Small microplastics (<100 μm), plasticizers and additives in seawater and sediments: Oleo-extraction, purification, quantification, and polymer characterization using Micro-FTIR

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HIGHLIGHTS

- Small microplastics and micro-litter were investigated in sediments and seawater.
- Pre-treatment and analytical method via Micro-FTIR were developed.
- Sediments showed higher concentrations than seawater.
- Additives and plasticizers can be good proxies for small microplastics' presence.

GRAPHICAL ABSTRACT
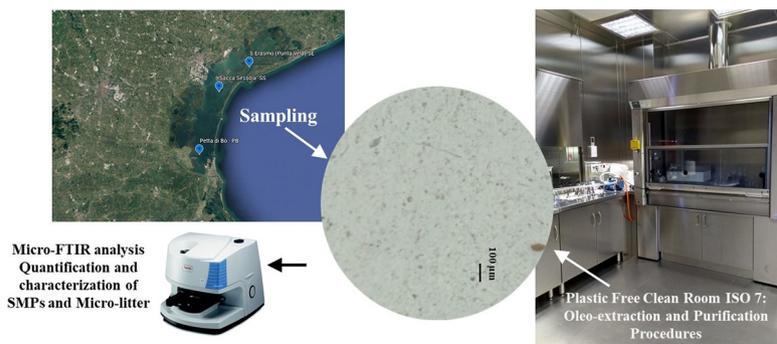
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ABSTRACT

In this study, the abundance and the distribution of small microplastics (<100 μm, SMPs) and of other components of micro-litter (i.e., additives, plasticizers, natural and non-plastic synthetic fibers, APFs) were investigated in sediments and seawater of three different sites of a transitional environment; different anthropogenic impacts and environmental features characterize these three sites. The pretreatment method developed (oleo-extraction and purification procedures) allowed the collection of particles (SMPs and APFs) in a wide range of densities, e.g., from low-density plastics to high-density plastics, avoiding further degradation/denaturation of polymers. An analytical method for quantification and simultaneous identification of SMPs and APFs via Micro-FTIR was developed. Higher abundances of SMPs were observed in sediments compared to the abundance observed in seawater. SMPs were not the major component of the micro-litter. With natural fibers and non-plastic fibers, additives and plasticizers were quantified and identified in sediments and seawater. These latter are employed to obtain specific characteristics of polymers; hence their presence can be a good proxy of these polymers' presence in the environment. Sources and pathways may influence the abundance and distribution of SMPs and APFs. Differences in abundance and distribution of these pollutants in sediments and seawater of the three sites investigated were statistically significant.

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1. Introduction

Microplastic particles and fibers (MPs) are persistent and ubiquitous in the environment. The term microplastics was coined in 2004 (Thompson et al., 2004), but there has not been an all-inclusive

definition until the ECHA report (2019), where size ranges for particles and fibers were given as well, and the need for polymer identification was strongly highlighted. MPs can be primary, i.e., manufactured to small sizes, and secondary, which are formed by the breakdown of any larger item of plastic (Alomar et al., 2016; Arthur et al., 2009; Efimova et al., 2018; Gigault et al., 2018; Hale et al., 2020; Rocha-Santos and Duarte, 2015; Waldman and Rillig, 2020).

MPs have been extensively investigated in marine environments, i.e., seawater and sediments (Kanhai et al., 2019; Lefebvre et al., 2019; Lindeque et al., 2020; Lyons et al., 2020; Rochman et al., 2016; Van Cauwenberghe et al., 2015; Woodall et al., 2014; Zobkov et al., 2019), but few studies were performed in transitional environments (Bayo et al., 2019; Lorenzi et al., 2020; Vianello et al., 2013 and 2018). MPs in transitional environments could originate from multiple diffuse or point sources (e.g., freshwater, agriculture, discharge inputs, diffuse pathways coming from extended areas) and the degradation of plastic items and larger plastic pieces (i.e., macroplastics; Coyle et al., 2020). Besides, only larger MPs have been comprehensively studied, while small microplastic particles (SMPs <100 μm) are always overlooked (Gillibert et al., 2019).

Sediments are generally the sink of inorganic and organic pollutants, and they are used to monitor spatial and temporal trends of these substances in the environment. However, due to their resuspension and re-mobilization, sediments can become a source of pollutants (Bancon-Montigny et al., 2019). Sediments are suggested to be an extended sink for MPs (Nel et al., 2018; Van Cauwenberghe et al., 2015) since more than 70% of the marine litter is estimated to end up in the seabed (Plastics Europe, 2018) and to be a source of MPs as well (Ballent et al., 2016; Näkki et al., 2019). Therefore, it is crucial to evaluate the occurrence of MPs in sediment as well as in water to comprehend the role of sediment as a source and sink for these pollutants. According to their sizes, high-density plastic fragments (e.g., fluorinated plastics) will sink to the sediments. In contrast, low-density plastic polymers (e.g., polyethylene (PE)) can float in the water column and eventually be deposited as a result of biofilm formation or fecal pellets of animals after ingestion (Avio et al., 2017; Andrady, 2011; Cole et al., 2013). It should be stressed that it is crucial to consider SMPs (particles and fibers) because they represent the majority of MPs (Peng et al., 2017; Vianello et al., 2013).

Plastic polymers have ubiquitous employments in industry, mechanics, agriculture, fishing, medical appliances (Ebnesajjad and Khaladkar, 2017; Gao et al., 2013; Gardiner, 2015; Geyer et al., 2017; Hidalgo-Ruz et al., 2012; Kutz, 2017; Vianello et al., 2013; Xie et al., 2020). Therefore, activities and tasks performed in the lagoon and the drainage basin may be relevant sources of these polymers.

Natural and non-plastic synthetic fibers, additives, and plasticizers are components of the microlitter, together with MPs and SMPs. Only few studies have dealt with additives and plasticizers associated with plastics (Campanale et al., 2020; Engler, 2012; Hermabessiere et al., 2017; Sundt et al., 2014; Wright and Kelly, 2017). Plastic polymers are usually mixed with various plasticizers and additives (e.g., bisphenol A, phthalates, polybrominated diphenyl ethers (PBDEs)); these compounds are employed during raw polymer synthesis either to improve or to obtain specific characteristics. The quantity of additives added can vary from 0% to more than 50% for electronic applications, while the average quantity of additives is 20% by weight of the polymer. Fillers are mainly employed to improve plastic performance or stabilize additives and decrease the material's flammability. Organic polymers are also blended with plasticizers or dyed with colorants. Like MPS, additives and plasticizers could also be toxic for biota. On the other hand, due to their ubiquitous presence and persistence in the environment, they can be significant proxies to estimate the sources and the presence of SMPs in the environment.

