

Contents lists available at ScienceDirect Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.journals.elsevier.com/spectrochimica-acta-part-amolecular-and-biomolecular-spectroscopy



# Detection of microplastics in sea salt using hyperspectral imaging and machine learning methods: Pollution control in the Mediterranean sea as a case study \*

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# HIGHLIGHTS

## Hyperspectral imaging (HSI) to monitor microplastics in Mediterranean Sea is proposed.

- Key methodology features to evaluate microplastic in natural salt samples are optimised.
- Multivariate analysis is applied to discriminate polluted and unpolluted salt samples.

## G R A P H I C A L A B S T R A C T



# ARTICLE INFO

Keywords: Microplastic sea pollution Hyperspectral imaging Machine learning Mediterranean Sea

# ABSTRACT

Microplastics represent 80% of the marine waste, becoming one of the main problems worldwide today, one of the reasons they have been categorised as the 10th greatest threat in the World Economic Forum's Global Risks Report 2024. To address this issue, many recognised organisations have developed action plans for monitorization, mitigation and prevention of microplastic contamination. This includes the development of analytical methods for the detection, characterisation and quantification of these contaminants. In this regard, this work presents a novel approach for the direct detection and analytical evaluation of microplastics in sea salt sampled from solar sea saltworks. These factories act as a natural 'pre-concentrator' of solid pollutants, and sea salt is thus a good indicator of their presence in the marine environment.

The developed methodology is based on the application of hyperspectral imaging a non-destructive/noninvasive analytical technique, in combination with machine learning methods, to detect five of the most common microplastics (PE, PET, PS, PP, PVC) in natural sea salt samples collected directly from a solar saltworks located on the Mediterranean coast of southern Spain. For this purpose, some key features were assessed to develop the methodology, including sample bank generation, particle size determination, imaging conditions,

\* This article is part of a special issue entitled: 'IASIM-2024 Special Issue' published in Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. \* Corresponding authors.

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https://doi.org/10.1016/j.saa.2025.126528

Received 7 March 2025; Received in revised form 16 May 2025; Accepted 2 June 2025 Available online 3 June 2025 1386-1425/© 2025 The Author(s). Published by Elsevier B.V. This is an open access article under

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and others. Finally, once the HSI analyses were performed directly on the solid salt samples, partial least squarediscriminant analysis was applied to develop a classification model capable of identifying salt-containing pixels and thus detecting  $\mu$ P pollution.

# 1. Introduction

Plastic can be defined as a material which contains as an essential ingredient a high polymer and which, at some stage in its processing into finished products, can be shaped by flow [1]. The global production of these materials started around 1950's, growing rapidly, reaching a staggering 413 million metric tons in 2023 and expecting to double this amount by 2039. This unprecedented growth is mainly explained by the exceptional characteristics of plastics, such as strength, lightness and durability, which have made them valuable in countless industry sectors. Nevertheless, their stability and resistance to degradation combined with a poor waste management has resulted in their accumulation across multiple ecosystems [2,3]. According to the last studies, 14 million tons of plastic enter the ocean system annually, representing the 80 % of the marine waste and becoming one of the main problems worldwide today [4]. Polluting plastics in the environment, particularly in the sea, are subject to degradation process due to the exposition to UV light, temperature, currents, waves and other natural factors which cause their fragmentation. These small-sized plastic fragments are called 'microplastics' ( $\mu P$ ) when their size ranges from 5 mm to 1  $\mu m$ , and 'nanoplastics' (nP) from 1 µm to 1 nm [5].

 $\mu$ P enter food chains when animals ingest them by mistaking them for food. These materials, although chemically inert, may absorb toxic substances found in the ecosystem and thus become toxic carriers. Once ingested by animals, they pass into the circulatory and lymphatic systems, even accumulating in fatty tissues, making them a potential health hazard [6,7]. This is one of the reasons why  $\mu$ P are ranked as the 10th greatest threat in the World Economic Forum's Global Risks Report 2024, for which concerted and urgent action is required [8]. To meet this challenge, several international organizations have developed action plans for monitoring, mitigating and preventing these pollutants. Among them, it is worth mentioning the action plan published by the European Commission in 2023 against  $\mu$ P. These actions include support for research into new methods for detecting  $\mu$ P in the sea and for harmonised monitoring and assessment methods for nano-, macro- and micro-plastic pollution [9].

Evidence of the growing concern for this issue is the drastic increase in scientific publications in recent years [10]. In this regard, several recent studies have focused their research on the development of analytical methods capable of detecting, typifying and/or quantify  $\mu$ P in different environmental samples, i.e., atmospheric air, natural water bodies (seas, lakes, rivers, etc.), sediments, and soils [11–15]. In addition, analytical monitoring of  $\mu$ P in food samples has recently gained rising interest as a response to the increasing occurrence of these contaminants in the food chain [16–18]. Despite the diversity of these samples, their preparation for analysis converges in the follow steps: filtration and/or density separation, digestion, often using H<sub>2</sub>O<sub>2</sub>, and, for solid samples, a water-based pre-dispersion step [19–22]. The shared purpose of these sample preparation methods is to separate  $\mu$ P (analytes) from the material matrix. Subsequently, they are analysed applying one of the analytical techniques mentioned bellow.

