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The seaweed *Chaetomorpha linum* cultivated in an integrated multitrophic aquaculture system: A new tool for microplastic bioremediation?

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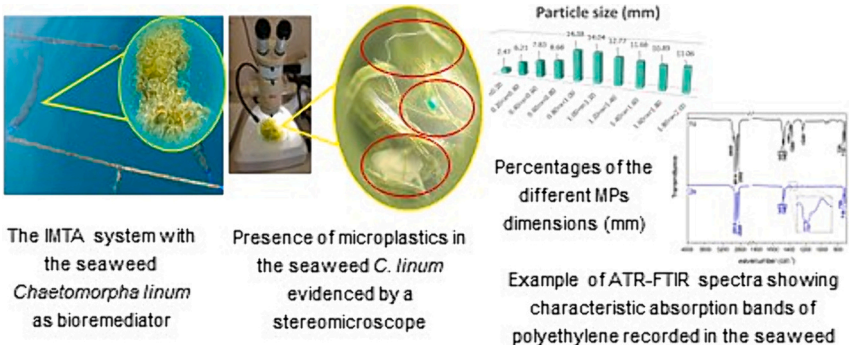
HIGHLIGHTS

- Microplastics (MPs) are emerging pollutants in the marine environment.
- The seaweed *Chaetomorpha linum* is able to trap microplastics.
- MPs were characterized in terms of size, colour, type and by ATR-FTIR spectroscopy.
- A simple procedure was used to remove MPs from *C. linum*
- The studied seaweed represents a new potential tool for MPs bioremediation.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MPs) are emerging pollutants with detrimental impacts on ecosystems and human health. Due to their adverse effects, new strategies to mitigate MP pollution in the marine environment need to be developed urgently. In this context, the capability of the seaweed *Chaetomorpha linum* (Chlorophyta, Cladophorales) to trap MPs, as well as the effectiveness of a simple washing procedure to clean up the harvested seaweed biomass, were investigated. This algal species was grown in an integrated multitrophic aquaculture system (IMTA), where bioremediator organisms such as macroalgae, polychaetes, sponges and mussels were farmed in the vicinity of the fish cages. MPs trapped in *C. linum* were classified based on shape and size, and representative samples of each shape were analysed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy to evaluate their chemical composition. Fibre MPs were the most abundant (97.3 %), while the size ranged from 0.025 to 2.00 mm, with most samples being in the size range 0.80–1.00 mm. MPs were composed mainly of polypropylene, polystyrene, and polyethylene. They were efficiently removed from the cultured seaweeds by a simple density separation procedure, consisting of three extractions with hypersaline solutions of sodium chloride. These results suggest that *C. linum* cultivated in an IMTA system can be proposed as a bioremediator to capture MPs from the surrounding environment. At the same time, harvested and cleaned green seaweeds may be considered a co-product of the bioremediation process and can find application in several biotechnological fields, including the use as a food source for human consumption.

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1. Introduction

The spread of plastic in the marine environment is one of the major environmental problems due to the increased global production of this material. (Horton et al., 2017; Rochman, 2018; Wang et al., 2020; Zhang et al., 2021; Alak et al., 2022; Alhusban, 2024). We cannot imagine today's life without plastic; its use has gradually replaced materials such as metals, glass, and paper, in a wide range of fields: construction, cosmetics, medicine, electronics, transportation, textile and food packaging (Kumar et al., 2021). Annual global plastic production increased from 348 million tonnes in 2017 to almost 400.3 million tonnes in 2022 and it is estimated that it will reach 1.2 billion tonnes by 2060 (Petersen et al., 2021; Petersen and Hubbart, 2021). Recent studies have shown that most of the plastic produced worldwide every year is disposed of into the environment (Shen et al., 2020). As a result, the oceans receive annually between 4.8 and 12.7 million tonnes of plastic waste that form marine debris, accounting for 60–80 % of marine litter (Jambeck et al., 2015; Lebreton et al., 2017; Koestner et al., 2023).

The concept of microplastics (MPs) was introduced in science in 2004. MPs are defined as plastic particles with a diameter of <5 mm (Thompson et al., 2004; Yang et al., 2015a, Yang et al., 2015b). They are mainly derived from plastic products made of synthetic polymer materials such as polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), and polypropylene (PP). They are referred as primary MPs when their microscopic size is of industrial origin. Larger plastic items subjected to specific biological, physical and chemical conditions, including weathering, UV radiation, high temperature and microbial action, can fragment into micrometre-sized particles that are referred as secondary MPs (Thompson et al., 2004; Yang et al., 2015a; Yang et al., 2015b). In addition, MPs could be degraded further and reach the size of nanoplastics (NPs) (Jambeck et al., 2015; Auta et al., 2017; Lambert and Wagner, 2018).

Once released into the marine environment, MPs negatively affect aquatic organisms. As highlighted by several studies, MPs can be ingested by invertebrate and vertebrate animals leading to adverse effects (Cole et al., 2011; Zhang et al., 2021; Alak et al., 2022; Köktürk et al., 2023; Alhusban, 2024). The impact of MPs on macroalgae and aquatic plants has been attracting a lot of interest in recent years (Podbielska and Szpyrka, 2023; Kim et al., 2024). Seng et al. (2020) detected microplastics in intertidal seagrasses such as *Cymodocea rotundata, Cymodocea serrulata* and *Thalassia hemprichii,* and in subtidal macroalgae such as *Padina* sp. and *Sargassum ilicifolium*, finding significantly higher MP densities in seagrasses than in subtidal macroalgae. The MP abundance in the green algae *Ulva prolifera*, the main species involved in the most famous green tide occurring in the Yellow Sea, was found 595–3917 times higher than in seawater (Feng et al., 2020). Moreover, Feng et al. (2020) unveiled four plastic trapping mechanisms (kinking, attachment, embedding, and wrapping) on or in *U. prolifera*, which were shown to account for the strong ability to immobilize MPs. Other studies have focused on new protocols for MP extraction from seaweeds, such as the one proposed by (López-Rosales et al., 2022) who extracted MPs from a mixture of 5 commercial macroalgae (*Undaria pinnatifida spp, Porphyra spp, Ulva spp, Laminaria ochroleuca* and *Himanthalia elongate*) via an enzymatic-oxidative treatment and a single filtration step.

