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Determination and classification of microplastics in sea salt of Spanish origin

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Abstract

Microplastics account for plastic less than 5 mm in their largest dimension. Their widespread presence in all environmental compartments, especially in the marine environment, has become an important environmental issue. In fact, the process of salt harvest in salt ponds is a susceptible process to be contaminated by microplastics which would be, originally, in the water column and would end up in the salt ponds.

Therefore, in the present study it was determinated and classificated the microplastics found in 15 different samples of Spanish sea salt, which were processed using vacuum filtration systems through filters of 50 and 25 µm. The determination and classification of microplastics was performed by direct count with a stereoscope. The analysis of particle composition was performed with µFTIR and for the packaging of the samples, with FTIR. Among the 1202 particles determined, 79.0% were fibres, 13.7% were fragments, 6.4% were films, 0.5% were foams and 0.4% were microbeads; and the colour proportion was 58% colourless, 21% blue, 6% black, 5% red, 4% green and 7% others. Microplastic content found was 10 – 190 microplastics/kg, being cellulosic the most frequently found polymer (57.1%), followed by polyethylene-terephthalate (20.4%), polypropylene (6.7%) and Polyvinyl chloride (2.5%). Lastly, it was determined that there are no significant difference depending on salt origin (*p* value = 0.6371), but further data was needed to establish a solid hypothesis.

Key words: *Microplastics, sea salt, µFTIR, Spain*.

Resumen

Los microplásticos son plásticos de tamaño inferior a 5 mm. Su amplia presencia en el medio natural y, especialmente, en el medio marino, se ha convertido en un importante problema ambiental. En este sentido, la producción de sal marina en las salinas es un proceso susceptible de sufrir contaminación por microplásticos que estarían, originalmente, en la columna de agua y quedarían retenidos en las salinas.

En el presente estudio se han determinado y clasificado los microplásticos encontrados en 15 muestras diferentes de sal marina de origen español, que fueron procesadas mediante sistemas de filtración al vacío a través de filtros de 50 y 25 µm. La determinación y clasificación de microplásticos se llevó a cabo mediante conteo directo con ayuda de un estereoscopio. El análisis de la composición de partículas se hizo con µFTIR y el del empaquetado de las muestras, con FTIR. Entre las 1202 partículas determinadas, el 79,0% fueron fibras, el 13,7% fragmentos, el 6,4% films, el 0,5% espumas y el 0,4% microesferas; y la proporción de color fue 58% incoloro, 21% azul, 6% negro, 5% rojo, 4% verde y 7% otros. El contenido de microplásticos encontrado fue de 10 a 190 microplásticos/kg, siendo la celulosa el polímero más frecuentemente encontrado (57,1 %), seguido del tereftalato de polietileno (20,4 %), el polipropileno (6,7 %) y el cloruro de polivinilo (2,5 %). Por último, se determinó que no existen diferencias significativas en función del origen de las sales (*p* valor = 0.6371), aunque más datos fueron necesarios para poder establecer una hipótesis sólida.

Palabras clave: *Microplásticos, sal marina, µFTIR, España.*

Introduction

The salt

Sodium chloride, an ionic compound which chemical formula is NaCl, forms the white crystalline solid product commonly known as salt. It is a widespread compound that appears naturally in rock formations or seawater, therefore being an abundant mineral on Earth.

It maintains a 1:1 ratio between its sodium and chlorine ions, which are essential elements for both animals and plants. Indeed, sodium chloride is essential to maintain the electrolyte balance of fluids in a human’s body (MedlinePlus, n.d.).

Regarding its chemical properties, sodium chloride is a soluble compound in water and other polar solvents, presenting a solubility of 35.7 g/100 mL at 0 ºC. When dissolved in a liquid, it directly affects the liquid boiling point by increasing it, and the freezing point, decreasing it. It decomposes only at high temperatures to produce toxic fumes of disodium oxide (Na_2O) and hydrochloric acid (HCl). Additionally, the molecular weight of NaCl is 58.44 g/mol (National Center for Biotechnology Information, n.d.).

As for sodium chloride uses, it could be said that almost every person in the world has either some direct or indirect contact with salt daily. It is used all around the globe in order to help preserve and enrich food products, or to habilitate many industry related procedures. Furthermore, it is present in a wide variety of household products concerning water conditioning, highway de-icing and various agricultural applications (National Center for Biotechnology Information, n.d.).

Besides, by treating sodium chloride with different procedures it can be shifted into many types of salt. Each country has a different classification of salts, while organizations like the World Health Organization (WHO) treat them equally for their investigations/studies (World Health Organization, n.d.).

As the samples from the present investigation are original from Spain, their classification follows the “Real Decreto 1424/1983”, of the 27th of April, in which is approved the official Technical-Sanitary Regulations for the obtaining, circulation and sale of salt and edible brines. Precisely, on the 3rd article it is stated these different types of salt classification:

-Rock salt. From natural saline deposits.

-Sea salt. From the evaporation of seawater.

-Spring salt. From saline springs by evaporation of brines.

-Refined salt. It is rock salt, sea salt or spring salt purified by washing or dissolution followed by crystallization.

-Salting salt. Edible salt that is tolerated a magnesium content higher than normal because it is intended for salting.

-Table salt. Refined salt whose grain size is equal to or less than 2.0 millimetres, moisture 0.5 per 100 maximum and which may contain authorised caking agents.

-Kitchen salt. It is refined salt defined in point 3.6 whose humidity does not exceed 5.0 per 100.

-Special salts. Defined as refined salts to which various authorized substances have been added and which are declared on the labelling of the containers. These include:

- Iodized salt. It is the salt to which potassium iodide, potassium iodate, or another iodinated derivative authorized by the General Directorate of Public Health has been added in the proportion suitable for the finished product to contain 60 milligrams of iodine per kilogram of salt, allowing a tolerance of 15 per 100.

- Fluoridated salt. It is the salt to which sodium fluoride or another fluorinated derivative authorized by the General Directorate of Public Health has been added in the proportion suitable for the finished product to contain between 90 and 225 milligrams of fluoride per kilogram of salt.

- Iodine fluoride salt. It is the salt that contains, together, the compounds of iodine and fluoride, authorized by the General Directorate of Public Health, within the limits set out in points 3.8.1 and 3.8.2.

- Nitrite salt. It is the salt to which sodium nitrite has been added in the maximum proportion of six grams per kilogram of salt.

- Other salts. They are those made with edible salt that can be added other food substances that may be authorized by the General Directorate of Public Health.

History of salt

The actual commonness and availability of salt make the importance of it in history overlooked by today's society. Nevertheless, it has been one of the most important pieces for the creation and evolution of many vital procedures and historical events of all time, profoundly influencing human thinking and lifestyle.

Regarding its importance in socioeconomics, throughout history salt has played a central role in the economies of many regions, representing a major source of revenue for those who managed to control the salt monopolies. For example, important salt sources used to be surrounded by religious centres and monasteries, which shared the proprietorship and production rights with the local nobility.

In fact, salt transportation was a catalyst for the creation of the very first roads (the *via salaria*, Ancient Rome); the establishment of the earliest cities was also motivated due to salt trading (Cirillo et al., 1994). Furthermore, the first tunnel in the Alps, Monte Viso, was built in 1480 to facilitate the transport of salt from Provence to Savoy (Ritz, 1996).

Dating back to the 16th century, coal replaced the use of wood as fuel, linking the salt industry to the coal industry and giving a huge impulse to the industrialization of salt farming procedures (Cirillo et al., 1994).

As for the Iberian Peninsula, due to its climate, topography, and geographical location it was one of the most important centres of salt during the 17th century, with salt being produced both in Portugal and Spain (Cirillo et al., 1994).

Salt has also appeared in history as a dispute factor. As for peasants salt was indispensable for curing food and raising cattle; government decisions like the implementation of taxation in the form of salt produced major conflicts that led to events such as the French revolution (Ritz, 1996) or the Indian War of Independence (Cirillo et al., 1994). Nevertheless, other countries such as Switzerland found in the preservation properties of salt a way of doing business peacefully, settling up as major producers of salted meat or cheese and supplying it to European armies (Ritz, 1996).

As for the medical history of salt, since ancient times, it has been known to counteract putrefaction, an effect due to the high osmolarity of saline solutions which arrests bacterial growth. Furthermore, saline solutions were advised in many health problems, as for gastrointestinal problems such as diarrhoea; or bruises and osteoarthropathies because of its capacity to reduce tissue swelling through osmotic pressure (Cirillo et al., 1994). Moreover, the Latin words for health and healthy, *salus* and *salubris*, are actually derived from salt, *sal;* or the common greeting in french, “salut”, is derived from salt as well (Ritz, 1996).

Additionally, salt has been invested with many cultural, symbolic and religious connotations. In connection with its main characteristics (purity, whiteness, obtainable from sun and sea, etc) it was treated like a divine gift and a sacred object, becoming an emblem in many cultures. In fact, it appears many times in the bible as a symbol of perpetuity, wealth, purity, or strength; or in the “Cenacolo” by Leonardo da Vinci, as a knocked-over saltshaker which symbolizes the imminent treason of Judas (Ritz, 1996) .

Salt role in the ocean

The system of prevailing currents around the globe constitutes an ocean conveyor belt which allows water masses circulation both in shallow and deep waters. Whereas wind and other factors contribute to the transport of water masses, the “engine” of this conveyor belt relies on the characteristic density of each water mass, which is in fact determined by temperature, pression and salinity.

Moreover, is the ocean circulation chain, influenced by the salinity variations, which helps distribute nutrients and regulate climate change (Figure 1). Therefore, without salinity gradient variations it would not be possible to maintain the ecosystems and climate around the globe.

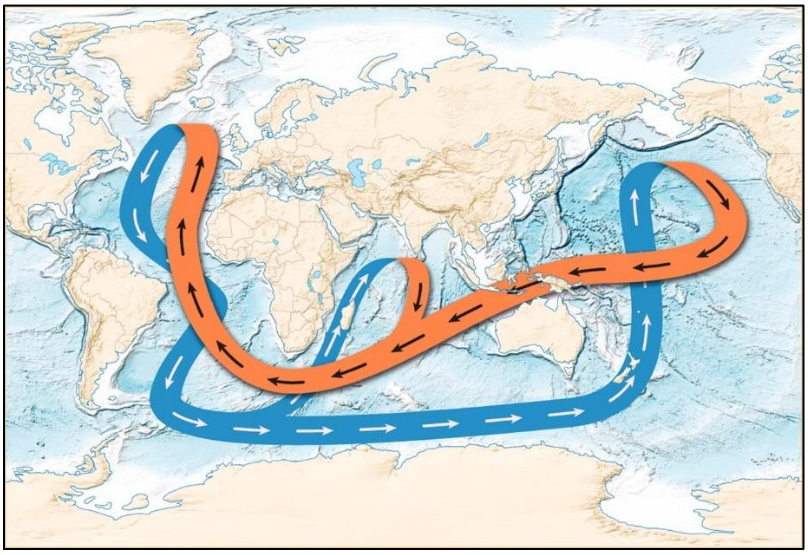


Figure 1: Ocean conveyor belt representation. Source: Lozier, 2010.

Nevertheless, natural seawater is a complex material as oceans are sources of life with constant chemical and physical reactions, where about 3.5% of seawater is dissolved salt. (*NASA Salinity: Overview*, n.d.).

Therefore, establishing an official and scientifically appropriate way of measuring salinity has been a challenging process throughout the years due to the complexity of the material of its composition and various imperfections in the existing measuring techniques. Nowadays, based on the Thermodynamic Equation Of Seawater-2010 (TEOS-10), salinity can be defined as:

- Absolute salinity (S_A): mass fraction of dissolved material in seawater.
- Practical salinity (S_p): salinity obtained from the measurement of the seawater conductivity ratio, which depends on temperature and pressure. All salinity values stored in scientific databases are in practical salinity.

Besides, ocean salinity varies geographically and with time. Freshening and salinification occur in certain places around the globe, commonly staged by the processes of evaporation, precipitation, river flows and ice formation. Consequently, salinity measures emerge as a useful way of tracking water masses.

For instance, in the North Atlantic and Indian Oceans, discharges from the highly evaporative Mediterranean and Red Seas cause a vertical salinity maximum at mid-depth (about 1000 to 2000 m deep). The relatively fresh but dense surface water from higher latitudes also enters the subtropical and tropical zones at mid-depth and generates a vertical salinity minimum, which is particularly common in the Southern Hemisphere and North Pacific (Talley et al., n.d.)

Methods of salt obtention

Salt is produced from salt mines or by evaporation either from seawater or mineral-rich spring water in shallow pools. These unrefined salts have various small amounts of minerals, sediments or even algae which can affect the colour and taste of the salt. Moreover, this characteristic composition reflects where the salt was harvested.

On the one hand, in salt mines the salt found in them is originated from the evaporation of ancient seas and can be mined directly or by pumping solvent (water) into the deposit.

On the other hand, salt in evaporation ponds evaporates naturally as a result of solar irradiation. These evaporations ponds are shallow basins (natural or artificial) designed to extract salt, being typically located near the coast to reduce the need for water transport and delivery. As they depend on the climatology, salt ponds are usually located in warm climates with high evaporation rates and low precipitations.

Regarding the process of salt extraction, the ocean water is firstly passed to the evaporation ponds. Once there, by solar evaporation the salinity level starts increasing from the initial 3%, which is typical for marine water, to levels near 25-30%. At these levels of salinity, the salt starts crystalizing and its harvest becomes available. The time spent in the entire process of evaporation and harvest varies depending on the climate, but it can take up to five years (*Evaporation Ponds for Salt*, n.d.) .

Nowadays, some safety measures have been implanted during this process. For instance, the seawater is often filtered in order to remove contaminants before solar evaporation and, if the evaporation ponds are built artificially, they must have impervious lining beneath to protect underlying aquifers.

Microplastics

Plastics are synthetic organic polymers mainly derived from hydrocarbons extracted from crude oil or natural gas. Their popularity comes from its properties as they are inexpensive, lightweight, strong and, most importantly, really durable (Pirsaheb et al., 2020).

This durability is what makes their destruction slow, becoming a persisting contaminant in the environments while accumulating in the long term. In fact, between 1950 and 2015, 6.3 billion tons of plastic waste were generated, of which 12% was incinerated, 9% recycled, and the remaining 79% was either stored in landfills or has been released into the different ecosystem compartments (Rhodes, 2018).

Handling some of the newest data, in 2021 about 390.7 million tons of plastic were produced (Figure 2), of which 90.2% was fossil-based and only 9.8% represented circular plastics. Regarding the production distribution, China accounts for one third of the world´s plastics production (32%), followed by the rest of Asia with a 17%. Lastly, for its uses, packing was the most common application (44%) followed by plastics used for building and construction (18%) (Plastics Europe, 2022).

