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An effective strategy for the monitoring of microplastics in complex aquatic matrices: Exploiting the potential of near infrared hyperspectral imaging (NIR-HSI)

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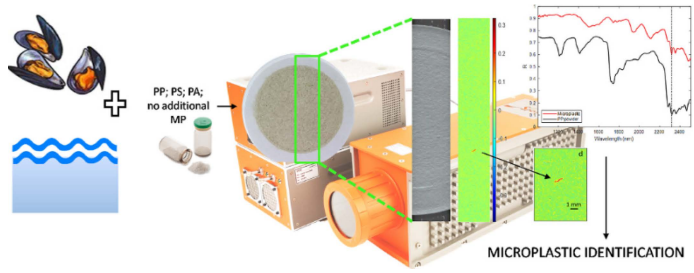
HIGHLIGHTS

- Near infrared hyperspectral imaging (NIR-HSI) to detect microplastics down to 50 μm.
- Polypropylene, polystyrene and polyamide detected in seawater and mussel digestate.
- Automated data processing strategy based on normalised difference image (NDI).
- NIR-HSI applied directly on filters without particles pre-sorting or purification.
- Useful tool for assessing the ecological risk related to microplastic contamination.

GRAPHICAL ABSTRACT



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ABSTRACT

Contamination by microplastics (MP) represents a critical environmental challenge with potential consequences at ecosystem, economic and societal levels. As the marine system is the final sink for MP, there is an urgent need to develop methods for the monitoring of synthetic particles in different marine compartments and sample matrices. Extensive evaluations are hindered by time and costs associated with to conventional MP spectroscopic analyses. The potential of near infrared hyperspectral imaging (NIR-HSI) has been recently evaluated. However, NIR-HSI has been poorly studied so far, limitedly to the detection of large particles (>300 μm), and its capability for direct characterization of MP in real marine matrices has not been considered yet. In the present study, a rapid near infrared hyperspectral imaging (NIR-HSI) method, coupled with a customised normalised difference image (NDI) strategy for data processing, is presented and used to detect MP down to 50 μm in environmental matrices. The proposed method is largely automated, without the need for extensive data processing, and

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enabled a successful identification of different polymers, both in surface water and mussel soft tissue samples, as well as on real field samples with environmentally occurring MP. NIR-HSI is applied directly on filters, without the need for particles pre-sorting or multiple sample purifications, avoiding time consuming procedures, airborne contaminations, particle degradation and loss. Thanks to the time and cost effectiveness, a large-scale imple-mentation of this method would enable to extensively monitor the MP presence in natural environments for assessing the ecological risk related to MP contamination.

1. Introduction

Due to the enormous production and mismanagement, the issue of plastic waste represents a critical and urgent social, economic and environmental challenge (He et al., 2018) and it was also included as one of the good environmental status (GES) descriptors in the Maritime Strategic Framework Directive (MSFD) Galgani et al., 2013.

Once in the environment, plastic wastes can be degraded by physical, chemical and biological factors (e.g., ultraviolet radiation, wind or water erosion, microorganisms) becoming small plastic debris charac-terized by reduced dimensions (between 1 μm and 5 mm) and commonly referred to as microplastics (MP). MP also include precursors of plastic products manufactured as virgin industrial pellets or synthetic micro-spheres found in detergents and cosmetics (i.e., exfoliates). Moreover, also rayon and synthetic cellulose microfibers represent a growing environmental concern (Cincinelli et al., 2021).

In the last years, MP presence has been reported in water and sedi-ments of numerous marine (Carbery et al., 2018), freshwater (Wagner et al., 2014) and terrestrial ecosystems (de Souza Machado et al., 2018) worldwide. Over 220 species were found as capable to uptake MP under natural environmental conditions with potential adverse consequences at toxicological level (Lusher et al., 2017).

To date, one of the major constrains and challenges in MP research is to conduct extensive environmental monitoring using an adequate number of replicated samples to detect relevant spatial and temporal variations in MP abundance and distribution patterns (Underwood et al., 2017). Further, the lack of methodological harmonisation in traditional analytical procedures, applied for MP detection, does not allow comparability among data from different studies and research labora-tories (O'Connor et al., 2020).

So far, different analytical methods with different degrees of preci-sion and resolution have been proposed to detect MP in field samples (for an updated systematic review the reader is referred to Lu et al., 2021). The most used methods are based on the visual sorting of MP followed by spectroscopic single-point techniques, such as the attenu-ated total reflectance-Fourier transform infrared (ATR-FTIR) or Raman microspectroscopy (e.g. Piarulli et al., 2019). Micro-Fourier transform infrared spectroscopy (μ-FTIR) imaging systems, coupled with a focal plane array detector (FPA), have been also developed and applied on different types of samples and heterogeneous environmental matrices (Cincinelli et al., 2021; Lorenz et al., 2019). In particular, FTIR FPA imaging systems permit the scan of the sample, with a very high spatial resolution (e.g. 5.5 μm pixel size as shown in Cincinelli et al. (2021) and Morais et al. (2020)), allowing the detection of MP characterized by very small dimension (ca. 11 μm).

However, all the traditional spectroscopic present common limita-tions: they are usually time and/or cost consuming, may require extensive sample manipulations and their sensitivity and selectivity strongly depend either on the initial visual pre-sorting of MP (for ATR-FTIR) or on the number of samples that can be analysed (for μ-FTIR-FPA). These limitations can prevent the applicability of these techniques for extensive monitoring programmes that require an adequate number of replicated samples to ensure the ecological relevance of the results (Underwood et al., 2017). As the marine system is considered as the final accumulation environment for MP, the development and use of cost-effective and easy applicable techniques to detect MP in different compartments is currently considered a research priority for assessing the risk posed by MP contamination (Gallo et al., 2018).

Near infrared hyperspectral imaging (NIR-HSI), was firstly described in Piarulli et al. (2020a) as a potential cost and time effective method, enabling a direct and fast (few minutes for the analysis of an entire filter of 5 cm diameter) detection of MP without heavy sample purification and manipulations, thus avoiding potential procedural bias related to particle pre-sorting steps. Further, Vidal and Pasquini (2021) showed the applicability of NIR-HSI to detect MP (ranging between 300 and 150 μm) in beach sediments samples.

However, the potentialities of NIR-HSI systems are still poorly investigated and limitedly used to properly address real ecological and analytical issues related to the detection of MP, including the effect of MP dimensions and the polymeric typologies.