In the present paper new insights on the capability of the green seaweed *Chaetomorpha linum* (O.F. Müller) Kützing (Chlorophyta, Cladophorales) to trap MPs are provided. The seaweed was collected in the natural environment (Mar Piccolo of Taranto, Ionian Sea Mediterranean) and then transferred and reared around fish cages together with other bioremediators such as Porifera, Polychaeta and Mollusca in an integrated multitrophic aquaculture system (IMTA). This activity was conducted in the framework of the REMEDIA Life project (REmediation of Marine Environment and Development of Innovative Aquaculture: Exploitation of edible/not edible biomass), financed by the European LIFE-ENV Program, under the Grant Agreement number LIFE16ENV/IT/000343, and of the National Biodiversity Future Center (NBFC- Spoke 2 Activity 3 Task 3.2) project, funded by European Union Next Generation EU (PNRR). Macroalgae were included as bioremediators since some species are able to lower the nitrogen and phosphorous load resulting from fish farming. Specifically, we focused on *C. linum* due to its well-established bioremediation ability (Aquilino et al., 2020). This species has already been proposed for the remediation of heavy metals in polluted sites and for the removal of nitrogen and phosphorus in nutrient-rich seawater (Jang et al., 2014; Liu et al., 2020). It is highly tolerant to a variety of environmental stressors such as changes in salinity, temperature, light intensity, pH, dissolved oxygen and nutrient concentration (Tsutsui et al., 2015). These ecological features make *C. linum* particularly suitable for seawater bioremediation purposes. Moreover, this species can give rise to blooms in suitable environmental conditions and is commonly reported as dominant in estuary and lagoon environments, mainly in the summer season and even in turbid water conditions (Lenzi et al., 2017).

The REMEDIA Life and NBFC-projects aim to demonstrate that the developed bioremediation technologies can be successfully applied to an industrial mariculture plant in a confined environment, with a positive impact on the specific sector and enabling the biotechnological valorisation and the zero-kilometre marketing of the biomass produced. As a co-product of bioremediation, the produced macroalgae biomass is an added value in the framework of the circular economy concept, since it can be used for the extraction of active ingredients with antibacterial action, as well as for human nutrition and to produce innovative fish feed (Stabili et al., 2019a; Stabili et al., 2019b). In addition, edible algae are increasingly being marketed as "functional foods" or as a source of "nutraceuticals". Although these terms have no legal significance in many countries, they describe foods or products derived from food sources, containing bioactive compounds that can provide health benefits beyond basic nutrition (e.g. anti-inflammatory, disease prevention) (Bagchi, 2006; Hafting et al., 2012; Kumari et al., 2023). In this framework, the antibacterial activity of the lipidic extract of *C. linum* against some vibrios has been recently established, suggesting its employment to control fish and shellfish diseases due to vibriosis, thus reducing the public health hazards related to antibiotic use in aquaculture (Stabili et al., 2019a; Stabili et al., 2019b). This extract also showed antioxidant activity, (170.960 ± 16 mmol Trolox equivalent/g (oxygen radical absorbance capacity assay — ORAC) and 30.554 ± 2.30 mmol Trolox equivalent/g (Trolox equivalent antioxidant capacity assay — TEAC)). Moreover, the chemical characterization of the extract revealed a high content of ω-6 and ω-3 polyunsaturated fatty acids (PUFAs), further highlighting the potential of this algal species in the production of fortified foods (Stabili et al., 2019a, Stabili et al., 2019b). In the present work we investigated the ability of *C. linum* to trap microplastics by removing them from the surrounding environment. Trapped microplastics were classified by size and morphology. Their chemical composition was assessed by Fourier transform infrared (FTIR) spectroscopy, thus gaining information on the main sources of plastics contamination in the area under investigation. Furthermore, to evaluate the safety of these algae for human consumption, the possibility of effectively removing microplastics from *C. linum*, once collected, was also verified.

2. Materials and methods

2.1. Study area and sampling

The seaweed *C. linum* was collected in the Mar Piccolo of Taranto (Ionian Sea) (Fig. 1) which is a semi-enclosed Transitional Water System located to the North of Taranto (Southern Italy, Ionian Sea, Mediterranean Sea) belonging to the LTER network (IT22 - Mar Piccolo of Taranto) (Capotondi et al., 2021). After collection, macroalgae were transported, transplanted, and cultivated for six months in plastic nets in the IMTA system (Fig. 1) set up in the mariculture farm of "Maricoltura

Fig. 1. The IMTA system investigated in this work with the seaweed *Chaetomorpha linum* as bioremediator.

del Mar Grande". The plant covers a surface of 0.06 Km^2 and is located about 600 m away from the coast in a semi-confined area of the Mar Grande. It consists of 15 cages (Ø 22 m), placed at a depth ranging from 7 to 12 m and producing about 100 tons/year of European seabass *Dicentrarchus labrax* (Linnaeus, 1758) and sea bream *Sparus aurata* (Linnaeus, 1758). Three replicates consisting of about 500 g of *Chaetomorpha linum* were collected from the nets of the IMTA system during the period of maximum growth (July) (Fig. 1).

All collected material was then transferred to the laboratory in plastic free containers and subjected to the analyses and treatments described below.

2.2. Prevention of MPs contamination during the analysis

Seaweed samples were prepared in a previously cleaned laboratory with limited access to prevent microplastic contamination. Moreover, all the procedures of seaweed samples processing were performed under a laminar flow hood. Clean, cotton laboratory coat and nitrile gloves were worn during all laboratory stages. All work surfaces were cleaned with 70 % ethanol before use and all chemical reagents were prepared in plastic free containers.

