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"Micro and nanoplastic: a growing threat to marine organisms, the case of the sea urchin *Paracentrotus lividus* "

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Abstract

It is common knowledge that the marine environment is facing a great challenge nowadays against the smallest fraction of marine litter known as microplastics (MP; < 5 mm) and nanoplastics (< 1µm). The Mediterranean Sea, due to its geographic conformation, is recognized as one of the main areas with a high accumulation of both MPs and nanoplastics. The most affected compartment seems to be the sediments, especially those bordering marine coastal areas, which act as a long-term sink of legacy and emerging contaminants of anthropogenic origin. How MPs and nanoplastics interact with marine species and in particular with benthic organisms is a research topic not yet completely understood. This knowledge-gap results in the absence of a fitting ecological risk assessment with consequent severe damages to the marine ecosystems. Among the benthic organisms of environmental concern, the sea urchin *Paracentrotus lividus* could be a target of MPs and nanoplastics with consequences on its ecological role and function in Mediterranean coastal areas. It is widely recognized as the key species of the Mediterranean benthic communities being grazer who defines structural complexity as well as the dynamic supply of energy, mass, and nutrients from detritus to higher trophic levels.

In order to fill current knowledge gaps on the impact and interaction of MPs and nanoplastics on benthic species populating Mediterranean coastal areas, the aim of the present study was to explore mechanisms of uptake, biodisposition and clearance, and toxicological responses of selected MPs and nanoplastics in different organs and cells of the sea urchin *P. lividus.* In addition, a special focus was reserved on the nanoscale dimension of plastics (<100 nm), by looking at the mechanisms of the bio-nano interactions between nanoplastics and the sea urchin immune cells. The research described here has been divided in 4 chapters in which findings on polystyrene (PS) MPs and nanoparticles (PS NPs) in *P. lividus* have been obtained by using 3 approaches: 1) an *in vivo* study has been designed in order to identify mechanisms of uptake, toxicity and immunological response of either virgin or colonized MPs of different sizes; 2) an *in vitro* study has been performed to examine the cellular trafficking of PS NPs having negative surface charges in immune cells coupled to the evaluation of immune function and ultrastructural properties; 3) a *field* study was performed to assess the occurrence of MPs in wild specimens collected from various locations in the Gulf of Naples (Italy). Concerning the *in vivo* study, adult specimen's sea urchins were exposed to two different sizes of PS MPs, 10 and 45 µm (10 MPs mL^{-1}), for 72h. A size-dependent MPs uptake in sea urchins was observed; while the smallest MPs resulted located in the aquifer system, the largest ones were mainly found in the digestive system. As far as the observed effects upon exposure, PS MPs regardless of their different size both affected the immune response in terms of quantity/quality of cells, antioxidant capacity, reactive oxygen and nitrogen species. Another important finding concerns the evidence that the

naturally occurring colonized MPs are higher internalized by sea urchins compared to virgin ones after 48h of exposure. Colonized microplastics were obtained by 1-week incubation in unfiltered natural sea water (NSW) collected from a coastal site in the Gulf of Naples. The biofilm formation on MPs surface targets immune cells in terms of counts and morphology as well as redox status. Therefore, it can be concluded that colonized PS MPs exhibit greater effects on sea urchin immune cells compared to virgin ones as those most commonly used in ecotoxicological studies. These results encourage the use of colonized rather than virgin MPs for a more environmentally relevant risk assessment, since once released in marine waters MPs are soon colonized both chemically and biologically.

Regarding the bio-nano interactions, our *in vitro* study with sea urchin's immune cells confirmed the role of surface charges (negative *vs* positive) as drivers of ecotoxicity. Precisely, immune cells were exposed to negatively charged PS NPs (PS-COOH; 50nm) (0, 5, 25 µg mL^{-1}) and to PS NPs (PS-NH_2; 50nm) (25 µg mL^{-1}) for 4h. In detail, our study showed a fast uptake by phagocytes of negatively charged PS NPs and sequestration into lysosomal compartments associated with low acute toxicity in comparison with their positively charged counterparts as amino-modified PS NPs (PS-NH_2).

In the *field* study, the occurrence of MPs, mostly microfibers (MFs), in adult sea urchin specimens sampled in four sites in the Gulf of Naples was demonstrated. MFs were found both in gonads, coelomic fluid and digestive system of wild sea urchins, although in the latter in higher amounts. Polyester-based fibers were the most abundant together with natural fibers such as cotton. Potential sources could be represented by fishing lines and textiles whole fibers could be released by the sewage treatment plants.

Overall these findings provided for the first time the evidence of the multisided interactions between MPs/nanoplastics and the sea urchin *P. lividus* with important ecological implications. This thesis provided key answers to the scientific existing gaps by establishing how MPs uptake and biodistribution occur and how these are transformed according to their surface biological characteristics. Furthermore, the key factors for estimating how the sea urchin immune response counteract exposure to MPs as well as toxicokinetic / toxicodynamic elements underlying the bio-nano-interaction have been revealed. The use of the adult sea urchin *P. lividus* as a suitable model has been proved to be instrumental in shedding light on the potential effects of MPs and nanoplastics on benthic marine grazer species thus stimulating future research in deepening the understanding of the potential impacts on marine communities and ecosystems.

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