Morphological analysis approach to detect microfiber contamination in Mytilus galloprovincialis

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Abstract. Microplastic pollution is a widespread threat for marine fauna. Mussels are good candidates for assessment of microplastic exposure in the environment because of their wide geographical and spatial distribution and their filtration system. In this work the 65% of analysed mussels assessed the presence of microfibres in their tissue, with an amount ranged from 0.0 to 4.3 MF/ g ww. This result confirm that mussels could be used as biomonitors of surrounding environment pollution.

Keywords: microplastic, mussel, microfiber, *Mytilus galloprovincialis* , morphological analysis

1. Introduction

Microplastic released from synthetic fabrics during washings have been esteemed to be the 35% of primary microplastics in oceans (Boucher & Friot, 2017). Recently, it has been reported that microplastics can be released form fabrics to air also as consequence of wearing (De Falco et al., 2020) thus indicating that the release of microplastics from textile strongly affect the environment. In light of the fact that microfibres were present in abundance in marine environments, evaluation of their accumulation in digestive tracts of fish and deep-sea organisms were conducted in several studies (Halstead et al., 2018; Palazzo et al., 2021; Remy et al., 2015; Taylor et al., 2016). Microplastic pollution was widespread in mussels, that could be used as indicator of level of microplastics and surrounding environments pollution because of their capacity of filtering large amount of water(Doucet et al., 2021; J. Li et al., 2018; Mankin & Huvard, 2020; Ward et al., 2019). This paper aims to determine the ability of microplastic of fibrous shape to accumulate in mussels as well as to correlate this type of pollution with mussel's living environment.

2. Methodology

Mytilus galloprovincialis from the Tyrrhenian Sea were collected in order to obtain n. 20 of samples, with a weight that ranged from 4.29 to 14.01 g ww (wet weight) and in length from 5.5 to 8.7 cm. Samples analysis was performed based on an extraction method according to (Foekema et al., 2013) and (Avio et al., 2015). The obtained samples underwent a digestion process with a 10% KOH solution and stored overnight at 45°C in an oven until the complete dissolution of the organic materials. After the digestion, each sample was added to 250 ml NaCl prefiltered hypersaline solution (1.2 g/cm^3), stirred, and decanted for 10 min. The overlying water was directly filtered over a pore size of 8µm, 47 mm diameter cellulose nitrate membrane filter. The membranes with retained materials were transferred in a Petri dish with a 15% H_2O_2 solution for the digestion of residual organic matter and allowed to dry in oven overnight at 45°C, before the microscopical observation. N.20 of filters were analysed with a LEICA M205C light microscope, with a magnification of 0.78 – 16x, with the purpose to discriminate the presence of natural and synthetic fibres, based on morphological characterization (Allievo, 1908; Quaglierini, 1989). In order to prevent contamination cotton lab coats and nitrile gloves were worn during all the observations and all of the liquids used were filtered with 0.45µm cellulose acetate filter before use. Blank extraction group without tissue was performed simultaneously to correct the potential procedural contamination. Approximately 30 minutes were spent examining each filter.

3. Results and discussion

The morphological observations allow the identification of microfibers on filter surface (Figure 1). The typical morphological features of the fibres were used to discriminate between synthetic and natural or artificial

ones. Fibres that showed a not uniform diameter, twisted upon themselves like ribbons, as the fibre appears in Figure 1a, were classified like natural fibres. Meanwhile, fibres with a smooth and shiny surface, with a cylindrical shape, were identified like synthetic fibres, like the red one in Figure 1b. The analysis of the optical micrographs acquired on the whole filter surface allow to determine that microfibres are present in the 65% of analysed mussels (Figure 2), while the presence of no-fibrous microplastics were considered negligible. 484 microfibres were found in all the analysed mussels of which about 50% were classified as synthetic fibres and 50% as natural/artificial fibres. The amount of microfibres per gram of mussels was ranged from 0.0 to 4.3 MF/ g ww, with a mean value of about 1.3 MF/g ww.

There is an increasing scientific evidence that marine species uptake and ingest microfibres thus indicating that this type of pollution represents the main fraction of marine pollutants (Doucet et al., 2021; Kolandhasamy et al., 2018; L. Li et al., 2019; Mankin & Huvard, 2020). Considering that many species of mussels are substantial commercial value as seafood items(Food and Agriculture Organization of the United Nations, 2017), microfibers present in mussels can represent a threat also for exposure in humans via ingestion (Rochman et al., 2015). It was probable that a large amount of fibres present in marine environments was released during the laundering process (De Falco et al., 2019; Napper & Thompson, 2016). Release of microfibres during the washing process is due to mechanical and chemical stress that the clothes undergo in washing machines, and due to their dimensions the majority of microfibres released cannot be blocked from WWTPs (De Falco et al., 2019), reaching seas and oceans, and consequently, they could be ingested from marine fauna.

Figure 1. Optical micrograph of a **a)** natural fibre and a **b)** synthetic fibre recovered from filter surface.

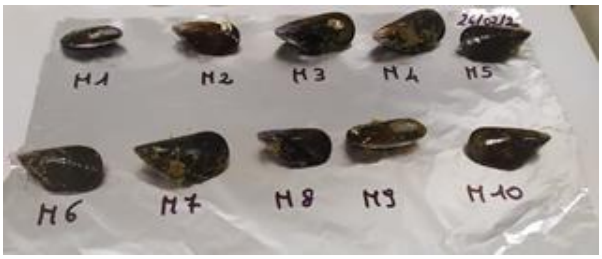

Figure 2. Samples before the extraction process.

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