2.3. Isolation of microplastics trapped in Chaetomorpha linum by density separation

The methodology developed by Thompson et al. (2004) for sediments was adapted, with some modifications, to macroalgae samples. Briefly, 250 mL of sediment of Thompson's protocol were replaced by 30 g of *C. linum* wet biomass, which were poured into a beaker with 500 mL of hypersaline solution of sodium chloride (NaCl - salinity 38.00 ‰). The mixture was kept under magnetic stirring at 400 RPM for 20 min, then it was left to rest for 2 h to allow floating of dispersed MPs. It was indeed assumed that MPs could float because of their density lower than the one of the hypersaline solution (1.026 g/cm^3). The supernatant was withdrawn with a glass micropipette, filtered through a glass microfibre filter (GF/F, 47 mm, 1 μm) using a vacuum pump and then dried in an oven at 40 °C for 24 h. This procedure was repeated three times for each sample and a total of nine filters were obtained. The use of NaCl in the above-described methodology proved advantageous in terms of ease of work, extraction time and cost, while also offering good abundance estimation (Claessens et al., 2011; Imhof et al., 2013; Nuelle et al., 2014). Moreover, to minimize the possibility of contamination of the samples with MPs from sodium chloride, the solution was filtered three times, given the ubiquitous nature of these pollutants in salt (Yang et al., 2015a, 2015b; Kim et al., 2018).

The filters were inspected with a stereomicroscope (Leica, MZ75), and any particles suspected of being MPs were photographed with a camera (Nikon, NIS–Elements BR 4.30.02) attached to the stereomicroscope. The particles were sorted and classified according to their shape and size following the categories suggested by Hidalgo-Ruiz et al. (2012); then the length of the particles supposed to be MPs was measured by using the open-source software ImageJ (Schneider et al., 2012).

2.4. Attenuated total reflectance fourier transform infrared (ATR-FTIR) spectroscopy

To assess the chemical nature of selected particles, the samples were analysed by attenuated total reflectance Fourier Transform infrared (ATR-FTIR) spectroscopy. A Spectrum One spectrometer (Perkin Elmer, Waltham, MA, USA), equipped with a universal ATR apparatus, was used for this purpose. Each sample was pressed onto the surface of a three-bounce diamond microprism representing the internal reflection element (IRE) and 16 interferograms were collected and averaged for each spectrum. The background spectrum was recorded with the bare IRE. A resolution of 4 cm^{-1} was employed for all measurements. The assignment of infrared absorption patterns to specific chemical compounds was performed by comparison with FTIR spectra of standard polymer materials, as reported in the literature (Jung et al., 2018) and in the National Institute of Standards and Technology (NIST) database.

2.5. Microplastics removal

To remove all trapped MPs from *C. linum* biomass, three replicates of each algal sample were washed with the hypersaline solution of NaCl, as described in paragraph 2.3, for three times. The algae were then examined with a stereomicroscope (Leica, MZ75) to verify the absence of MPs.

3. Results

3.1. Presence of microplastics in Chaetomorpha linum

Several kinds of MPs were isolated by density separation from the seaweed *C. linum*. Following the categories identified by Hidalgo-Ruiz et al. (2012) we proceeded by cataloguing the MPs based on their shape and size. Out of a total of 245 collected MPs, 237 were fibres (97.3 %), 4 fragments and 4 films.

The smallest and the largest fibres had a size of 0.025 mm and 1.99 mm respectively, while the average particle size was 0.96 mm. Based on the prevailing dimensions, ten size classes were determined, each 0.20 mm wide. The size class with the largest number of MPs was the one from 0.80 mm to 1.00 mm. The data were normalized by dividing the number of MPs found in each size class by the sum of the entire set of identified MPs and reported as percentages. The graph in Fig. 2 was obtained using the normalized data.

3.2. Chemical analyses and characterization of microplastics

The main representative fibres (Fig. 3), fragments (Fig. 5) and films (Fig. 7) isolated by density separation were photographed under a stereomicroscope and analysed by ATR-FTIR spectroscopy. The acquired spectra allowed to identify the main polymer of which each sample consisted as well as any added component (i.e. fillers) or contaminant agent.

The ATR-FTIR spectra acquired on the five selected representative fibres (1a, 2a, 3a, 4a, 5a) are reported in Fig. 4. They showed very similar absorption patterns indicating the same bulk chemical composition of analysed samples. The typical bands of polypropylene (PP) appeared clearly in all spectra allowing these fibres to be identified as PP microplastics. Marker bands ascribed to PP were highlighted in black colour in Fig. 4. Specifically, the signal due to the symmetric bending mode of methyl groups (-CH_3) was well evident at 1376 cm^{-1}, while the broader band at 1455 cm^{-1} was attributed to a superimposition of methyl asymmetric bending and methylene (-CH_2-) scissoring modes. In addition, the region characteristic of aliphatic C—H bond stretching signals (3000–2800 cm^{-1}) presented multiple absorption bands arising from methyl, methylene and methine (>CH-) moieties, in agreement

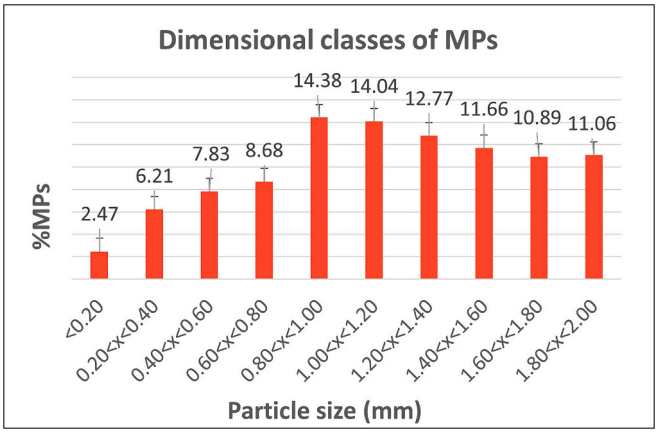

Fig. 2. Size distribution of collected MPs (mm).