Pretreatment procedures are essential to analyze MPs and SMPs in environmental matrices. Density separation (i.e., flotation) is a widespread method to recover MPs from sediments, and it is based on the differences in density between plastic particles and/or fibers and sediment particles (Coppock et al., 2017; Hahn et al., 2019; Hidalgo-Ruz

et al., 2012; Van Cauwenberghe et al., 2015; Zhang et al., 2020). Hypersaline solutions are generally employed. Sodium Chloride (NaCl) solution was employed (Thompson et al., 2004; Vianello et al., 2013; Quinn et al., 2017) due to low cost, but only plastic particles with density ≤ 1.2 g cm^{-3} floated and were retrieved for the analysis; all polymers with density ≥ 1.2 g cm^{-3} (e.g., polyarylamide (PARA), fluorinated polymers) were not identified resulting in a possible underestimation of MPs. Other hypersaline solutions were employed, such as sodium bromide (NaBr, 1.4 g cm^{-3}), sodium iodide (NaI, 1.6 g cm^{-3}), zinc bromide (ZnBr$_2$, 1.7 g cm^{-3}), zinc chloride (ZnCl$_2$ 1.7 g cm^{-3}), sodium polytungstate (Na$_2$WO$_4$·2H$_2$O, 3.1 g cm^{-3}), with different densities and different costs. Besides, NaCl and NaBr have low recovery rates (<90%) and high error rates, while both NaI and ZnBr$_2$ have higher recovery rates (99%) and low error rates (Quinn et al., 2017). NaI and Na$_2$WO$_4$·2H$_2$O are the most expensive. ZnCl$_2$ is a good compromise for efficient density separation.

Pretreatment procedures (i.e., extraction and purification procedures) should be matrix-specific, but they must be standardized, avoiding or minimizing any degradation/denaturation of polymers. Particulate organic matter, biological matter, and other impurities are present in environmental matrices and can be adsorbed on the surface of MPs and SMPs and other microlitter components; these plastic particles and fibers should be separated (extracted) and purified without further degradation/denaturation so that they can be adequately quantified and identified. However, in scientific literature, these procedures are often poorly documented and differ from studies. It should be stressed that strong oxidizing agents and/or aggressive reagents (e.g., strong acids, alkaline agents; Carr et al., 2016; Cole et al., 2014; Klein et al., 2015; Nuelle et al., 2014) to remove organic matter and other interferences during the extraction/separation of MPs and SMPs from the matrix may contribute to the denaturation-degradation of polymers investigated (Cole et al., 2014; Corami et al., 2020a, 2020b; Hanvey et al., 2017; Hurley et al., 2018; Prata et al., 2019). These agents may break MPs and SMPs into smaller particles, reduce the weight, altering the shapes of particles and fibers, and increasing their abundance (Hanvey et al., 2017; Hurley et al., 2018; Lu et al., 2021; Zhang et al., 2019). Besides, temperature > 50 °C and a prolonged time of extraction procedure may denature polymers, especially those with a low pH tolerance (e.g., PA; Claessens et al., 2013; Jin and Su, 2009). Glass transition temperature (Tg) is the critical temperature at which a material changes its features from "glassy material" to "rubbery material"; when a plastic polymer is gradually heated, it develops a degree of softness or flexibility at a specific Tg. The temperature employed in the extraction procedure can affect the characteristics of a polymer according to its Tg (Andrady, 2017; Corami et al., 2020a, 2020b).

One aim of this study is to develop a pretreatment method (i.e., extraction and purification) suitable for SMPs (<100 μm), additives, plasticizers, and other components of micro-litter (APFs) which can be present in seawater and sediments, without further denaturation/degradation of particles and fibers. Besides, density separation with ZnCl$_2$ will be evaluated for gathering SMPs and APFs and the subsequent analysis via Micro-FTIR.

Another goal of this study is to develop an analytical method for the quantification and the simultaneous identification of SMPs and APFs via Micro-FTIR. Micro- Fourier Transformed Infrared Spectroscopy (Micro-FTIR, also known as μ-FTIR) is a non-destructive technique and allows the quantification and the simultaneous polymer identification of MPs > 100 μm, SMPs, and other components of microlitter (Corami et al., 2020a, 2020b; Iannilli et al., 2019; Liu et al., 2020).

2. Materials and methods

2.1. The study area

Water and sediments samples were collected in three different sites of the Venice Lagoon (Fig. 1), a broad, shallow area located in the

Adriatic Sea Coast in northeastern Italy, to develop the pretreatment method and the analytical method for the quantification and the simultaneous polymer identification of SMPs (<100 μm). The lagoon includes islands, tidal marshes, and fishing farms. The present lagoon-scape results from human modifications throughout the centuries; various human activities can affect pathways and biogeochemical cycles of nutrients and pollutants. Sant'Erasmo (SE) is in the northern basin of the lagoon; agricultural activities, the presence of a built-up area, and a sewage treatment plant on the island may be the principal sources of microplastics and other components of microlitter. Sacca Sessola (also known as Island of Roses, SS) is in the central basin of the lagoon, close to the historic city center; tourist activities related to the presence of a luxury hotel complex may be sources of microplastics and microlitter. Petta di Bò (PB) is in the southern basin of the lagoon; recreational boating and harbor activities related to the proximity of the port of Chioggia may be the primary sources of microplastics and microlitter in this area. Fishing activities may be other significant sources of SMPs and microlitter in these sites. Also, the drainage basin can be a relevant pathway of SMPs and other components of microlitter (i.e., additives, plasticizers, natural and non-plastic synthetic fibers, APFs) from the mainland towards the lagoon.

2.2. Sample collection

All containers, bottles, and tools were rinsed with ultrapure water (UW; Elga Lab Water, Veolia, High Wycombe, UK) and decontaminated with methanol (205 Superpurity Solvent >99.9%, Romil, Cambridge UK) and ethanol (absolute, for HPLC, ≥99.8%, Sigma Aldrich, Merck, Darmstadt Germany).

Standardized sampling methods of MPs in water are lacking (Karlsson et al., 2019). The most employed samplers for MPs in water are a manta trawl or a neuston net (mesh size of the net between 390 and 300 μm); plankton nets are also employed (mesh size of the net 100 μm; Baldwin et al., 2016; de Lucia et al., 2018; Prata et al., 2019; Schönlau et al., 2020). Larger mesh sizes are employed to collect MPs > 100 μm, avoiding clogging on the surface of nets by SMPs (Liu et al., 2020). Because SMPs are the most abundant fraction of plastic litter, and along the other components of microlitter were the subject of this study, surface seawater was collected 30 cm beneath the water surface employing

decontaminated steel containers (20 L capacity). While sampling, all the operations were carried out to avoid plastic contamination. After stirring to obtain homogenous subsamples, seawater subsamples for analysis of SMPs and APFs were then stored in previously decontaminated 2,5 L amber glass bottles at 4 °C until the pretreatment.