The most widely applied analytical techniques are visual inspection [23], Raman spectroscopy and Fourier transform infrared spectroscopy (FT-IR) [24], including the use of portable miniaturised instruments [25]. However, when higher quantification reliability is required, methods combining thermal decomposition with gas chromatography coupled to mass spectrometry (GC–MS) are more convenient [26]. Depending on the analytical technique applied, the result of the  $\mu$ P content quantification is expressed differently. Visualisation-based methodologies such as optic microscopy and spectroscopic techniques

report it as  $\mu$ P 'items' or particles per gram (or per litre) of sample, according to the material's original physical state. In contrast, when a chromatographic method is used, results are usually expressed in  $\mu$ g/g or  $\mu$ g/L of material. This dichotomy makes it very difficult to compare results as the equivalence between the two units is not easy to establish since it is necessary to know the density and size of each  $\mu$ P 'item' or particle. Detection capabilities vary significantly: optical microscopy can reveal  $\mu$ P particles up to 50  $\mu$ m in size, Raman spectroscopy detects particles as small as 1  $\mu$ m and FT-IR reaches 10  $\mu$ m. GC–MS, in comparison, has detection limits of 100 to 1000  $\mu$ g of  $\mu$ P [11,27].

Notwithstanding this range of options, or perhaps as a result of it, there is concern about the lack of harmonisation and standardisation of test procedures used by different laboratories, which all too often leads to inconsistent results [28]. Nevertheless, efforts are being made to mitigate this disadvantage, and some standardised methods have been published or are in preparation by recognised standardisation bodies, e. g. ASTM [29,30] or ISO [31–33]. In addition, the European Union has recently reported a directive setting a methodology for sampling and characterising  $\mu$ P in water intended for human consumption [34]. These initiatives have recently been reinforced with the EU funding of a COST Action, titled 'ISO compatible, efficient and reproducible protocols/ equipment for micronanoplastic detection through machine-learning (ICPLASTIC)' (https://www.cost.eu/actions/CA23131/).

Despite these successes, a critical challenge persists: no analytical method has vet been developed to analyse environmental samples directly, without requiring a prior preparation. The steps of sample preparation procedure, which involve separating µP from solid or liquid matrices, may be complex and resource-intensive, relying on solvents, filters, and other materials. Imaging analytical techniques, particularly hyperspectral imaging (HSI), which is able to perform non-invasive, in situ analysis and real-time monitoring, has great potential to address this issue [35-38]. In this context, there are studies that address the optimal spatial resolution and the most suitable spectral ranges in an integrated manner. For example, Huang et al. [39] conducted a review in which they highlighted that most of the studies employed reflectance mode for their analyses, covering a spectral range from 370 nm to 2500 nm. All of them applied principal component analysis (PCA) as an exploratory analysis and the classification methods were support vector machine (SVM) or partial least squares-discriminant analysis (PLS-DA). The review also highlights the relationship between detection limits and spatial resolution, emphasizing that increasing spatial resolution can lower the minimum detectable particle size. The smallest reported detection limit (LOD) was greater than 0.2 mm, achieved with a spatial resolution of 30 µm. Faltynkova et al. [40] conducted another literature review in which they highlighted that the spectral range covered in the reviewed studies spans from 375 to 2500 nm, identifying spatial resolution as a critical factor influencing the results. The best performance was achieved with a spectral resolution of 3 nm, while the average spectral resolution across most studies was approximately 6 nm. Serranti et al. [41] proposed an optimal analytical protocol for characterizing microplastics. They evaluated three kind of plastic materials that were cut into three size ranges and disposed in a black background. Their capture device had spatial resolutions or either 150 µm or 30 µm, and the spectral ranges studied were 1000-2500 nm or 1000-1700 nm. Finally, they concluded that for particles larger than 250  $\mu m,$  PLS-DA showed superior class recognition at a spatial resolution of 150  $\mu$ m/pixel within the 1000-1700 nm range, offering a cost- and time-efficient solution with high accuracy and faster processing. For particles smaller than 250  $\mu m,$  a spatial resolution of 30  $\mu m$  combined with nonlinear methods yielded better results, especially within the broader 1000-2500 nm

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 343 (2025) 126528

spectral range. Similarly, other researchers have also conducted studies focused on the detection of microplastics in soil and aquatic matrices, primarily utilizing spectral ranges between 1000–2500 nm and 387–1034 nm, achieving spatial resolutions ranging from 6.45  $\mu$ m to 21  $\mu$ m [42–44].

Note that the previously mentioned studies are focused on pretreated environmental samples or directly on  $\mu P$  sample characterisation. This restricts their capacity to simplify workflows and leverage the advantage of being environmentally friendly.

Solar saltworks act as a natural 'pre-concentrator' of solid pollutants, including  $\mu$ P, presents in the source water to be tested. In both cases, water from the sea, natural deposits or from mineral-rich springs, enters the saltworks by pumping and it is deposited in crystallisers, which constitute the storage ponds. Different sequential precipitation processes take place in them. The final one happens in the crystallisation tank where the sea salt precipitates and is finally collected. Solid pollutants present in water are in turn deposited on the salt, which is an indicator of their presence in the medium [45]. The occurrence of  $\mu$ P in salt has attracted significant scientific attention, either as an indicator of environmental contamination or as a food product that could pose risks to human health.