Within this scenario, the present research is aimed at assessing and boosting the capabilities and the analytical robustness of NIR-HSI for the detection of MP in complex marine environmental matrices.

In the present study, NIR-HSI in the range of 1100–2500 nm was tested and successfully applied to detect MP, down to 50 μm in size (according to the pixel size of the hyperspectral images recorded), of different polymers in surface water and mussels' soft tissue samples. Additionally, a new and automated normalised difference image (NDI) strategy for data processing was here introduced, also significantly reducing the time required for data processing and evaluation.

To establish the methodological potentialities of NIR-HIS in envi-ronmental monitoring, the set of seawater and biota samples included both spiked (with different commercially available MP) and field collected samples (without any plastic addition, potentially containing environmental MP).

2. Materials and methods

2.1. Sample collection

Mussels and surface water samples were collected in Marina in Ravenna, North Adriatic Sea, Italy (44_29032.600 N, 12_17015.200 E), a coastal semi-enclosed area characterised by numerous anthropogenic activities and, thereby, a potential hot-spot for MP contamination (the interest reader is referred to Airoldi et al., 2016 for a detailed description of the sampling area).

In July 2020, naturally occurring specimens of the mussel *M. galloprovincialis* (4–7 cm in length) were collected with a stainless steel wall-scraper from the artificial jetties, seawalls, while surface water samples were collected, approx. 50 cm below the surface, within 5 L standard-sampling jars secured into the box of the stainless-steel scraper, to allow water collection to be performed at the same depth, without significant oscillations. Mussels, were chosen as model organisms as they typically filter large quantities of water and capture suspended natural and anthropogenic particles of a wide range of sizes, including MP (Ward and Shumway, 2004). Further, recent research highlighted a quantitative and qualitative correlation between MP in mussels and in surrounding waters and, therefore, pointing these organisms as potential bioindicator species for monitoring MP contamination in natural coastal habitats (Bråte et al., 2018; Li et al., 2019).

2.2. Samples preparation and processing

After collection, both mussels and water samples were brought, as rapidly as possible, to the laboratory and processed.

Each mussel was, then, scrubbed and rinsed to remove any adhering material (including epibiota and environmental MP) and depurated for 24 h in aerated aquaria filled with pre-filtered (0.7 μm, Whatman ® glass microfibre filters) artificial seawater without adding any source of nutrition. This was done to allow mussels to egest any of previously ingested MP.

After the depuration time, each mussel was rinsed with ultrapure (0.2 μm)-filtered water and separated from the shell. All isolated tissue samples were pre-weighed and processed with 1 M potassium hydroxide (KOH; 40 mL per sample) following the alkaline digestion protocol described in (Piarulli et al., 2019). After the KOH addition to each mussel tissue, pre-weight MP particles of different polymers (poly-propylene = PP; polystyrene = PS and polyamide = PA), and at high (≅ 1.5 mg), medium (≅ 1 mg) and low (≅ 0.5 mg) content, were added separately to 3 replicated individual mussel (total samples n = 27) samples and incubated for 48 h at 40 °C. Three individual mussel digestates were kept without any MP addition to be used as procedural control treatments. Concerning the see water samples, no digestion pre-treatment was applied and MP were added before the filtration. Similarly to mussel samples, the three types of MP at the three quantity levels (high, medium and low) were individually added to 3 replicated water samples (1 L each, total samples n = 27). Three water samples were left without any MP addition as controls.

Commercially available PP and PS cryogenically grinded fragments (100–200 μm as median grain size) from pristine pellets (REF.no CTR200.00V0.0 and CTR300.00V0.0 for PP and PS reference materials) were provided by CARAT GmbH (Harderhook 20, 46395 Bocholt, Ger-many; www.carat-lab.com), while PA fragments of a median grain size of 41 μm were provided by Environmental Tracing Systems (Bannachra, Helensburg, G84 9 EF U.K.).

Fragments were chosen to mimic as much as possible environmentally-relevant MP typologies, and PP, PS and PA were chosen as three of the most common MP polymers found in Mediterranean waters and in biota, including *M. galloprovincialis*, in our sampling area (Piarulli et al., 2020b; Suaria et al., 2016).

Both mussel and water MP-spiked samples and controls after their respective processing were filtered through cellulose filters (d = 5 cm; pore size = 11 μm; Whatman®) and, then, dried in closed glass petri dishes at room temperature. The choice of using a cellulose filter was performed according to Piarulli et al. (2020a), reporting that due to hydrophilicity and porosity of cellulose filters, the efficiency of filtrati on of water-based suspensions is enhanced and the risk of MP aggregation during the filtration is reduced compared to other filters. Also, these filters are relatively cheap and easily commercially available. However, as has been demonstrated in the previous research, different type of filters ca be also used (such as the Nylon© filter).

To evaluate the applicability of the NIR-HSI method to real samples at environmentally relevant MP amounts, the same processing without the addition of external MP was applied to 3 real samples of mussels not subjected to prior depuration. Given that the MP content per mussel, in this close coastal area, was estimated to be lower than 1 MP particle per individual (Piarulli et al., 2020b), a pool of soft tissue from 5 mussels was analysed following the same protocol of the spiked samples, but using 100 mL of 1 M KOH, as the quantity of tissue to be digested was higher. Additionally, to allow methodological comparability with the spiked test samples, the total amount of digestate to be analysed with

NIR-HSI was split onto 5 filters, which. Similarly, 3 seawater samples (≅ 5 L each) without any plastic addition were analysed for environmental MP detection.

Overall, a total number of 66 samples were analysed. For the sake of conciseness, only a selected number of representative results will be shown.