A steel Van Veen Grab sampler was employed to collect sediments, and sediments were stored in decontaminated glass boxes, which were kept at 4 °C until the arrival at the laboratory. Sediments were homogenized by quartering and subsampled once at the laboratory; sediment aliquots were then stored at 4 °C until the pretreatment. Sediments' characteristics are reported in the supplementary material.

2.3. Flotation of SMPs and other micro-litter components(additives, plasticizers, natural and non-plastic synthetic fibers) in sediment samples

Flotation with zinc chloride (ZnCl$_2$, density up to 1.7 g cm^{-3}; anhydrous, free-flowing, Redi-Dri™, ACS reagent, ≥97%, Sigma Aldrich, Merck, Darmstadt, Germany) was employed to recover polymers with a wide density range (from light SMPs to heavy SMPs). The procedure was applied to 3 aliquots of the same sediment sample to recover SMPs and other components of the micro-litter. First, an aliquot of 10 g was placed in a previously decontaminated Erlenmeyer flask with 10 mL of hydrogen peroxide 30% (H$_2$O$_2$, ACS reagent, Sigma Aldrich, Merck, Darmstadt, Germany), and 50 mL of ZnCl$_2$ solution and stirred for 15 min at 150 rpm on an orbital shaker. Then the mixture was left to rest for 2 h and 30 min until the liquid phase and sediments were separated. Then, the supernatant was carefully recovered (Crichton et al., 2017) and placed into a previously decontaminated glass separatory funnel. This procedure was repeated two more times with fresh aliquots of the hypersaline solution to retrieve all SMPs and APFs without adding other aliquots of H$_2$O$_2$. All the supernatants were carefully recovered and placed in the same separatory funnel, where 5 mL of H$_2$O$_2$ were added. After shaking, the solution was left to rest for 12 h to allow further separation between possible residual sediments and other impurities and the ZnCl$_2$ solution. After this time, all the impurities were discarded, and the solution was recovered and then vacuum-filtered on aluminum oxide filters (0.2 μm, 47 mm diameter, ANODISC (Anopore Inorganic Membrane), Whatman, Merck Darmstadt Germany). Reagent blanks and procedural blanks were performed.
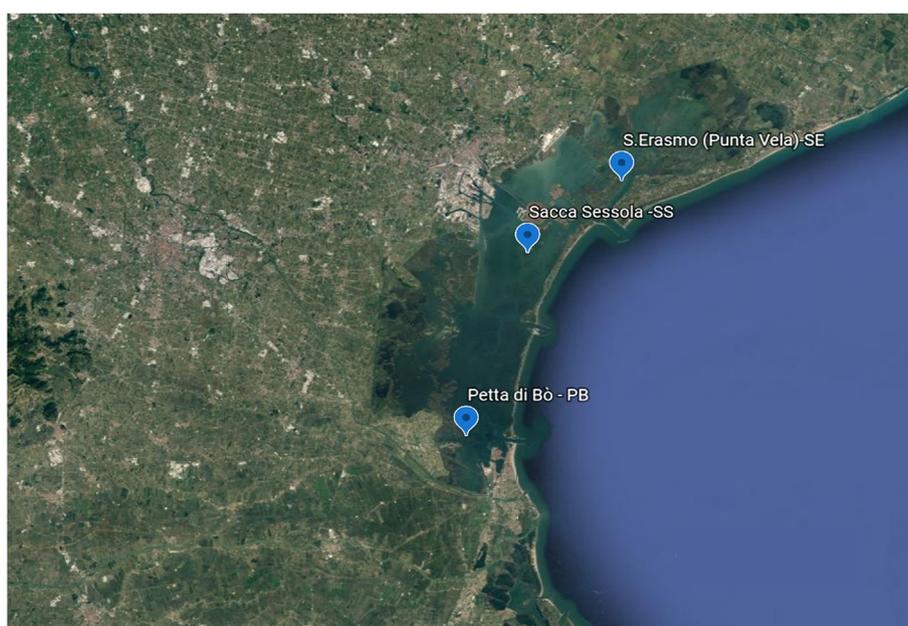

Fig. 1. Map of the three sites in the Venice Lagoon, where abundance and characterization of SMPs were investigated in sediments and seawater, SE Sant'Erasmo (Punta Vela), SS Sacca Sessola, PB Petta di Bò.

2.4. Oleo-extraction of SMPs and other components of micro-litter (additives, plasticizers, natural and non-plastic synthetic fibers) from sediments

Oleo-extraction to collectively retrieve SMPs and APFs was harmonized and optimized from the method illustrated by Crichton et al. (2017). Several tests were performed, and each test was run in triplicate. A sediment subsample was homogenized again by quartering, and aliquots of 10 g were placed inside separating funnels (500 mL capacity).

In the first test, 50 mL of UW, 5 mL of Triton X (BioUltra, for molecular biology, ~10% in H_2O, Sigma Aldrich, Merck, Darmstadt, Germany), and 5 mL of organic solvent-free and cold-pressed sunflower seeds oil (SSO, Crudolio, Camisano Vicentino (VI), Italy) were added to sediments. Triton X was employed to cleanse SMPs and other components of the micro-litter, exploiting its surfactant capacity.

Organic cold-pressed SSO was chosen because it was certified solvent-free; besides, it had a minor content of pigments (e.g., chlorophyll, carotenoids, etc.) compared to other oils (e.g., canola oil, castor oil, extra virgin olive oil, rapeseed oil, etc.; Lechthaler et al., 2020; Mani et al., 2019; Scopetani et al., 2020). These characteristics could allow a clear separation of the two phases.

In the second test, 5 mL of SSO, 10 mL of H_2O_2, and 50 mL of UW were added to the aliquots of sediments. In the subsequent tests, volumes of reagents were modified as follows: in the third test, 7 mL of SSO, 15 mL of H_2O_2, and 75 mL of UW, while in the fourth test, 7 mL of SSO, 20 mL of H_2O_2, and 100 mL of UW, respectively.