Despite its relatively low direct consumption compared to other daily food, salt is used in a wide variety of products, making it necessary to subject salt to quality and safety controls, including assessing its contamination. Exposure to µP through salt intake has been shown to be significant, with levels reaching  $(1.4-2.0) \times 10^4$  items/kg of salt in regions such as Croatia, which has reported the highest contamination levels [46]. Similar studies conducted in Spain have revealed µP concentrations in Spanish table salt ranging from 50 to 280 particles/kg. The most prevalent plastics identified were polyethylene (PE), polyethylene of terephthalate (PET) and polypropylene (PP), consistent with their widespread use in various food industry applications. At these levels of contamination, it is estimated that the Spanish population could annually ingest between 91 and 500 µP particles only through salt consumption [47–50]. Other polymers such as polyvinyl chloride (PVC), polyamide-6 (nylon-6), polystyrene (PS) or polyacrylonitrile (PAN) have also been detected in analyses of salts from other countries [51,52].

This study aims to address the development of an environmentally friendly analytical methodology based on the application of HSI, which enables the direct evaluation of µP in natural salt samples jointly with machine learning methods, to detect µP presence in environmental sea salt samples. For this, raw salt samples from coastal and inland solar saltworks were sampled and analysed. Particularly, this study has focused on the determination of the five most common kind of µP present in sea salt from the Mediterranean Sea: PE, PET, PP, PVC and PS, as indicator of µP pollution [53-55]. The novelty of this strategy is its ability to perform direct analysis in solid samples, without any sample preparation involving water, filters, digestion or solvents to perform any separation or pre-concentration of µP from the sample matrix. This is significant, because it simplifies the analytical process, reduces analysis times and the risk of laboratory contamination, which otherwise may compromise the representativeness and reliability of the results when detecting these pollutants.

## 2. Methodology

To design a direct analytical method based on HSI for the detection of  $\mu$ P in sea salt samples, key issues of the methodology were carefully assessed. These included: (i) plastic standards and salt sample bank; (ii) simulated  $\mu$ P-free sea salt; (iii) equalisation of sample particle size and sample preparation; (iv) set-up HSI capturing system; and (v) data analysis. This section provides a detailed discussion of each one of the key issues considered.

## 2.1. Natural salt samples and $\mu P$ standards

Three different material types were identified as essential to develop the analysis methodology. Firstly, representative  $\mu$ P-free natural salts. Secondly, natural salts suspected of being contaminated and, finally, representative specimens of the contaminating  $\mu$ P to be tested.

Natural salts were sampled from two types of solar saltworks: (i) inland saltworks located in Granada (Spain) and (ii) coastal saltworks located in Almería (Spain), both located on the Mediterranean watershed. On the one hand, inland salt samples were considered unpolluted since these rely on underground brines or evaporation of water from mineral-rich springs, which are usually protected from anthropogenic debris, being its only source of pollution the environmental and given that the salt production period is shorter than that of coastal salt works and the latter are exposed not only to the environment but also to plastic waste present in the sea, inland salts have been considered in this study "blank salt samples".

A total of 232 natural salt samples were analysed in this study, 212 from inland saltworks and 36 from coastal saltworks. Note that, in the case of natural salt from coastal saltworks, two types of salt were sampled, common salt and 'flower of salt'. The later differs from common salt in the process of evaporation of the water and its subsequent collection, which is left in smaller tanks, where a small layer of crystals is created and floats on the surface of the water, which are then collected daily in a traditional way due to the fragility of the crystals.

Five chemically-pure standards of the most commonly used plastics in industry, and whose presence has been previously reported in the Mediterranean Sea (PE, PET, PP, PVC and PS) [52,53], were purchased from a recognised supplier (Sigma Aldrich, Darmstadt, Germany). The relevant information about these samples is shown in Table 1.

In addition, small pieces of the same plastics were manually retrieved from everyday objects made of these materials (bottles, caps, cups, etc.). Samples of everyday plastic items are representative of common-life materials, which together with the polymer incorporate additives commonly found in final products. Specimens from both origins (Sigma

#### Table 1

Key properties and structural characteristics of the plastic polymers considered in the study.



\* Density values extracted from ISO 4484-2 standard[31]

Aldrich and common objects) were considered as  $\mu$ P standards. Details about the preparation of these specimens are given in section 2.3.

Most standard  $\mu P$  samples appeared white after grinding, while commercial packaging samples ranged from amber to green, blue, or transparent. Although visible color can affect reflectance within the VIS range, its influence in the SWIR is minimal. However, additives used to generate visible colors may cause slight spectral shifts near the VIS-SWIR boundary, though the overall spectral shape remains mostly unchanged.

# 2.2. µP-free simulated sea salt reference

Given that the composition of blank salt samples is not the same as sea salt, and to have a reliable reference that would resemble the composition of the salt obtained in the Mediterranean Sea, it was decided to prepare a mixture of pure inorganic salts bringing together the major components of sea salt. This simulated salt emulating the chemical composition of coastal salt was prepared by mixing the following solid chemical compounds: NaCl (62.9 g/100 g), MgCl<sub>2</sub> (11.4 g/100 g), CaCl<sub>2</sub> (2.1 g/100 g), KCl (2.1 g/100 g), Na<sub>2</sub>SO<sub>4</sub> (17. 7 g/100 g), MgSO<sub>4</sub> (2. 9 g/100 g) and CaSO<sub>4</sub> (1.2 g/100 g). The composition of the blend was fitted based on the data provided in reference [56].