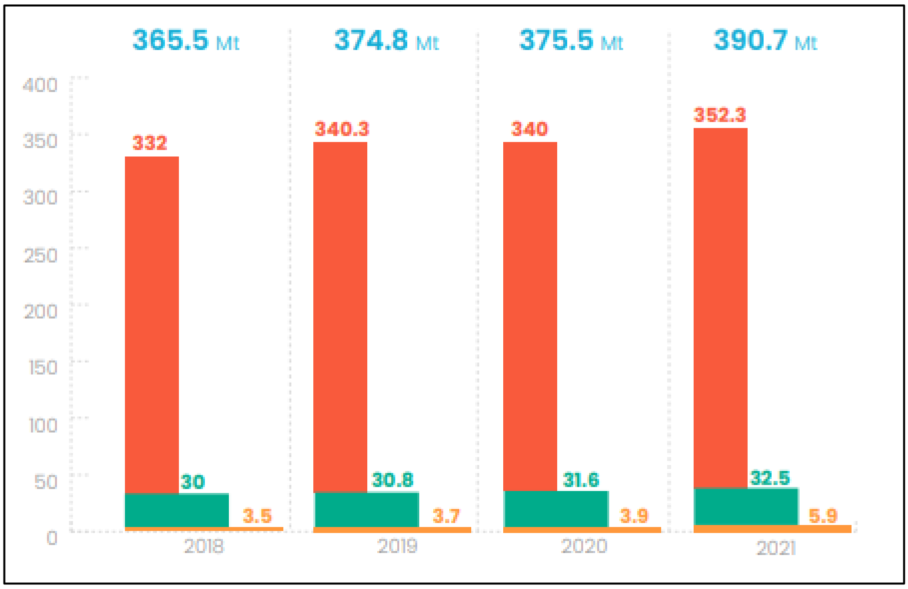


Figure 2: World plastic production evolution. Mt: million tonnes. Red: Fossil-based plastics; green: Post-consumer recycled plastics, Yellow: Bio-based plastics. Source: Plastics Europe, 2022.

Within the term “plastics” microplastics are found, denomination which, according to the US National Oceanic and Atmospheric Administration (NOAA), accounts for plastic less than 5 mm in their largest dimension (U.S. Department of Commerce, 2023).

These microplastics can be divided into primary and secondary. Primary microplastics are directly crafted less than 5 mm in size mainly for industrial and domestic use, while secondary microplastics were larger plastics that due to progressive fragmentation via different agents ended up breaking into that size. Nevertheless, regardless of the origin, they are mainly divided into fibres, fragments, foams, pellets and microbeads (Crawford & Quinn, 2017) **.**

On account of the impact of microplastics as pollutants, their importance comes from their widespread presence in all environmental compartments and their wide range of sizes, chemical composition, density, shape, and colour.

Concerning the marine environment, plastics distribute along the oceans following the path of the five large systems of circulating ocean currents. Therefore, these gyres, apart from driving the oceanic conveyor belt, are drawing all the floating pollution that is released into the ocean and forming what are called as “garbage patches”.

Precisely, the North Pacific Subtropical Gyre is the one with most marine debris concentration, being mostly plastic. This is attributed to substantial oceanic plastic sources in Asia and the fishing activity in the Pacific Ocean. The plastic mass estimation calculated using aerial survey data, in situ observations and trawl devices, is around 79 000 tonnes, it being mostly made of polyethylene (PE) and polypropylene (PP) pieces (Lebreton et al., 2018).

Consequently, plastics of small size and microplastics pieces can be easily mistaken as food by a wide range of marine biota (zooplankton, fish, molluscs, etc) and they can accumulate in their guts or even in other tissues such as gills or the circulatory system, ultimately affecting enzymatic activity and cell metabolism. Furthermore, a study published this year introduced the term "*plasticosis*", regarding a new plastic-induced fibrotic disease in seabird stomach tissues, which could be affecting other marine organisms (Charlton-Howard et al., 2023).

Moreover, other pollutants such as persistent organic pollutants (POPs) and polychlorinated biphenyls (PCBs) are able to adsorb and concentrate onto the microplastics, making them potential conveyors of this type of pollutants into the tissues of animals that ingest these plastic particles (Pirsaheb et al., 2020).

Microplastics present in sea salt

As mentioned previously, salt plays a fundamental role in various biochemical reactions and the proper functioning of vital physiological processes. Therefore, it becomes an indispensable element in the human diet.

Regarding salt intake, it is not until the 19th century that it started to get into consideration that although an adequate amount of salt is indispensable for life, a too elevated salt intake could be unhealthy.

So, despite the fact that sodium chloride has been verified as of low concern based on experimental and modelled data (U.S. Environmental Protection Agency, n.d.), it has been proved that elevated levels of dietary sodium (consumed as common salt) are associated with raised blood pressure and adverse cardiovascular health (Brown et al., 2009).

In fact, individuals of the most developed countries are often unaware of the amount of salt consumed on a daily basis, as the majority of it is hidden in processed foods. Concretely, when referring to European and Northern American diets, about 75% of the sodium intake comes from processed or restaurant-prepared foods, while the remaining 25% is equally distributed in the sodium naturally composing foods and the one used discretionary at home. So, although the WHO recommends that sodium intake of adults should be <85 mmol/day, most adult population have a mean sodium intake that excess 100 mmol/day (WHO and FAO, 2003). For instance, lean beef or roast beef have a sodium content of 2.1 mmol/100g, while corned beef or canned beef have a sodium content of 41.3 mmol/100g; and the same happens for marine products as raw or steamed salmon have a sodium content of 4.8 mmol/100g while smoked salmon has a concentration of 81.7 mmol/100g (Brown et al., 2009).

Nevertheless, sodium intake is not the only problem attached to the over ingestion of sea salts as other trace elements are present, as well as food contaminants. Concretely, as of the high disposal and environmental stability of microplastics, they are nowadays considered emerging food contaminants (Rubio-Armendáriz et al., 2022), and its presence in the food web has been demonstrated in a wide variety of studies.

Although not enough investigation about the cumulative human exposure to microplastics has been conducted, it has been reported that there is potential immunotoxicity through immunosuppression and immune activation, also causing disruption of the oxidative stress control or genetic expression. Even so, the knowledge gaps are still a risk characterization barrier and further research is needed (Rubio-Armendáriz et al., 2022).

As a result of the growing concern about the presence (or ingestion) of microplastics in sea salt, different investigations have emerged that approach this topic, so to sum up the following table (Table 1) displays a bibliography research on previous projects related to microplastics in salts:

Table 1: Summary of the bibliographical research of microplastic presence in samples of marine salt. PP: Polypropylene, PE: Polyethylene, PES: Polyester, Ry: Rayon, NC: Nitrocellulose, CP: copolymer, PA: Polyamide, PET: polyethylene terephthalate, PS: Polystyrene, N: Nylon, PVA: polyvinyl acetate., PVC:, C: Cellulose Own elaboration.

Origin (n=number of samples)	Concentration items/kg	MPs type	Average length	Size range	Colours	Composition	Reference
Europe (n=13)	540 ± 152	Fibres (75.6%) & fragments (24.4%)	17,5 µm ± 6,1	12,6 – 2054 µm	Red (24.4%), blue (13.5%), black (7.9%), other (54.2%)	PE, PP, Ry & PES (-)	(Thiele et al., 2023)
Lebanon (n=16)	159,6 ± 157,6	- (-)	-	-	Mainly transparent, white, blue, yellow & black (-)	PP, PE & PES (-)	(Nakat et al., 2023)
Italy (n=3)	1653 ± 29	Fibres (80.6%), fragments (18.9%) & spheres (2.7%)	≤ 500 µm	3 – 5000 µm	Blue (67%), red (14%), black (10%) & white (9%)	PP, PA & PE (-)	(Di Fiore et al., 2023)
India (n=12)	1400-1900 ± (-)	Fragments (44%), fibres (39%), film (13%) & pellets (4%)	10 – 1000 µm (≥50%)	20 – 5000 µm	White (20%), transparent (18%), blue (11%) & others	PE (26%), PP (20%) & PET (20%)	(Yaranal et al., 2021)
Bangladesh (n=8)	137 ± 21	Fragments (48%), films (22%), fibres (15%), pellets (9%)	500 – 1000 µm (40%)	250 – 5000 µm	White (37%), black (17%), blue (15%) & other	PET (48%), PP (20%), PE (17%), PS (15%)	(Rakib et al., 2021)

India (n=12)	2133 ± 153	Fibres (100%)	-	-	Mainly white, blue, red & black (-)	Ny (53%), PE (27%), PP (15%) & PET (5%)	(Nithin et al., 2021)
Vietnam (n=16)	133.62 ± (-)	Fibres (-)	(-)	20 – 500(+) µm	(-)	PET, PE, PP & PS (-)	(Khuyen et al., 2021)
Africa (n=23)	38.42 ± 24.62	Fibres (93.8%)	(-)	3,3 – 4660 µm	(-)	PVA, PP & PE (-)	(Fadare et al., 2021)
South India (n=25)	(-)	Fragments (55%), fibres (42%), films (3%)	≤500 µm (60%)	0 – 2000 µm	White (45%), colourless (25%), blue (17%) & green (13%)	PE (42%), PP (23%), C (11%) & Ny (9%)	(Selvam et al., 2020)
Italy (n=6) & Croatia (n=5)	170-320 ± (-) (Italy) 70-200 ± (-) (Croatia)	Fibres (-)	(-)	10 – 150 µm	Mainly green & black (-)	Italy: PET & PVC (-) Croatia: PA & PP (-)	(Renzi et al., 2019)
Spain (n=21)	50-280 ± (-)	(-)	(-)	30 – 3500 µm	Mainly black, red, blue, white & transparent (-)	PET (83.3%), PE (3.3%) & PP (6.7%)	(Iñiguez et al., 2017)

Objectives

The main objective of this Final Degree Project is the determination and classification of microplastics in samples of sea salt of Spanish origin.

To accomplish the main objective, the following specific objectives have been set:

1. Discussion of a proper extraction and determination method for microplastics contained in different packages of commercial sea salt.
2. Performance of the morphological characterization and measurement of the microplastics found.
3. Analysis of the polymer composition of the microplastics found.
4. Determination of plastics composing the packaging of the samples to evaluate possible contamination.
5. Discussion of the geographical distribution of the microplastics found in the samples.
6. Measurement of the main ingestion of microplastics per person according to the identified samples.

Material and methods

Reagents and solutions

For dissolving the salt, double filtrated Milli-Q water was used. Also, just for one of the samples, H_2O_2 at 33% w/v was used in order to reduce the organic matter content.

Materials

Vacuum filtration systems (VWR® International) were used for sample filtration.

1000 mL glass beakers were used for sample storage and 1000 mL glass bottles were used to facilitate Milli-Q water filtration.

Metallic tweezers were used during all the sample treatment, for example for filter accommodation or particle selection for µFTIR analysis.

Washing bottles were used to maintain the cleaning protocol and to clean the material during sampling.

MF-Millipore™ 0.45 µm MCE filters were used for Milli-Q water filtration, and stainless-steel mesh filters (25 µm and 50 µm) of 4.5 cm in diameter were used to filtrate the samples in order to collect the particles in them.

Glass Petri dishes were used for storing stainless-steel mesh filters before its use. On the other hand, PP Petri dishes were used for storing stainless-steel mesh filters after its use.

Laboratory instruments

Mettler Toledo balance SB32000 (error: ±1g) was used to weigh the samples.

Fourier-transform infrared (FTIR) spectrometer Cary 630 equipped with a single reflection diamond Attenuated Total Reflectance (ATR) module (Agilent Technologies, California, USA) was used to determine the polymeric nature of the original sample packaging.

µFTIR: Thermo Scientific ™ Nicolet™ iN10 Infrared Microscope was used for polymer identification of the particles selected from the samples.

Laboratory apparatus

Various IKA® Hot plate magnetic stirrers were used in order to dissolve the salt added in the samples.

NexiusZoom EVO stereo microscope with trinocular lights was used for determination and classification of microplastics.

Two Nabertherm muffle furnaces Model L 24/11 with Flap Door were used to previously heat laboratory material in order to calcinate any contamination in it.

A Levenhuk digital camera M1400 PLUS-14Mpx attached to the stereo microscope was used for photographing all plastic particles found in the samples.

All processes performed on the samples were done inside of a sealed methacrylate glove box, which dimensions were 1x0.5x0.65 m (length x width x height). The manipulation space designed for hand introduction is 15 cm in diameter, and the glove box has a lateral door for material introduction.

Programs

Levenhuk Lite software version x64, 4.10.17659.20200906 was used for measurement of plastic particles photographed in the samples.

Microsoft Excel® was used for data processing and creation of representation graphics.

Mendeley Reference Manager version 2.89.0 was used for bibliography management.

OMNIC Picta software (ThermoFisher Scientific Inc., Massachusetts, USA) was used to operate the μFTIR: Thermo Scientific ™ Nicolet™ iN10 Infrared Microscope and identify spectra using polymers libraries.

Agilent MicroLab PC FTIR software was used to operate the Fourier-transform infrared (FTIR) spectrometer Cary 630 and identify spectra using polymers libraries.

siMPle software version 1.1 and the siMPle database version 1.0.1 were used as a second check of the spectrophotometry identification of polymers.

Open Specy online software was used as a third check of the spectrophotometry identification of polymers.

GraphPad Prism version 9.5.1 for Windows was used to make all the statistical comparisons.

Sample collection

In this study a total of 15 samples of sea salt of Spanish origin have been treated: 6 from the Canary Islands and 9 from mainland.

As the samples consist of ordinary packs of sea salt, they were bought directly from the supermarket were anyone could buy them. Characteristics of each sample are stated in Table 2. Nevertheless, to maintain confidentiality any identifying information was not made available to or accessed by anyone but the project team.

750 grams of each sample were processed, resulting in 3 replicates of 250 grams.

Table 2: Sample classification. For M2 and M9 samples no further data was available on salt origin apart from Atlantic sea origin.

Sample number	Salt origin	Salt type	Packaging composition
M1	Gran Canaria	Fine	Plastic
M2	Atlantic sea, Spain*	Coarse	Plastic
M3	Marismas de Odiel (Huelva)	Coarse	Plastic
M4	Marismas de Odiel (Huelva)	Fine	Plastic
M5	Fuencaliente (La Palma)	Fine	Plastic
M6	Lanzarote	Fine	Plastic
M7	Gran Canaria	Coarse	Plastic
M8	Murcia	Fine	Plastic
M9	Atlantic sea, Spain*	Fine	Plastic
M10	San Pedro del Pinatar (Murcia)	Fine	Plastic
M11	Murcia	Coarse	Plastic
M12	Fuencaliente (La Palma)	Coarse	Plastic
M13	Lanzarote	Coarse	Plastic
M14	San Pedro del Pinatar (Murcia)	Coarse	Plastic
M15	Villena (Alicante)	Coarse	Plastic-free

Sample treatment

First, 250 grams of salt from the desired sample were weighted inside of a glass beaker in a balance inside of the cleanroom, covering it with aluminium except when necessary for sample weighting. This process was done for each replicate. The procedural blank was kept empty but without the aluminium for the same time as the replicates in order to recreate possible airborne contamination.