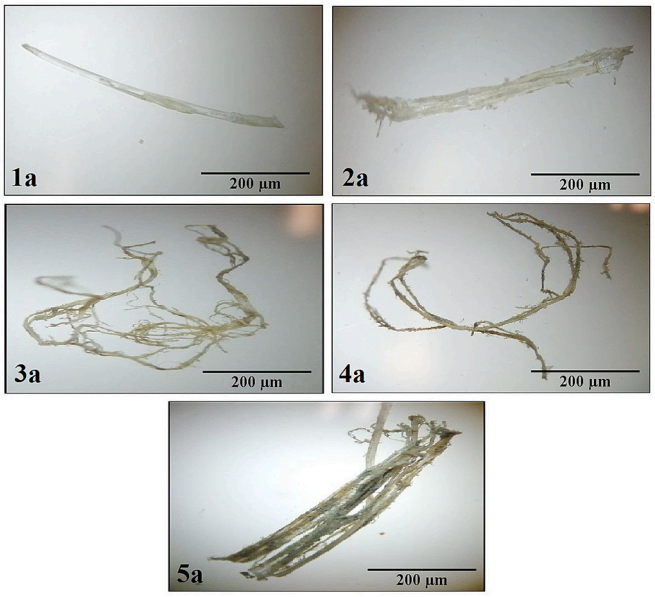

Fig. 3. Fibres isolated by density separation, removed from filters and photographed by the digital camera attached to the stereomicroscope (1a - white fibre, 2a - white fibre, 3a - white fibre, 4a - white fibre, 5a - white fibre).

with PP structure. The spectra of fibres 3a, 4a and 5a showed an additional sharp signal at 873 cm^{-1} (highlighted in orange colour in Fig. 4) assigned to the out-of-plane bending mode of calcium carbonate (Xu and Poduska, 2014). The specific frequency of this band is characteristic of calcite, the most stable polymorph of $CaCO_3$ (Boulos et al., 2014). It is well known that carbonate anion vibrations are also responsible for another typical absorption band, much broader, falling in the wavenumber range 1500–1400 cm^{-1}, which arise from CO_3^{2-} asymmetric stretching mode. Samples 3a, 4a and 5a presented indeed a shoulder at around 1420 cm^{-1} (highlighted in orange colour too), whose intensity was proportional to the intensity of the signal at 873 cm^{-1}, further confirming the presence of calcium carbonate in these fibres. Another feature common to samples 3a, 4a and 5a was the broad and intense signal located at around 1050 cm^{-1}. Since Si—O bond vibrations absorb infrared light in this spectral region, it is likely that, besides a $CaCO_3$ source such as chalk, these samples also contained glass fibres (Etcheverry and Barbosa, 2012). Finally, blue labels in Fig. 4 highlighted two signals at 1643 and 1537 cm^{-1} detectable in the spectra of all samples. These peak frequencies are compatible with specific absorptions by peptide bonds of proteins (Amide *I* and Amide II bands). Protein signals likely arise from biofilms adhering to the MPs. This hypothesis is strengthened by O—H and C—O stretching signals appearing at around 3300 cm^{-1} and 1000 cm^{-1} respectively, which can be ascribed to alcoholic and hemiacetal groups of bio-produced exopolysaccharides. Hence, based on the infrared analysis, the five representative microfibres isolated from *C. linum* biomass can be referred to residues of biofilm-coated PP items.

Fig. 6 shows the ATR-FTIR spectra of the four fragments whose micrographs are reported in Fig. 5. Polystyrene (PS) was identified as the polymer of fragment 1b, since its infrared spectrum showed both the characteristic bands of both monosubstituted benzene ring and methylene moieties (Jung et al., 2018). Specifically, the multiple bands at frequencies higher than 3000 cm^{-1} were ascribed to aromatic C—H stretches, the peaks at 2921 cm^{-1} and 2850 cm^{-1} were typical of asymmetric and symmetric stretches of aliphatic -CH_2- groups, the signals at 1600 cm^{-1} and 1493 cm^{-1} were assigned to benzene ring vibrations, and the band at 755 cm^{-1} was referred to aromatic out-of-plane C—H bends for monosubstituted benzene rings. Moreover, the bending mode of -CH_2- was the main component of the band at 1452

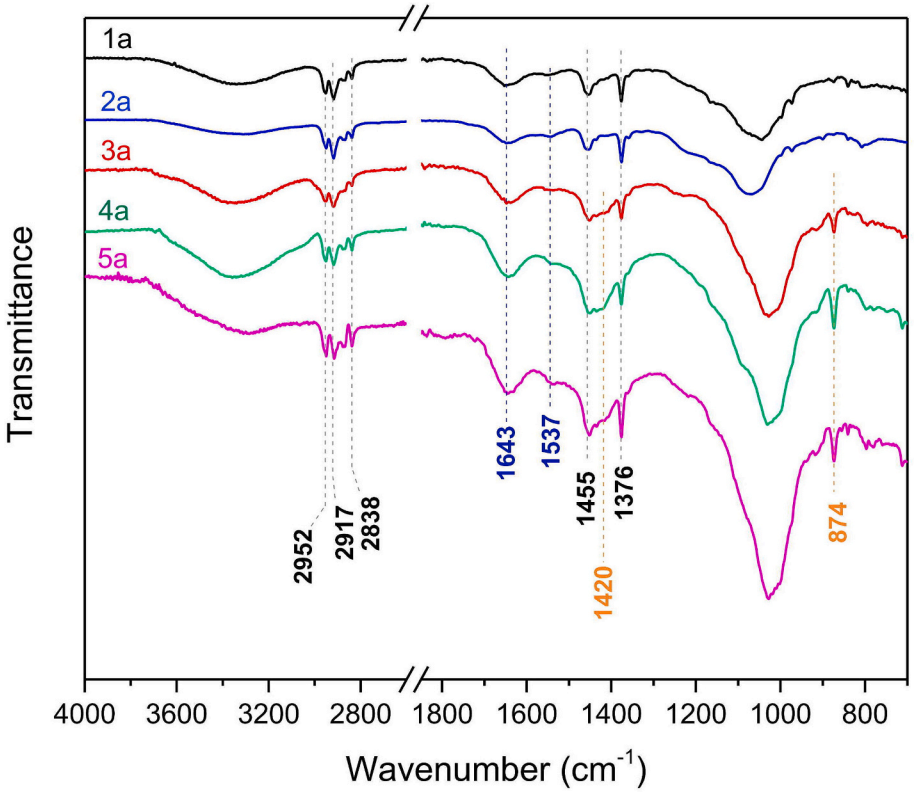

Fig. 4. ATR-FTIR spectra acquired on the selected MP fibres: 1a - white fibre, 2a - white fibre, 3a - white fibre, 4a - white fibre, 5a - white fibres. Black labels highlight signals ascribable to PP polymer, orange labels point out signals ascribable to calcium carbonate and blue labels indicate Amide I and Amide II signals ascribable to a biological component (biofilm).