## 2.3. Specimen size uniformity

The hypothesis underlying this study suggests that unpolluted natural salt is a homogeneous solid matrix, consisting largely of small white crystals of sodium chloride (>60 %) along with other mineral salts. In contrast, when salt contains solid contaminants such as  $\mu$ P these occur as dispersed particles throughout the crystalline material, resulting in discernible heterogeneity. These exogen particles may be detected applying HSI.

As described above,  $\mu P$  range in size from 5 mm to 1  $\mu m$  and occur in forms such as spheres, pellets and fibres. To achieve this size range on the material fragments it is necessary to equalise the sample particle sizes to ideally achieve a one-particle-per-pixel configuration, whether the particle belongs to the salt matrix or to the plastic pollutants. This equalisation was carried out by milling the samples in a laboratory milling device (IKA A10 basic, Staufen, Germany). Then, a particle size study was carried out to examine the relationship between milling time and µP particle size, ensuring a uniform particle sizing. This also made possible to fine-tune the HSI image capturing settings to align with the target particle size. Four milling times were selected for the study: 0 (original packing size), 5, 10 and 15 s. Samples of PVC and PE, sourced from manufacturers, were milled accordingly to evaluate the impact of milling time on particle size distribution. A granulometry study was performed using a Mastersizer 2000LF (Malvern Panalytical, Malvern, UK) particle size analyser.

To ensure the evenness of the particle size of all natural and simulated salt samples, all of them were equalised using the mill described above. For this purpose, around 20 g of each of the salts under study were milled for each sample. For imaging, test portions of the salt samples were placed in colourless glass Petri circular dishes of 60 mm diameter and 15 mm depth, which were filled to the brim.

## 2.4. Study of optimal capture set-up parameters for HSI measurement

The first step for HSI measurement was the identification and selection of the optimal spectral range which depends on the components of the material to be measured: both analytes ( $\mu$ P) and the matrix material (natural salt). To achieve this, a thorough literature review was conducted to identify the chemical structural features of the five selected  $\mu$ P (see Table 1) and their characteristic IR spectral fingerprints. Note that, density values of each polymer presented in Table 1 were extracted from the ISO 4484–2 standard [31] which provide guidelines to perform qualitative and quantitative  $\mu$ P analysis in textile industry. This information provided the basis for selecting the most relevant spectral range for detecting and distinguishing each one of these materials.

Several studies have focused on the  $\mu$ P detection and identification by HSI in the visible-short wavelength (400–2500 nm) spectral region with great success [40,57,58]. In particular, Vidal & Pasquini [57] identified some combinations of bands associated with C–H bones of  $\mu$ P structure in the region of 2000–2500 nm. However, the first and the second overtone of C–H stretching vibration were observed in 1600–1800 nm and 110–1220 nm region. For PET, the stretching vibration of C–H aromatic bonds was observed in 2100–2200 nm while, ester group in 1800 to 2000 nm and C-O stretching third overtone in 2082 nm. Methyl groups absorb near to 1195 nm and methylene to 1210–1220 nm. Phenyl groups, present in polystyrene were observed at 2170 nm. These characteristics explain why most of the published studies on this topic have selected the SWIR (short-wave infrared) spectral region, typically ranging from 1000-2500 nm, as the optical working spectral range.

Given this, it was decided to focus this study on the SWIR spectral range (from 900 to 1700 nm in our case, due to the capture device available: Resonon PikaNIR + camera from Resonon Inc, Canada). Furthermore, an additional HSI camera (Resonon PikaL) which measures in visible-near infrared (VNIR, from 400 to 1000 nm) spectral region was used to assess whether the primary differences observed in this region were only related with characteristics such as colour rather than chemical composition of the  $\mu P$ .

Once the working spectral range was selected, a study was performed to determine the optimal imaging settings. The key features to consider are spatial resolution and capturing time. The spatial resolution is directly related to the minimum plastic particle size that can be detected within the crystalline salt matrix. Besides, capturing time needs to be minimised specially for a long image monitoring campaign with hundreds of measurements yet ensuring enough amount of signal for a robust image capture.

A critical imaging setting which significantly influences both spatial resolution and capturing time is the imaging distance (i.e., the distance between the camera and the material sample being measured). Capturing at a closer distance to the sample (i.e. reducing imaging distance), reduces the linear field of view (FOV or swath), but increases the spatial resolution. This happens because the image size and the angular field of view are constant while the object area spanned by the image gets smaller. Hence, each pixel is imaging a smaller region achieving higher spatial resolution (i.e., gaining ability to discriminate smaller details in the captured image). If only considering this, one may think that then, getting as close as possible to the sample would be the best practice. This idea however has some limitations. One of the limitations could be thought of as a mechanical and optical restriction. The optical components in the imaging equipment have limits regarding the closest possible distance to be focused, and object distance also critically influences depth of focus (i.e. the range of distance that result focused in the final image). In addition to this effect, the mechanical components including the illumination lamps, the scanning stage device, or the size and shape of the samples mechanically limit the closest achievable distance too. As it can be seen in Fig. 1, the scanning device has a horizontal linear stage with a vertical column. This column is where both the camera and the illumination system are attached, giving the possibility to change the illumination and imaging distances.