Then, in order to prepare the dissolution of the samples, Milli-Q water was stored in glass bottles and brought to the glove box. Once inside of the glove box, a vacuum filtration system was prepared and a 0.45 µm filter was incorporated. The Milli-Q water was filtrated through the vacuum filtration system and used for sample preparation inside of the glove box. Once both the blank sample and the 3 replicates are prepared, Milli-Q water is also filtrated to fill a whole wash bottle.

Altogether, three replicates were done for each one of the samples, each consisting of 800 mL of Milli-Q Water previously filtrated through a filter of 0.45 µm and 250 g of the sample. The dissolution was driven by a hot plate magnetic stirrer at 500 rpm and 35 ºC until full dissolution of the salt.

Then, the samples were introduced inside the glove box, where a vacuum filtration system was prepared for use. The vacuum filtration system was equipped with a waterfall-design 3D piece which allowed accommodation for both 50 µm filters and 25 µm filters.

Before starting the procedure with the 3 replicates of the sample, the procedural blank was filtered.

After filtering the black sample in the vacuum filtration system, the entry part was rinsed 3 times with Milli-Q filtrated water. Then, while dismantling the system, the filters were picked carefully with clean metal tweezers and accommodated in previously labelled plastic Petri dishes, all done inside the glove box.

Finally, the vacuum filtration system was dismantled and cleaned whole with Milli-Q filtrated water, and the dissolution left was discarded. This procedure was common for both the procedural blank and the 3 replicates of each sample.

Additionally, only for sample 6 (M6) between 5 and 7 drops of H_2O_2 at 33% w/v were added to each filter before its removal of the vacuum filtration system in order to reduce the organic matter laying on the filter.

Once the sample treatment was finished and all the filters were inside of plastic Petri dishes, they were carried out of the cleanroom into a desiccator with silica gel in order for them to dry out for future observation.

*μ*FTIR measurements of the microplastics

After visualization and identification of microplastics in the samples, certain particles were selected for μFTIR analysis. Precisely, colourless particles were selected randomly and all colour particles were selected for analysis.

In order to ensure proper particle selection and avoid the loss of particles the whole process was carried out in the clean room while maintaining the air filters in low flow.

In order to successfully pick the particles specialized metal tweezers and a metal punch were used.

The particles were then accommodated in a 12 well plate, fitting a close range (1-3) of particles in each well depending on the size of the particles and always considering the potential risk of particle loss.

The plate was then introduced in the μFTIR and using the OMNIC Picta software (ThermoFisher Scientific Inc., Massachusetts, USA) each particle was closely analysed until achievement of a satisfactory spectrum and its proper polymer library match.

The parameters used for micro-transmission mode were as follows: spot 50 μm width/height, 16 scans, high spectral resolution, and spectral range 675 – 4000 cm^{-1}.

As recommended in the bibliography, at least 10% of each sample was analysed (MSFD Technical Subgroup on Marine Litter, 2013)

FTIR measurements of the packaging

A Fourier-transform infrared (FTIR) spectrometer Cary 630 equipped with a single reflection diamond Attenuated Total Reflectance (ATR) module (Agilent Technologies, California, USA) was used to determine the polymeric nature of the packaging of the samples, with a ZnSe beamsplitter and a 1.3 mm diameter thermoelectrically cooled deuterium triglycine sulphate (dTGS) detector.

32 scans per spectrum were applied to obtain FTIR spectra (Happ-Genzel apodization function was applied) at a resolution of 8 cm^{-1} in the range 4000 and 650 cm^{-1}. Agilent MicroLab PC FTIR software was used in order to identify spectra using polymers libraries.

The packaging of all the samples was analysed both in the outside part and in the inside part in order to compare possible differences.

Sample identification

Once dry, filters were ready for observation and identification of the microplastics with the NexiusZoom EVO stereo microscope. Each microplastic found was written down on a sheet were form, length/width, colour, and placement were listed.

In order to keep track of the microplastics found in each sample, the program Levenhuk Lite (software version x64, 4.10.17659.20200906) with its respective Levenhuk digital camera M1400 PLUS-14Mpx incorporated in the stereo microscope was used to picture, measure, and store all records of the samples.

Particles less than 50 µm were not considered unless solid reasons were stated.

Finally, following the indications of the Guidance of Marine Litter in European Seas of the European Commission, µFTIR and FTIR analysis were only definitive when a good match was obtained, which was not always possible. The suitable approach specified was to automatically accept any match >70% similarity, to individually examine matches between 60-70% similarity rejecting any samples which did not show clear evidence of peaks corresponding to known synthetic materials and to routinely reject any samples which produce spectra with a match <60%. Nevertheless, instead of rejecting completely the particles with a <60% match, they were compared with other libraries present in the siMPle software or in the Open Specy online software (MSFD Technical Subgroup on Marine Litter, 2013).

Statistical treatment

In order to properly manage the data recorded all the information was stored in different excel documents classified by samples. Moreover, a centralized excel document was created where results related to sample analysis were processed.

The data already processed was introduced in the GraphPad Prism software, where statistical comparations of the desired topics were obtained by first doing the Shapiro-Wilk normality test, and then *t* student tests.

Cleaning and environmental pollution control protocols

In order to minimize the contamination during sample treatment different strict measures were applied to the procedure. Firstly, all procedures were done inside of a cleanroom, which dimensions were 5 x 4.25 x 3.20 m, (length x width x height) 21.25 m^2 or 68 m^3.

All the material used during the application of the methodology can-not be made of plastic; therefore, glass material was used. It was first cleaned with Milli-Q water, then covered in aluminium and lastly subjected to 550 ºC during 4 hours in a chamber furnace in order to eliminate any remains of contamination in it (Figure 3). Finally, without retiring the aluminium, it was stored in a cupboard reserved for this type of material. Additionally, the stainless-steel mesh filters used in the vacuum filtration systems were introduced in glass Petri dishes and subjected to 500 ºC during 4 hours in a chamber furnace in order to eliminate any contamination that could later interfere with results.

The Milli-Q water used in the washing bottles was renewed every day, previously rinsing the bottle three times to rule out possible contamination deposited inside of it.

While working with the samples, both in the cleanroom or outside, an orange lab coat was worn, so if any contamination happened it was easily recognisable by its orange colour.

Nevertheless, before entering the cleanroom, a lint roller was dragged all around the lab coat and trousers in order to retain any fibber or other contaminant which could detach from them.

Inside of the cleanroom there were 2 Rowenta air filtration systems (model PU3030) which were working continuously in order to eliminate any contamination present in the air.

Moreover, inside the cleanroom the sample treatment was done inside a glove box (Figure 4). All the working area and both the inside and outside of the glove box were carefully cleaned before and after sample treatment with a microfibre cloth and Milli-Q water.

Regarding the control of contamination during the sample treatment inside the glove box, a procedural blank was also analysed within every batch of samples. Furthermore, an ambient contamination sample consisting of an open Petri dish with a stainless-steel mesh filter of same width as the ones used for the samples was left inside of the glove box during the whole sample treatment, thus when the procedure was finished the filter could be checked in order to evaluate the contamination present in the air.

Finally, for sample observation the Petri was always kept close. In case its necessary to open it for any procedure, an ambient blank Petri dish with a filter of the same width was kept open next to the open sample Petri dish.

Figure 3: Nabertherm muffle furnace filled with material for sterilization. Source: own elaboration.

Figure 4: Empty glove box ready for use. Source: own elaboration.

Results and discussion

Extraction method

Before the start of the present study, the experimental procedures of 11 different studies were considered in order to help in finding a proper extraction method for microplastics contained in packages of commercial sea salt. These studies with its results are presented in Table 1.

After comparison, certain procedural errors were noted down in order to avoid misinterpretation of the results.

Starting with contamination protocols, there is little evaluation of the possibility of external contamination in the samples, usually not applying any contamination control or mitigation apart from procedural blanks. In fact, avoidable mistakes in the procedural method derive from this topic as, for example, in the latest study conducted in European salts (Thiele et al., 2023) transparent fibres were not considered due to constant contamination in blank samples.

In relation to particle size, there is a lot of misinterpretation related to microplastic abundance in samples due to the fact that there is no standardized classification method. For instance, in the Lebanese study (Nakat et al., 2023) the results accounted only particles identifiable with FTIR, while not describing any information related to size. Differences go as far as that in the Bangladesh study (Rakib et al., 2021), microplastics tinier than 250 µm were not accounted in the final results; while in the study conducted in Italian and Croatian salts (Renzi et al., 2019) only microplastics in the range of 10 – 150 µm were considered as it is stated that if bigger, they could not be absorbed by mammals. Therefore, it is evident that there is yet to set an unified nomenclature and a standardized quantifying method.

Sample treatment is not standardized either. For instance, H_2O_2 digestion, which can affect in colour degradation of particles, is used during different time periods and at different temperatures.

In relation to the present study, the abundant presence of sediment in many samples difficulted the determination of colourless, white and brown fragments, as well as the identification of films.

Ambient and procedural blanks analysis

In order to control the possible contamination happening during the extraction procedure both ambient and procedural blanks were carried out.

For each working day a different ambient blank was used, having a total of 11 procedural blanks, which results are showing in Table 3.

Table 3: Ambient blanks results

Sample	Microplastic type	Lenght (µm)	Colour
M2 BA	Clean	-	-
M3 BA	Fibre	417	Colourless
M4 BA	Clean	-	-
M5 BA	Clean	-	-
M6 BA	Clean	-	-
M7 BA	Clean	-	-
M8 BA	Fibre	281	White
M9/10 BA	Fibre	236	Colourless
M11/12 BA	Clean	-	-
M13/14 BA	Clean	-	-
M1/15 BA	Clean	-	-

Of the 11 different ambient blanks only 3 presented particles, all of them being white or colourless fibres.

This results evidence that the 72.7% of the time the contamination protocol was 100% effective against possible air pollution in the samples. Furthermore, if contamination was present, it only accounted for 1 fibre.

On the other hand, procedural blanks were carried out to evaluate possible contamination during the extraction method in each set of samples, replicating the whole filtration process using both 50 and 25 µm stainless-steel mesh filters. A sum up of the results is shown in Figure 5, while the extended determination and classification is specified in Annex I.

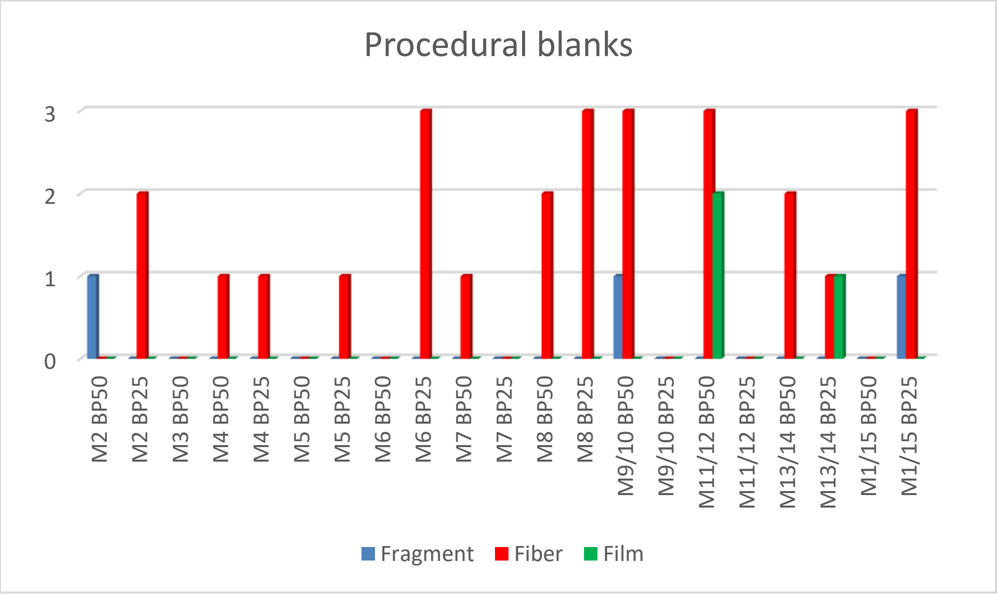


Figure 5: Summary of the results of particle presence in procedural blanks. M3 BP25 is not present due to a procedural mistake where it was contaminated.

The results evidence the presence of contamination in every procedural blank. Specifically, the appearance of fibres is the most common form of contamination, being in 13 out of the 21 procedural blanks (61.9%), but not stacking for more than 3 units per sample. The appearance of fragments (14.3%) and films (9.5%) is less common in comparison, always ranging between 1 to 2 units per sample.

A higher tendency of contamination can be appreciated when conducting more than one sample filtration at a time, possibly being because of the extra material being manipulated during a more elongated period of time.

Moreover, μFTIR analysis of 7 of the particles found in the procedural blanks (21.8% of the total) showed a distribution of 57.1% cellulosic and 42.9% PET.

In total, 32 particles were found between the 11 procedural blanks, accounting for an average contamination rate of 2.9 particles/sample.

The most similar particles found in the samples to the particles found in the blanks were subtracted from the analysis as they were considered as contamination.

Sample analysis

Regarding sample analysis, 14 out of the 15 samples were completely analysed. Results on particles per sample can be appreciated in Table 4. Sample 7, which was sea salt hand-harvested, contained a higher amount of sediment and a notable higher proportion of plastics (Annex II), but due to time limits the complete characterization of the sample was not achievable.

Table 4: Results summary of particles per sample. N1, N2 and N3 account for replicate 1, 2 and 3 respectively. NC: not counted.

	Number of particles														
Sample **Replicate**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**	**M14**	**M15**
N1	2	41	20	22	42	32	NC	39	19	44	47	35	12	19	29
N2	6	30	17	18	62	38	NC	48	18	32	32	23	18	24	28
N3	4	23	23	22	47	27	NC	44	19	36	41	27	16	33	43
Mean	4	31	20	21	50	32	-	44	19	37	40	28	15	25	33
SD	2	9	3	2	10	6	-	5	1	6	8	6	3	7	8

Regarding the results, it is observed that there are no average particle per sample notably higher, but sample 1 does present a scarce presence of particles per sample. Nevertheless, a Shapiro-Wilk normality test and t student analysis in GraphPad Prism were performed in order to determine possible differences between fine and coarse salts (Table 2 for classification). Results showed that data was normal (alpha=0.05), and in the t student a p value of 0.7599 was obtained, stating that the comparison of results between fine and coarse salts was not significantly different ($p < 0.05$).

