Detection and identification of microplastics in biota using Nile red and machine learning: validation of an innovative, cost-effective approach

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Plastic pollution has become one of the most ubiquitous threats the oceans are facing nowadays. Microplastics (MPs) are of special concern as it has been shown these are ingested by a wide range of marine species from different trophic levels [1]. However, the environmental implications of MPs ingestion, coupled to the toxicological relevance of different MP polymers and their chemical composition, remain poorly understood. This highlights the need for standardised, cost- and time-effective monitoring procedures to accurately and routinely determine the abundance, composition and distribution of MPs in the marine environment, to allow for effective management strategies.

An innovative approach for MP analysis in marine biota was developed, thereby combining the advantages of both high-throughput screening and automation. We evaluated the method in mussel samples. The presented method combines RGB-color quantification of emitted fluorescence of Nile red (NR)-tagged MPs with a simplified supervised machine learning (ML) classification model, which has been developed in an open source environment [2]. Our approach further enhanced the Nile red technique for the fast detection of MPs and included the identification of the polymer types.

For this MP analysis in biota, a two-step digestion was applied within the sample preparation to remove all lipid-rich animal tissue and plant and algae materials present. An alkaline digestion with potassium hydroxide (KOH, 10%) and an oxidative digestion with hydrogen peroxide (H_20_2, 15%) was implemented, each for 48h at 50°C on a magnetic stirring plate. In between both digestion steps, the sample was filtered over a stainless steel filter and sonicated to loosen all particles adhering to the filter surface. The sample was then filtered over a PTFE-filter, stained with the fluorescent dye NR (1µg/ml acetone), and left to dry for 24h.

The automated detection of MPs and the polymer type identification was based on two machine learning decision models, the Plastics Detection Model (PDM) and the Polymer Identification Model (PIM), which both use recursive binary splits through simple decision rules inferred from emission spectra features [2]. Both models were trained and validated using stained reference MPs. The first model predicted with high accuracy whether a particle is plastic or of natural origin. The second model allowed to identify the polymer type of MPs. Both models use RGB colour data, extracted from the stained particles photographed through a fluorescence microscope under blue, green and UV filters.

The accuracy and precision of the analytical method was calculated using recovery tests. Six MP types were spiked into the mussels samples, varying in the combination of polymer type (polyacrylonitrile - PAN, polyethylene terephthalate - PET, polypropylene - PP, polystyrene - PS and polyvinyl chloride - PVC), size (250 – 1000 µm), and shape (particle/fibre). Next to accuracy and precision, the validation included the determination of the limit of quantification and detection (LOQ and LOD), selectivity, specificity, and robustness. Selectivity and specificity were assessed based on the performance of both ML models to detect plastic particles and to identify the polymer types, respectively. To assess the robustness of the overall procedure, sample type was varied across the experiments.

Preliminary results showed high recovery rates and good model performance. The combination of the emitted fluorescence of particles photographed under all three filters is unique for each particle type (plastic/ non-plastic as well as plastic polymer type) assessed in our analyis, not just in terms of fluorescence intensity but also in terms of colouration. In this way, the new approach solved the issue of co-staining of natural materials by Nile Red and allowed for the accurate, precise and selective determination and identification of MPs in mussel matrices.

References
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