PROJECT PERIODIC REPORT

Direct and indirect ecotoxicological impacts of microplastics on marine organisms

Project acronym: PLASTOX

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PUBLISHABLE SUMMARY (MAX. 2 PAGES)

The PLASTOX project investigates the ingestion, food-web transfer, and ecotoxicological impact of microplastics (MPs), together with the persistent organic pollutants (POPs), metals and plastic additive chemicals associated with them, on key European marine species and ecosystems. It also studies the temporal dynamics of microplastic colonization by microbial communities in the field and the influence of microbial biofilms on ingestion rates and POP toxicity. The influence of MP physicochemical properties (size, shape, surface area and composition) on these processes are evaluated. PLASTOX combines field-based observations, laboratory tests and manipulative field experiments to study the ecological effects of MPs. The use of common references material, including a marine litter-derived MP, allows a meaningful comparison of data generated by different partners and across the different activities of PLASTOX.

During the last period, multi-component marine litter-derived MPs have been prepared and characterized. Plastic litter was collected along the northern Dutch North Sea coast, sorted by polymer type, milled and homogenized to produce a mixture of marine litter-derived MPs representative of those found in the environment. Nearly 60% of the plastic items found on beaches were ropes and nets, with plastic sheets (bags and industrial) accounting for 12 % and foams for 5%. The most common polymer types found were polyethylene PE (61%), polypropylene PP (28%) and polyamide PA (3%). Two additional single-component MP reference materials have been prepared from beached fish-boxes and characterized. Standard operating procedures (SOPs) for a long-term field deployment test have been developed, allowing all participants to purchase materials, build the deployment apparatus, and implement the field test in a comparable manner. Further SOPs for the “adsorption of POPs on MPs”, the “adsorption of metals on MPs”, the “adsorption of POPs on ML-derived MPs” and the “desorption of plastic additives from MPs” have also been developed and benchmarked. In the field tests, partners across Europe have selected 13 stations (each comprising 1-2 polluted and a clean reference sites) and samples have been deployed for up to 24 months. The selected stations represent the Mediterranean Sea, the North Sea, the European Atlantic coast and a wastewater treatment plant. Deployment has been completed at some sites whilst others will be completed during early 2017. At the laboratory scale, a solid phase extraction method for the extraction of a broad range of POPs and additives from seawater has been developed. Furthermore, a series of adsorption tests have been conducted to investigate the influence of the MP size, production method (LDPE from two different producers), batch-to-batch variation (LDPE from same producer), and additive content (e.g. lubricant, colorants) on the adsorption behavior of selected PAHs. Metal adsorption tests under laboratory conditions have also been performed using PVC pellets.

A KOH-based digestion method and an enzymatic digestion method have been developed and evaluated in the PLASTOX project for the extraction of MPs present in a broad range of marine organisms. Although the enzymatic approach is a gentle process that results in minimal damage to MPs, it involves multiple steps, a longer processing time and can leave traces of proteins on the MPs surface (complicating their identification). The KOH method ultimately selected for use throughout the PLASTOX project is slightly more aggressive, but quicker and generates cleaner MPs for characterisation. Digestion was then used to study the occurrence and types of MPs present in a range of marine organisms, including crabs, fish inhabiting different levels of the water column and salps. In a sample of 90 crabs (*Carcinus aestuarii*) collected from three lagoons in the Italian Adriatic, only 6 MPs were found, all of which were fibres (2 polyester, 3 nylon and 1 unknown polymer). Similarly, 95 fish representing species living close to the seafloor in Mondego Estuary (Portugal) contained 157 MPs, of which 141 were fibres. Analysis indicated that the MPs were dominated by polyester (32%), rayon (17%), nylon (14%) and polypropylene (12%). The data generated so far suggest that the MP content in wild-caught organisms may be lower than first expected. Furthermore, results indicate that fibres comprise a very high proportion of ingested MPs in marine organisms.

Laboratory-based MP uptake, accumulation and toxicity studies have been conducted using polystyrene (PS) and polyethylene (PE) MPs representing a broad range of sizes from 45 µm down to the nano-scale (50 nm). These studies have been conducted with a range of planktonic organisms (algae and zooplankton) and mussels. Preliminary MP uptake experiments performed with the planktonic species *Rhodomonas baltica* (micro algae), *Oxyrrhis marina* (a dinoflagellate) and *Calanus finmarchicus* (a copepod). Results show that *R. baltica* can attach to PS and PE MPs (~10

µm), whilst the same PS MPs are readily ingested by *O. marina* and *C. finmarchicus*. Additionally, an unspecified fluorescent MP (~10 µm) has been investigated for its use in tracking MP uptake and accumulation by planktonic species. In the case of mussels, studies have used adults, early life stages and cells. In studies with adult mussels (*Mytilus galloprovincialis*), ingested PS MPs are found in the digestive tract (stomach and digestive gland), smaller PS MPs (4.5 µm) are found in the digestive gland diverticula, surrounding gills and in selected connective tissues (in the digestive gland and gonads). After a 3-day depuration period, some PS MPs were still present in the tissues. Furthermore, both 3 µm and 45 µm PS MPs are found in the haemolymph (fluid equivalent to blood in invertebrates) indicating transfer from the digestive system. In many cases, a dose-dependent accumulation of PS MPs was observed. Mussel larvae also exhibited significant uptake and retention of PS MPs for up to 192h (8d) after a 24 h exposure period. This indicates mussel larvae are a potential vehicle for MPs along the trophic chain. Adult mussels exposed for 3 days to PS MPs (4.5 µm) coated in benzo(a)pyrene (BAP) showed higher BAP accumulation than control organisms. In contrast, Cadmium (Cd) accumulation was not detected in mussels exposed to PS MPs coated with Cd (1 µM). These short-term studies provided evidence that PS MPs are differentially taken up and internalized by mussels depending on their size, and that MPs act as carriers for organic persistent pollutants, such as benzo(a)pyrene. Importantly, ecotoxicological effects on adult mussels appear to be limited for pristine PS MPs in the sizes and concentrations evaluated in the current studies, but evidence of inflammatory responses and a decrease in cell membrane stability were observed. Furthermore, no effects on mussel embryo-larval development were observed following PS MP exposures under the conditions tested. However, PS MPs did produce alterations on molecular and cellular parameters which are involved in the mussel embryo larval development (at 500 MP mL^{-1}). Furthermore, exposure of cells to PS NPs and MPs produced an increase in reactive oxygen species but no decrease in cell viability. The uptake of BAP adsorbed to PS MPs and NPs indicates their potential to act as carriers of organic pollutants and to cause deleterious effects on mussel immune cells.

In studies investigating the biotransformation of MP-associated polychlorinated biphenyls (PCBs) by marine microbial communities, a procedure for MP sterilization and contamination with the Aroclor 1254 PCBs reference mixture has been selected and adapted, and contaminated MPs prepared. A protocol for the extraction of Aroclor 1254 PCBs from contaminated MPs has also been tested. A site in Piallassa Baiona (Ravenna, Italy) has been selected for the *in situ* incubation of MPs.