In relation to microplastics characterization, Figure 6 illustrates the relative abundance of each microplastic type found in the total of all samples while Table 5 presents both a classification of plastic type as well as the summatory of the total amount of particles analysed.

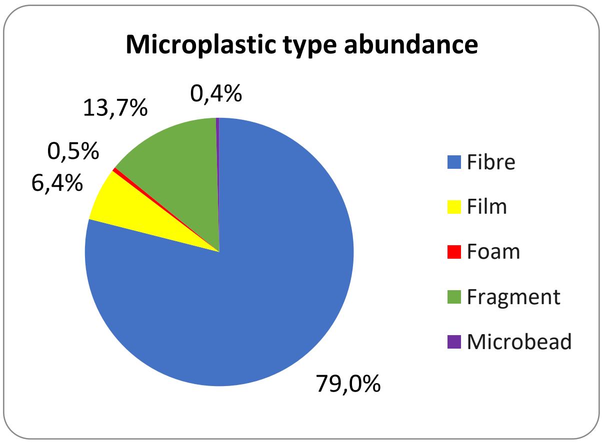


Figure 6: Microplastic type abundance in the analysed salts.

Table 5: Particle distribution based on microplastic type, according to the total count.

	Number of particle					
MPs type / **Sample**	**Fibre**	**Film**	**Foam**	**Fragment**	**Microbead**	**ΣParticles**
M1	8	4	0	0	0	12
M2	82	2	1	9	0	94
M3	51	2	2	5	0	60
M4	56	1	0	5	0	62
M5	101	16	0	31	3	151
M6	78	14	3	2	0	97
M8	104	5	0	22	0	131
M9	42	6	0	8	0	56
M10	100	3	0	9	0	112
M11	68	6	0	45	1	120
M12	71	2	0	12	0	85
M13	43	2	0	0	1	46
M14	64	3	0	9	0	76
M15	81	11	0	8	0	100
ΣParticles	949	77	6	165	5	1202
Mean	67,8	5,5	0,4	11,8	0,4	
SD	26,6	4,8	0,9	12,7	0,8	
%	79,0%	6,4%	0,5%	13,7%	0,4%	

In Table 5, it can be appreciated that out of the 1202 particles found in the samples, the majority of them were fibres accounting for a 79.0%. The second majority group is fragments with a 13.7%, and the third is films, with a 6.4%. The least represented microplastic types are foams and microbeads, with a 0.5% and 0.4%, respectively.

The presence of fibres as the most abundant microplastic type matches the bibliographical research stated in Table 1, where 6 of the 9 (66.6%) articles sharing the proportions of microplastic types state fibres as the most abundant.

Regarding fragments, a standard variation of 12.7 (higher than the mean value) is observed. Looking deeply in these results, while 10 out of the 14 samples (71.42%) have less than 10 fragments between the 3 replicates, samples M5, M8, M11 and M12 have values which even quadruplicate this amount (M11 has 45 fragments). By looking at Table 2 it can be appreciated that M5 – M12 and M8 – M11 share the same origin. In fact, the 2 highest values are for M8 and M11 which both origins are Murcia, and the following 2 higher values are for M5 and M12, which both origins are Fuencaliente (La Palma). This results state that further research should be conducted for both salt ponds.

Length and colour of each particle were also determined, results showing in Figure 7 (see Annex III for detailed results).

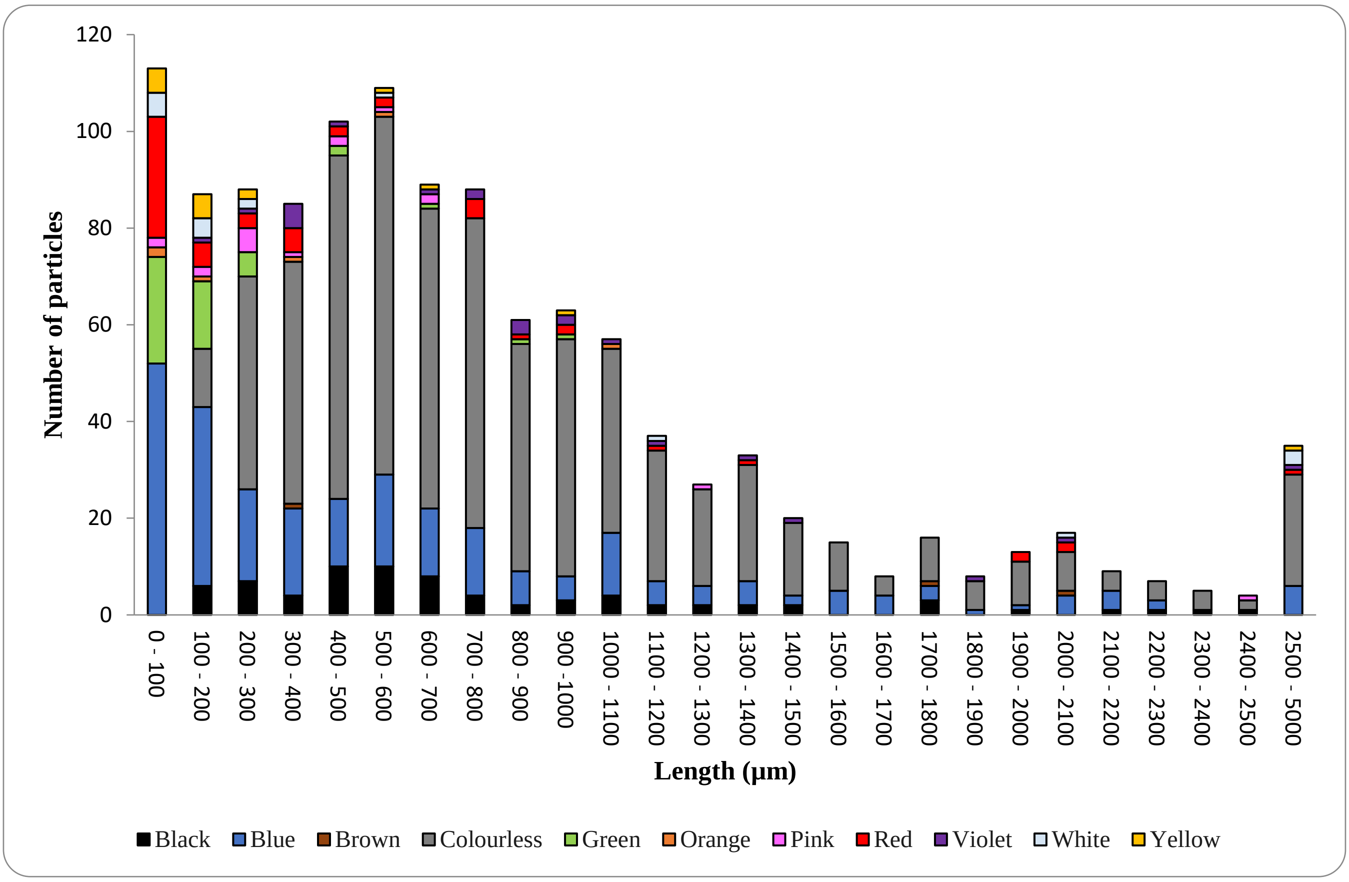


Figure 7: Colour and length of the total particles found among the samples.

Regarding length, in Figure 7 it can be seen that the most common range is 0 to 100 micrometres, which if extended to 300 micrometres would also be the one with greater colour variety. This result is linked to fragment presence, as they present a wider variety of colours while their average length is 127 ± 117 µm.

In general, length distribution concentrates in the range between 0 and 1000 µm, accounting for 74% of the total particles. In addition, only 3% of the particles were larger than 2500 µm.

The smallest particle found was a blue fragment in M8 which maximum width was 32 µm. On the other side, 6 fibres were found to be bigger than 5000 µm therefore not being able to be determined as microplastic.

At first impression, colours might not seem like a crucial data to note down. Nevertheless, colour, as well as the size, can reflect the degradation stages of a particle and act as a hint for tracking its exposure time in the environment (Martí et al., 2020). In Figure 8 are presented the colour proportions of all the particles analysed in the present study.

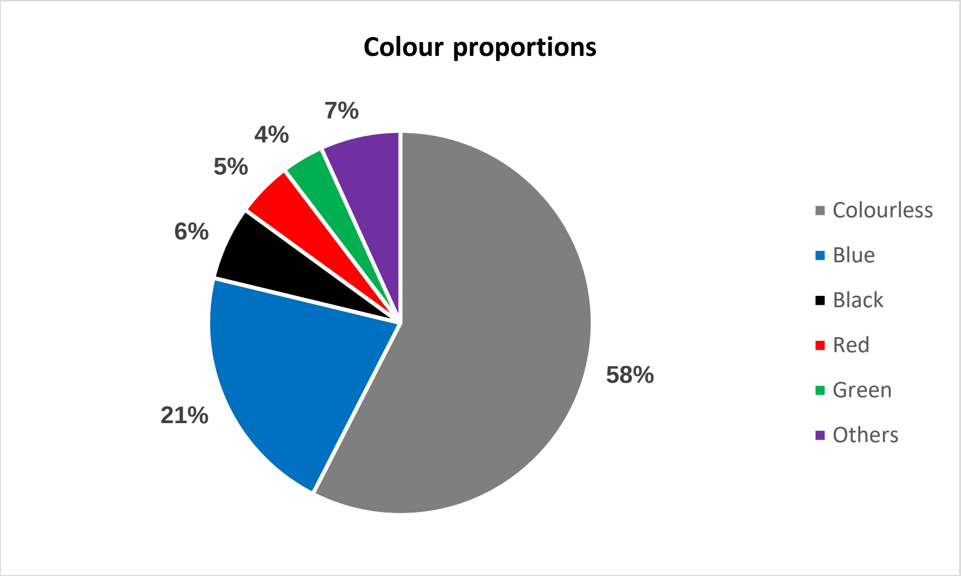


Figure 8: Colour proportions of all the particles analysed.

Colourless was the most abundant proportion, representing the 58% of the total particles. The colour with higher presence was blue, which accounted for 21%, followed by black, which accounted for 6%.

As previously mentioned, colour can be linked to particle degradation. In fact, it is proven that blue pigments absorb long-wavelength light with lower energy than red/yellow pigments, which makes light energy transmitted to blue plastics higher and accelerates plastic degradation (Zhao et al., 2022). This explains why a higher proportion of blue microplastics is found commonly in studies, as for instant in the present document blue represents the majority coloured group (21%).

Examples of particle identification are presented in Figures 9, 10 and 11.

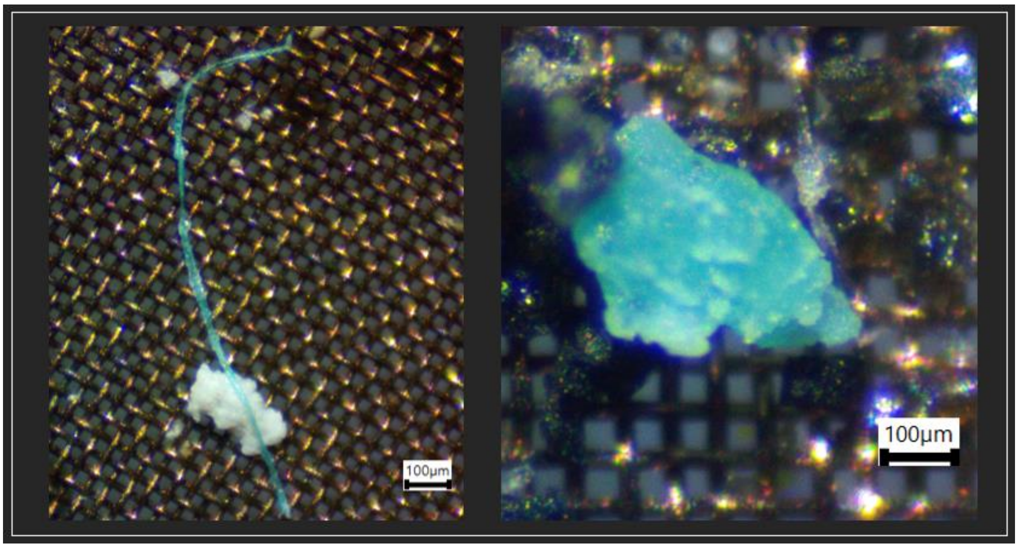


Figure 9: Blue fibre (left) and blue fragment (right).

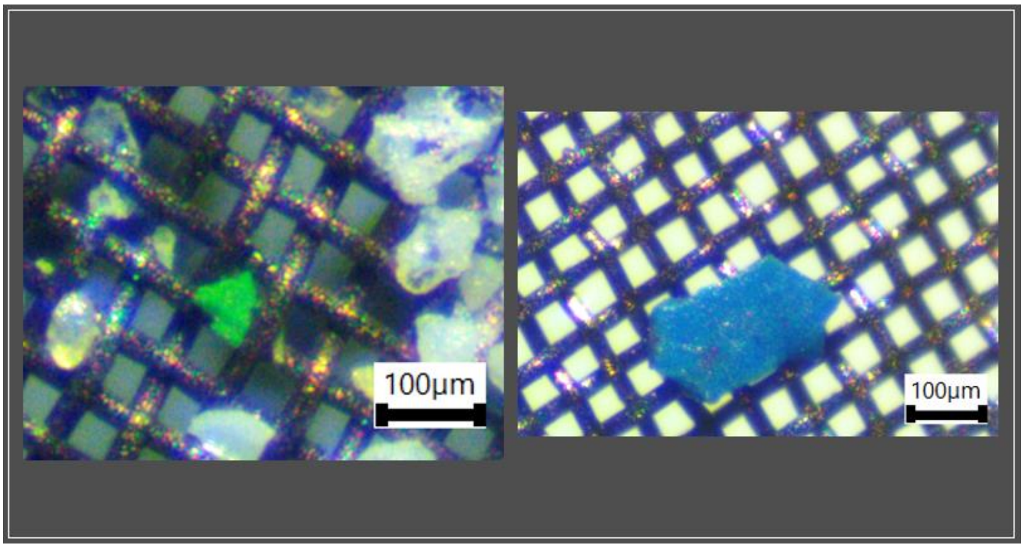


Figure 10: Green fragment (left) and blue fragment (right).

