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Ecotoxicity of discharged plastic materials on marine organisms

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The PhD project, under an agreement with the Department of Biology of the University of Naples Federico II, was carried out at the Department of Ecosustainable Marine Biotechnologies of the Stazione Zoologica Anton Dohrn in Naples (SZN) and the Ischia Marine Center, under the supervision of Dr. Maria Costantini and Dr. Valerio Zupo. The research was supported by SZN technicians: Davide Caramiello, Antonia Chiarore, Alberto Macina, and Alice Mirasole.

Collaborations:

For preparation of test solutions in nominal concentrations and support in exposure of sea urchin (Dr. Loredana Manfra)

For the preparation of microplastics from five biodegradable polymers (polybutylene succinate, polybutylene succinate-cobutylene adipate, polycaprolactone, poly-3-hydroxybutyrates, polylactic acid), and polystyrene disposable plates and virgin (Dr. Mariacristina Cocca)

For the preparation of microplastics from commercial cups made from polypropylene and from the biodegradable polymer polylactic acid (Dr. Ermelinda Prato, Dr. Francesca Biandolino)

For the preparation of polylactic acid nanoplastics labeled with the fluorescent dye rhodamine and histological samples from sponge tissue for fluorescence microscope (Prof. Antonietta Santoro, Prof. Giovanna Della Porta, Dr. Erwin Pavel Lamparelli)

For sampling of marine sponges and their identification (Prof. Marco Bertolino)

For three-month period abroad under the supervision of Prof. Patrick Fink (with Dr. Julian Wagenhofer, Dr. Frank Nitsche, Dr. Rosita Bieg)

Abstract

Since its introduction to the global market, plastic production has grown rapidly due to its affordability, durability, light weight, and insulating properties. Nowadays, plastic is widely used with applications in a variety of sectors, from packaging to foodservice, from biomedical compounds to the toy industry. The challenging concern is that the lack of adequate end-of-life waste management, which leads to the dispersion of plastic into the environment, with toxic impacts on organisms and also with negative consequences for human health. Within the various ecosystems, the aquatic environment is considered one of the most impacted and polluted, taking into account that the ocean surface represents approximately 70% of the Earth. Among the main issues related to plastic pollution in aquatic environment, those of greatest concern are: *i*) fragmentation and dispersion of micro- and nano plastics (MPs and NPs); *ii*) polymers are not pure substances and contain additionally dangerous chemical additives; *iii*) over-use of biodegradable polymers (BPs) proposed as a solution to the environmental impact. Marine invertebrates, which occupy a key position in food chain as the primary food source for higher trophic levels, are considered particularly at risk from human pressures, such as plastic pollution. In this scenario, the main objective of the present PhD project was to evaluate the potential toxic effects of these emerging contaminants on different aquatic invertebrates, characterized by different feeding strategies: *i*. the sea urchin *Paracentrotus lividus* (opportunistic omnivore); *ii*. the isopod *Idotea balthica basteri* (herbivore-detritivore); *iii*. the decapod *Hippolyte inermis* (opportunistic herbivore); *iv*. the amphipod of sea and brackish water *Gammarus aequicauda*; *v*. the freshwater amphipod *Gammarus pulex* (omnivores-detritivores-decomposers). All have proven to be excellent model organisms for use in eco-toxicity testing.

In this PhD project various conventional and non-conventional plastics were used, and in particular as virgin material and commercially-product derived:

- Virgin biodegradable polymers (BPs):

poly(butylene succinate) (PBS) (193.10 ± 148.40 μm)

poly(butylene succinate-co-butylene adipate) (PBSA) (207.70 ± 131.40 μm)

poly(ε-caprolactone) (PCL) (164.90 ± 99.20 μm)

poly(3-hydroxybutyrate) (PHB) (0.64 ± 0.30 μm)

polylactic acid (PLA) (335.00 ± 182.01 μm).

- Conventionally synthesized virgin polymer:

polystyrene (PS) (199 ± 139 μm)

PS (10.0 μm).

- Commercial product-derived, biodegradables or not:

commercial cups based on PLA (38 ± 212 μm)

commercial cups based polypropylene (PP) (38 ± 212 μm)

commercial disposable plates based on PS (318 ± 187 μm).

Specifically, the analyses conducted provided a broad overview of the toxic effects of monomeric polymers, such as morphological alterations, mortality rates, and changes in the expression levels of genes involved in various functional pathways (stress, detoxification, development, differentiation, and skeletal formation). All the polymers studied demonstrated a detrimental effect on model organisms. More in detail, among BPs, PBSA, PCL, and PLA were found to be the strongest in a larger number of model organisms. Among MPs obtained from commercial products, the three polymers (PLA, PP and PS) had comparable and rather toxic effects.

These findings produced results that can expand the understanding of the effects of MPs on aquatic organisms, and consequently, in a One Health perspective.

On the other hand, based on the "*from science to action*" concept, the second aim of the project was to propose a solution to the problem of plastic contamination in the marine environment. According to the scientific literature and exploiting their enormous filtering capability, we propose using marine sponges as bioremediators of contaminants in aquatic ecosystems. To achieve this goal, we first farmed several species of marine sponges starting in April 2023. We then tested different types of diets to determine which would best support the sponge survival

within the mesocosms. The feeding regimes that exhibited the greatest efficacy in relation to our experimental goals were based on selected algal species (*Rhinomonas* sp. and *Dunaliella* sp.), as well as the mussel *Mytilus galloprovincialis* smoothie. Our results have allowed us to keep several sponge species alive in a farm for the first time for a period of three years.

Some of these marine sponge species will be used to evaluate the possible filtration and incorporation of 250 nm PLA NPs, and therefore, their possible use in plastic bioremediation.