Separating funnels were then stirred for 15 min at 100 rpm on an orbital shaker in all the tests performed. Then, separating funnels were filled with UW and left to rest for 72 h for the complete separation of the three phases. After that time, all the oil phase and about 75% of the water phase were carefully poured into another previously decontaminated separating funnel, where another 10 mL of H_2O_2 were added. These separating funnels were stirred again for 15 min at 100 rpm on an orbital shaker and then left to rest for 24 h for the complete separation of the two phases. The water phase was discharged, and the oil phase was utterly recovered by adding 20 mL of hexane and 20 mL of ethanol and stored in a previously decontaminated Erlenmeyer flask.

The oleo-extraction procedure of the fourth test was adopted for all samples of sediments. An additional oleo-extraction was performed on each previously extracted sediment aliquot in the fourth test; this additional oleo-extraction was analyzed separately to check the extracting efficiency. Reagent blanks and procedural blanks were performed.

2.5. Oleo-extraction of SMPs and other components of micro-litter (additives, plasticizers, natural and non-plastic synthetic fibers) from seawater

The oleo-extraction procedure for seawater samples was developed by the harmonized and optimized oleo-extraction procedure for sediments. Oleo-extraction of each subsample was run in triplicate. After stirring to obtain homogenous aliquots from seawater subsamples, an aliquot of 200 mL was diluted with UW in a 500 mL separating funnel to diminish salinity and avoid spectral interferences (1:1 ratio). Then 5 mL of H_2O_2 and 5 mL of SSO were added.

The separatory funnel was stirred for 15 min at 100 rpm on an orbital shaker and then left to rest for 3 h, to achieve a complete separation of phases. After this time, water was collected in another previously decontaminated 500 mL separating funnel, while the oil phase was wholly recovered with 20 mL of hexane and 20 mL of ethanol and placed in a previously decontaminated Erlenmeyer flask. Then, the oleo-extraction was repeated by adding 5 mL of SSO and 5 mL of H_2O_2 again to the water in the second separating funnel. After 3 h, water was discarded, and the addition of 20 mL of hexane and 20 mL of ethanol allowed a complete recovery of the oil phase, which was then poured in the same Erlenmeyer flask. A third oleo-extraction on the three identical aliquots of the same sample was assessed and analyzed

separately to test the extracting efficiency. Reagent blanks and procedural blanks were performed.

2.6. Filtration and purification procedure

Filtrations of supernatants from flotation with $ZnCl_2$ and oil phases from sediments and seawater samples were performed under a plastic-free fume hood in a plastic-free Clean Room ISO 7. A decontaminated glass vacuum filtration system (VWR International, Milan, Italy) and aluminum oxide filters (0.2 μm, 47 mm diameter, ANODISC (Anopore Inorganic Membrane), Whatman, Merck Darmstadt Germany) were employed.

Every filter was cleansed before the filtration by pouring 50 mL of a solution of ethanol-methanol (7:3), and 20 mL of ethanol. Then, the supernatants from the flotation were poured several times, alternating the ethanol-methanol solution (ratio of 7: 3) and then ethanol alone. Finally, ethanol and 100 mL of UW were employed to rinse the filters.

The oil phases from sediments and seawater samples were poured several times, alternating with hexane and ethanol to allows the oil to be filtered more efficiently and quickly, without leaving traces of oil on the filter and the particles. Finally, an ethanol-methanol solution (7: 3) and ethanol were employed to rinse the filter. After cleansing the filter as described above, reagent blanks (including UW) and procedural blanks were filtered accordingly.

During filtration, the filtering funnel was covered with aluminum foil to minimize contamination. All filters were stored in previously decontaminated glass Petri dishes, coated with aluminum foil to prevent contamination. Filters were left to dry at room temperature for 72 h in the cleanroom until the analysis via Micro-FTIR.

2.7. Quantitative analysis and polymer identification of SMPs and other micro-litter components (additives, plasticizers, natural and non-plastic synthetic fibers) using Micro-FTIR

A Micro-FTIR Nicolet™ iN™ 10 (Thermo Fisher Scientific), equipped with an ultra-fast motorized stage and liquid nitrogen cooled MCT detector (mercury cadmium telluride detector), was employed. Abundance and polymer identifications were evaluated according to Corami et al. (2020a). Briefly, for the microscopic counting, at least 20 known-sized areas (i.e., count fields) were randomly chosen with no overlapping (Huppertsberg and Knepper, 2018; Mazziotti et al., 2013; Oßmann et al., 2018; Tong et al., 2020; Vianello et al., 2013). On each count field, an average of 250 particles was selected by employing the WIZARDS section of the Omnic™ Picta™ software. 64 co-scans were collected (spatial resolution 100 μm, aperture 100 μm × 100 μm, spectral range 4000–1200 cm^{-1}) on transmittance mode for each particle; particle sizes (length and width) were retrieved employing the imaging of the Omnic™ Picta™ software. Full details regarding microscopic counting and selection of particles using Omnic™ Picta™ Wizards, analysis, and identification of FTIR spectra are presented in the supplementary material.

Each polymer spectrum was then compared with specific polymer reference libraries (see the list of libraries employed in supplementary material); SMPs and APFs were counted and identified only when the identification match percentage was ≥65%.

The abundance (N_{SMPs}/Kg ww and N_{SMPs}/L) of SMPs and APFs in sediments and seawater were calculated according to equations modified from Corami et al., 2020a, 2020b.

$$N_{SMPs}/Kg = \frac{n * 1000 * F}{W} \tag{1a}$$

$$N_{APFs}/Kg = \frac{n * 1000 * F}{W} \tag{1b}$$

$$N_{SMPs}/L = \frac{n * 1000 * F}{V} \tag{2a}$$

$$N_{APFs}/L = \frac{n * 1000 * F}{V} \qquad (2b)$$

where n = SMPs or APFs counted on every field, V = volume of seawater, W = weight of sediments, and F = count factor, calculated as follows:

$$F = \frac{Filter\,area}{Count\,field\,area * n\,count\,fields} \qquad (3)$$

The weight of each microplastic particle and/or fiber is calculated according to its volume (V) and its density (ρ); the volume is calculated according to the aspect ratio AR. The total weight of SMPs ($\mu g/Kg$ ww and $\mu g\ L^{-1}$) was calculated, as well. All the equations are reported in the supplementary material.

2.8. QA/QC, blank analyses, and statistical analysis

All pretreatment procedures and filtration were performed under a decontaminated fume hood in a plastic-free cleanroom ISO 7. This laboratory is characterized by controlled air circulation and limited access; hence it is suitable for minimizing any source of contamination for the analysis of organic compounds, including SMPs. The laboratory is free of plastic materials, even in the air filtration systems; workbenches, cabinets, fume hoods, and all the rest of the cleanroom are made of stainless steel.