2.3. NIR-HSI analyses and data processing

All the filters were subjected to NIR-HSI analysis. NIR HSI data were acquired by a push-broom system composed by a SWIR3 hyperspectral camera working in the 1000–2500 nm spectral range, at 5.6 nm spectral resolution (Specim Ltd, Finland). The instrumental setting is characterized by three halogen lamps (35 W, 430 lm, 2900 K, each) as illumination sources and a horizontal line scanner (40 × 20 cm moving stage) on which samples are laid down. The number of pixels per line is equal to 384. The system is controlled by the Lumo Scanner v. 2.6 software (Specim Ltd, Finland). Prior to each measurement, dark (closed shutter) and white (99 % reflectance Spectralon® rod) images were automatically recorded and used to compute the spectral reflectance value (R) for each pixel and wavelength. For the acquisition, the scan parameters were set as follows: scan rate equal to 0.7 mm/s, frame rate equal to 50.00 Hz and exposure time equal to 16.00 ms; manual focus was tuned before the scan. Few minutes were required for the analysis of an entire filter of 5 cm diameter. To obtain a high spatial resolution and ensure an efficient chemical characterisation of the MP, a macro lens (OLES MACRO, 73.3 mm focal length, 10 cm working distance) with a nominal pixel size of 21 μm was used. The collected image data were organized in a three-dimensional data matrix, often called hypercube or, alternatively, spectral cube. The first two dimensions of the 3D array are vertical and horizontal spatial coordinates (pixels), while the third dimension represents the wavelengths (spectral dimension). To identify the diagnostic spectral bands of the selected polymers (PP; PS and PA), the MP used for spiking mussels and seawater samples were used as reference material and analysed. Chemical maps were obtained by calculating the normalised difference image (NDI) (Malegori et al., 2020), according to the following formula:

$$NDI = \frac{R_{\lambda f} - R_{\lambda p}}{R_{\lambda f}}$$

where $R_{\lambda f}$ and $R_{\lambda p}$ are the reflectance values at wavelengths characteristic for the filter and the polymer, respectively. To maximise the analytical performance in terms of selectivity and detection capability of the method, a different band for the cellulose filter was selected for the NDI analysis of each MP typology, enhancing differentiation between cellulose and the given MP (Table 1).

NDI were computed on the raw spectra (without any row pre-processing) because computation of reflectance ratios minimised the contribution of baseline deviations, such as shifts and global intensity effects.

For each pixel, the NDI value was coded by a chromatic scale ranging between red (maximum value) and blue (minimum value). A RGB chemical map was thus obtained, showing the presence of MP. In more detail, pixels corresponding to the detected MP particles maximised the NDI ratio and, therefore, were encoded in red in the map. Regions of interest (ROIs) at predominant red colour were automatically selected by defining a numerical selection range of the colour values of the pixels in the resulting NDI maps. Namely, an automatic segmentation of the background was performed by setting a cut-off that identified as MP all the pixels with value higher than 220 for the red channel, value lower than 120 for the green channel, and value lower than 120 for the blue channel, simultaneously.

Table 1
Wavelengths characteristic for the polymer (Rλp) and the cellulose filter (Rλf), used for the NDI analysis.

Polymers (Rλp)			Cellulose (Rλf)	
	nm	cm^{-1}	nm	cm^{-1}
PP	2321	4308	2110	4739
PS	1692	5910	1636	6112
PA	1697	5892	2110	4739

2.4. QA/QC procedures

Specific precaution procedures were applied at all the experimental phases, from the field set-up and sampling to the sample processing and MP analyses, by following the protocol described in (Piarulli et al., 2019), to avoid airborne contamination from external plastics. Briefly, when possible, all the equipment was made of metal or glass and, if plastic was necessary few polymers were used (polyethylene, polyvinyl chloride or polytetrafluorethylene). The use and properties of any plastic equipment was annotated to be, in case of necessity, eventually tracked in the samples.

All the processes involving the direct exposure and handling of the samples were performed in a clean laboratory, where the use of a cotton laboratory coat was mandatory, and with minimised external air circulation.

All the field and laboratory equipment were rinsed with ultrapure filtered water (0.2 μm) before the use and all laboratory surfaces were regularly decontaminated from airborne synthetic fibres.

Contact with air and plastic surfaces during all the laboratory procedures was minimised for samples and instruments by covering them with aluminium foils before and after use. After filtrations, filters were kept covered in glass petri dishes, minimising exposure to air, until analysis.

To validate the effectiveness of the contamination prevention approach, 4 air filters for each batch of samples were processed and then inspected at the microscope to monitor the presence of particles, particularly airborne fibres. Only white fibres from lab coats were found on the air filters, while none of the polymers used for the sampling or laboratory processing was found in the real samples.

3. Results and discussion

A representative set of samples (three replicates for each type of sample, characterised by a different MP polymeric typology, quantity and sample matrix) was analysed with NIR-HSI to assess the repeatability and robustness of the method. Each chemical map was acquired in few seconds, guaranteeing an affordable application of the method for a routine characterisation of a high number of samples.

To investigate the potential of the HSI method, 3 different amounts of MP for each type of polymers (Figure SI1), were considered and added to the two different matrices (marine surface water and mussel soft tissue). In this section, only the results obtained from filters with the lowest quantity of MP are presented, while results on samples with a higher MP amount are reported in SI (Figure S2-S11), showing the high efficiency of the proposed approach in the detection of higher MP amounts. .

Figs. 1a and 2a report the NDI false colour chemical maps obtained on the filter related to the two matrices added with the lowest amount of PP. A red (high NDI values) to blue colour scale (low NDI values) was used to represent the MP distribution (Fig. 1a). Thanks to the automatic segmentation of the background, performed by setting a cut-off on the RGB channels, as detailed in the Materials and Methods section, Fig. 1b shows only the MP particles (red pixels) detected by the algorithm, against a black background. In attempt to make data evaluation efficient and fast, a targeted approach based on the use of an automated extraction of the most relevant spatial and spectral information embedded in the complex datacube, was proposed. Specifically, the method provides a direct visualisation of the MP particles detected (as red pixels in Figs. 1b and 2b) as well as an automatic extraction of the associated average NIR spectrum (Figs. 1c and 2c). Evaluation of the spectral profile is highly functional to a proper characterisation of MP polymers, achievable through the visual inspection of the diagnostic absorption bands by comparison with reference spectra. As an example, the NIR spectrum extracted from the filter with marine water (Fig. 1c) showed PP distinct bands at 2321 nm (CH bend (3δ) 2nd overtone) and 2360 nm (CH and CC stretch combination). Additionally, the spectrum presented a band at 2282 nm (CH stretch + CH_2 deformation + CH bend (3δ) 2nd overtone) and a broad and weak band at 1742 nm (antisym- metric methylene CH stretch (2ν) 1st overtone) (Salzer, 2008). Inter-estingly, the above-mentioned bands of PS resulted to be clearly differentiated from the cellulose NIR bands that, in turn, are visible in the extracted spectrum at 2121 nm (OH stretch + OH bend combination) and at 1948 nm (OH combination band of water) (Popescu and Popescu, 2013; Schwanninger et al., 2011).

