowing in-situ MP monitoring on beaches was also developed and launched and is available for free download on both the ANDROID and iOS platforms. Cost-effective analysis techniques based on automated image analysis using hyperspectral imaging in the 1100-1700 nm wavelength or using fluorescence microscopy for NR-stained particles filters were also elaborated by WP2, both techniques allowing for polymer identification. Several organic compounds such as benzothiazoles, guanidines, 6PPD and other PPDs were identified and proposed as potential markers. Efforts have been made in WP2 for exercises of cross-validation and cost-effectiveness evaluation. Two WP2 oceanographic cruises dedicated to surface water sampling were organised in the coastal area of Marseille in the Mediterranean Sea in Spain/Atlantic and an evaluation of cost-effectiveness of MP analysis methods was done. This led to the development of a cost-effectivity assessment tool, which can be used by other research groups. It should be noted that the imaging methodology and the smart phone application open the door to citizen science in Europe, and open up prospects for large-scale use in Europe.

This Andromeda work in WP3 has shown the importance of using reference materials for both calibration and biodegradation experiments. They also make it easier to work together and compare results. Work on the preparation of tire wear particle (TWP) RMs from car tires has also been initiated to familiarise the project partners. The results of WP3 include the use of auto-coding networks to reconstruct FTIR and Raman spectra, particularly when targeting specific particles, as in monitoring approaches. It was also demonstrated that the analytical combination of FFF and pyGC-MS could be an interesting approach for the analysis of polymers in heterogeneous samples. Similarly, our efforts have led to the development of a deep learning method for signal enhancement of FTIR and Raman spectra in the analysis of environmental samples. Similarly, certain techniques (LC-HRMS, pyGC-MS, SEM) have proved highly effective for the analysis of TWPs in the marine environment. The use of platinized membranes proved particularly effective for the analysis of MPs (1 to 10 µm) by Raman microscopy, while SEM-Raman proved suitable for the identification of NPs.

Research dealing with the biodegradation of plastics has shown that degradation processes vary greatly depending on the nature of the plastic and the importance of external factors on the degradation of plastics. Material has been degraded in situ in the Mediterranean and Baltic Seas, or in the laboratory under different conditions of light pressure, and subsequently studied in the laboratory. The work carried out demonstrated the production of dissolved compounds delivered by bacteria from various polymers such as TWPs, PET and PVC. Sediments were also shown to be a significant source of toxic organic additives for the entire water column. Clearly, the degradation of plastic polymers induces the production of other plastic fragments and dissolved compounds that are toxic to organisms in the marine environment.

WP5 focused on communicating and disseminating project activities and outputs and stakeholder engagement. This work amplified project partner scientific work and efforts to reach intended target audiences and interact with key stakeholders by applying classic communication tools (website and social media) to increase visibility of the project and its research and by collaborating with project partners in the development of information-rich documents for the general public on microplastic pollution in the marine environment and in support of the use and dissemination of the WP2 smartphone application. Furthermore, WP5 stakeholder workshops ensured dialogue and mutual learning between WP2 researchers and key stakeholders around the cost effectiveness of microplastic analysis methods for seawater samples, which specifically focused on engaging policy and decision makers. Relevant communication material and documents such as the project brochure, ANDROMEDA factsheets and the stakeholder workshop reports are all available for download from the project website. The dedicated communication, dissemination and stakeholder engagement work, developed as part of Andromeda demonstrated the benefits of WP5, which ensured a direct link between scientific discoveries and dedicated science communication, dissemination and stakeholder engagement to and with diverse audiences.

Specifically, by developing new techniques for detecting MPs, Andromeda's work has helped to reduce pollution from litter, plastic and microplastic, in the Mediterranean Sea basin and other European waters in line with the EU Zero Pollution Plan objectives and the Convention for the protection of the Mediterranean Sea against pollution. Andromeda helped facilitate beach prevention and clean-up operations, as well as an awareness-raising campaign for communities and tourists urging them to "reduce the damage caused to the Mediterranean by beach litter to a sufficiently precautionary level, by adopting the threshold value, developed by the MSFD's Marine Litter Technical Group, for shoreline litter of 20 litres/100 m of beach length". Andromeda's partners have been in frequent contact with local public authorities and the Plastic Pirate program involved in reducing plastic waste on European riverbanks. Andromeda's partners contributed to raising general awareness by regularly presenting the progress made thanks to the project's implementation and making these results accessible to the public. Several of Andromeda's results are in line with the EU's action plan for zero pollution of air, water and soil, including: 1) marine pollution due to plastic waste and microplastics discharged into the environment; 2) the use of plastics and the subsequent risk of hazardous chemicals such as micro, nano-plastics and organic endocrine disruptors such as phthalates or/and toxic materials such as bisphenols and OPEs, which are organic plastic additives, and their consequent impact on health. In addition, are contributing elements to other European Green Deal initiatives, including the strategy on chemicals for sustainability, for the period 2021-2024. Reducing plastic and other waste and contaminants, as well as marine discharges from ships, will have an impact on the health and pollution-free status of oceans, seas, and waters. It should be noted that the participation of all stakeholders at all levels will be encouraged to carry out collective action and collective change for zero pollution.