The ultrapure water (ELGA system), ethanol -methanol solution (7:3 ratio), and ethanol were employed to clean and decontaminate benches, glassware, and steelware. Decontaminated steel tweezers were employed to put aluminum oxide filters on the filtering unit before filtration and put them in decontaminated Petri glass dishes after filtration. Before the analysis, filters were mounted on the stage for analysis under a decontaminated fume hood in the cleanroom and carried to the Micro-FTIR laboratory, carefully covered with aluminum foil to avoid any external contamination. Samples of seawater and sediments were extracted and filtered in batches; for each batch, reagent blanks and procedural blanks were prepared and then analyzed to evaluate the possible interference of propylene caps, manipulation, and airborne contamination. Operators wore cotton lab coats and nitrile gloves.

No plastic particles and/or fibers were detected on reagent blanks and procedural blanks. Contamination was then minimized at any step of pretreatment and analysis.

Microplastic abundance data are count data and follow a Poisson distribution ((Filella, 2015; Courtene-Jones et al., 2017; Karlsson et al., 2019); Poisson's confidence interval was calculated accordingly. Kruskall-Wallis non-parametric test ($p < 0.05$) was employed to evaluate significant differences in abundances of polymers and APFs in seawater and sediments of the three sites studied. Cluster analysis was performed to identify differences among polymer distribution in seawater and sediments samples from the three sites under investigation. Statistical analyses were performed using STATISTICA software (TIBCO, Palo Alto, CA, USA).

3. Results and discussion

3.1. Development of extraction and purification procedures for sediments and seawater

Density separation was performed on sediment samples; the resulting supernatant was filtered as described above (Section 2.6). However, the analyses using Micro-FTIR could not be performed; despite rinsing the filters with UW at the end of filtration, the filter surfaces were thoroughly covered by a crystalline salt layer (Fig. S2a, in supplementary material), and quantification and identification of SMPs and APFs via Micro-FTIR were not possible.

Regarding oleo-extraction, Triton X employed in the first series of tests did not allow removing all particulate organic matter and other interferences. On the contrary, it dramatically slowed the filtration. The purification procedure was almost useless; the filter could be viewed under the Micro-FTIR microscope objective, but particles could not be analyzed with Micro-FTIR, i.e., spectra could not be collected and identified (Fig. S2b in supplementary material).

Increasing the amount of UW for sediment resuspension and H_2O_2 in the subsequent tests was not effective (Fig. S2c in supplementary material) until the fourth test. In this last set, oleo-extraction and purification were significantly effective (Fig. S2d in supplementary material). The filter could be viewed under the Micro-FTIR microscope objective; particles could be analyzed with Micro-FTIR, i.e., spectra could be collected and identified (see details of Micro-FTIR analysis of SMPs and other micro-litter components in supplementary materials). Furthermore, identification match percentages of spectra significantly increased ($\geq 65\%$, even close to 95% for some polymers, Figs. S5 and S6 in supplementary material; Corami et al., 2020b). Hence, the oleo-extraction and purification procedures of this set of tests were employed for all the samples of sediments.

Although flotation is the most employed pretreatment procedure for MPs >100 μm in seawater samples (Cincinelli et al., 2017; Cutroneo et al., 2020; Dyachenko et al., 2017; Gallagher et al., 2016; Maes et al., 2017), it was not employed to investigate SMPs and AFPs in seawater samples for the reasons given above for sediment samples. Oleo-extraction and purification procedures developed and applied to sediment samples were optimized for seawater. The volumes of seawater (Koelmans et al., 2019; Oßmann et al., 2018) and sediment (Bayo et al., 2016; Frias et al., 2016) aliquots analyzed in our study were adequate to obtain robust data of abundance and distribution SMPs and other microlitter components.

The efficiency of oleo-extraction was tested in sediment and seawater samples. The first oleo-extraction in sediments was efficient (> 95%), while the two consecutive extractions in seawater samples allowed an efficiency of ≫95%.

Solvents employed removed any oil residue and other interferents from the filter and the SMPs, avoiding degradation/denaturation of these particles. Particle sizes are measured by the imaging of the WIZARDS section of the Omnic™ Picta™ software. The instrumental limit of detection (LOD) of particle size in length is 10 μm; thanks to extraction and purification procedures developed in this study, the LOD of particle size in length was 5 μm.

3.2. Abundance and characterization of SMPs in sediments

By analyzing filters via Micro-FTIR, SMPs were quantified and characterized (polymer identification), sizes (length and width) of each particle were retrieved. The complete list of polymers identified in the three sites studied, together with their acronyms, is reported in Table 1. The spectra of some polymers observed are shown in the supplementary material (Figs. S5 and S6). AR, volume, and then the weight of SMPs were calculated.

The average length of SMPs in sediments is <50 μm, while the average width is <26 μm (the average length and width for each site studied were shown in Table S1 in supplementary material). The shapes of SMPs were evaluated as spherical, elliptical/elongated, or cylindrical; the most frequent shape in each site was elliptical (AR ≥ 2). Fibers and/or cylindrical SMPs were observed as well, especially in the sediments from PB. Our findings are in agreement with the data of sediments in lagoons or enclosed bays (Teng et al., 2020; Yuan et al., 2019; Zheng et al., 2019).

The abundance and distribution of polymers in sediments are shown (Fig. 2). SMPs followed Poisson distribution, and the confidence limit (error) for the abundance of each site was calculated (Fig. 2). While the abundances of SMPs in SS and PB sediments were similar, SE showed a 16% lower abundance than that observed in SS. The abundances observed in sediments of the three sites studied are higher

Table 1
List of polymers and their acronyms identified in the sediments and the seawater of the three sites studied.