The illumination system was placed as close as possible to the object yet allowing the scanning of the samples in a safe way, and the cameras had some freedom of movement up and down across the vertical column. The SWIR PikaNIR + camera is bigger than the VNIR PikaL, so that its range of movement in terms of object distance went from 40 cm (the furthest) to 3.5 cm (the closest), using an optical extension tube to allow for very close focusing distances as done in [59]. For the VNIR camera, the moving interval went from 8.5 cm to 60 cm.

The second limitation when deciding what imaging distance to be used is capturing time. If a horizontal:vertical ratio of 1:1 is to be retained for the final images (i.e., images neither stretched nor



**Fig. 1.** Benchtop scanning system with the SWIR spectral camera and some salt samples protected from external contamination prior to image capture process. (1) Resonon PikaIR + camera; (2) halogen lamps; (3) linear stage with white reference placed on it; (4) salt samples; (5) computer.

compressed horizontally), the closer we set the camera to the sample, the slower the image capture must be (for a given framerate), since in each frame (i.e. each captured image line), a smaller portion of the sample image is captured the closer we get. Even if optimizing the imaging framerate, exposure time and scanning speed to achieve the fastest possible image captures at this distance, using the closest possible positions in both cameras ended up in very slow capturing speeds which took more than a minute of capturing time for a portion of 1 cm of the object in the case of the SWIR camera. This capturing time could be allowed for imaging a few samples, but it is not practical for measuring hundreds of samples in monitoring campaigns continuously receiving samples.

On the other extreme, using the furthest positions for both cameras, resulted in very fast image captures, but the spatial resolution was underexploited, since the FOV of the cameras allowed imaging the material samples closer while framing them completely into the final image. Hence, it was decided to place the cameras to obtain for both a linear FOV as similar possible, with the additional constraints of being able to image most of the sample container area (measuring around 28 cm<sup>2</sup>). The imaging distance was then 21.5 cm for the VNIR camera and 16 cm for the SWIR camera. A theoretical spatial resolution can be calculated using the FOV covered by each camera and the corresponding sensor sizes, by simply dividing the FOV by the sensor size (see Table 2. for the exact data used in this estimation). The estimated spatial resolutions were 55.5  $\mu$ m/pixel for the VNIR camera, and 95.3  $\mu$ m/pixel for the SWIR camera. As explained before, this constitutes a theoretical limit for the spatial resolution.

## Table 2

Instrumental settings selected for capturing hyperspectral images using each spectral camera.

Camera	Pika L	Pika IR+	
Spectral range	VNIR (400 nm - 1000 nm)	SWIR (900 nm - 1700 nm)	
Number of bands	121	161	
Wavelength step	5 nm	5 nm	
Pixels/line	900	640	
Exposure time	1.26 ms/line	9.22 ms/line	
Frame rate	249.4 fps	87.18 fps	
Capturing time	8.66 s	14.45 s	
Imaging distance	21.5 cm	16 cm	
Focal length	23 mm	25 mm	
Vertical FOV	$13.1^{\circ}$	$21.7^{\circ}$	
Swath	5 cm	6.1 cm	
Scanning speed	1.39 cm/s	0.83 cm/s	
Number of lines	2250	1350	

However, more factors are to be considered like the fact that the spectral signal from a single pixel could be not smooth enough to achieve a good identification as pointed out in [59]. These are not necessarily the smallest particle sizes detectable by each system, but at least we can know that bigger particle sizes should be represented by more than a single pixel in the final images.

## 2.5. Image capturing strategy

Once the optimum conditions for analysis have been selected as indicated in the previous section, it should be pointed out that spectral reflectance images were used instead of raw images. This is done since their image values are only depending on the characteristics of the sample and neither the spectral power distribution of the illumination nor the spectral sensitivity of the cameras has an impact on them. Hence, in order to work in reflectance mode, reference white and dark images are captured as well, using the same capturing parameters as for the samples, to perform dark noise subtraction and flat-field correction according to equation (1).

$$\rho_{sample}(\mathbf{x}, \mathbf{y}, \lambda) = \frac{I_{sample}(\mathbf{x}, \mathbf{y}, \lambda) - I_{dark}(\mathbf{x}, \mathbf{y}, \lambda)}{I_{white}(\mathbf{x}, \mathbf{y}, \lambda) - I_{dark}(\mathbf{x}, \mathbf{y}, \lambda)} \rho_{white}(\lambda)$$
(1)

In this equation,  $\rho_{sample}$  (x,y, $\lambda$ ) is the spectral reflectance value of the sample at pixel position (x,y) and wavelength  $\lambda$ . I<sub>sample</sub>, I<sub>white</sub> and I<sub>dark</sub> are the raw spectral images of the sample, the reference white target and the dark field (camera lens covered to block light in) respectively. A 90 % reflectance standard reference white tile was used as the white target (model Zenith Lite, by SphereOptics, Herrsching, Germany).

The HSI images were captured over batches of samples. Each batch included 2 samples placed in different Petri dishes on a white platform for spectral scanning. The first sample of each batch was the same and was consistently kept in the same position throughout all captures as a quality control.

#### 2.6. Data analysis

#### 2.6.1. Sample data handling

HSI images were stored as a 'band interleaved by line' file (.bil) and converted to MATLAB file format later (.mat). The Matlab version used was R2019a version (Mathworks Inc., Natick, MA, USA).

