UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

A rapid method for detection and quantification of microplastics in bivalves molluscs: preliminary results

Cristian Bernardi*, Tirloni Erica*, Stella Simone*, Matteo Ottoboni*, Pinotti Luciano*, Giusti Alice°, Armani Andrea°

* Dipartimento di Scienze Veterinarie per la Salute, la produzione Animale e la Sicurezza Alimentare, Università degli Studi di Milano.

°FishLab, Dipartimento di Scienze Veterinarie, Università di Pisa.

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Introduction

Microplastics (MPs) are considered a high concern topic because widespread in all environmental compartments. They are also found in many marine seafood species used for human consumption. MPs, and in particular fibers, have been reported in the digestive tract of filter feeding organisms such as bivalves molluscs (BM) that are consumed whole. However, analytical procedures for MPs detection and quantification in BM are still standardized and a wide range of protocols is currently available. In particular, sample processing considerably differs among studies. Many researchers applied a two-step sample pretreatment (Dehaut et al; 2016), consisting in an acid or alkaline digestion of BM and a density separation step followed, after decantation, by the filtering of the top layer of the solution, through a filter with a setting porosity. However, this two-step approach is time consuming and greatly increase the risk of airborne fibers contamination throughout the analysis. Therefore, in this preliminary work, based on an ongoing project, a one-step method consisting in alkaline digestion and direct filtration without any flotation step was developed and then used for detecting MPs in three species of BM (*Mytilus galloporovincialis, Chamelea gallina, Ruditapes philippinarum*) of great commercial interest on the Italian market. In addition, with the aim to validate the method, lab-made internal standard fibers were produced and used to contaminate some samples.

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Method

After preliminary trials aimed to define the best condition for the digestion of BM tissues, the following one-step protocol was used for the detection and quantification of MPs in *M. galloporovincialis, C. gallina* and *R. philippinarum*. After collection, MB shells were washed with ultrapure water to prevent contamination.

Ten grams of internal tissues and water were digested in 100 ml of 35% potassium hydroxide solution at 40°C for 48h.

The digested samples were then passed through a glass fibers filter (pore size: 1.6 µm) that was subsequently observed at stereomicroscope.

Two blanks (atmospheric control and reagents control) were tested for the presence of microplastic particles/fibers.

Internal control standards were also produced starting from monofilament polyamine suture for surgery. These were included in paraffine and then, the paraffine block was cut with microtome in 4 µm slices. The number of fibers in each slice was checked at stereomicroscope before addition to samples. Internal standards were then used to contaminate BM samples that were analyzed as previously described. The recovery was calculated as the percentage of the number of spiked microplastic fibers recovered after treatment. All preparation and measurement steps were conducted in a lab, where the surfaces were cleaned carefully with 70% ethanol solution. Cotton lab coats and nitrile gloves were always used. All plastic materials were replaced by metal or glassware, when possible. All materials were carefully cleaned and rinsed three times with ultrapure water (18.2 MΩ*cm, Elix5, Millipore) prior to use.

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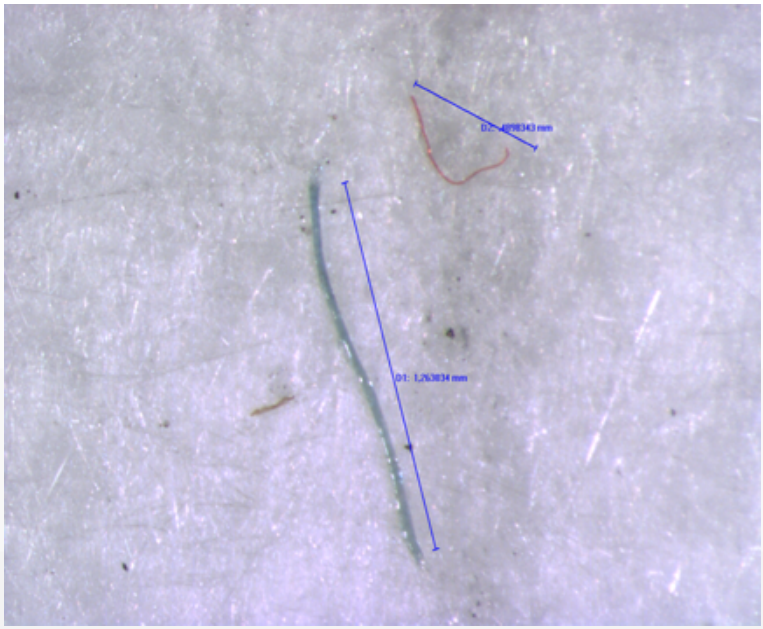
Results

The one-step protocol developed in this study allowed to completely digest the bivalve tissues in all the species analyzed. The maximum concentration of MPs g^{-1} was found in the *R. philippinarum* with values of 2.12 MPs g^{-1}. The lowest values were instead recorded in the *M. galloprovincialis* with a value of 0.8 MPs g^{-1}, and in *C. gallina* with 0.30 MPs g^{-1}.

Overall, a high proportion of fibers was observed (95% of total microplastics), while fragments were the second most abundant morphotype (5%). In blank controls fibers were 84% of total microplastics while fragments were 16%. It is also recommended that operators should register colors of clothes worn underneath the lab coats. Regarding the color of MPs in samples, blue and green fibers were the most abundant, followed by black and red. The most frequent length range of MPs was 200-800 µm (66% of observed MPs). In the control blanks, the number of MPs observed in each blank ranged between 1 and 14 MPs $filter^{-1}$, with a mean of 5.33±4.59 MPs $filter^{-1}$. The fibers recovery was 100% and no alteration in shape and color was observed.



In figure, two fibers with measure (green and red) filtered by a sample of *C. gallina;* the green one is the internal standard.

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Discussion

Based on the data produced in the present study, the digestion performed using 35% KOH at 40°C for 48h, can be considered a suitable technique for MB tissues processing. The 100% recovery of internal standard fibers confirm no effects on microplastic fibers confirming previous findings. In fact, also the recent work of Thiele et al., (2019) recommend the utilization of KOH as the most viable extraction method. In fact, it is not only representing the most economical and least time-consuming method but it also does not affect microplastics recovery. Therefore, the developed method, after further validation on a higher number of samples, could be considered as a possible standard method for BM tissue digestions in order to extract microplastics. Also in this study major issues arises from the airborne fibres contamination, as already reported by Woodall et al. (2015) and Nuelle et al. (2016). The airborne fibres contamination was in agreement with Reguera et al. (2019), who observed 4.29±4.68 MPs filter^{-1} in the procedural blanks. In fact, despite the application of procedures aimed at preventing samples contamination, background concentration of airborne fibres reached a significant level with similar numbers both in microfibres from laboratory blanks both in environmental samples. To reduce or eliminate airborne contamination, analyzes should be implemented in a plastic-free clean room ISO 7.

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