Andromeda

Analysis techniques for quantifying nano- and microplastic particles and their degradation in the marine environment

Context

This report summarizes the results obtained from ILVO and VLIZ within the JPI oceans project Andromeda. A wide range of methods is available to analyse microplastics (MPs) in marine environment matrices, ranging from simple light microscopy and particle-by-particle spectroscopy to imaging spectroscopy and various statistical algorithms and image analysis tool packages. In this myriad of options, the Andromeda project identified a clear need for further optimization, validation and harmonization of current methods , in order to tune the methods available with monitoring and research needs.

The work of ILVO and VLIZ focuses on the optimization of cost-effective methods for microplastic analysis. Specific focus was on the application of Nile Red (NR) staining-based techniques to be used as reliable alternatives for spectroscopic techniques such as µFTIR or µRaman spectroscopy, both being expensive and time consuming. The development and optimization of cost-effective methods is challenging, as cost-effective techniques should, compared to the state of the art benchmarking methods, combine savings in time and cost whilst reaching an acceptable level of accuracy and precision, a reliable detectability of a large variety of polymers, a low probability for contamination. .

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. NR staining has proven efficient at distinguishing MPs from non-plastic materials such as amphipod carapaces, algae, seaweeds, wood, feathers, mollusc shells, chalk and sand particles . Moreover, the interaction of NR with different polymers varies according to the chemical characteristics of the plastic, which has led to the successful use of NR staining of particles for polymer identification based on fluorescence.

The automation of inexpensive fluorescence staining methodologies as an alternative to often used, spectroscopy-based methodologies has arised as a promising alternative for the development of cost-effective screening methods for MPs analysis. Most of these approaches however focused on MP detection only, and use black-box models rather than white-box models which have a transparent inner structure and represent information in a visual and clear way. Furthermore, at the start of the project, the interaction between NR and polymers based on their chemical characteristics has not yet been thoroughly explored, and shows potential for the development of more advanced high-throughput, cost-effective analysis methods that allow for both MP detection and polymer identification.

Objectives

The main objectives of ILVO and VLIZ within the Andromeda project were:

1. Microplastic analysis by fluorochromes

- To develop a straightforward, semi-automated, ex-situ methodology to detect and identify MPs, based on NR staining, which allows to identify also the most abundantly produced plastic polymers.

- To optimize the sample processing protocols for MP extraction with NR-based methods from seawater, sediment and biota, as well as validating the complete MP analysis workflow.

- To test the applicability of the developed sample processing and analysis protocols for real environmental samples and meanwhile provide an update of MP pollution of the Belgian Part of the North Sea (BPNS), with focus on the macrolitter hotspot dredge disposal site Zeebrugge Oost.

- To perform an exploratory study to verify the potential of combining flow cytometry with fluorescent staining of MPs to develop a time-efficient, semi-automated, in-situ analysis method to observe smaller-sized MPs in seawater on board of a research vessel, without the need for an extensive sample processing step.

2. Cost-effectiveness analysis

- To perform a cost-effectivity analysis (CEA) of commonly used methods for the analysis of MPs in seawater based on survey data gathered from experts in the field and to provide concrete and useful recommendations of MP analysis techniques in terms of their cost-effectivity. This will be done to support researchers, policy makers and other stakeholders when having to choose between different MP workflows.

Conclusions

The main outcome of the ILVO-VLIZ collaboration in this project is the optimization and validation of a NR-based analysis method for MPs in different matrices of the marine environment. A two-step semi-automated classification method was created which combines (RGB)-color 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) 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).

Additional tests with artificially weathered plastics showed decreased predictive accuracies of the developed CT models. After expanding existing training datasets from 2 x 60 to 2 x 500 particles (PDM), and from 7 x 30 to 7 x 200 particles (PIM), the best performing models for artificially weathered MPs were based on random forest algorithms rather than classification trees (> 90 % and > 80 % accuracy for PDM and PIM, respectively). Consequently, it was decided to use the improved models for all

further MP analyses. Determining the cut-off size of the random forest algorithm-based models showed that pristine MP polymers as of 10 µm could be accurately identified using the developed method. A second set of random forest models was built using images acquired with a fluorescence stereomicroscope (FSM) rather than a FM, which allows whole filter image acquisition. Interlaboratory testing of the models showed lower accuracies for MP photographed using a different microscope than the one used to develop the models. These results demonstrate that lab-specific training datasets are a requirement for reliable MP analysis. Next, a detailed analysis protocol was written for the detection of MPs and identification of their respective polymer types using the two developed random forest models.

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. MP pollution analysis in these areas is currently ongoing.

Experiments combining flow cytometry with the fluorescent dye NR showed potential for future development of an in-situ MP analysis method where MPs down to 1 µm can be detected, and where no sample processing is required. Exploratory results showed that the obtained fluorescent signals allow to differentiate MPs from organic material using the k-nearest neighbors algorithm, a simple, supervised machine learning algorithm.

Lastly, a predictive tool was developed using the obtained survey data on cost-effectivity of MP analysis methods. In the future, this tool could help science, policy and industrial actors to gain a holistic insight on which workflows provide the greatest value for money for specific seawater samples and could act as baseline data. Furthermore, it could allow for more informed decision-making regarding policy and management.

Keywords
Microplastics, Fluorescence microscopy, Nile red, Machine learning, Artificial intelligence, Weathered plastics, Validation, Cost-effectiveness analysis.