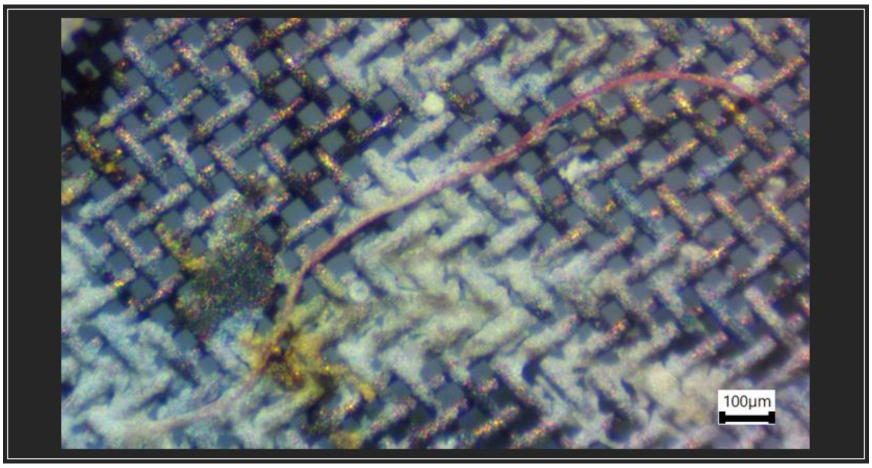


Figure 11: Red fibre, partially covered by crystalised salt.

Finally, despite only analysing plastic particles, a wide variety of other items were found inside the samples. On the one hand, big amounts of sediment composed of minerals, sand, variety of rocks and crystalized salt were present in most of the samples; as well as algae. On the other hand, more rare finds involve a wide variety of foraminifera, remains of tiles, or what was considered artemia cysts. Visual examples are presented in Figures 12 and 13.

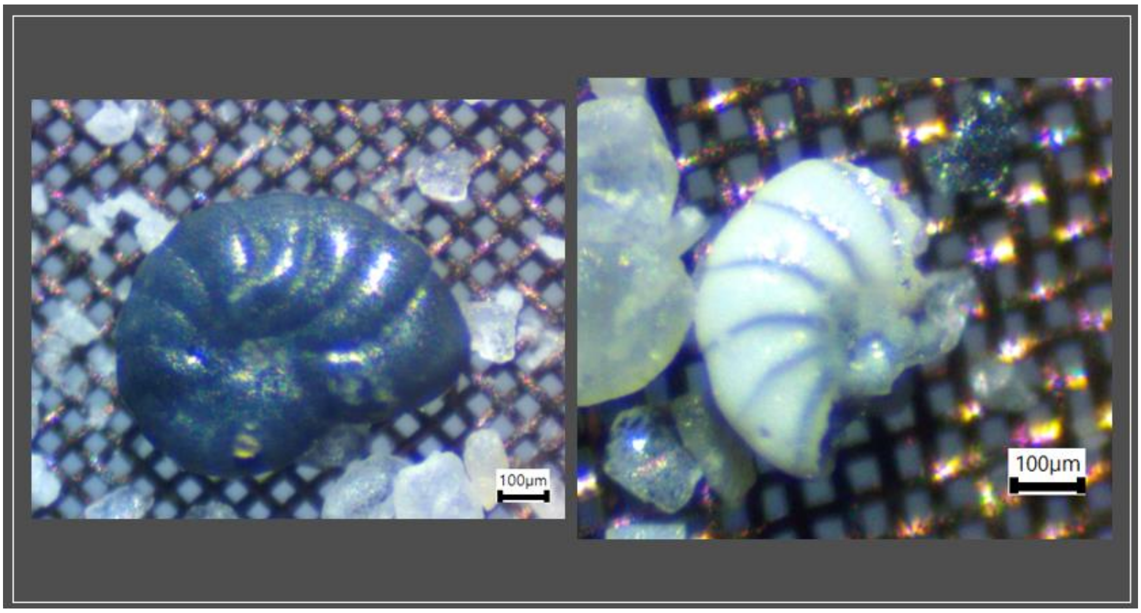


Figure 12: Black coloured foraminifera (left) and broken white foraminifera (right)

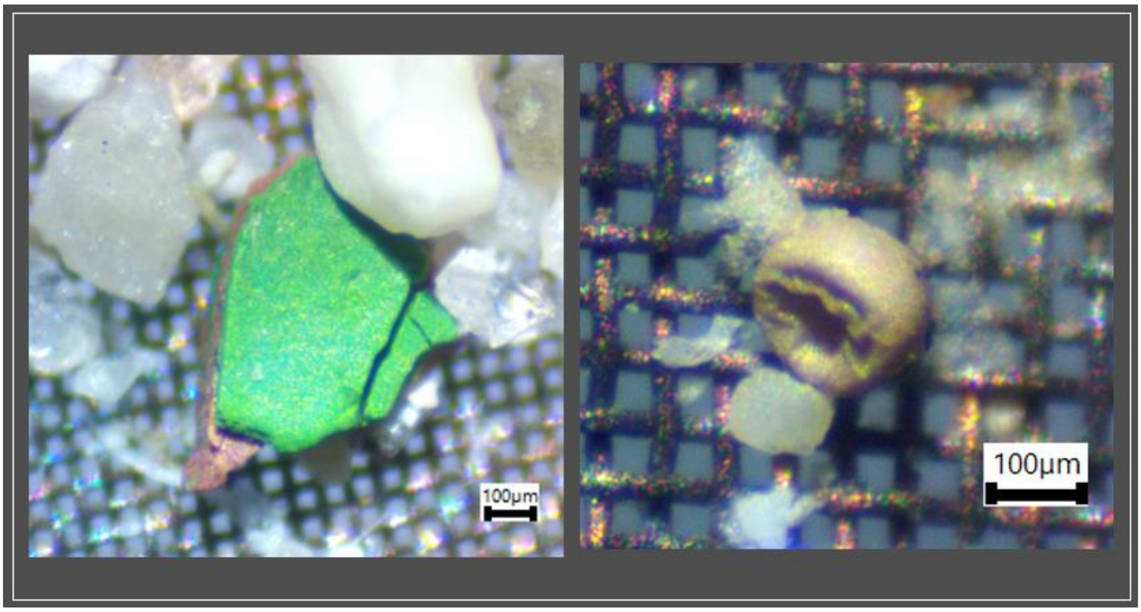


Figure 13: Alleged piece of broken green tile (left) and alleged artemia cyst (right)

µFTIR analysis

As indicated in the bibliography (MSFD Technical Subgroup on Marine Litter, 2013), at least 10% of each sample was analysed. Precisely, 19.1% of the total particles (230/1202), involving every microplastic type except microbeads, were analysed by µFTIR (see Annex IV). Results are showing in Figure 14.

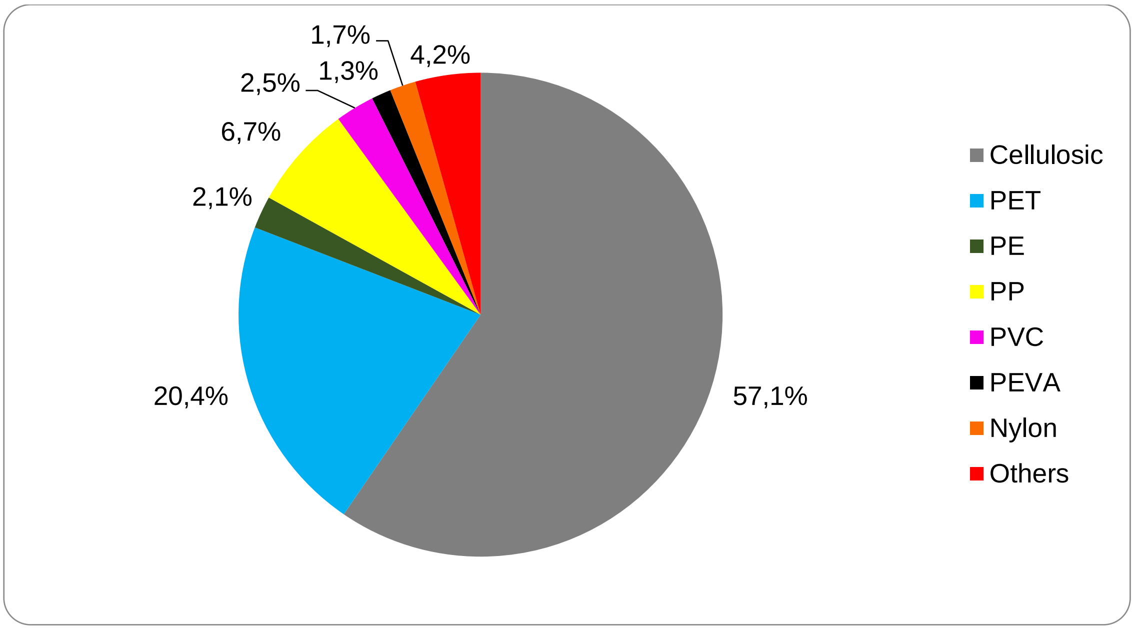


Figure 14: Composition distribution of the particles analysed. PET: Polyethylene Terephthalate, PE: Polyethylene, PP: Polypropylene, PVC: Polyvinyl chloride, PEVA: Polyethylene vinyl acetate.

If cellulosic particles are not considered, PET appears as the majority group (20.4%), followed by PP (6.7%), PVC (2.5%) and PE (2.1%). Nevertheless, it is important to clarify that PET includes both polyester and PET results obtained in the µFTIR analysis. This aggrupation was done because they are the same polymer, it is just that polyester is the common name used in the context of textile applications, while PET is commonly used when talking about packaging.

The least represented groups are Nylon (1.7%) and PEVA (1.3%). The group “others” (4.2%) accumulates the results of acrylic, polynorbornene, polystyrene and phenoxy resin.

When compared to the latest data related to plastics production (Plastics Europe, 2022), it is stated that the major plastic types produced in Europe are PP (16.6%), PE (14.7%) and PVC (11.4%). In addition, PET accounts for 5.3%.

Despite further identification of particles should be made in order to properly compare the results of the present study to the plastic production in Europe, is interesting to note that PET proportions are higher than expected when compared to the Europe yearly production (47.6% of plastic particles > 5.3%). This result may be linked to the polyester fibres, as little effort is necessary for them to detach from clothes and can end up contaminating all departments of the biosphere, or even the air we breathe (Salthammer, 2022).

Regarding the 57.1% of particles made of cellulose, a graphic developing the result by including colour proportions is shown in Figure 15:

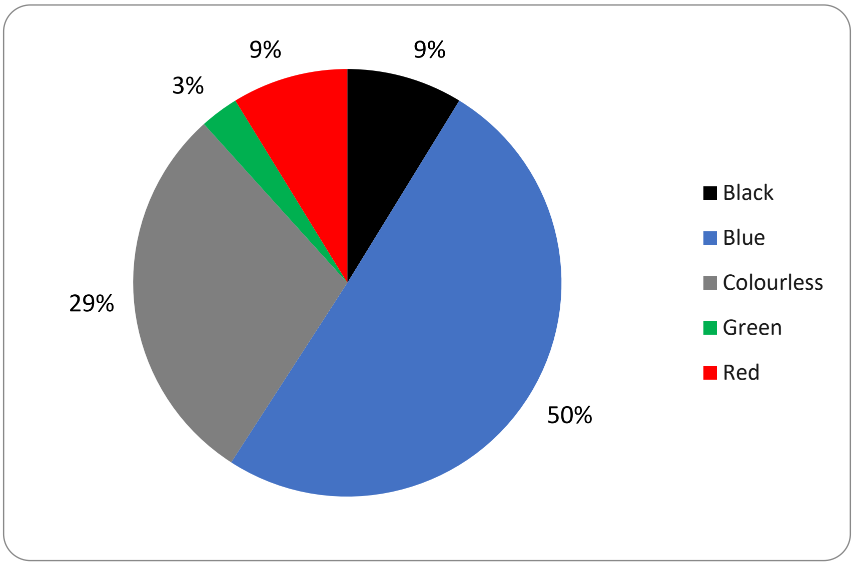
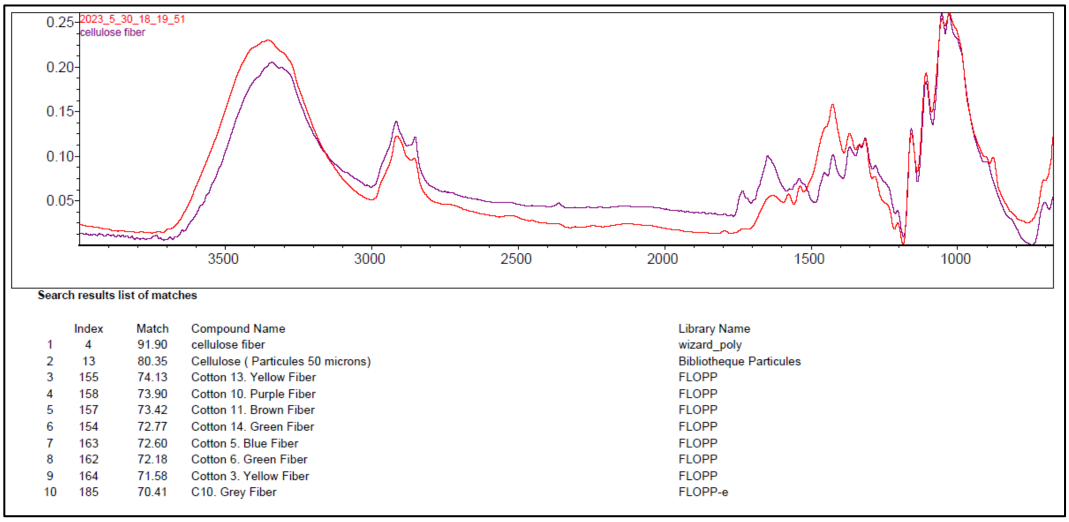


Figure 15: Colour proportions in the cellulose fibres analysed by μFTIR.

Results in Figure 15 demonstrate that, despite particle composition being cellulosic, the majority of particles analysed had been anthropologically modified to add colours as 50% of the particles present colour blue, scarce in nature (Lowe, 2019). Furthermore, it can be hypothesised that instead of pure cellulosic fibres, the composition of the particles could be viscose or rayon, which are in fact textiles materials made from cellulose. This misidentification of the particles might happen because of the close similarity between the mass spectrum of cellulose and viscose, as improvement in the methodology is needed to provide a proper identification.