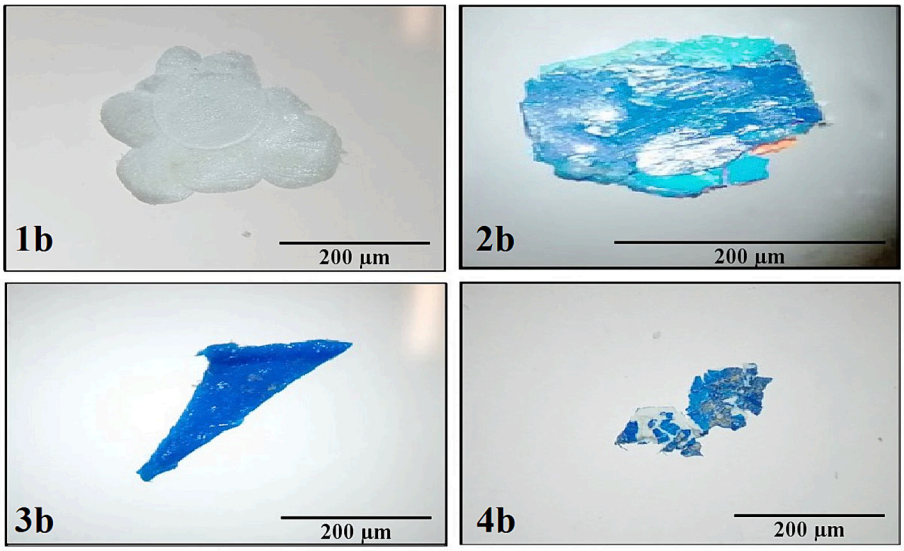

Fig. 5. Some fragments isolated by density separation, removed from the filters and photographed by a digital camera attached to the stereomicroscope (1b - white fragment, 2b - blue fragment, 3b - blue fragment, 4b - blue fragment).

cm^{-1}, although further vibration modes of PS accounted for the enhanced intensity of this signal (Liang and Krimm, 1958).

Fragments 2b and 4b presented very similar infrared absorption patterns, indicating that they arose from the same plastic source. The characteristic peak at 1728 cm^{-1} appearing in their spectra (blue and green traces in Fig. 6) is ascribable to an ester C═O stretching vibration, suggesting that a polyester resin was one the main constituent of the fragments. The ester functional group was responsible also for the signal centred at 1256 cm^{-1}, which arose from CO-O-C ester linkage. The peaks at 2924 and 2852 cm^{-1} were referred to methylene group stretches, while the broad peak at 1415 cm^{-1} and the sharp peak at 873 cm^{-1} were both assigned to carbonate vibrations, indicating that a calcium carbonate filler was likely added to the original polymer matrix. The broad absorption signal, appearing between 1150 and 950 cm^{-1} and overlapped to sharper bands, was ascribed to Si—O vibrations and was compatible with the presence of glass fibre as further reinforcing agent.

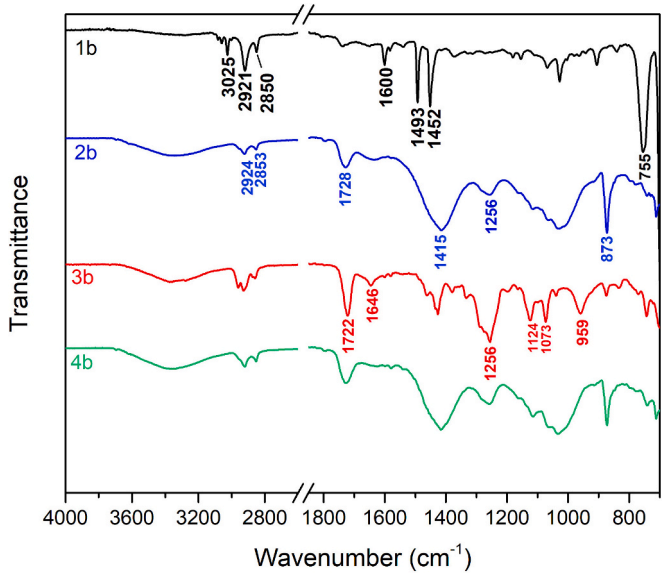

Fig. 6. ATR-FTIR spectra acquired on the MP fragments: 1b - white fragment, 2b - blue fragment, 3b - blue fragment, 4b - blue fragment.

The spectrum of fragment 3b (red trace in Fig. 6) did not show signals ascribable to reinforcing agents. Nevertheless, signals arising from an analogous polyester bulk were clearly present and were more resolved. The signals at 3360, 1646, 1124, 1073 and 959 cm^{-1}, which were assigned to O—H stretch, alkenyl C═C stretch, secondary alcohol C—O stretch, alkyl substituted C—O ether, and aromatic C—H bend vibrations respectively, were indicative of a bulk material made of either a vinyl ester resin or an unsaturated polyester resin (Ardhyananta et al., 2017). These assignments would be in line with the relatively low frequency of the ester C═O stretching band (1722 cm^{-1}), typical of a conjugated carbonyl.

The Fig. 7 shows MP films isolated by density separation. Relevant ATR-FTIR spectra of these films are presented in Fig. 8 and showed both the characteristic absorption bands of polyethylene (PE) (Charles and Ramkumaar, 2009). Specifically, the bands at 2914 and 2852 cm^{-1} in trace 1c and at 2915 and 2849 cm^{-1} in trace 2c were assigned to asymmetric and symmetric stretching modes of methylene groups, which are characteristic of the repeating unit -$(CH2\text{-}CH2)_n$- of this polymer. The doublets at 1472/1462 cm^{-1} and 729/718 cm^{-1} were due to further vibration modes of the same methylene unit, i.e. scissoring and rocking deformations respectively. The splitting was due to a certain crystallinity degree of the polymer matrix (Smith, 2021).