Once the spectral reflectance image was created according to equation (1), a region of interest (ROI) was selected for each sample using a MATLAB graphical user interface (GUI). This GUI automatically detected the centre of the circular petri dishes and extracted a centred square area. The ROI was both big enough to have a large number of pixels and small enough to avoid edge or background pixels. The imaging conditions were the same for all samples and the size of the petri dishes did not change across captures. The ROI size was then fixed at 273  $\times$  273 pixels for the SWIR camera and 607  $\times$  607 for the VNIR camera. Each pixel captured by the SWIR camera contained a spectrum with 161 spectral bands, and for the VNIR camera the number of bands was 121. As a result, each ROI consisted of a 3D data structure (ROI data pseudo cube, which is indeed a data parallelepiped) of 273  $\times$  273  $\times$  161 elements, which is a total of 74,529 spectra per salt sample or µP standard when SWIR camera was employed and of 607  $\times$  607  $\times$  121 elements which is a total of 368,449 spectra per salt sample or µP standard when VNIR camera was used.

For each ROI data pseudo cube, the representative average spectrum of each salt sample and  $\mu P$  standard was computed and stored separately. Fig. 2 shows a schematic view of this process.

As it is shown in Fig. 2, three spectral bands were selected to create a false colour image (false RGB), using them as red, green and blue image channels respectively. The purpose of these false colour images was only for visually inspecting that both the full images were correctly captured, and the ROIs were correctly retrieved containing only sample pixels and not edge of background areas. The spectral data used for the classification models were retrieved from the spectral images.

Moreover, in order to verify that the imaging conditions remained unchanged throughout the capturing process, a similarity analysis was performed on the average spectra of the batch-to-batch control samples. In this regard, the nearness index (NEAR) was calculated between each pair of average spectra [60]. Once the consistency of the quality control samples was ensured, the analysis of the data salt samples and microplastic standard was carried out.

#### 2.6.2. Multivariate discrimination models

All multivariate data treatment was carried out using PLS\_Toolbox (Eigenvector Research Inc. MA, USA, version 8.6.1) working under the MATLAB framework. Hierarchical clustering analysis (HCA) was employed as exploratory analysis in order to detect natural grouping of all the samples.

PLS-DA was selected as discrimination machine learning method in order to identify if the natural salt from coastal saltworks was polluted with microplastics and thus confirm that such samples can be used as a strategy to control the presence of these plastic materials in the Mediterranean Sea. To train and validate the PLS-DA model, the data set was split into training set (70 %) and validation set (30 %) subsets using the CADEX algorithm, i.e., Kennard-Stone's method [61]. Model outcomes are assessed by quality performance metrics for classification including sensitivity, specificity and precision [62].

## 3. Results and discussion

This section describes and discusses the results obtained after carrying out the steps explained in section 2.

## 3.1. Specimen size uniformity study

Fig. 3 displays the particle size distribution from samples took from PVC and PE standards before and after 15 s of milling. Although additional milling times (5 and 10 s) were assessed, their results are not included in the figure, as it was found that particle size distribution remained unchanged beyond 10 s. Consequently, only relevant data points representing the initial and final distribution are shown.

The particle sizes of original PVC were more uniform or homogeneous than PE. PVC particles ranged from 70 to 400  $\mu$ m, with most of the population concentrated around a medium size of 170  $\mu$ m. In contrast,



Fig. 2. Scheme of the ROI extraction from the hyperspectral reflectance full images and the false colour RGB images generated using bands 1600, 1200 and 1000 nm from both the full images and the retrieved ROIs. Petri dishes contain inland saltworks (left) and sea PVC (right). The mean spectra from the two ROIs are shown in the bottom.



**Fig. 3.** Size distribution curves resulting from the granulometric study on the original standard plastic material and after grinding for 15 s: (A) PVC, and (B) PE.

PE exhibited a broader particle size range in their original state (280  $\mu$ m medium size), spanning from approximately 10 to 1000  $\mu$ m. Thus, the size range of PE was significantly wider. However, after 15 s of milling,

the size distribution of both plastics became more similar. This effect was less significant in PVC, suggesting that its initial particle size was already close to the minimum size achievable with the milling method used. Upon completing the milling procedure, the bulk of PVC particles were close to 170  $\mu$ m in size, approximately, whereas the distribution of PE particles' size peaked around 230  $\mu$ m. Note that these sizes refer to diameters, considering that the particles are spherical in shape.

Based on these findings, it can be confirmed that the milling enabled the control of particle size across different plastics, irrespective of their composition or original size.

## 3.2. Natural and simulated reference salts and $\mu P$ standards imaging

Fig. 4 shows the average spectra of all the pixels within the ROI, extracted from the five plastic standards and from the three salt samples:  $\mu$ P-free simulated sea salt reference samples prepared in the laboratory and the salts sampled from inland (blank) and coastal saltworks. Significant differences can already be found between the average spectra from the  $\mu$ P standards and the salt samples, as well as among the spectra of the different types of  $\mu$ P standards. Likewise, there are slight differences between the spectra of the three types of salt samples, with the reference salt having a higher reflectance up to 1500 nm. However, within the VNIR range (not shown in Fig. 4) these differences are less marked, and the result is that they are not easily discernible to the naked eye. This highlights that using the SWIR range is a priori a better option for the purpose of separating salt from  $\mu$ P.