Comparable results were obtained in the filters of the MP-spiked mussel tissues (Fig. 2c), even though this matrix may potentially present a higher variability of interferences due to the presence of undigested inorganic and organic residues of mussel shells and tissues, such as calcium carbonate, lipidic and proteinaceous materials. Indeed, these materials do not present significant interfering absorption bands in the

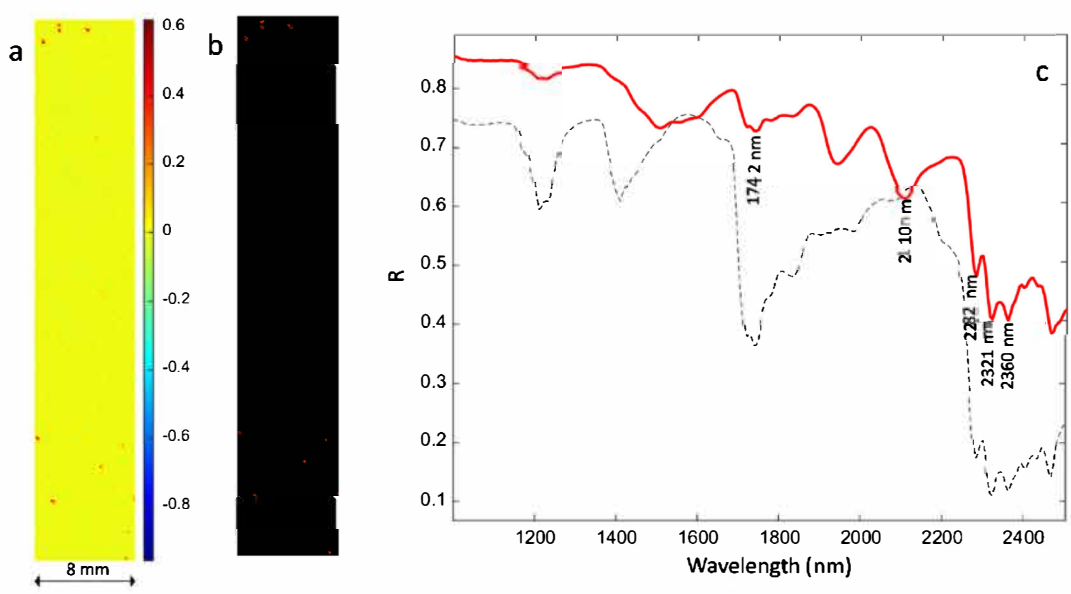

Fig. 1. Cellulose filter after the filtering of polypropylene MP at the lowest quantity investigated (0.5 mg) in seawater (1 L). a) NDI; b) segmented NDI; c) comparison between the average NIR spectrum of the NDI red pixels (red full line) and the NIR spectrum of standard polypropylene (black dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

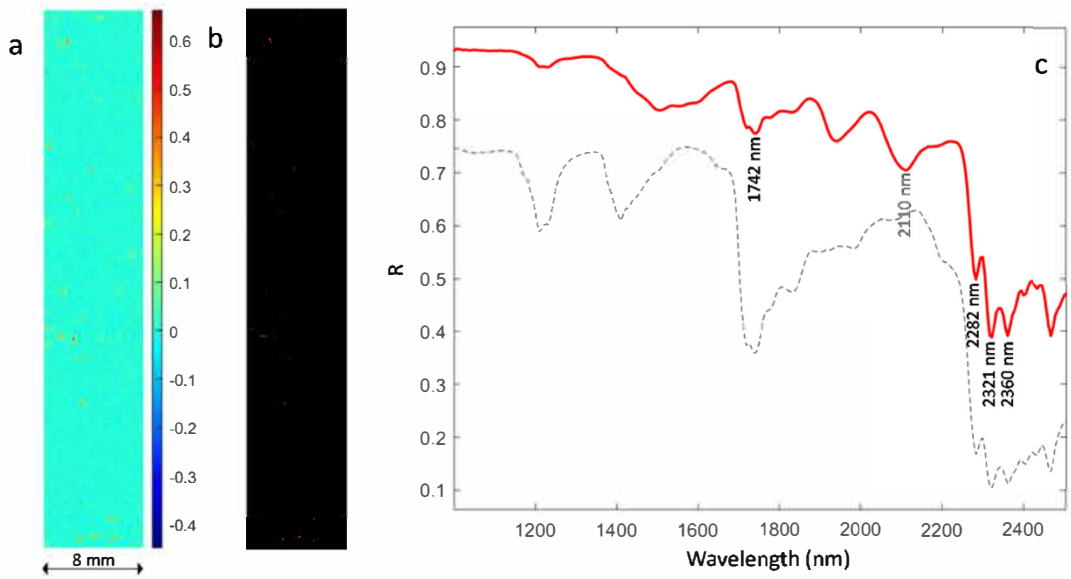

Fig. 2. Cellulose filter after the filtering of polypropylene MP at the lowest quantity investigated (0.5 mg) in a mussel digestate. a) NDI; b) segmented NDI; c) comparison between the average NIR spectrum of the NDI red pixels (red full line) and the NIR spectrum of standard polypropylene (black dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

NIR regions selected for NDI computation, as it is evident from the spectral profiles reported in (Bartlett et al., 2018; Sciutto et al., 2014).

These outcomes demonstrated that NIR-HSI coupled with the NDI strategy can be successfully applied also on heterogenous sample matrices and it does not require strong or multiple sample purifications, which are usually time consuming as well as a potential source of degradation and/or loss of particles.

Figs. 3a and 4a show the NDI chemical maps obtained from filters of marine water and digested mussel tissue, respectively spiked with PSMP particles are highlighted as red pixels, clearly identifiable in the extracted map (Figs. 3b and 4b). In the corresponding averaged NIR spectra (Figs. 3c and 4c) the diagnostic bands, ascribable to PS at 1692 nm (aromatic CH stretch (2ν) 1st overtone), 2171 nm (CH bend (3ν) 2nd overtone) and at 2355 (CH stretch + CH_2 deformation combination), were easily identified and differentiated from those of the cellulose filter (Salzer, 2008).

The filter of digested mussels (Fig. 4c) shows a higher accumulation of MP in the area subjected to HSI analysis, possibly due to an aggregation effect carried out by the biological materials present in the sample.

Differently from what observed for PP and PS samples, it was not possible to detect PA particles in samples containing the lowest amount of MP (*data not shown*). The evident difficulties encountered in detecting PA in all samples examined may be tentatively justified both by the smaller size of these particles, which were of the same magnitude of the porosity of the filter, and by the relatively lower intensity of the NIR absorption bands of PA, when compared with the bands of cellulose (Lipik and Yang, 2018; Piarulli et al., 2020a).

