Pollutants bioavailability and toxicological risk from microplastics to marine mussels

Carlo Giacomo Avio [a], Stefania Gorbi [a], Massimo Milan [b], Maura Benedetti [a], Daniele Fattorini [a], Giuseppe d'Errico [a], Marianna Pauletto [b], Luca Bargelloni [b], Francesco Regoli [a, *]

[a] Dipartimento di Scienze della Vita e dell'Ambiente (DiSVA), Università Politecnica delle Marche, Ancona, Italy
[b] Dipartimento di Biomedicina Comparata e Alimentazione (BCA), Università di Padova, Italy

ARTICLE INFO

ABSTRACT

Microplastics represent a growing environmental concern for the oceans due to their potential of adsorbing chemical pollutants, thus representing a still unexplored source of exposure for aquatic organisms. In this study polyethylene (PE) and polystyrene (PS) microplastics were shown to adsorb pyrene with a time and dose-dependent relationship. Results also indicated a marked capability of contaminated microplastics to transfer this model PAH to exposed mussels Mytilus galloprovincialis; tissue localization of microplastics occurred in haemolymph, gills and especially digestive tissues where a marked accumulation of pyrene was also observed. Cellular effects included alterations of immunological responses, lysosomal compartment, peroxisomal proliferation, antioxidant system, neurotoxic effects, onset of genotoxicity; changes in gene expression profile was also demonstrated through a new DNA microarray platform. The study provided the evidence that microplastics adsorb PAHs, emphasizing an elevated bioavailability of these chemicals after the ingestion, and the toxicological implications due to responsiveness of several molecular and cellular pathways to microplastics.

1. Introduction

The global production of plastic dramatically increased in the last decades, from 0.5 million tons/yr in 1960 to 280 million tons in 2012 (Plastic Europe, 2012). Almost 10% of the annual production ends up into the oceans, and plastic debris accumulation has been reported as a global scale phenomenon for the marine environments, including polar areas and abyssal regions (Barnes et al., 2009).

Adverse effects of plastics have been documented in terms of entanglement and physical damages to locomotory, respiratory or digestive appendages in marine mammals, turtles, seabirds and crustaceans (Andrady, 2011). In addition, since plastics degrade very slowly, they also act as floating substrates for several organisms, and thus contribute to long-range transport of alien species, representing an additional risk to local biodiversity (Andrady, 2011).

In the recent years, a great scientific interest is being directed toward microplastics, i.e. fragments with a grain size lower than 5 mm, which are manufactured ex novo for their use in cosmetics, industrial or medical applications, or derive from macroscopic debris after chemical, physical and biological fragmentation (Barnes et al., 2009).

Ingestion of microplastics has been demonstrated in various marine organisms with different feeding strategies; this phenomenon may negatively influence both the feeding activity and nutritional value of a plankton-based diet, particularly in those species which can not discriminate the food source (Moore et al., 2001; Browne et al., 2008).

Recent evidences also suggest the potential role of microplastics as vectors of chemical pollutants, either used as additives during the polymer synthesis, or adsorbed directly from seawater (Rios et al., 2007; Teuten et al., 2009; Engler, 2012). The hydrophobicity of organic xenobiotics and the large surfaces of floating polymers facilitate the adsorption of these chemicals on microplastics at concentrations orders of magnitude higher than those detected in seawater (Ogata et al., 2009). The possibility for plastic particles to adsorb chemical pollutants from the surrounding environment has

* Corresponding author.
  E-mail address: f.regoli@univpm.it (F. Regoli).

been also characterized in laboratory conditions. Different particles polymers, like polyvinyl chloride, polyethylene, polypropylene, polystyrene, were shown to have a high sorption capacity for DDTs, polycyclic aromatic hydrocarbons (PAHs), hexachlorocyclohexanes and chlorinated benzenes (Bakir et al., 2012; Lee et al., 2014). Consistent with these studies, several persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs), organo-halogenated pesticides, nonylphenol, PAHs and dioxins have been detected in plastic pellets stranded on different beaches of the world (Endo et al., 2005; Ogata et al., 2009; Hirai et al. 2011; Heshett et al., 2012).

Despite the importance of microplastics in adsorption and transport of hydrophobic pollutants, it is still unclear whether they also represent a potential source of chemical exposure within marine food webs. Various evidences, including the use of a thermodynamic approach and of models simulating physiological conditions in the gut, suggested that both adsorbed pollutants and chemical additives of plastics might be released to organisms (Gouin et al., 2011; Tanaka et al., 2013; Bakir et al., 2014a).

In laboratory conditions, microplastics have been shown to be ingested by amphipods, barnacles, and lugworms (Thompson et al., 2004); in mussels, *Mytilus edulis*, plastic particles (3–9.6 μm) were accumulated in digestive tissues and translocated to haemolymph (Browne et al., 2008). In the same organisms, the uptake of microplastics caused notable histological changes in digestive cells with strong inflammatory responses, formation of granulocytomas and lysosomal destabilization which increased with exposure time (Von Moos et al., 2012).