In the case of trace 2c, the analysis of characteristic bands assignable to methylene and methyl groups in the 1400–1300 cm^{-1} range (see inset in the Fig. 8) allowed concluding that low-density polyethylene (LDPE) was the main constituent of the microplastics film 2c. Gulmine et al. (2002), have shown indeed that it is possible to identify the three most important types of commercial polyethylene, namely high density polyethylene (HDPE), LDPE, and linear low density polyethylene (LLDPE), by looking at the relative intensity of the band at 1377 cm^{-1} with respect to the band at 1366 cm^{-1}. Specifically, the peak at 1377 cm^{-1}, ascribable to -CH_3 was absent in HDPE due to the very low branching degree, while the intensity of this peak in LLDPE was lower than the one at 1366 cm^{-1}. LDPE, with the highest branching degree, presented the band at 1377 cm^{-1} slightly stronger than the one at 1366 cm^{-1} as is the case of the microplastics 2c. The same region could not be investigated in the spectrum of microplastics 1c, because of the superimposition of more intense bands at 1389 and 1364 cm^{-1}, which probably arose from additional substances in the polyethylene film. These compounds might be additives or contaminant species responsible also for the bands at 2955 and 1230 cm^{-1}.

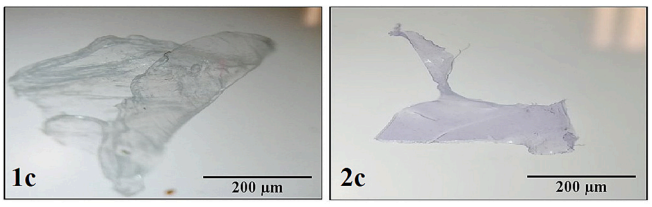

Fig. 7. Some films isolated by density separation, removed from filters and photographed by a digital camera attached to the stereomicroscope (1c - white film, 2c – purple film).

3.3. Removal of microplastics from Chaetomorpha linum

The algal biomass subjected to the washing procedure was examined by a stereomicroscope and no MPs were detected. The density separation procedure, repeated three times, thus provided *C. linum* biomass free of microplastics with a size from 0.025 mm to 2.00 mm.

4. Discussion

MP contamination has become a major environmental problem worldwide due to its numerous sources, widespread, persistence, and harmful impacts on ecosystems and human health (Ahmed et al., 2022; Puri et al., 2023; Hong et al., 2023). Furthermore, it has been reported that MP particles have the ability to absorb harmful substances such as heavy metals present in the environment and to concentrate several hydrophobic pollutants (Bhuyan, 2022). Thus, they represent an ecotoxicological risk for marine organisms (Hermabessiere et al., 2017, 2019) and pose harmful effects such as developmental toxicity, neurotoxicity, reproductive toxicity, cytotoxicity, immunotoxicity, and phytotoxicity (Behera and Das, 2023; Alqahtani et al., 2023). Therefore, it is a global challenge to remove MPs from the intake of living bodies. Many strategies have been developed to remove MPs. Conventional approaches include physical, chemical and biological methods. Microalgae have recently been proposed as a means to remove MPs from aquatic environments (Padervand et al., 2020). The present paper represents an attempt to introduce a new perspective in the field of MP bioremediation by investigating the trapping of MPs on the green macroalga *Chaetomorpha linum* cultivated at a preindustrial level in an IMTA system established in the Gulf of Taranto. The estimated production of algal biomass in this IMTA system is of about 0.84 t for each annual cycle (Giangrande et al., 2020) and it represents a co-product of bioremediation with possible applications in several biotechnological fields including human nutrition. We selected *Chaetomorpha linum* because this thinly structured, filamentous alga produces a dense mat with very small gaps in which MPs can be trapped. The potential of macroalgae, in addition to microalgae, for MP removal from aquatic environment is a relatively unexplored topic and suggests a promising avenue for future research and application in the field of bioremediation. The key role played by aquatic organisms in MP sequestration was further highlighted by the detection of MPs in the filter-feeding organisms present in the same IMTA system, such as the polychaete *Sabella spallanzanii*, the mussel *Mytilus galloprovincialis* and the sponge *Paraleucilla magna,* similarly investigated for their ability to remove MPs from the environment (Fraissinet et al., 2023).

The density separation methodology with a hypersaline NaCl solution has been selected for the separation of MPs in this study since the Marine Strategy Framework Directive (MSFD) Marine Litter Technical group recommends NaCl as the most suitable salt for this purpose, being cheap, non-toxic, easily available and environmentally friendly (Galgani et al., 2011; Han et al., 2019; Li et al., 2020). Sodium chloride was first used by Thompson et al. (2004) for microplastic density separation and is still used worldwide (Klein et al., 2015; Frias et al., 2016). Some studies have also shown that the treatment with a NaCl solution and the

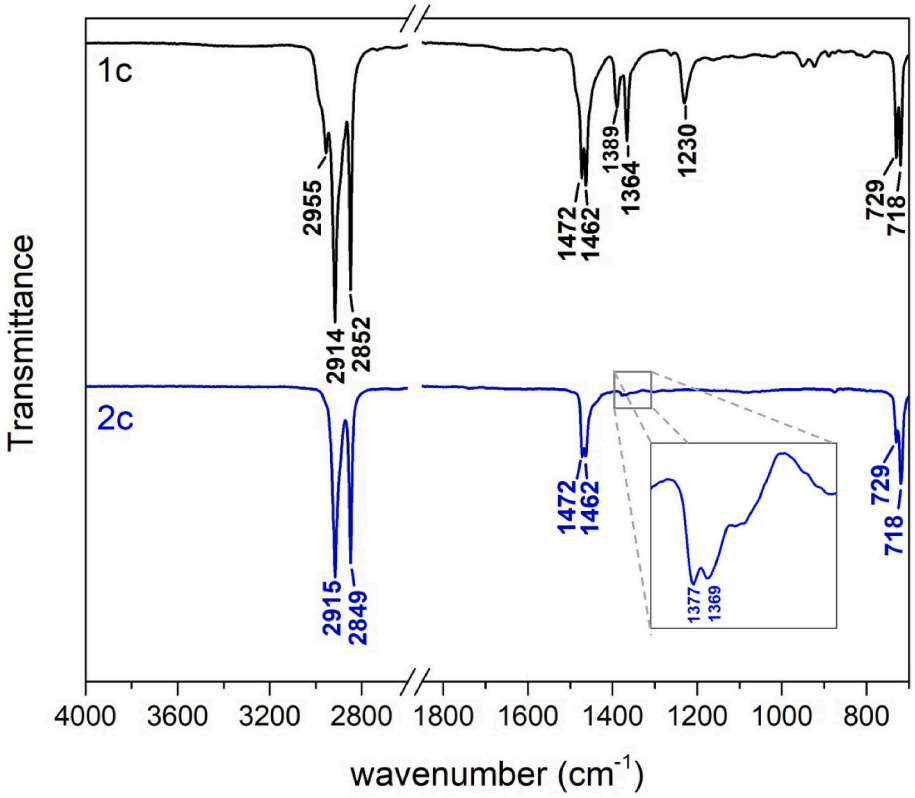

Fig. 8. ATR-FTIR spectra acquired on the selected MP films: 1c - white film, 2c – purple film.

subsequent filtration do not affect the identification of MP particles under Raman and FT-IR observation (Lares et al., 2019). By the density separation methodology, we isolated mainly MPs in the form of fibres, fragments, and films, in agreement with other studies carried out using the same approach (Mayoma et al., 2020).