ANNEX I:

IDENTIFICATION OF PROJECT PARTICIPANTS/ BENEFICIARIES
(FIGURE 15)

- Coordinator: Dr. Richard Sempéré, CNRS, Aix-Marseille University, France
- Dr. Bavo De Witte, Flanders Research Institute for Agriculture and Fisheries Aquatic Environment and quality, Belgium
- Dr. François Galgani, IFREMER ODE/LER-PAC, France
- Dr. Andy Booth, SINTEF Ocean AS Environment and New Resources, Norway
- Dr. Dorte Herzke, Norwegian Institute for Air Research Environmental Chemistry, Norway
- Dr. Gert Everaert, Flanders Marine Institute, Research department, Belgium
- Prof. Alan Deidun, University of Malta, Malta
- Prof. Martin Hasselov, University of Gothenburg Marine Sciences, Sweden
- Dr. Stephan Wagner, Hemholtz-Centre for Environmental Research-UFZ, Department of analytical chemistry, Germany
- Mrs Kathrin Kopke, University College Cork, MaREI Centre, ERI
- Dr. Jesus Gago, Instituto Espagnol de Oceanografia, Spain
- Prof. Urmas Lips, Talinn University of Technology, Department of marine systems
- Prof. Nathalie Tufenkij, McGill University, Chemical Engineering, Canada
- Prof. Bart Koelmans, Wageningen University, Aquatic Ecology and Water Quality Management
- Dr. Nicolas Toupoint, Merinov, Canada

CONSORTIUM

Organisation	Acronym	Country
Université d'Aix-Marseille - Mediterranean Institute of Oceanography	AMU-MIO	FRANCE
Flanders Research Institute for Agriculture and Fisheries	ILVO	BELGIUM
French Research Institute for Exploitation of the Sea	Ifremer	FRANCE
SINTEF Ocean AS	SINTEF	NORWAY
Norwegian Institute for Air Research	NILU	NORWAY
Flanders Marine Institute	VLIZ	BELGIUM
University of Malta	UM	MALTA
University of Gothenburg	/	DENMARK
Helmholtz-Centre for Environmental Research	UFZ	GERMANY
University College Cork	UCC	IRELAND
Instituto Español de Oceanografía	IEO	SPAIN
Tallinn University of Technology	TALTECH	ESTONIA
McGill University	/	CANADA
Wageningen University	WUR	THE NETHERLANDS
Merinov	/	CANADA

FIGURE 15: ANDROMEDA brings together 15 international partners from 11 countries across europe and canada in an expert consortium to tackle challenges in characterising & quantifying microplastics.

ANNEXE 2

Andromeda-JPI peer review publication list: See also update on the Andromeda web site (https://www.andromedaproject.net/publications).

- Alimi, O., Claveau-Mallet, D., Lapointe, M., Biu, T., Liu, L., Hernandez, L., Bayen, S., Robinson, S., Ghoshal, S. and Tufenkji, N., 2023. Effects of Weathering on the Properties and Fate of Secondary Microplastics from a Polystyrene Single-Use Cup. *Journal of Hazardous Materials*, 2023, 131855.
- Alimi, O.S., Claveau-Mallet, D., Kurusu, R.S., Lapointe, M., Bayen, S. and Tufenkji, N., 2021. Weathering pathways and protocols for environmentally relevant microplastics and nanoplastics: What are we missing? *Journal of Hazardous Materials.* Volume 423, Part A.
- Alkan, N., Alkan, A., Castro-Jimenez, J., Royer, F., Papillon, L., Ourgaud, M. and Sempere, R., 2020. Environmental occurrence of phthalate and organophosphate esters in sediments across the Gulf of Lion (NW Mediterranean Sea). *Science of the Total Environment.*
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- Brandt, J. Mattsson, K., Hasselov, M. (2021). Deep learning for reconstructing Low-quality FTIR and Raman spectra: A case study in Microplastic analyses. Analytical chemistry 93(49), 16360-16368 (doi: 10.1021/acs.analchem.1c02648)
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- Fauvelle, V., Garel, M., Tabburini, C., Nerini, D., Castro-Jiménez, J., Schmidt, N., Paluselli, A., Fahs, A., Papillon, L., Booth, A. M. and sempéré, R., 2021. Organic Additive Release From Plastic To Seawater Is Lower Under Deep-Sea Conditions. *Nature Communications* 12 (4426).
- Foscari, A., Schmidt, N., Seiwert, B., Herzke, D., Sempéré, R., Reemtsma, T. (2023). Leaching of chemicals and DOC from tire particles under simulated marine conditions. Frontiers in Environmental Science, 11, 1206449. [doi: 10.3389/fenvs.2023.1206449].
- Gondikas, A., Mattsson, K., & Hassellöv, M. (2023). Methods for the detection and characterization of boat paint microplastics in the marine

environment. Frontiers in Environmental Chemistry, 4, 1090704 [doi: 10.3389/fenvc.2023.1090704].

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