Sant'Erasmo - SE	Sacca Sessola - SS	Petta di Bò - PB
Sediments		
Polyethylene- PE	Polyethylene- PE	Polyethylene- PE
Polyestere - PES	Polypropylene - PP	Polyestere - PES
Polyolefin - PO	Polyolefin - PO	Polyphtalamide - PPA
Nylon - PA	Nylon - PA	Polyarylamide PARA
Trichlorotrifluoroethane Polytetrafluoroethylene Blend – PTFE blend*	Trichlorotrifluoroethane Polytetrafluoroethylene Blend – PTFE blend*	Trichlorotrifluoroethane Polytetrafluoroethylene Blend – PTFE blend*
Polytetrafluoroethylene - PTFE*	Polytetrafluoroethylene - PTFE*	Polytetrafluoroethylene - PTFE*
Polyarylamide PARA	Polyarylamide PARA	Fluoroelastomer*
Tetrafluoroethylene-Perfluoro alcoxy vinyl ether copolymer - PFA*	Tetrafluoroethylene-Perfluoro alcoxy vinyl ether copolymer - PFA*	Tetrafluoroethylene-Perfluoro alcoxy vinyl ether copolymer - PFA*
Acetal copolymer - POM	Acetal copolymer - POM	Acetal copolymer - POM
Ethylene Methyl Acrylate copolymer - EMA§	Ethylene Tetrafluoroethylene copolymer - ETFE*	Polyethylene co ethylene acrylate – EEA§
Poly methyl acrylate - PMA§	XT Polymer 375* Fluoroelastomer*	XT Polymer 375*
Polyphenyloxide - PPE	Polyphenyloxide - PPE	
	Polyphtalamide - PPA	
Seawater		
Polyethylene- PE	Polyethylene- PE	Polyethylene- PE
Polyolefin - PO	Polyolefin - PO	Polyolefin - PO
Polyestere - PES	Polyestere - PES	Ethylene Methyl Acrylate Copolymer- EMA§
Polyarylamide PARA	Polyarylamide PARA	Polyarylamide PARA
Polyphtalamide - PPA	Polyphtalamide - PPA	Polyphtalamide - PPA
Polytetrafluoroethylene - PTFE*	Polytetrafluoroethylene - PTFE*	Polytetrafluoroethylene - PTFE*
Polyphenyloxide - PPE	XT Polymer 375*	XT Polymer 375*
Polyacrylate - PA§	Acetal copolymer - POM	Acetal copolymer - POM
Nylon - PA	Nylon - PA	Nylon - PA
Polyvinylidene fluoride - PVDF*	Fluoroelastomer *	Polyacrylate - PA§
Polypropylene - PP	Polypropylene - PP	Polypropylene - PP
Poly(Ethylene Vinyl Alcohol) - EVOH	Trichlorotrifluoroethane Polytetrafluoroethylene Blend – PTFE blend*	Trichlorotrifluoroethane Polytetrafluoroethylene Blend – PTFE blend*
		Polystyrene-PS
		Tetrafluoroethylene-Perfluoro alcoxy vinyl ether copolymer - PFA*

§ acrylic polymers.
* fluorinated polymers.

compared to data in the scientific literature (Qi et al., 2020; Vianello et al., 2013; Zheng et al., 2019), because SMPs are often overlooked (Gillibert et al., 2019) and MPs >100 μm are the most studied.

The concentration by weight (Figure S7: supplementary information) of SMPs in PB was the highest (491 μg/kg ww), while those in SS and SE were similar (99 μg/kg ww and 81 μg/kg ww, respectively). It should be noted that large fibers of PES (> 400 μm in length) were present in sediments from PB; although they were only 25% of all the PES present in sediments, these fibers represented a very significant contribution in terms of weight.

FPs were the majority of SMPs in all three sites (Fig. 2). PE was the second most abundant polymer in SE and SS, while its abundance in
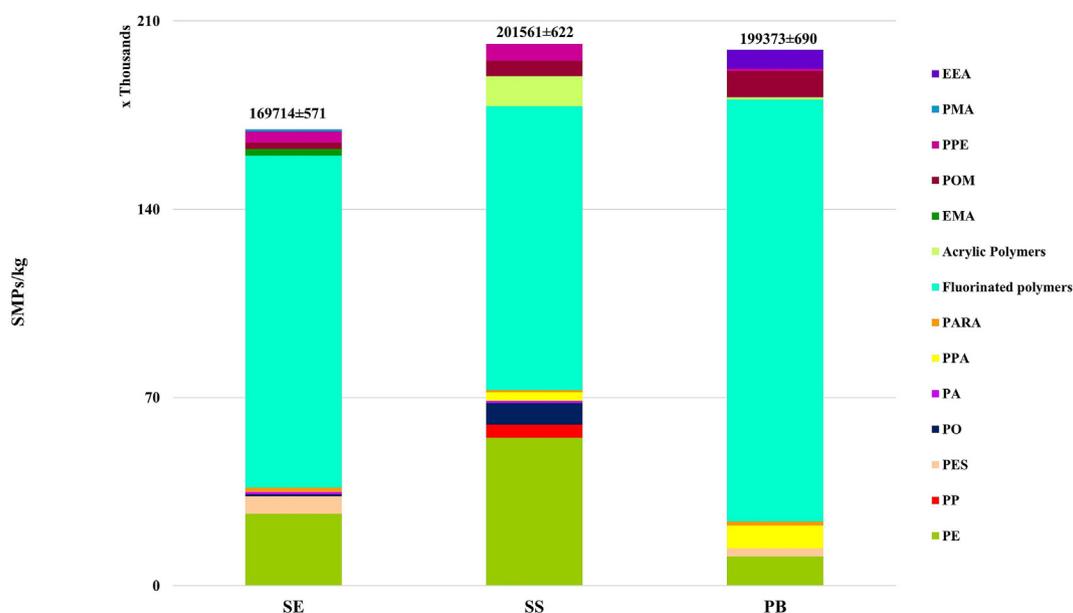

Fig. 2. Abundance (SMPs/kg ww) and polymer distribution of SMPs in sediments of the three sites investigated. Abundances and errors (fiducial interval according to Poisson Distribution) are reported. Weight (μg SMPs/kg ww) of SMPs in sediments of the three sites investigated: SE 81 μg/kg ww, SS 99 μg/kg ww, PB 491 μg/kg ww.

PB was quite similar to that of acetal copolymer (5%, POM), followed by polyethylene co ethylene acrylate and polyphtalamide (4%, EEA and PPA, respectively). POM is employed for automotive, medical equipment and healthcare, fluid handling applications, and consumer goods. PPA is often molded into parts to replace metals in applications requiring high-temperature resistance, while EEA is often employed with other thermoplastics, i.e., PE, polypropylene (PP), and polyvinylchloride (PVC).

Densities of fluorinated polymers (> 2 g cm^{-3}) are higher than that of seawater (1.020–1.029 g cm^{-3}) and are the highest among all the polymers found; these particles tend to sink quickly into the sediments. PE density is lower than seawater (0.91–0.94 g cm^{-3}); these particles should float on seawater, but marine aggregates, biofouling, and tide processes have been proposed as possible causes to increase the apparent density of these particles, which then sink in deep waters or into the sediments (de Haan et al., 2019). Therefore, floating in surface waters will only be a temporary phase of their fate before they sink (Andrady, 2017; de Haan et al., 2019). Due to their wide use, diffuse rather than point sources influence the distribution of fluorinated polymers and PE in the Venice Lagoon.