Of the total 245 retrieved microplastics, the majority had the fibrous form (97.3 %). This finding could arise from the filamentous shape of *C. linum* which makes it easier to trap the fibres. This phenomenon has also been observed in other filamentous macroalgae as reported by Sanchez-Vidal et al. (2021). These authors observed that MP fibres enter the blades of seagrasses. Furthermore, Peller et al. (2021) provided further evidence that microfibers can adhere to *Cladophora* cell wall. Zhang et al. (2022) reported that the macroalga *Sargassum horneri* was more prone to trap fibres (93.85 %). The observed predominance of MP fibres on the surface of *C. linum* is also in line with the ubiquitous and prevalent presence of this type of MPs in aquatic environment, as commonly reported for a variety of matrices from freshwater to marine environments (Cole et al., 2011; Andrady, 2017). This is presumably related to the extensive use of synthetic fibres and their release into coastal areas, domestic wastewater, and textile industries (Browne et al., 2011; Salvador Cesa et al., 2017). Cotton, wool, and linen textile fibres have been gradually replaced by synthetic fibres since the 1950s (Napper and Thompson, 2016) and numerous studies usually report microfibres in sediment, water column and biota samples (Browne et al., 2011). In the study by Napper and Thompson (2016), the authors estimated that >700,000 fibres could be released from an average wash load of 6 kg of acrylic fabric. Due to their small size, a large fraction of synthetic microfibres can pass through wastewater treatment screens and can thus enter the aquatic environment directly from washing machine effluent (Browne et al., 2011).

Regarding the sizes of the MPs in this study, they ranged from 0.025 to 1.99 mm and the prevailing size class was 0.80–1.00 mm. This finding is in agreement with the study by Feng al. (2020) who observed that in the algal species *Ulva prolifera* the size of MPs ranged from 0.13 to 4.00 mm. The size interval 0.13–1 mm accounted for 38.2–72.3 % of total MPs and the size interval 4.00–4.90 mm for only 0.0 % – 8.8 %. A further research carried out by Esiukova et al. (2021) on seven macroalgal species (*Chlorophyta - Cladophora glomerata, Ulva intestinalis; Ulva prolifera, Urospora penicilliformis, Rhodophyta- Polysiphonia fucoides, Ceramium tenuicorne; Phaeophyta - Pylaiella littoralis*) and seawater samples taken in the south-eastern part of the Baltic Sea - Russian sector, revealed that MPs were distributed mainly (37 %) in the size range 0.50–1.00 mm, while MPs in the size range 1.00–2.00 mm accounted for 33.4 %.

In the present work we employed the ATR-FTIR spectroscopic approach for MP analysis since it was demonstrated to be non-destructive and very effective in the identification of the chemical composition of MPs (Xu et al., 2019; Lynch et al., 2022). The infrared absorption pattern is indeed unique for each sample and allowed the simultaneous detection of both organic and inorganic components. Our measurements allowed not only to identify the bulk polymer of MPs, but also to highlight the presence of inorganic reinforcing agents, especially in the case of polypropylene fibres. Since binary salts are among the few inorganic compounds transparent to the mid-infrared radiation, any trace of the washing agent NaCl did not affect the quality of our measurements. Moreover, being a surface sensitive technique, the ATR mode allowed to get information also on MP coating, which could have arisen from a biological or chemical contamination of the original polymer particle.

The most abundant polymers in the marine environment are polyethylene (PE), polypropylene (PP), polystyrene (PS), nylon (PA), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and cellulose acetate (CA) (Andrady, 2011; Jambeck et al., 2015; Fraissinet et al., 2023). Most of these polymers are used in manufacturing of several plastic materials, widely used in the marine aquaculture sector (e.g., boats, fish cages, buoys, nets, ropes etc.) and pointed out as a source of MPs. Once released into the marine environment the fate of plastic materials depends mainly on their physicochemical properties, such as

the density, the shape, the position in the water column and the possible interaction with various organisms (Browne et al., 2011), which influence their buoyancy.

Polymers with density lower than seawater (e.g. PE and PP) mainly float on the surface, while denser polymers (such as PVC) tend to sink, changing their position in the water column. In addition, biofouling increases the specific weight of the particles, influencing their buoyancy and accelerating their sinking to the bottom sediments (Thompson, 2006; Coyle et al., 2020). In agreement with these observations, the MPs recovered from *C. linum* biomass were made of lightweight polymers such as polypropylene (PP), polystyrene (PS), polyethylene (PE), and low-density polyethylene (LDPE). The typical bands polypropylene (PP) appeared clearly in all spectra acquired on fibre MPs. Common plastics retrieved in marine environments are low-density polyethylene, high density polyethylene, and polypropylene, which account for 62 % of global plastic production (Andrady, 2011). These plastics, even in presence of additives, usually have a lower density than seawater and float on the surface (Kreczak et al., 2021). The samples 3a, 4a and 5a analysed in the present study are made of PP reinforced with some calcite-rich filler agent, such as chalk (Psyanchin et al., 2021). Although the attribution to a reinforcing agent is the most probable, it cannot be excluded that the detected calcium carbonate arises from a secondary process, i.e. precipitation and surface deposition of $CaCO_3$ in the marine environment. Another feature common to the spectra of samples 3a, 4a and 5a is the broad and intense signal due to Si—O bond. Thus, it is likely that, besides chalk, these samples also contained glass fibres, which are the reinforcement agent most used in PP-based composites (Etcheverry and Barbosa, 2012). It is likely that the samples 1a and 2a, whose spectra did not show signals due to reinforcing agents, arose from plastic bags, fishing nets, or 100 % PP ropes, which are often used in aquaculture. Fibres 3a, 4a and 5a were probably released from ropes too, but the presence of inorganic fillers suggests that these ropes were employed for practices requiring enhanced resistance to warpage, stiffness, and strength. The presence of Amide *I* and Amide II bands, typical of proteins, in the spectra of all fibre samples suggests that a biotic component is associated to these PP microplastics. This finding is not surprising considering that the formation of biofilms onto the surface of plastic contaminants in aquatic environment is a well-known issue of ecological concern (He et al., 2022). Well-developed biofilms are indeed characterized by a polysaccharide matrix, which strengthens adhesion abilities of cells and protects them from adverse environmental conditions. In addition to protein marker bands, all the spectra of samples 1a, 2a, 3a, 4a and 5a showed C—O vibration signals ascribable to saccharides, strongly supporting the hypothesis that all analysed microfibers are coated by a biofilm. The fibrous nature of these particles likely plays a role in favouring cell adhesion and biofilm development.