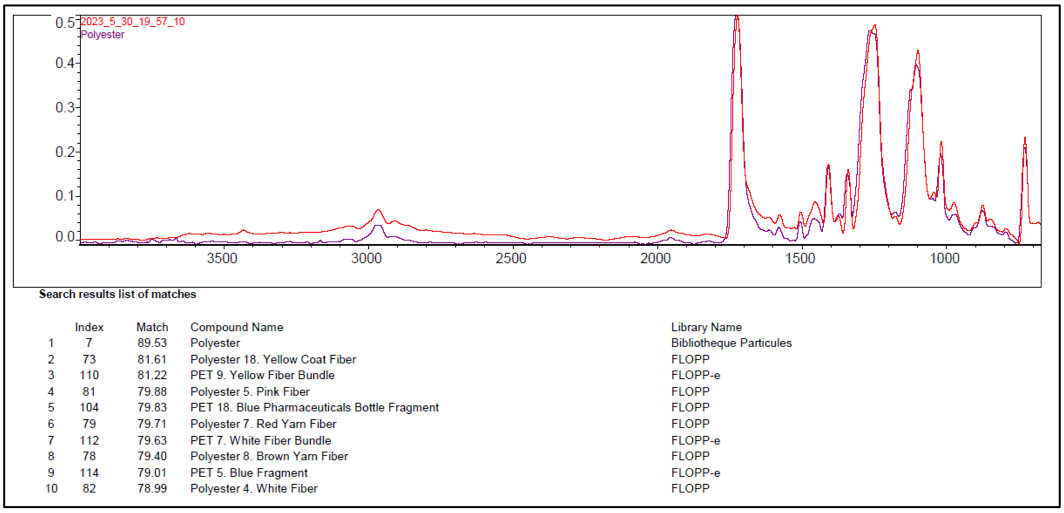
Examples of particle identification are presented in Figure 16, 17, 18, 19 and 20.



Search results list of matches

	Index	Match	Compound Name	Library Name
1	4	91.90	cellulose fiber	wizard_poly
2	13	80.35	Cellulose (Particules 50 microns)	Bibliotheque Particules
3	155	74.13	Cotton 13. Yellow Fiber	FLOPP
4	158	73.90	Cotton 10. Purple Fiber	FLOPP
5	157	73.42	Cotton 11. Brown Fiber	FLOPP
6	154	72.77	Cotton 14. Green Fiber	FLOPP
7	163	72.60	Cotton 5. Blue Fiber	FLOPP
8	162	72.18	Cotton 6. Green Fiber	FLOPP
9	164	71.58	Cotton 3. Yellow Fiber	FLOPP
10	185	70.41	C10. Grey Fiber	FLOPP-e

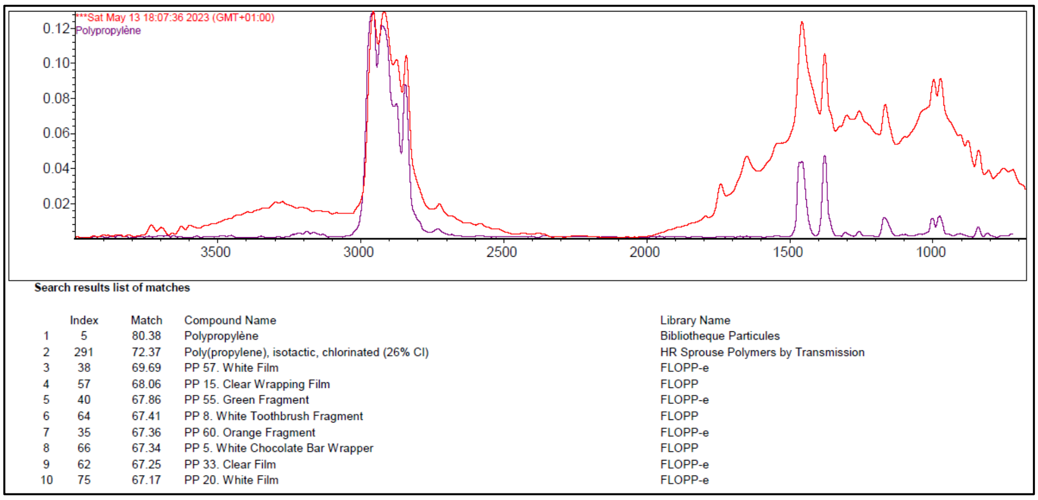
Figure 16: Example of particle identification (cellulose) with µFTIR. Red: particle´s spectrum; Purple: library´s spectrum.



Search results list of matches

	Index	Match	Compound Name	Library Name
1	7	89.53	Polyester	Bibliotheque Particules
2	73	81.61	Polyester 18. Yellow Coat Fiber	FLOPP
3	110	81.22	PET 9. Yellow Fiber Bundle	FLOPP-e
4	81	79.88	Polyester 5. Pink Fiber	FLOPP
5	104	79.83	PET 18. Blue Pharmaceuticals Bottle Fragment	FLOPP
6	79	79.71	Polyester 7. Red Yarn Fiber	FLOPP
7	112	79.63	PET 7. White Fiber Bundle	FLOPP-e
8	78	79.40	Polyester 8. Brown Yarn Fiber	FLOPP
9	114	79.01	PET 5. Blue Fragment	FLOPP-e
10	82	78.99	Polyester 4. White Fiber	FLOPP

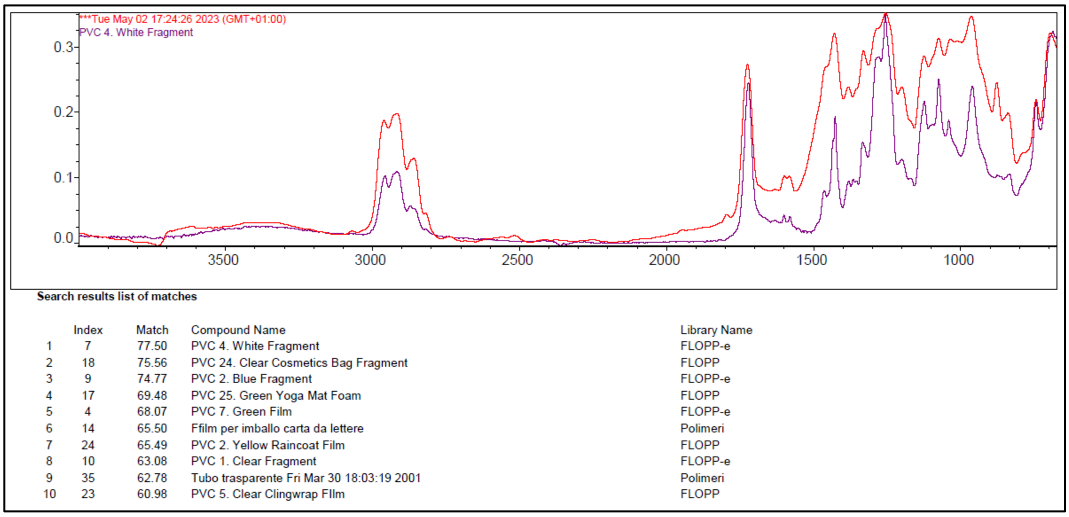
Figure 17: Example of particle identification (PET) with µFTIR. Red: particle´s spectrum; Purple: library´s spectrum.



Search results list of matches

	Index	Match	Compound Name	Library Name
1	5	80.38	Polypropylène	Bibliotheque Particules
2	291	72.37	Poly(propylene), isotactic, chlorinated (26% Cl)	HR Sprouse Polymers by Transmission
3	38	69.69	PP 57. White Film	FLOPP-e
4	57	68.06	PP 15. Clear Wrapping Film	FLOPP
5	40	67.86	PP 55. Green Fragment	FLOPP-e
6	64	67.41	PP 8. White Toothbrush Fragment	FLOPP
7	35	67.36	PP 60. Orange Fragment	FLOPP-e
8	66	67.34	PP 5. White Chocolate Bar Wrapper	FLOPP
9	62	67.25	PP 33. Clear Film	FLOPP-e
10	75	67.17	PP 20. White Film	FLOPP-e

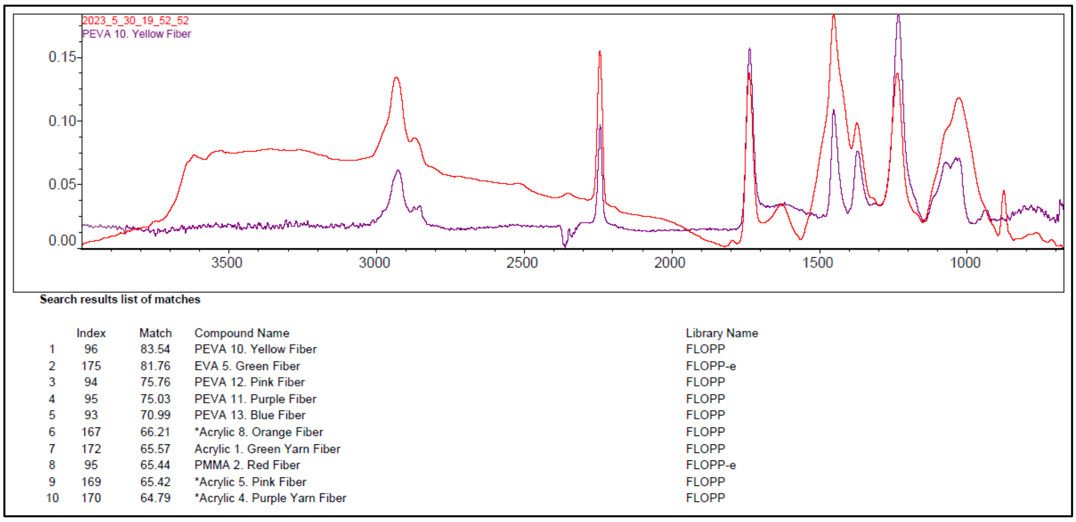
Figure 18: Example of particle identification (PP) with µFTIR. Red: particle´s spectrum; Purple: library´s spectrum.



Search results list of matches

	Index	Match	Compound Name	Library Name
1	7	77.50	PVC 4. White Fragment	FLOPP-e
2	18	75.56	PVC 24. Clear Cosmetics Bag Fragment	FLOPP
3	9	74.77	PVC 2. Blue Fragment	FLOPP-e
4	17	69.48	PVC 25. Green Yoga Mat Foam	FLOPP
5	4	68.07	PVC 7. Green Film	FLOPP-e
6	14	65.50	Ffilm per imballo carta da lettere	Polimeri
7	24	65.49	PVC 2. Yellow Raincoat Film	FLOPP
8	10	63.08	PVC 1. Clear Fragment	FLOPP-e
9	35	62.78	Tubo trasparente Fri Mar 30 18:03:19 2001	Polimeri
10	23	60.98	PVC 5. Clear Clingwrap FIlm	FLOPP

Figure 19: Example of particle identification (PVC) with μFTIR. Red: particle´s spectrum; Purple: library´s spectrum.



Search results list of matches

	Index	Match	Compound Name	Library Name
1	96	83.54	PEVA 10. Yellow Fiber	FLOPP
2	175	81.76	EVA 5. Green Fiber	FLOPP-e
3	94	75.76	PEVA 12. Pink Fiber	FLOPP
4	95	75.03	PEVA 11. Purple Fiber	FLOPP
5	93	70.99	PEVA 13. Blue Fiber	FLOPP
6	167	66.21	*Acrylic 8. Orange Fiber	FLOPP
7	172	65.57	Acrylic 1. Green Yarn Fiber	FLOPP
8	95	65.44	PMMA 2. Red Fiber	FLOPP-e
9	169	65.42	*Acrylic 5. Pink Fiber	FLOPP
10	170	64.79	*Acrylic 4. Purple Yarn Fiber	FLOPP

Figure 20: Example of particle identification (PEVA) with μFTIR. Red: particle´s spectrum; Purple: library´s spectrum.

FTIR analysis

The packaging of each sea salt sample obtained from the supermarket was analysed by FTIR to specify the material of which it is composed. The results are summed up in Table 6, and specific spectra can be consulted in the Annex V.

Table 6: FTIR results of sample packaging. PP: Polypropylene, PE: Polyethylene, CAB: Cellulose acetate butyrate, CP: Cellulose propionate

Sample number	Inside composition	Outside composition
M1	PP	PE
M2	PP	PP
M3	PP	PP
M4	PP	PP
M5	PP	PP
M6	PP	PE
M7	PP	PE
M8	PP	PP
M9	PP	PP
M10	PP	PP
M11	PP	PP
M12	PP	PP
M13	PP	PE
M14	PP	PP
M15	CAB	CP

Results show that while 10 out of the 15 packaging analysed have the same composition in both sides of the envelope (66.6%), 5 out of 15 had different polymers (33.3%). In fact, leaving apart the “plastic-free” envelope in M15, the 4 plastic envelopes shared the same pattern of polypropylene in the inside and polyethylene in the outside.

Regarding the plastic envelopes, PP appears in the inside side of all the 14 samples made of plastic (100%). This is because PP is known to keep humidity at static levels, therefore being the perfect material for salt preservation. On the other hand, the presence of polyethylene in the outside of 4 of the samples (28.57%) can be linked to its better elasticity when compared to polypropylene, therefore improving the endurance of the packaging. (Soroushian et al., 1992).

The plastic free packaging was made of CAB and CP, two cellulose modified materials.

Not enough samples with “plastic-free” properties were analysed to develop a statistical evaluation to observe if the average particle appearance was dependent on the composition of the packaging. Nevertheless, if the average particle appearance on sample M1 to M14 is calculated, it is similar to the one in sample M15 (26 – 33, respectively).

Geographical distribution comparison

Average particle appearance for samples in the mainland and in the Canary Islands are shown in Figures 21 and 22, respectively.

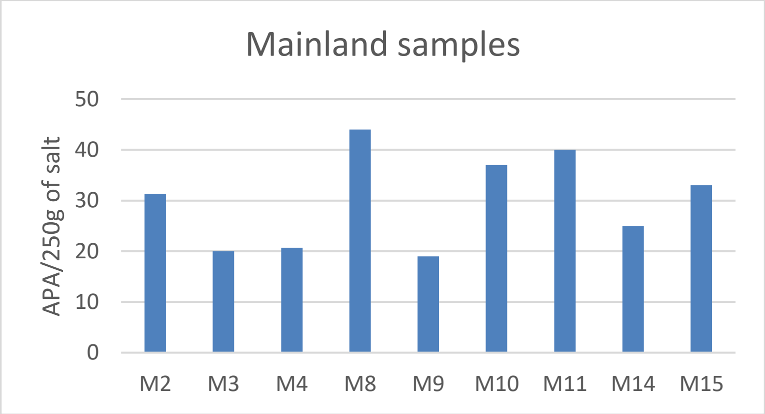


Figure 21: Average particle appearance (APA) in mainland samples.

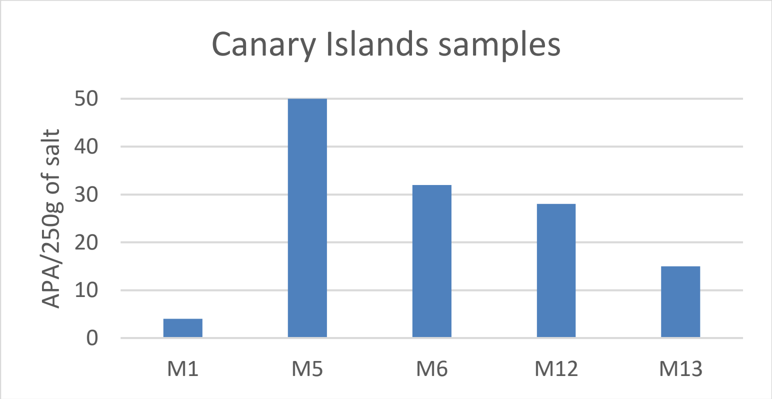


Figure 22: Average particle appearance (APA) in the Canary Islands.