Nevertheless, NDI results revealed the possibility to detect PA in samples with higher amounts of MP added (high ≅ 1.5 mg, medium ≅

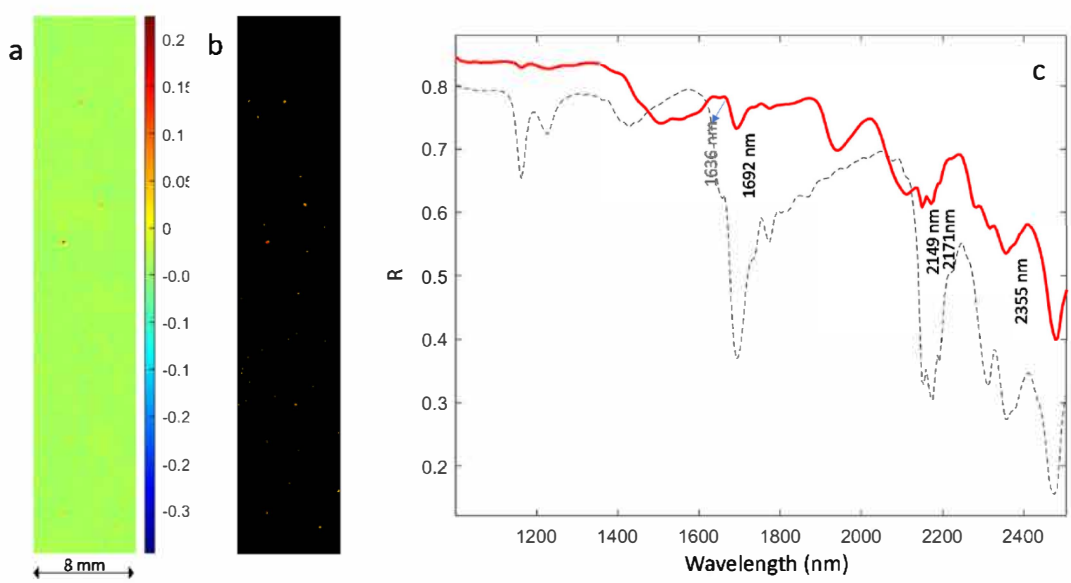

Fig. 3. Cellulose filter after the filtering of polystyrene MP at the lowest quantity investigated (0.5 mg) in seawater (1 L). a) NDI; b) segmented NDI; c) comparison between the average NIR spectrum of the NDI red pixels (red full line) and the NIR spectrum of standard polystyrene (black dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

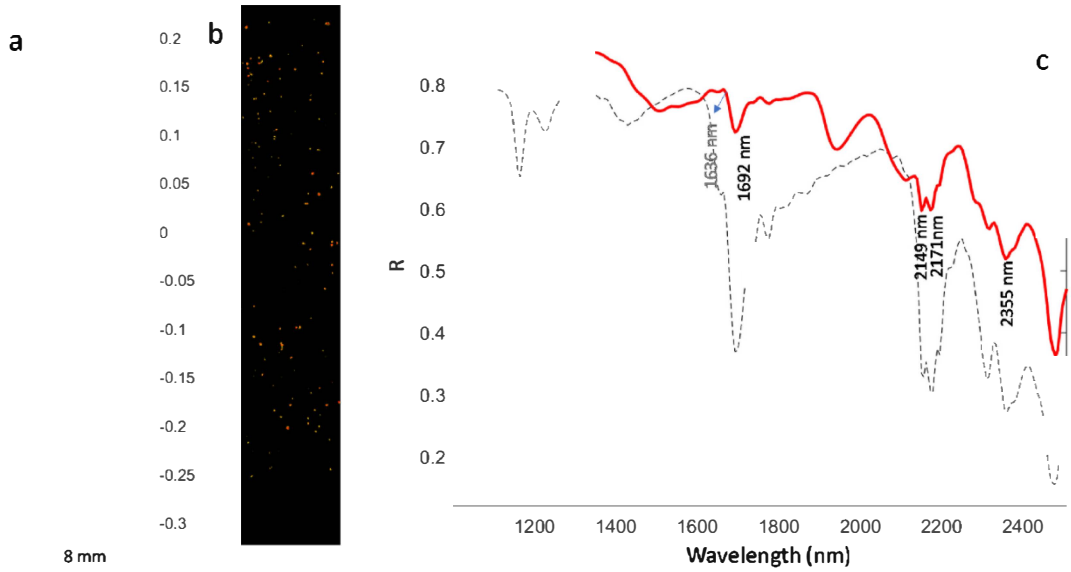

Fig. 4. Cellulose filter after the filtering of polystyrene MP at the lowest quantity investigated (0.5 mg) in a mussel digestate. a) NDI; b) segmented NDI; c) comparison between the average NIR spectrum of the NDI red pixels (red full line) and the NIR spectrum of standard polystyrene (black dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

1 mg). Results from marine water samples with 1 mg of PA (Fig. 5), showed the presence of very small and isolated red spots, ascribable to the MP particles, as confirmed by the presence of the weak bands of PA at 1697 nm (aromatic CH stretch (2ν) 1st overtone), at 2149 nm, 2180 nm (C●NH_2 as combination of amide modes) and bands at 2316 nm and 2377 nm (CH antisymmetric/symmetric stretch + CH bend combination). The same bands were also identified in the samples with mussels and medium content of MP added, despite their very low intensity (Fig. 6). Interestingly, the method provided an effective identification of PA and their differentiation from the mussel spurious residues, as shown in Fig. 6b, in which, thanks to automatic background segmentation, it was possible to visualize polymer particles with dimensions starting from 50 μm.

Finally, the NIR-HSI method was applied on real seawater samples with a potential content of MP environmentally occurring, without any artificial addition.

In one of the 3 replicas, obtained by filtering 5 L of water for each filter, it was possible to identify a single MP (Fig. 7). The particle was well localised in the hyperspectral image of the filter, presenting a peculiar filament shape with the approximate dimension of 1 mm × 0.1 mm. Fibres and filaments are one the most frequent particles type detected in samples from the coastal area of Ravenna possibly deriving from urban wastewater effluents or from the degradation of nets and ropes used for fishing purposes (Piarulli et al, 2019, 2020b).