## 3.3. Multivariate analysis: Detection of $\mu P$ in sea salt

A preliminary exploratory analysis was conducted by building an HCA model in order to evaluate the spectral similarities between blank salt samples and  $\mu$ P standards. The goal was to determine whether the signals acquired by both VNIR and SWIR cameras yielded similar results or if one of them showed fewer promising ones and could be excluded from subsequent steps.

Fig. 5 displays the results of the HCA model through dendrograms,



Fig. 4. Example of ROI average spectra of the five µP standards (top), and the three types of salts: µP-free simulated reference, sea coastal and inland (bottom).



**Fig. 5.** Dendrogram of the ROI average spectra from inland salt samples (red shaded), and μP standards samples (green shaded). The measurements correspond to VNIR (A) and SWIR spectral ranges (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

which were generated using the ROI average spectra of blank salt and  $\mu P$  reference standards as input data. The spectra captured using the VNIR camera did not provide a clear grouping, leading to an unclear separation between samples. However, the signals obtained using the SWIR camera clearly show a split into two clusters corresponding to the spectra of salts and plastics, showing a variance-weighted distance between their centroids of approximately 200 arbitrary units. These preliminary results confirm the observations extracted from the observation of the spectra shown in Fig. 5.

The most noticeable differences are observed within the  $\mu P$  standards cluster, which is expected given the diverse chemical compositions of the different types of plastic materials. The spacing between the  $\mu P$  standard first subsets is about twice as large as for the salt samples. This can indicate the  $\mu P$  exhibit notable intra-cluster variability due to their distinct chemical composition, while the blank salt samples are more evenly matched. The findings from this preliminary analysis led to the decision to develop the classification models using only the signals captured from the SWIR camera as input data.

The development of the multivariate model to detect  $\mu P$  in salt samples consisted of the following steps: first, a PLS-DA model aiming to discriminate between  $\mu P$ -free salt samples and plastic materials was developed. The model was trained and externally validated using two sets of samples: blank/simulated reference sea salt and different plastic materials from pre-milled commercial product packaging/  $\mu$ P standards (see section 2.1). The model was built with 5 latent variables (LVs) which explained a 99.52 % of the variance, using mean-centering as the preprocessing method.

Two input classes (2iC) were defined: ' $\mu$ P-free salts' (class 1) and 'microplastics' (class 2). A crucial aspect of the model development was the selection of the spectral signals to be used for each of the two classes. For representing Class 1, the average spectra of the ROIs of the blank salt samples and the spectra from several randomly selected pixels of the ROI of the simulated sea salt were used (see Table 3 for details). Class 2 was represented by the spectra of several pixels randomly selected from the ROIs of each of the standard and packaging plastic samples (see Table 3). Although this model training strategy is slightly unusual, it is nevertheless valid since a spectral reflectance image from a particulate solid material is nothing more than a set of independent spectra, one for each pixel, which are acquired simultaneously, so that the spectral information of each pixel is unique. The use of the average spectrum of the blank salt can mitigate the influence of the presence of some pixels that might be polluted with  $\mu$ P or other exogenous substances commonly found in

#### Table 3

Spectra employed to build the PLS-DA model.

Class	Sample/specimen		Number of spectra	
			Training set	Validation set
Class 1 (µP-free salts)	Blank salt Simulated sea salt		149 200	63 30
Class 2 (microplastic)	μP standards	PE	14	6
		PET	14	6
		PP	14	6
		PVC	84	6
	·	PS	14	6
	Plastics from commercial	PE	25	5
	packaging	PET	71	19
		PP	63	47
		PS	44	16
TOTAL			692	210

salt mines, since their effect on the average spectrum is cancelled out by the large number of uncontaminated pixels. Thus, the average spectrum reliably represents the composition of most of the salt pixels.

The model results are summarized in the score plot showed in Fig. 6A. The decision criterion established for the classification of the samples was a threshold value of 0.65, i.e. all the spectra with scores greater than 0.65 were classified to class 1 and spectra with scores lowers than 0.65 were assigned as belonging to class 2. It is noteworthy that all spectra in the external validation set were correctly classified; this means the model is able to reliably discriminate between µPpolluted and uP-free salt samples. The classification performance of the PLS-DA model with respect to Class 1 ('µP-free salts') was evaluated through training, cross-validation, and external validation [62]. During the training step, the model achieved an RMSEC of 0.18, with sensitivity, specificity, precision, F1-score and accuracy all equal to 1.0. Crossvalidation results yielded an RMSECV of 0.19, keeping all classification metrics (sensitivity, specificity, precision and F1-score) equal to 1.0. In the external validation step, the model reached an RMSEP of 0.23, with sensitivity, specificity, precision, F1-score and accuracy also equal to 1.0, confirming the robustness of the model. A confusion matrix corresponding to the external validation results is shown in Fig. 6B.

Additionally, a second evaluation was conducted to corroborate the initial findings. In this case, the test samples were some of the  $\mu$ P-free





Fig. 6. (A) PLS-DA classification plot showing both training and external validation set. Class 1 ('µP-free salts ') and class 2 ('microplastics'). The red line marks the 0.65 threshold level. (B) External validation contingency chart of the PLS-DA classification model. Results are classified according to class 1 (µP-free salts) and class 2 (microplastics), considering class 1 as the target class. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

simulated sea salt reference samples that were previously contaminated by sprinkling on the surface around 20  $\pm$  2 mg of standard PVC microparticles. Then, the spectra of all the pixels making up the ROI, totalling 74,529, were classified by the previously developed model. The results are showed in Fig. 7. The spectra are distributed above and below the classification threshold, differentiating pixels that contain  $\mu P$  (below the threshold) from those that do not (above the threshold). Notice that previously, when unpolluted salt was tested in this same classification model (Fig. 6), all pixels were found to be free of contamination. This suggests that the model could identify spectra with a higher contribution of  $\mu P$ , classifying them into class 2 ('microplastics').