A Shapiro-Wilk normality test and *t* student test were conducted to find if the average particle appearance in the samples were significantly different between the two regions. The normality test showed that data was normal (alpha=0.05); and in the *t* student test, a *p* value of 0.6371 was obtained, therefore indicating there is not significant difference between the 2 groups of samples (p<0.05).

Microplastic intake caused by salt

In order to calculate microplastic exposure through salt, an estimation of particles per gram of salt was calculated. Results are shown in Table 7:

Table 7: Result summary of the present study on particle intake per gram of salt. NC: Not counted.

	[Particles·g^{-1} salt]														
Sample **Replicate**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**	**M14**	**M15**
N1	0,01	0,16	0,08	0,09	0,16	0,13	NC	0,16	0,08	0,18	0,19	0,14	0,05	0,07	0,11
N2	0,01	0,16	0,08	0,09	0,17	0,13	NC	0,15	0,07	0,18	0,19	0,14	0,05	0,08	0,11
N3	0,01	0,16	0,08	0,09	0,17	0,13	NC	0,16	0,08	0,18	0,19	0,14	0,05	0,08	0,12
Mean	0,01	0,16	0,08	0,09	0,17	0,13	-	0,16	0,08	0,18	0,19	0,14	0,05	0,08	0,11
SD	0,00	0,00	0,00	0,00	0,00	0,00	-	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

From the results obtained it can be deduced that the concentration of particles in the present study ranges from 10 to 190 particles per kilogram of salt. These results are similar to Iñiguez (2017) experiments with Spanish salts, where it was concluded concentrations between 50 to 280 particles/kg.

The World Health Organization (WHO) recommended salt intake is less than 5 g/day (*Salt Intake - PAHO/WHO | Pan American Health Organization*, n.d.); nevertheless, recent studies indicate that the average intake in Europe is 7 to 12 grams of salt per day (Kloss et al., 2015).

If this data is compared to the present study, depending on the sample a minimum average intake of 0.07 to 0.12 or a maximum of 1.33 to 2.28 particles of microplastic are ingested per day. Theorizing the results into particles ingested per year, in the worst case presented by the analysed samples a maximum of 832 particles would be ingested per year.

However, data on the destination of microplastics in the human body following their ingestion is lacking, although it has been reported that is probable that microplastics bigger than 150 µm are not absorbed by mammals (Lusher et al., 2017). If this hypothesis is applied to the results of the present study, 13.64% of the particles found would be absorbed (164/1202 particles).

Finally, it is important to highlight the presence of microbeads in the samples. Plastic microbeads are used in personal care products and, although some countries have settled a legislative ban on them, many other countries have not taken any legal action and hence they are still being fabricated. The problem is that, after personal care products are used, microbeads end up in wastewater treatment plants where they can pass the filtration system and reach the marine environment. Regarding their composition, about 93% of microbeads produced worldwide are made of PE (Miraj et al., 2021). Example of microbead found in the sample is shown in Figure 23.

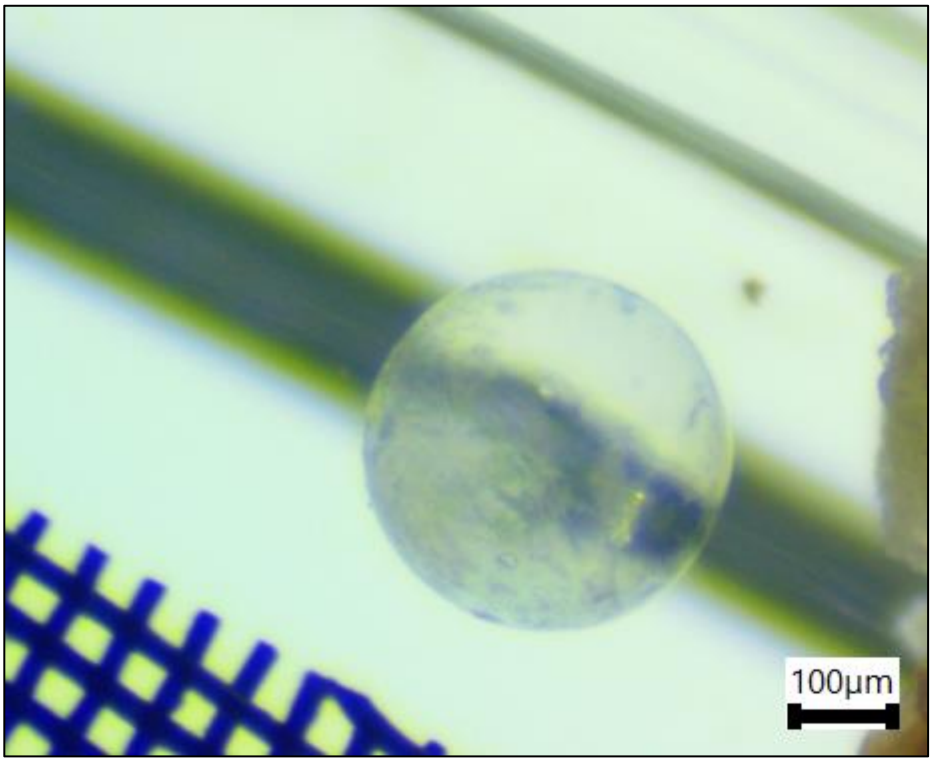


Figure 23: Colourless microbead. Located on the surrounding of the filter due to the microbead rolling properties.

Conclusions

1. The methodology used was effective, being able to clearly determine the abundance of microplastics in each sample while presenting the results in a transparent and referable way. However, the methodology used can be optimized in order to obtain more precise results regarding fragment and film count.
2. The total amount of microplastics found in the samples is 1202 particles, of which 79.0% were fibres, 13.7% were fragments, 6.4% were films, 0.5% were foams and 0.4% were microbeads. Regarding length, the size range found in the samples is 31.83 – 5000 µm, and in the range 0 – 1000 µm are found 74% of the particles. To conclude, colour distribution is 58% colourless, 21% blue, 6% black, 5% red, 4% green and 7% others.
3. µFTIR analysis of the 19.13% of the total microplastics found between the samples showed that particles found were made of cellulosic (57.1%), PET (20.4%), PP (6.7%), PVC (2.5%), PE (2.1%), Nylon (1.7%), PEVA (1.3%) and others (4.2%). Despite cellulosic being the highest percentage, it was theorized that viscose might be the proper identification of the particles, but further improvement of the methodology is needed for it.
4. Results showed a 100% PP composition in the inside of the plastic packaging. Comparisons between the average particle appearance in plastic envelopes and the plastic-free envelope declined possible contamination caused in the samples because of the packaging. Nevertheless, only the average particle appearance of one plastic-free packaging was analysed so further samples are needed in order to properly do a statistical comparison.
5. After statistical comparison of the samples from the mainland and the samples of the Canary Islands, no significant difference was found. Nevertheless, as sample 7 was not determined, and the number of samples analysed in each group was not the same, further investigations should be made to confirm this statement.
6. Concentration of particles in the samples analysed ranged from 10 to 190 particles per kilogram. This implies an exposure to the average European citizen of 0.07 to 2.28 particles per day.

Future implications

Microplastic investigations have become a rising aspect of environmental research in recent years. This is linked to the growing awareness of the detrimental effects of microplastics on ecosystems, which promotes the urge to understand their sources, distribution and real impacts. Moreover, the exposure to microplastics during human´s lifetime is inevitable, so it is urgent to call for a better understanding of the potential hazards of microplastics to human health.

The present investigation on microplastics in marine salt samples sheds light on the extent to which these contaminants have entered our daily lives and offers a glimpse into the magnitude of human exposure to them. In fact, due to the commonness of use of the salt, this present line of study can act as an important scientific divulgation topic to increase public awareness on the plastic environmental issue.

Nevertheless, in order to develop the present study, some measurements can be made. First, continue the process of methodology development as samples with sediment can suppose a difficulty in particle count.

Secondly, try to do a µFTIR identification of most of the particles found in the samples to have a better understanding of the contamination composition.

Thirdly, increase the number of samples analysed to have a better representation of geographical distribution of microplastics.

To conclude, a good way to expand the investigation would be to add other types of salt such as rock salt to be able to evaluate the extent of marine contamination in the samples.

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Annexes

Annex I. Procedural blanks

Table I. 1: Procedural blanks

Sample	MPs type	Lenght (µm)	Colour
M2 BP50	Fragment	92	Colourless
M2 BP25	Fibre	720	Colourless
M2 BP25	Fibre	879	Colourless
M3 BP50	Clean		
M3 BP25	XXX		
M4 BP50	Fibre	2102	Blue
M4 BP25	Fibre	1423	Colourless
M5 BP50	Clean		
M5 BP25	fibre	2573	Blue
M6 BP50	Clean		
M6 BP25	Fibre	1454	Colourless
M6 BP25	Fibre	877	Colourless
M6 BP25	Fibre	1445	Colourless
M7 BP50	Fibre	560	Colourless
M7BP25	Clean		
M8 BP50	Fibre	1573	Colourless
M8 BP50	Fibre	1119	Colourless
M8 BP25	Fibre	996	Colourless
M8 BP25	Fibre	654	Colourless
M8 BP25	Fibre	985	Colourless
M9/10 BP50	Fibre	1024	Colourless
M9/10 BP50	Fibre	293	Colourless
M9/10 BP50	Fibre	1268	Colourless
M9/10 BP50	Fragment	391	white
M9/10 BP25	Clean		
M11/12 BP50	Fibre	876	Colourless
M11/12 BP50	Fibre	828	Colourless
M11/12 BP50	Fibre	1257	Colourless
M11/12 BP50	Film	322	Colourless
M11/12 BP50	Film	326	Colourless
M11/12 BP25	Clean		
M13/14 BP50	Fibre	407	Colourless
M13/14 BP50	Fibre	221	Colourless
M13/14 BP25	Film	400	Colourless
M13/14 BP25	Fibre	268	Colourless
M1/15 BP50	Clean		
M1/15 BP25	Fragment	83	White
M1/15 BP25	Fibre	1034	Colourless
M1/15 BP25	Fibre	434	Colourless
M1/15 BP25	Fibre	535	Colourless

Annex II. Visual examples of organic and inorganic matter present in sample 7

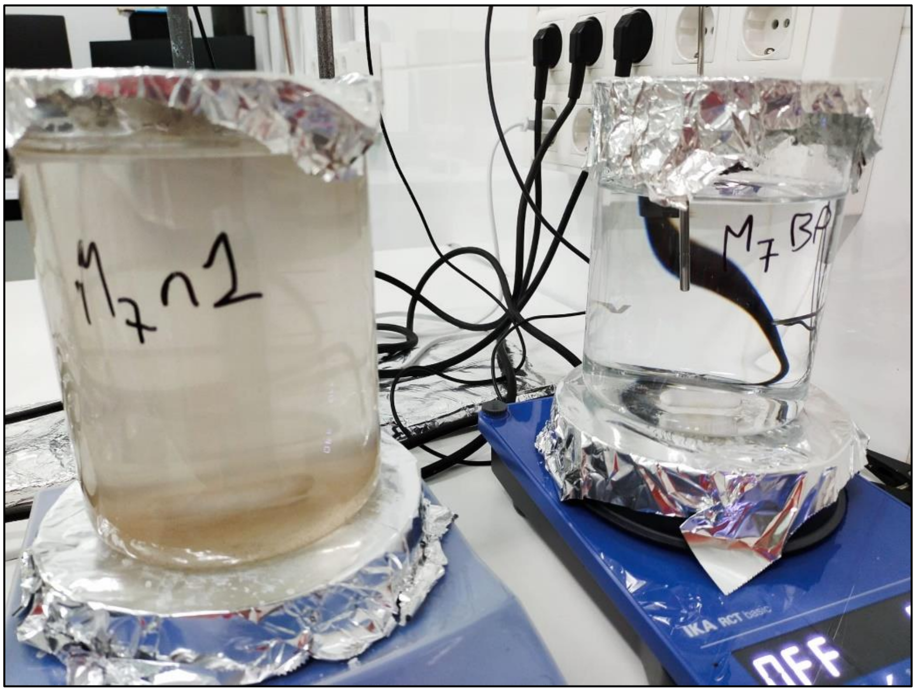


Figure II. 1: Comparison between replicate 1 of sample 7 and its respective procedural blank.

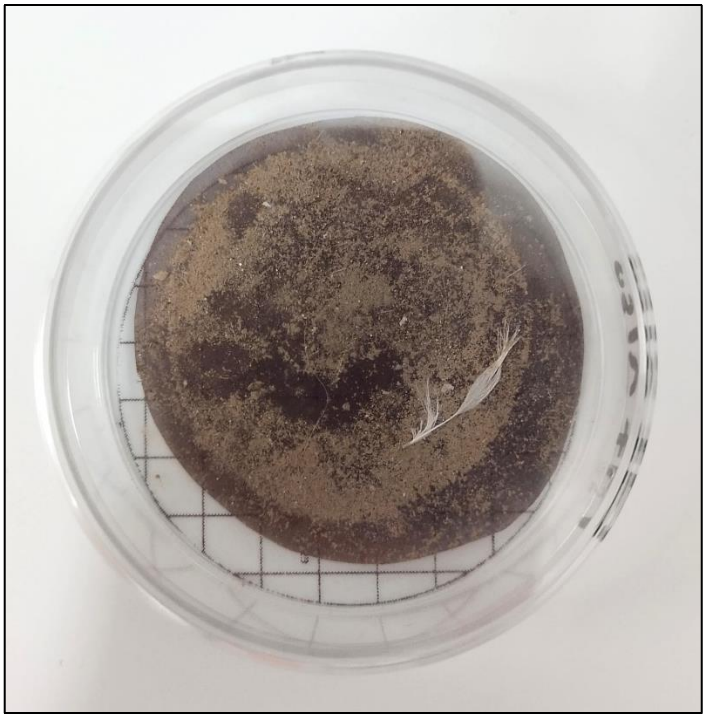


Figure II. 2: Plan view of 50 µm filter of replicate 1 of sample 7. The amount of organic/inorganic matter can be easily appreciated without stereoscope.

Annex III. Extended results on colour and length of the total particles found among the samples

Table III. 1: Colour and length of the total particles found among the samples.