Regarding the Kruskall-Wallis test, differences in the abundances and polymeric distribution observed in the sediments of the three sites are statistically highly significant ($p < 0.01$). Regarding the size of SMPs (length and width), two major clusters (Fig. 3) can be distinguished, i.e., PP particles as one group (average length 67 μm) and all the other SMPs as the other group (average length 40 μm); PP particles were observed only in SS. SMPs' characteristics (shape and size) may significantly change about aging, mechanical erosion, and/or photodegradation, but sources and pathways might affect them as well. Hence, PP particles might come from different sources than other particles.

3.3. Abundance and characterization of SMPs in seawater

As for SMPs in sediments, these particles were quantified and characterized (polymer identification) in seawater, and sizes (length and width) of each particle were retrieved. AR, volume, and then the weight of SMPs were calculated. The list of polymers identified in the three sites studied, together with their acronyms, is reported in Table 1. The average length is <60 μm, and the average width is <27 μm (the average length and width for each site studied were shown in supplementary material). The shapes of SMPs were evaluated as spherical, elliptical/elongated, or cylindrical. The spectra of some polymers are shown in the supplementary material (Figs. S5 and S6).

Polymer distribution and abundance of SMPs in the water (SMPs L^{-1}). of the three sites studied, with respective confidence limits, are shown in Fig. 4. Poisson distribution allowed the calculation of the fiducial interval, i.e., the error. Compared to those in sediments, the abundance of SMPs in seawater was always lower. The lowest abundance was observed in SE, an increase (34%) in abundance was observed in PB, while the highest abundance was observed in SS; the latter is in the central-southern lagoon, close to the historic center of Venice, which is an urbanized area subject to intense touristic activity. SMPs may not be retained by the filters of the wastewater treatment plants (Sun et al., 2019; Talvitie et al., 2017), but it should be noted that in some areas of the historic center of Venice, urban wastewater sewer discharges directly into the lagoon. Together with the inputs from the industrial activities in Porto Marghera and the drainage basin, these inputs can contribute to the abundance observed in SS. Higher abundances of SMPs in sediments highlight an accumulation process (Ballent et al., 2016; Wang et al., 2019; Zheng et al., 2019), and sediments are sink, but they can become a source of SMPs, due to resuspension.

PE and fluorinated polymers are among the most abundant polymers observed in seawater. Due to its wide range of applications, thinking of a pointed source for PE is complex; instead, a diffuse source of this polymer is highly probable. An identical scenario can be envisioned for
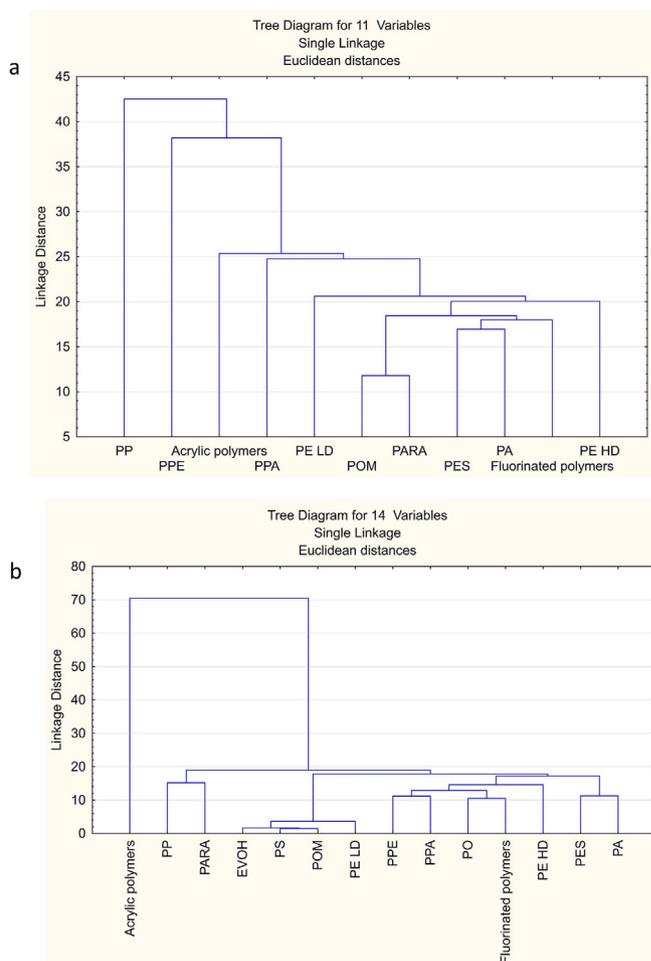
Fig. 3. Cluster analysis of a) SMPs in sediments of the three sites investigated and b) SMPs in seawater of the three sites investigated.

fluorinated polymers, which have a broad range of usages and are present in similar concentrations in the water of the three sites.

Another abundant polymer in SE water is polyester (PES); its presence is related to fiber release in washing discharges with the possibility to reach the environment (Belzagui et al., 2019; Corami et al., 2020a), and it can also be related to the washout from agricultural areas, such as SE. This polymer can be commonly found in soil amending agents, plant support, and wind protection netting (Crossman et al., 2020). The presence of nylon (PA) in SE seawater can be related to the same PES sources and the breakdown of fishing gears, nets, and ropes (Vianello et al., 2018; Wang et al., 2018).

It is also worth highlighting the presence of acrylic polymers in PB seawater; these polymers are employed for ship and boat paints due to their resistance to abrasion and washing; thus, they could be connected to the maritime activities near the harbor area of PB.

Regarding the weight of SMPs in seawater (Fig. S8), the highest value was observed in PB (83.8 μg L^{-1}), while SS and SE showed similar values (45.0 μg L^{-1} and 49.9 μg L^{-1}, respectively). Weight is related to the polymer density and AR. The elliptical/elongated shape was preeminent in all sites for most of the polymers identified; however, cylindrical particles were observed in PB, and they could account for the highest weight observed. The shape of SMPs depends on the degradation process and the residence time in the environment (Chubarenko et al., 2016); thus, fibers could be broken up into smaller pieces and originates more elongated or cylindrical fragments.