Once the model was externally validated as explained above, 36 salt samples from the coastal saltworks were analysed individually to assess the presence of  $\mu$ P pollution in the Mediterranean Sea. The 74,529 spectra within each sample's ROI were classified to evaluate the  $\mu$ P presence. 20 samples corresponded to common salt and 16 to flower of salt, and significant differences were found between the two salt types.

For common salt samples, the model detected less presence of pixels polluted with microplastics, classifying most of them in class 1 (µP-free salts). However, for the flower of salt samples, the model detected polluted pixels in higher amounts. The classification results on the 16 samples showed that in 4 samples there were 2–3 % of polluted pixels, in 2 samples were around 0.4 % while in 6 samples this number was below 0.1 %. In the remaining 4 samples, no polluted pixels were detected. In Fig. 8, three common salt samples are shown as an example: one unpolluted, one with approximately 3 % of polluted pixels, and the third with around 0.4 % of polluted pixels. Moreover, another observation that can be formulated from Fig. 8 is that, in some natural salt samples, there are pixels identified as polluted whose classification scores lie close to the decision threshold. This may be explained since the pixel size exceeds that of the microplastics, a pixel with a high contribution from plastic and a minimal presence of natural salt is still correctly recognised by the model as polluted, but its output probability is pushed nearer to the class discrimination threshold.

The differences observed between flower of salt and common salt samples might be due to the different collecting and producing methods. The flower of salt is produced in the cold of the night and is left floating on the surface of the water in the salt ponds. It is collected manually at dawn before the water is heated by the sun. In this way, any solid pollutants floating on the water are collected together with the flower of salt. On the contrary, common salt is collected from the bottom of the ponds once all the water has evaporated. Based on these findings, it could be assumed that flower of salt is a good candidate for further detailed study linking the presence of  $\mu P$  in salt from coastal solar saltworks to  $\mu P$  contamination in the sea.

# 4. Conclusions and future perspectives

The use of hyperspectral imaging together with machine learning methods has allowed the development of a sustainable analytical methodology to directly detect the presence of  $\mu$ P in solid sea salt samples. A multivariate classification method using SWIR range spectra was developed and validated using three different sample subsets. This methodology is in line with the 12 principles of green analytical chemistry (GAC) and its subsequent evolution to detect microplastics in solid samples such as salt. Likewise, the proposed analytical methodology has demonstrated its potential for the evaluation of the presence of pollution from microplastics in marine waters of the Andalusian Mediterranean coast, taking as an indicator of pollution the  $\mu$ P content in sea salt sampled directly from coastal saltworks in the area under study. To our knowledge, this is the first time that such strategy has been applied for this purpose.

The next stage of the study will aim to relate the percentage of pixels reported as contaminated to the overall  $\mu$ P content present in the salt sample. This task is not straightforward, as it is not possible to directly relate the concentration of pollutants with the abundance of contaminated pixels. Since the salt is partially translucent in the SWIR range, using the abundance of polluted pixels found in the surface of the sample might lead to overestimating the amount of  $\mu$ P present. Nevertheless, it is reasonable to assume that there is a correlation between the abundance of polluted pixels and the concentration of pollutants in the sample.

The proposed method involves direct testing of samples collected in the saltworks and could prove to be more practical than the previous analytic procedures involving collection of water samples and several preparation steps, with a view to continued monitorization of the  $\mu P$  content in the sea.

# Funding

Project (PCM-00042) is funded by the «Next Generation EU» Recovery Fund through the 'Recovery, Transformation and Resilience



Fig. 7. PLS-DA classification plot of 74,529 pixel-spectra embedded in the ROI extracted from the reflectance image of the µP-free simulated sea salt reference sample which was polluted with PVC.



Fig. 8. PLS-DA classification plots of the 74,529 pixel-spectra within the ROI extracted from the reflectance images of the different flower of salt samples: (A) unpolluted salt; (B) polluted salt (3% of pixels) and (C) polluted salt (0.4% of pixels).

Plan' and co-financed by the Consejería de Universidad, Investigación e Innovación de la Administración de la Junta de Andalucía (Regional Ministry of University, Research and Innovation of Andalusia). The viewpoints and opinions expressed herein are exclusively the author(s)' and are not necessarily linked to those of the European Union or the European Commission. Neither the European Union nor the European Commission are responsible for them.

## 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.

# Acknowledgements

AMJC acknowledges the Grant (RYC2021-031993-I) funded by MICIU/AEI/501100011033 and "European Union NextGeneration EU/ PRTR". In addition, authors acknowledge to Salins Ibérica S.L for supplying sea salt samples from the sea coastal saltworks located in Cabo de Gata (Almeria), and to Salinas de La Malahá S.L (Granada) for supplying salt samples from the inland saltworks. Funding for open access charge: Universidad de Granada / CBUA.

#### Data availability

Data will be made available on request.

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