Another interesting issue is that polystyrene (PS) was identified as the polymer constituent of only one fragment. PS is generally considered non-biodegradable due to its high molecular weight and high structural stability and there are various types such as: general purpose polystyrene (GPPS), expanded polystyrene (EPS), high impact polystyrene (HIPS) and syndiotactic polystyrene (SPS). Among them, EPS is widely used in aquaculture and take-out packaging boxes and causes serious pollution in coastal areas (Gao et al., 2020). Moreover, PS plastics tend to float with current and gather into plastic garbage patches even in oceanic areas (Kaiser, 2010). The detection of only one PS fragment suggests that the investigated seaweed has a poor capability to trap this kind of pollutant.

Fragments 2b and 4b present the infrared absorption pattern of a polyester resin as well as the signals of calcium carbonate indicating that this inorganic compound was likely added as a filler to the original polymer matrix. Moreover, in fragments 2b and 4b the signals ascribed to Si—O vibrations were observed, pointing out the presence of glass fibre as further reinforcing agent. Polyester resins reinforced with calcium carbonate and glass fibres are widely employed as excellent composites in several sectors (Setyanto et al., 2022) including the marine industry (Visco et al., 2008). Polyester resins have been indeed used to build and repair boats since fiberglass boats were first invented (Rubino et al., 2020a). The presence of this type of resin in the algae sampling site can be explained by considering that it is traversed by various service boats, involved in the aquaculture plant activities. The spectrum of fragment 3b was slightly different from the other ones and the recorded signals were indicative of a bulk material made of either a vinyl ester resin or an unsaturated polyester resin (Ardhyananta et al., 2017). This hypothesis was also supported by the specific absorption frequency of the carbonyl group, which is compatible with a conjugated ester group. Glass fibre reinforced vinyl ester resin is increasingly being used for military and commercial transport applications, e.g. in ship and boat construction, owing to good toughness, excellent resistance, good mechanical properties and minimal maintenance requirements (Iskander et al., 2001; Santos et al., 2009; Rubino et al., 2020b). The detection of this plastic material is in line with the characteristics of the sampling area. The city of Taranto is indeed an important commercial and military port that hosts the site of the maritime military arsenal and is involved in the activities of steel iron factories, oil refineries, chemical factories, shipyards, and food-processing plants (Cardellicchio et al., 2007, 2008).

Polyethylene and low-density polyethylene were identified as bulk polymers of films 1c and 2c respectively. Polyethylene has become an essential part of the day-to-day activities of human life. Accounting for almost 30 % of the yearly demand for plastics in the European Union, polyethylene is the most widely used type of plastic. Based on density, it can be found in two forms; low density polyethylene (LDPE), used for the production of reusable bags, trays, containers, agricultural film and food packaging film, and high-density polyethylene (HDPE), used as a manufacturing material for films, toys, bottles, pipes, houseware, etc. However, its excessive use and disposal led to accumulation in the environment, causing marine pollution. It has been reported that polyethylene represents 79 % of MP polymers sampled in the marine environment (Hidalgo-Ruiz et al., 2012).

Finally, in the present work we employed a simple washing with NaCl solution, repeated three times, to remove MPs from harvested macroalgae. This procedure proved to be satisfactory since the inspection of treated *C. linum* under the stereomicroscope did not highlight any MPs thus resulting as a MP-free sample. This outcome paves the way to the employment of the algal biomass obtained in the IMTA system for the formulation of bio-products with various biotechnological applications in medicine, cosmetics, pharmaceutics, nutraceutics and human food production (Stabili et al., 2012, 2014, 2019a; Freile-Pelegrín and Tasdemir, 2019). In fact, to be marketable as a source of animal feed and human food, *C. linum* biomass must be free of contaminants, including MPs, to avoid that loaded pollutants could be transmitted to animals and humans through the food web.

Therefore, the results obtained in the present study pointed out two interesting issues relevant to the double use of cultivated macroalgal species as i) MP bioremediator in an aquaculture scenario and ii) source of animal feed and human food, after a prior washing step, consisting of the density separation methodology here described, which proved to be effective to remove captured MPs with a size detectable by stereo microscopy (>0.025 mm).

5. Conclusion

Our results highlighted the presence of microplastics trapped in the seaweed *C. linum* grown in an IMTA system and the possibility of efficiently removing them with a simple procedure. Hence, our findings encourage the use of the studied algae in an aquaculture scenario where *C. linum* can play a dual role as a bioremediator and as a source of bioactive compounds including fatty acids useful for animal and human nutrition and for the production of fortified food. The MP bioremediation strategy proposed here based on MP trapping by the cultivated seaweed is an in-situ method for restoring the marine environment. It is

worthy to mention that 0.84 t of macroalgae are cultivated in the plant for each annual cycle and consequently their MP trapping capacity is remarkable. Therefore, in the present scenario, bioremediation using macroalgae represents an intriguing tool to remove pollutants, contaminants, or undesirable compounds, such as MPs, from aquaculture wastes. In conclusion, the outcome of this investigation not only highlighted the importance of MP contamination assessment in different marine sites, but also encourages further progress in this crucial area of environmental remediation, pointing out potential research directions and opportunities.

CRediT authorship contribution statement

Loredana Stabili: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Elisa Quarta:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Livia Giotta:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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