Since the polymeric nature of the MP fibre detected was unknown, all the combinations of absorption bands (for PS, PP, and PA) reported in materials and methods section were tested for the creation of the NDI. However, it is worth to underline that different polymeric materials may present analogous bands in the NIR region, such as those associated with CH stretching and bending combination bands (between 2300 and 2400

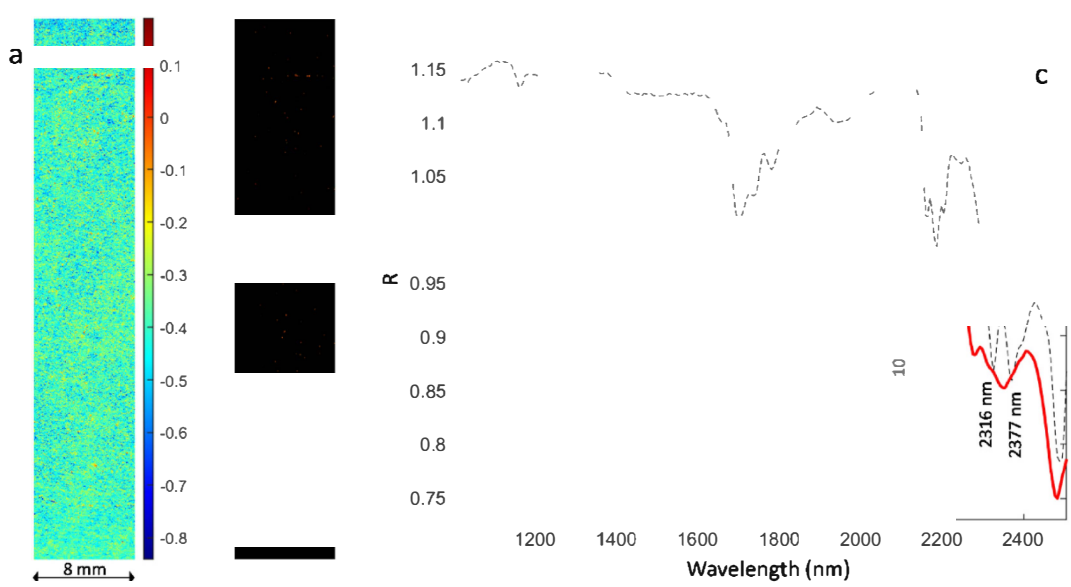

Fig. 5. Cellulose filter after the filtering of polyamide MP at the lowest quantity detected (1 mg) in seawater (1 L). a) NDI; b) segmented NDI; c) comparison between the average NIR spectrum of the NDI red pixels (red full line) and the NIR spectrum of standard polyamide (black dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

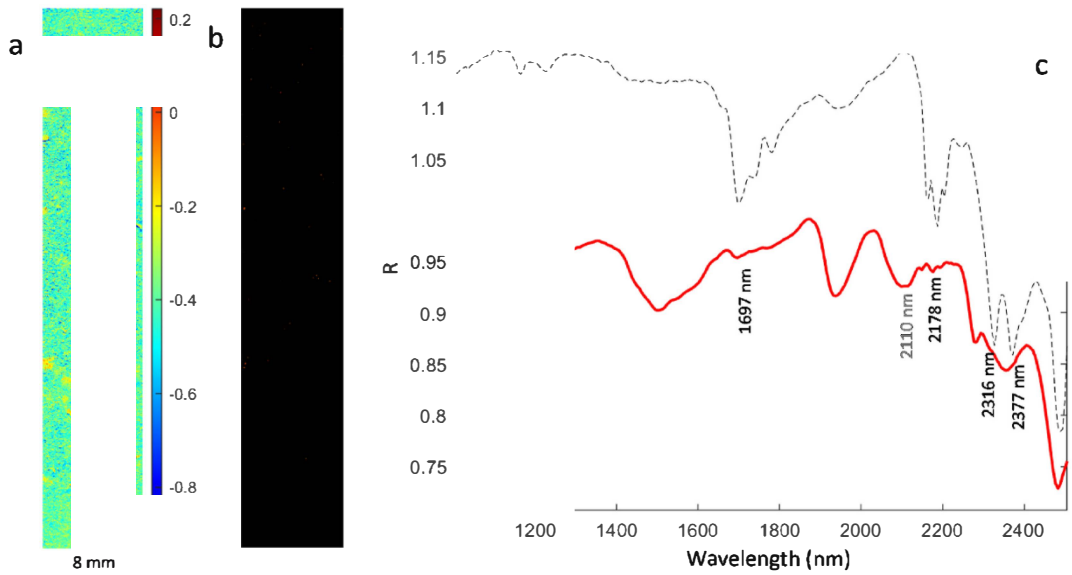

Fig. 6. Cellulose filter after the filtering of polyamide MP at the lowest quantity detected (1 mg) in a mussel digestate. a) NDI; b) segmented NDI; c) comparison between the average NIR spectrum of the NDI red pixels (red full line) and the NIR spectrum of standard polyamide (black dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

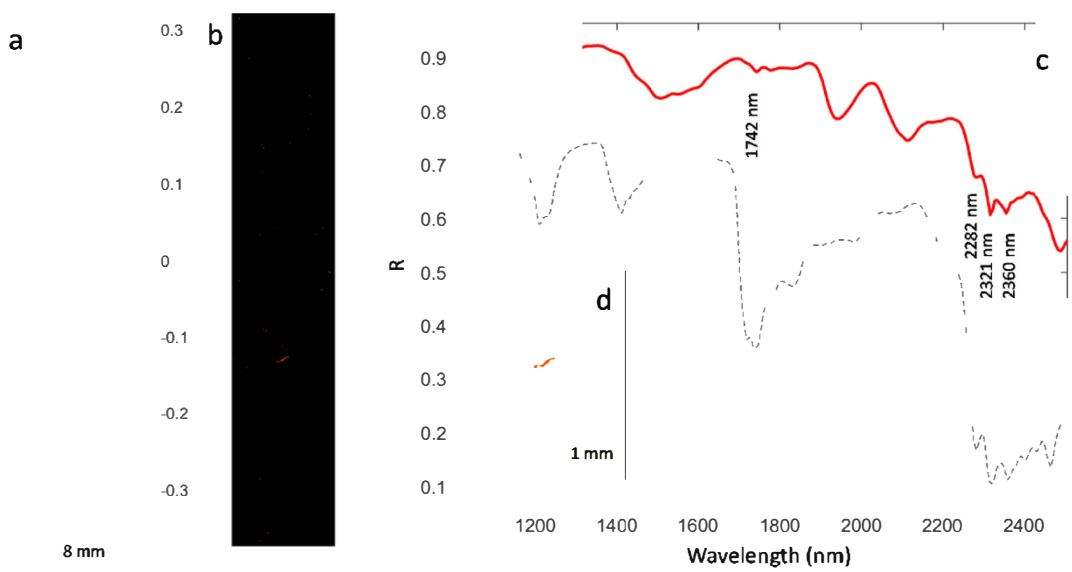

Fig. 7. Cellulose filter with polypropylene MP identified after the filtering 5 L of seawater. a) NDI; b) segmented NDI; c) comparison between the average NIR spectrum of the NDI red pixels (red full line) and the NIR spectrum of standard polypropylene (black dashed line); d) magnification of the NDI portion containing the MP fiber detected. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