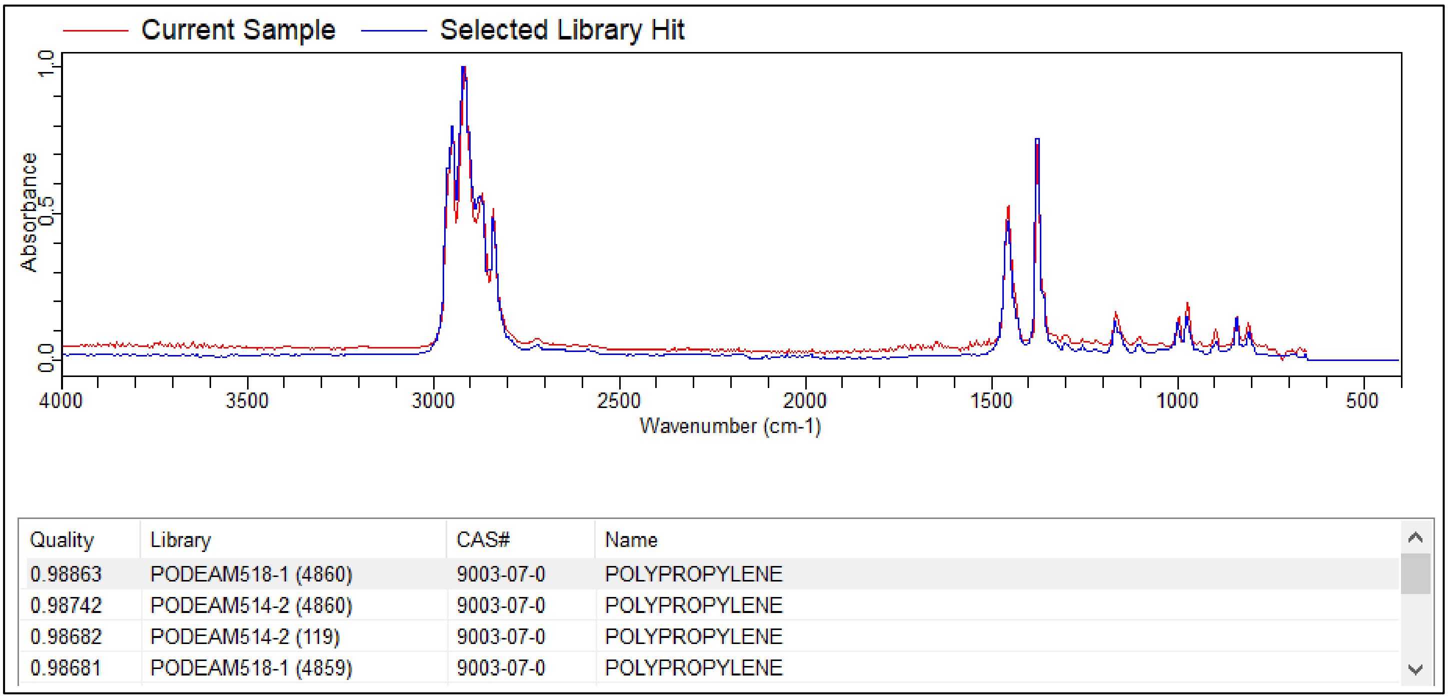
	Number of particles											
Colour **Length (µm)**	**Black**	**Blue**	**Brown**	**Colourless**	**Green**	**Grey**	**Orange**	**Pink**	**Red**	**Violet**	**White**	**Yellow**
0 - 100	0	52	0	0	22	0	2	2	25	0	5	5
100 - 200	6	37	0	12	14	0	1	2	5	1	4	5
200 - 300	7	19	0	44	5	0	0	5	3	1	2	2
300 - 400	4	18	1	50	0	0	1	1	5	5	0	0
400 - 500	10	14	0	71	2	0	0	2	2	1	0	0
500 - 600	10	19	0	74	0	0	1	1	2	0	1	1
600 - 700	8	14	0	62	1	0	0	2	0	1	0	1
700 - 800	4	14	0	64	0	0	0	0	4	2	0	0
800 - 900	2	7	0	47	1	0	0	0	1	3	0	0
900 -1000	3	5	0	49	1	0	0	0	2	2	0	1
1000 - 1100	4	13	0	38	0	0	1	0	0	1	0	0
1100 - 1200	2	5	0	27	0	0	0	0	1	1	1	0
1200 - 1300	2	4	0	20	0	0	0	1	0	0	0	0
1300 - 1400	2	5	0	24	0	0	0	0	1	1	0	0
1400 - 1500	2	2	0	15	0	0	0	0	0	1	0	0
1500 - 1600	0	5	0	10	0	0	0	0	0	0	0	0
1600 - 1700	0	4	0	4	0	0	0	0	0	0	0	0
1700 - 1800	3	3	1	9	0	0	0	0	0	0	0	0
1800 - 1900	0	1	0	6	0	0	0	0	0	1	0	0
1900 - 2000	1	1	0	9	0	0	0	0	2	0	0	0
2000 - 2100	0	4	1	8	0	0	0	0	2	1	1	0
2100 - 2200	1	4	0	4	0	0	0	0	0	0	0	0
2200 - 2300	1	2	0	4	0	0	0	0	0	0	0	0
2300 - 2400	1	0	0	4	0	0	0	0	0	0	0	0
2400 - 2500	1	0	0	2	0	0	0	1	0	0	0	0
2500 - 5000	0	6	0	23	0	0	0	0	1	1	3	1

Annex IV. μFTIR results extended

Table IV. 1: μFTIR results shorted by composition and colour.

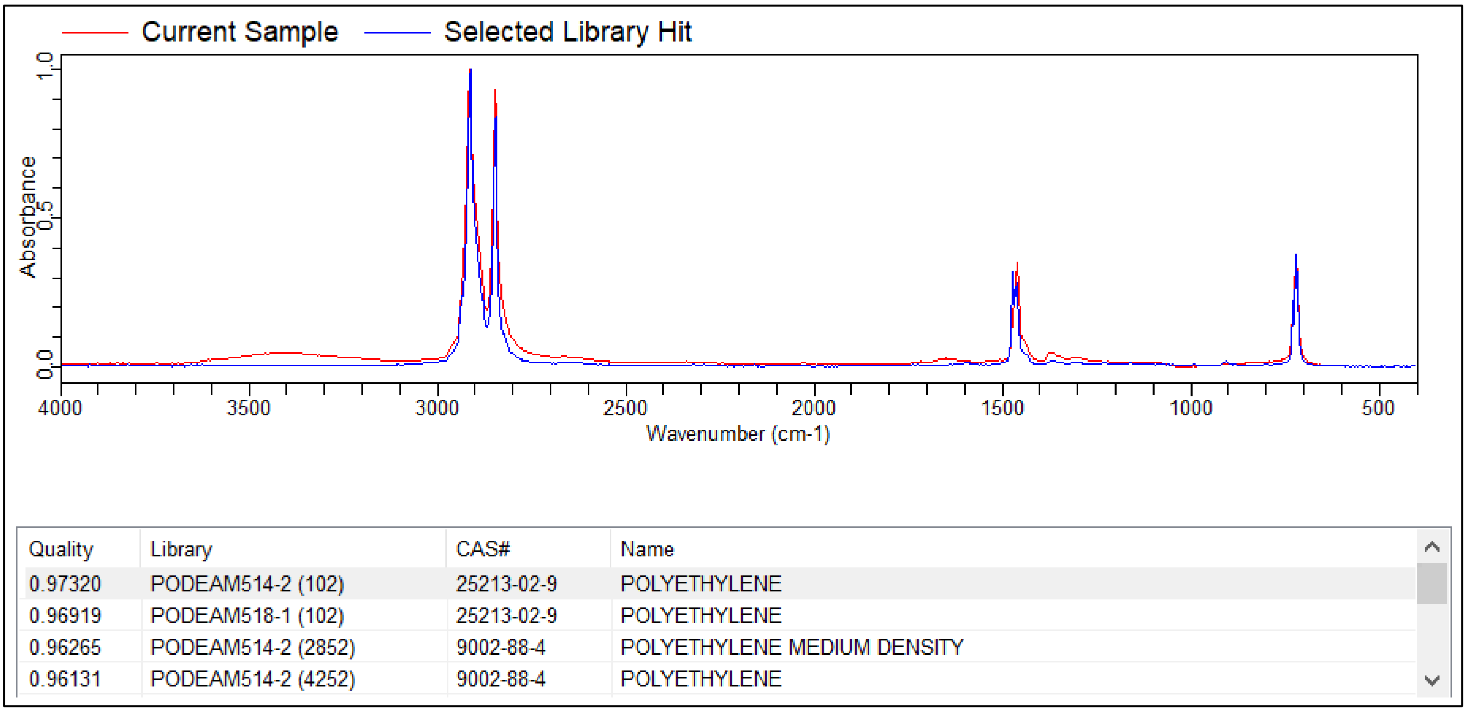
	Number of particles											
Composition **Colour**	**Cellulosic**	**PET**	**PE**	**PP**	**PVC**	**PEVA**	Nylon	Acrylic	Polynorborene	Polyvinyl alcohol	PS	Phenoxy resin
Black	12	4	0	2	1	0	0	0	0	0	1	0
Blue	69	7	1	3	4	1	4	0	3	1	0	0
Brown	0	0	0	0	0	0	0	0	0	0	0	0
Colourless	40	28	3	7	0	1	0	0	0	0	0	0
Green	4	0	0	0	1	0	0	1	1	0	0	1
Grey	0	0	0	0	0	0	0	0	0	0	0	0
Orange	0	0	0	0	0	0	0	0	0	0	0	0
Pink	0	3	1	4	0	0	0	0	0	0	0	0
Red	12	1	0	0	0	1	0	0	0	0	0	0
Violet	0	3	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0	0	0
Yellow	0	3	0	0	0	0	0	2	0	0	0	0
ΣParticles	137	49	5	16	6	3	4	3	4	1	1	1

Annex V. Examples of specific spectra obtained by FTIR analysis



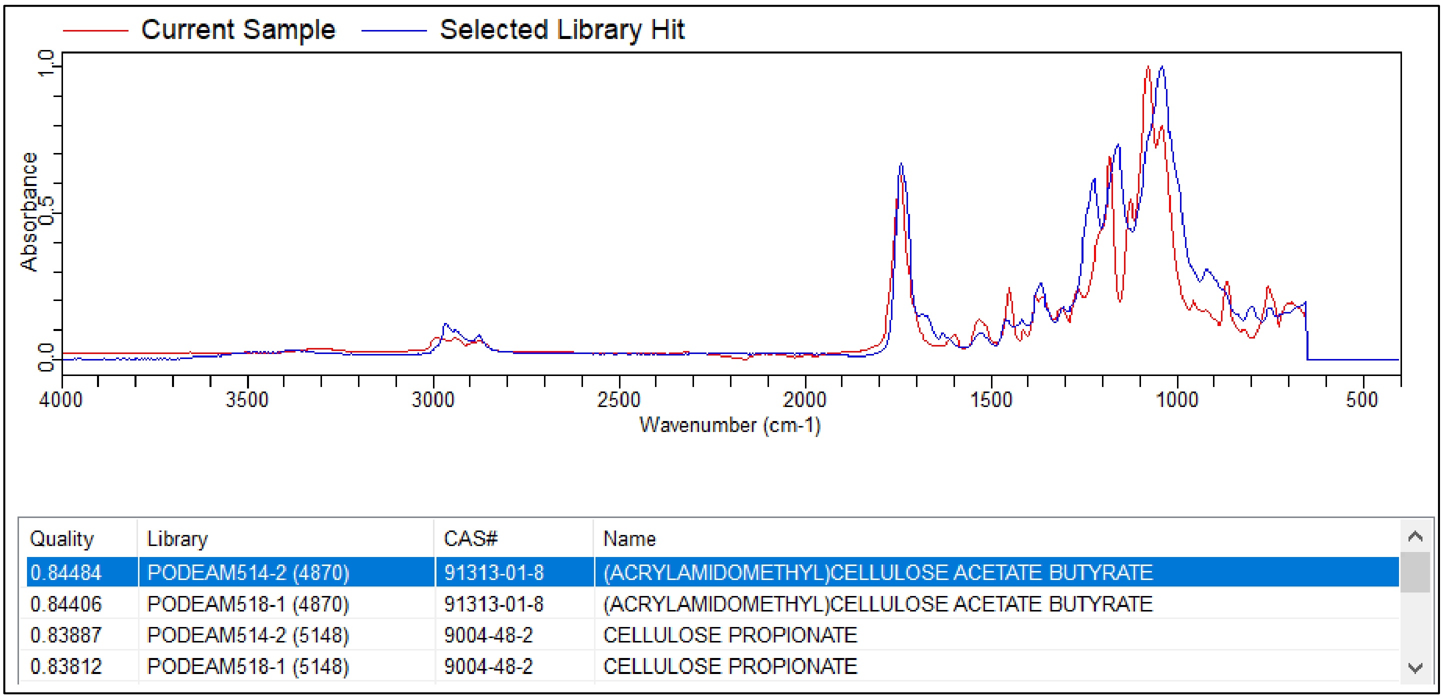
Quality	Library	CAS#	Name
0.98863	PODEAM518-1 (4860)	9003-07-0	POLYPROPYLENE
0.98742	PODEAM514-2 (4860)	9003-07-0	POLYPROPYLENE
0.98682	PODEAM514-2 (119)	9003-07-0	POLYPROPYLENE
0.98681	PODEAM518-1 (4859)	9003-07-0	POLYPROPYLENE

Figure V. 1: Comparison example of envelope of sample 1 with the polypropylene library spectrum.



Quality	Library	CAS#	Name
0.97320	PODEAM514-2 (102)	25213-02-9	POLYETHYLENE
0.96919	PODEAM518-1 (102)	25213-02-9	POLYETHYLENE
0.96265	PODEAM514-2 (2852)	9002-88-4	POLYETHYLENE MEDIUM DENSITY
0.96131	PODEAM514-2 (4252)	9002-88-4	POLYETHYLENE

Figure V. 2: Comparison example of envelope of sample 13 with the polyethylene library spectrum.



Quality	Library	CAS#	Name
0.84484	PODEAM514-2 (4870)	91313-01-8	(ACRYLAMIDOMETHYL)CELLULOSE ACETATE BUTYRATE
0.84406	PODEAM518-1 (4870)	91313-01-8	(ACRYLAMIDOMETHYL)CELLULOSE ACETATE BUTYRATE
0.83887	PODEAM514-2 (5148)	9004-48-2	CELLULOSE PROPIONATE
0.83812	PODEAM518-1 (5148)	9004-48-2	CELLULOSE PROPIONATE

Figure V. 3: Comparison example of envelope of sample 15 with the Cellulose acetate butyrate library spectrum.