To further assess the possible risk of microplastics as environmental contaminants, the present investigation aimed at a multi-disciplinary approach to characterize the chemical adsorption of hydrophobic pollutants, as well as bioaccumulation, chemical release and onset of potential health effects in the filter feeding mussels *Mytilus galloprovincialis*. Two different polymers, poly-ethylene (PE) and polystyrene (PS) were exposed to various doses of pyrene, selected as one of the more commonly represented PAHs adsorbed on plastic marine debris (Rios et al., 2007); virgin and contaminated PE and PS were then used in a trophic transfer experiment with mussels. Tissue localization of microplastics was integrated with measurement of pyrene bioaccumulation and a wide battery of cellular biomarkers to detect the early onset of adverse effects. Such analyzed responses included immunological parameters, lysosomal membrane stability, peroxisomal proliferation, antioxidant defences and oxidative stress biomarkers, neurotoxic effects and onset of genotoxicity; for the first time, effects of microplastics were also investigated at the transcriptomic level through a new *M. galloprovincialis* DNA microarray platform, to better elucidate pathways and molecular mechanisms of action (MOA).

Obtained results have been elaborated with a classical Weight Of Evidence (WOE) approach that combine and differently weight various typologies of data, or lines of evidence (LOEs), providing multidisciplinary characterization of hazard indices and risk evaluation (Chapman et al., 2002; Chapman, 2007). WOE methods are considered as key components of Ecological Risk Assessment (ERA) procedures, according to recent European Directives which require member states to evaluate and classify the ecological status of water bodies integrating different quality elements. Among the available WOE procedures, the Sediqualsoft model elaborates data from sediment chemistry, bioavailability of pollutants and onset of adverse effects at different levels of biological organization (Piva et al., 2011; Benedetti et al., 2012); the computational rules have been successfully validated in filed conditions for the characterization and classification of risk from industrial and harbour sediments, natural hydrocarbon seepage in coastal areas or the recent Costa Concordia wreck at Giglio Island (Piva et al., 2011; Benedetti

et al., 2012, 2014; Regoli et al., 2014). In this study we have applied the flow-charts and mathematical algorithms developed for elaborating data and summarizing the hazard index for bioavailability and biomarker responses, thus providing a synthetic judgment on the biological relevance of these observed effects.

The overall results of this study were expected to increase our knowledge on the potential toxicological risk of microplastics in the marine environment.

2. Materials and methods

2.1. Experimental design

Polyethylene (PE) and polystyrene (PS) powders were obtained from a private plastic company. Particles were size-sorted in a 1000–100 μm group used for characterization of the pyrene adsorbing capacity, and in a <100 μm group for the exposure of mussels to virgin and contaminated polymers.

The adsorption of pyrene to PE and PS was assessed by mixing solutions of microplastics (20 g/L in seawater) with pyrene dosed at final concentrations of 0.5 μg/L (low, L), 5 μg/L (medium, M) and 50 μg/L (high, H). While the L and M treatments are environmentally realistic for pyrene, the H dose is uncommon but still possible, i.e. after heavy oil spill or in highly contaminated sewage (Neff, 2002). The mixing solutions were maintained in continuously rotating 50 mL glass tubes for 6 days; water was changed and pyrene re-dosed after 3 days. Levels of pyrene adsorbed on polymers were measured after three and six days of treatment.

For the exposure of mussels to microplastics, specimens of *M. galloprovincialis* (5 ± 1 cm shell length) were obtained from a local farm (Numana, Ancona, Central Adriatic Sea) and acclimatized for 10 days to laboratory conditions with aerated seawater, at 18 ± 1 °C and 35‰ salinity. Contaminated plastics were prepared according to the above description, by maintaining a solution of <100 μm microplastics with pyrene (50 μg/L) in rotating conditions for 6 days. A total of 150 organisms were distributed into fifteen 6 L glass-beakers and exposed to virgin or pyrene-contaminated plastics for 7 days with three replicates for each of the 5 following treatments: Control (CNTR), Polyethylene (PE), Polystyrene (PS), Pyrene-treated Polyethylene (PE-PYR), Pyrene-treated Polystyrene (PS-PYR). Water was changed daily and both virgin and pyrene-treated particles re-dosed at a nominal concentration of 1.5 g/L.

No mortality of mussels was observed during the experiments. After the exposure period, haemolymph, digestive glands and gills were rapidly removed from 30 specimens for each treatment, pooled in 10 samples (each with tissues of 3 specimens), frozen in liquid nitrogen and maintained at −80 °C for chemical, biochemical and histochemical analyses; for haemolymph samples, an aliquot was also immediately processed for lysosomal neutral red retention time assay (NRRT), phagocytosis activity, and DNA damage, and another aliquot fixed in Carnoy's solution (3:1 methanol, acetic acid) for the microscopic evaluation of granulocytes and chromosomal alteration. Four additional pools, each with digestive glands of three specimens, were prepared from CNTR, PS and PS-PYR groups for DNA microarray analysis.

2.2. Chemical analyses of pyrene on plastics and exposed mussels

Pyrene adsorbed on microplastics (PE and PS) or accumulated in mussels tissue (gills and digestive glands) was determined after extraction of samples in 0.5 M potassium hydroxide and methanol (1:10 w:v) with microwave at 55 °C for 15 min (Benedetti et al., 2014). After centrifugation for 5 min at 1000 × g, the methanolic solutions were concentrated in speedvac and purified with solid