nm). The similarity in the spectral profile may support the identification of an unknown MP by direct application of the NDI strategy, simply by considering the common bands for polymers. Indeed, once extracted, the averaged NIR spectrum associated to the particles detected, can be examined entirely and compared with spectra of reference standards, for a more robust identification of the polymer. Applying this procedure, PP was identified in the extracted sea water sample (Fig. 5c). This is in line with previous findings from the same study area, where PP has been recognised as the most abundant MP in seawater samples, mussel soft tissue and mussel biodeposits (Piarulli et al., in prep). PP is, indeed, widely used as a constituent polymer for nets and ropes (Frère et al., 2017), and thereby is not surprising its presence in a harbour area such as the one where the sampling was performed. No MP were identified in real mussels' tissue samples, although this should not be considered as a methodological issue but as a common trend in MP distribution in biota. In fact, MP content in soft tissue of organisms can be very variable be-tween different species and even between single individuals of the same species (Piarulli et al., 2010b, 2019). Particularly, MP quantification in suspension-feeding bivalves can be very challenging as these organisms can egest synthetic particles > 1 0 μm (MP dimensional range often detectable in field samples) in few hours (Piarulli and Airoldi, 2020; Ward et al., 2019). Further, previous findings shown in Piarulli et al. (2020b) reported an average value of 0.03 MP per organism in mussels from the same area.

The results presented in this work, together with the complementary findings highlighted in Piarulli et al. (2020a) and Vidal and Pasquini (2021) for other polymer typologies and sample matrices, demonstrate and empirically strength the potentialities of NIR–HSI– based methods as cost and time effective techniques for the MP detection in various and heterogeneous environmental samples.

4. Conclusions

The present study demonstrates the wide applicability and efficiency of the NIR-HSI method for the qualitative detection of MP in complex environmental matrices, addressing the still open research issues related to the identification of MP in aquatic systems.

The successful spectral identification of the MP in real samples, boosted by information obtained on particle morphology and shape, demonstrates the suitability of the method for the monitoring of envi-ronmentally occurring MP.

It is worth noticing that the spatial resolution of the NIR HSI system is not as high as those of other spectroscopic techniques implemented at the microscopic scale, which are able to detect very small MP down to 11 μm. Nevertheless, the fast and largely automatable NIR-HSI analytical procedure provides an efficient and cost-effective method for the analysis of a large number of environmental samples, with complex and heterogeneous matrices, directly on filters and without any extensive sample manipulations. Further, the NDI procedure, coupled with the automatic background segmentation, permits to sensibly reduce the time needed for the processing and the evaluation of data, boosting the potentialities of the HSI method. Future methodological implementations will be required to conduct qualitative and quantitative analyses on large and diverse sets of samples and environmental matrices, as well as to test the performance of NIR-HSI on a wider range of MP shapes, including microfibres, and different polymers. The benefits of a large-scale implementation of the method include the possibility to conduct extensive, and rather fast, monitoring of spatial and temporal distribu-tion of MP in the aquatic environment and biota which are essential knowledge for assessing the environmental risks associated to MP contamination in natural marine systems.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.131861.

Credit author statement

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References

Airoldi, L., Ponti, M., Abbiati, M., 2016. Conservation challenges in human dominated seascapes: the harbour and coast of Ravenna. Reg. Stud. Mar. Sci. 8, 308–318. https://doi.org/10.1016/j.rsma.2015.11.003.

Bartlett, J.K., Maher, W.A., Purss, M.B.J., 2018. Near infra-red spectroscopy quantitative modelling of bivalve protein, lipid and glycogen composition using single-species versus multi-species calibration and validation sets. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 193, 537–557. https://doi.org/10.1016/j.saa.2017.12.046.

Bråte, I.L.N., Hurley, R., Iversen, K., Beyer, J., Thomas, K.V., Steindal, C.C., Green, N.W., Olsen, M., Lusher, A., 2018. *Mytilus* spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: a qualitative and quantitative study. Environ. Pollut. 243, 383–393. https://doi.org/10.1016/j.envpol.2018.08.077.

Carbery, M., O'Connor, W., Palanisami, T., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environ. Int. 115, 400–409. https://doi.org/10.1016/j.envint.2018.03.007.

Cincinelli, A., Scopetani, C., Chelazzi, D., Martellini, T., Pogojeva, M., Slobodnik, J., 2021. Microplastics in the black sea sediments. Sci. Total Environ. 760, 143898. https://doi.org/10.1016/j.scitotenv.2020.143898.

De Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. Microplastics as an emerging threat to terrestrial ecosystems. Global Change Biol. 24, 1405–1416. https://doi.org/10.1111/gcb.14020.

Frère, L., Paul-Pont, I., Rinnert, E., Petton, S., Jaffré, J., Bihannic, I., Soudant, P., Lambert, C., Huvet, A., 2017. Influence of environmental and anthropogenic factors on the composition, concentration and spatial distribution of microplastics: a case study of the Bay of Brest (Brittany, France). Environ. Pollut. 225, 211–222. https:// doi.org/10.1016/j.envpol.2017.03.023.

Galgani, Hanke, Werner, De Vrees, 2013. Marine litter within the European Marine Strategy Framework DirectiveICES. J. Mar. Sci. 70, 1055–1064. https://doi.org/10.1093/icesjms/fst122.

Gallo, F., Fossi, C., Weber, R., Santillo, D., Sousa, J., Ingram, I., Nadal, A., Romano, D., 2018. Marine litter plastics and microplastics and their toxic chemicals components: the need for urgent preventive measures. Environ. Sci. Eur. 30 https://doi.org/ 10.1186/s12302-018-0139-z.

He, D., Luo, Y., Lu, S., Liu, M., Song, Y., Lei, L., 2018. Microplastics in soils: analytical methods, pollution characteristics and ecological risks. TrAC Trends Anal. Chem. (Reference Ed.) 109, 163–172. https://doi.org/10.1016/j.trac.2018.10.006.

Li, J., Lusher, A.L., Rotchell, J.M., Deudero, S., Turra, A., Bråte, I.L.N., Sun, C., Shahadat Hossain, M., Li, Q., Kolandhasamy, P., Shi, H., 2019. Using mussel as a global bioindicator of coastal microplastic pollution. Environ. Pollut. 244, 522–533. https://doi.org/10.1016/j.envpol.2018.10.032.

Lipik, V.T., Yang, G., 2018. Carbon containing material in non-woven fabrics for thermoinsulation of Winter apparel. IOP Conf. Ser. Mater. Sci. Eng. 460 https://doi.org/10.1088/1757-899X/460/1/012045.

Lorenz, C., Roscher, L., Meyer, M.S., Hildebrandt, L., Prume, J., Löder, M.G.J., Primpke, S., Gerdts, G., 2019. Spatial distribution of microplastics in sediments and surface waters of the southern North Sea. Environ. Pollut. 252, 1719–1729. https://doi.org/10.1016/j.envpol.2019.06.093.

Lu, H.C., Ziajahromi, S., Neale, P.A., Leusch, F.D.L., 2021. A systematic review of freshwater microplastics in water and sediments: recommendations for harmonisation to enhance future study comparisons. Sci. Total Environ. 781 https:// doi.org/10.1016/j.scitotenv.2021.146693.

Lusher, a.L., Welden, N.a., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Method. 9, 1346–1360. https://doi.org/10.1039/C6AY02415G.

Malegori, C., Alladio, E., Oliveri, P., Manis, C., Vincenti, M., Garofano, P., Barni, F., Berti, A., 2020. Identification of invisible biological traces in forensic evidences by hyperspectral NIR imaging combined with chemometrics. Talanta 215. https://doi.org/10.1016/j.talanta.2020.120911.

Morais, L.M.S., Sarti, F., Chelazzi, D., Cincinelli, A., Giarrizzo, T., Martinelli Filho, J.E., 2020. The sea anemone Bunodosoma cangicum as a potential biomonitor for microplastics contamination on the Brazilian Amazon coast. Environ. Pollut. 265, 114817. https://doi.org/10.1016/j.envpol.2020.114817.

O'Connor, J.D., Mahon, A.M., Ramsperger, A.F.R.M., Trotter, B., Redondo-Hasselerharm, P.E., Koelmans, A.A., Lally, H.T., Murphy, S., 2020. Microplastics in freshwater biota: a Critical review of isolation, characterization, and assessment methods. Glob. Challeng. 4, 1800118. https://doi.org/10.1002/gch2.201800118.

Piarulli, S., Airoldi, L., 2020. Mussels facilitate the sinking of microplastics to bottom sediments and their subsequent uptake by detritus-feeders. Environ. Pollut. 266 https://doi.org/10.1016/j.envpol.2020.115151.

Piarulli, S., Scapinello, S., Comandini, P., Magnusson, K., Granberg, M., Wong, J.X.W., Sciutto, G., Prati, S., Mazzeo, R., Booth, A.M., Airoldi, L., 2019. Microplastic in wild populations of the omnivorous crab *Carcinus aestuarii*: a review and a regional-scale test of extraction methods, including microfibres. Environ. Pollut. 251, 117–127. https://doi.org/10.1016/j.envpol.2019.04.092.

Piarulli, S., Sciutto, G., Oliveri, P., Malegori, C., Prati, S., Mazzeo, R., Airoldi, L., 2020a. Rapid and direct detection of small microplastics in aquatic samples by a new near infrared hyperspectral imaging (NIR-HSI) method. Chemosphere 260. https://doi. org/10.1016/j.chemosphere.2020.127655.

Piarulli, S., Vanhove, B., Comandini, P., Scapinello, S., Moens, T., Vrielinck, H., Sciutto, G., Prati, S., Mazzeo, R., Booth, A.M., Van Colen, C., Airoldi, L., 2020b. Do different habits affect microplastics contents in organisms? A trait-based analysis on salt marsh species. Mar. Pollut. Bull. 153 https://doi.org/10.1016/j.marpolbul.2020.110983.

Popescu, C.M., Popescu, M.C., 2013. A near infrared spectroscopic study of the structural modifications of lime (Tilia cordata Mill.) wood during hydro-thermal treatment. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 115, 227–233. https://doi.org/10.1016/j.saa.2013.06.002.

Salzer, R., 2008. Practical Guide to Interpretive Near-Infrared Spectroscopy. Von Jerry Workman, Jr. und Lois Weyer. Angew. Chem. 120, 4706–4707. https://doi.org/10.1002/ange.200885575.

Schwanninger, M., Rodrigues, J.C., Fackler, K., 2011. A review of band assignments in near infrared spectra of wood and wood components. J. Near Infrared Spectrosc. 19, 287–308. https://doi.org/10.1255/jnirs.955.

Sciutto, G., Prati, S., Bonacini, I., Oliveri, P., Mazzeo, R., 2014. FT-NIR microscopy: an advanced spectroscopic approach for the characterisation of paint cross-sections. Microchem. J. 112, 87–96. https://doi.org/10.1016/j.microc.2013.09.021.

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup : synthetic polymers in Mediterranean surface waters. Nat. Publ. Gr. 1–10 https://doi.org/10.1038/srep37551.

Underwood, A.J., Chapman, M.G., Browne, M.A., 2017. Some problems and practicalities in design and interpretation of samples of microplastic waste. Anal. Method. 9, 1332–1345. https://doi.org/10.1039/c6ay02641a.

Vidal, C., Pasquini, C., 2021. A comprehensive and fast microplastics identification based on near-infrared hyperspectral imaging (HSI-NIR) and chemometrics. Environ. Pollut. 285, 117251. https://doi.org/10.1016/j.envpol.2021.117251.

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ. Sci. Eur. 26, 12. https://doi.org/10.1186/s12302-014-0012-7.

Ward, J.E., Shumway, S.E., 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 300, 83–130. https://doi.org/10.1016/j.jembe.2004.03.002.