As already observed in the sediments, the different abundance and distribution of polymers in the waters of the three test sites were statistically significant (Kruskall-Wallis, $p < 0.05$). By applying cluster
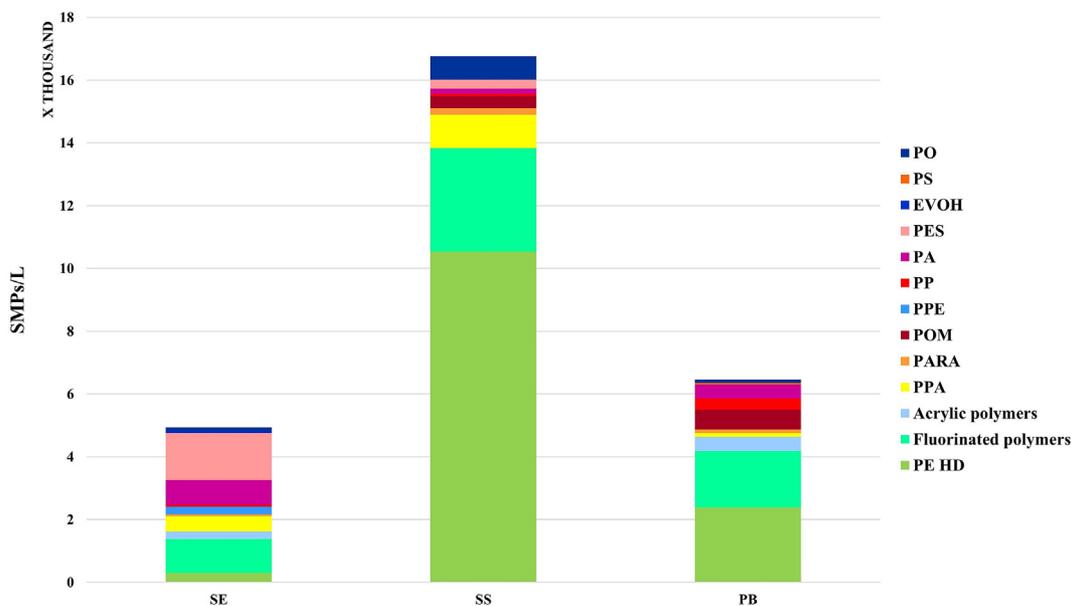
Fig. 4. Abundance (SMPs/L) and polymer distribution of SMPs in seawater of the three sites investigated. Abundances and errors (fiducial interval according to Poisson Distribution) are reported. SE 4937 ± 97 SMPs/L; SS 16759 ± 179 SMPs/L ww; PB 6653 ± 113 SMPs/L.

analysis, two major clusters can be observed (Fig. 3 b), i.e., acrylic polymers (APs) as one group (average length 123 μm) and all the other SMPs as the other group (average length 45 μm). APs were observed in SE and PB and might come from different sources than other particles, while usage, sources, and pathways for other SMPs might be relatively similar.

3.4. Other components of micro-litter: additives, plasticizers, and natural fibers

Besides SMPs, additives, plasticizers, natural and non-plastic synthetic fibers are components of micro-litter. These components were simultaneously collected and filtered together with SMPs from sediments and seawater, using the same pretreatment method; then, they were

simultaneously quantified and identified. Compared to SMPs in sediments (Fig. 2), these other components accounted for 55% in PB (Fig. S10), 41% in SS (Fig. 5a), and 54% in SE (Fig. S9); in seawater, these components accounted for 41% in PB (Fig. S10), 28% in SS (Fig. 5b), and 71% in SE (Fig. S9). Except for SS seawater, SMPs were not significant micro-litter components; hence, to avoid overestimating SMPs in environmental matrices, polymer identification is compulsory and strictly essential.

For example, rayon is a synthetic textile fiber produced from cellulose, is not a plastic polymer, and is employed in textiles and fabrics (Peng et al., 2018). It is often among the main components of micro-litter, marine organisms can ingest this fiber, and the ecological effects should be carefully considered; in some studies, it was wrongly considered as plastic, and then MPs' abundance was overestimated (Dong
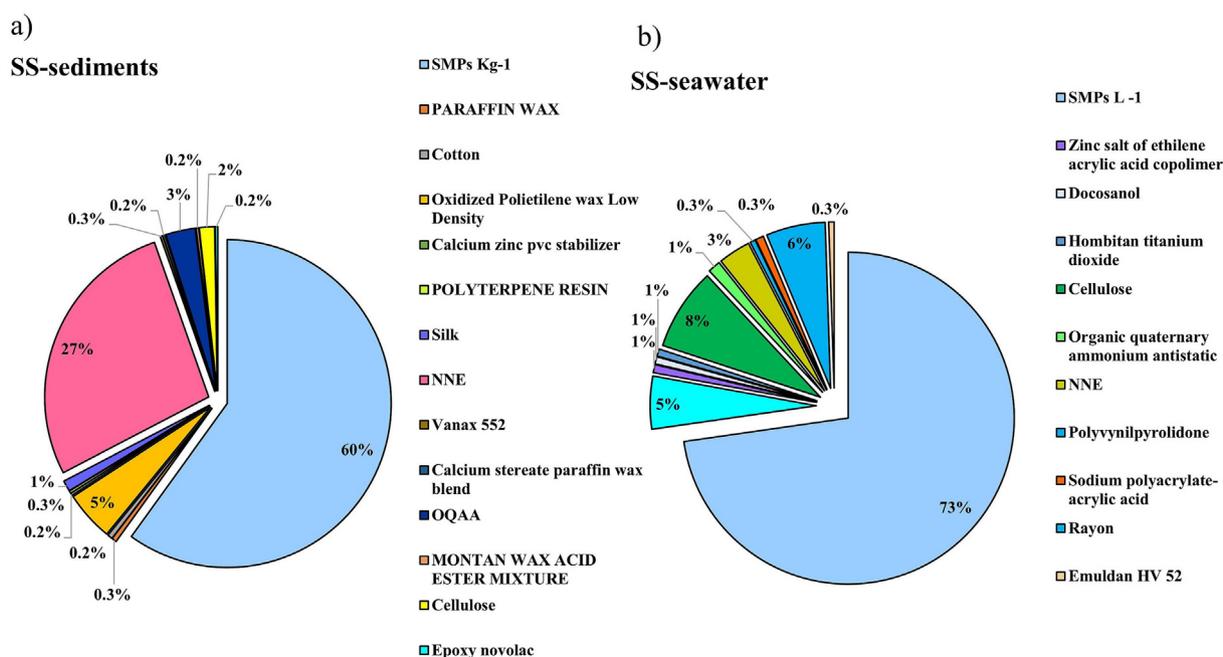

Fig. 5. Other components of micro-litter: additives, plasticizers, natural and non-plastic synthetic fibers a) in the sediments of SS and b) in the seawater of SS.

et al., 2020; Qi et al., 2020). Rayon cannot be distinguished from other MPs in a microscopic investigation (Peng et al., 2017); thus, polymer identification by IR spectrometry allows the best proper quantification.

Cellulose is the most abundant natural fiber, followed by rayon and cotton, in seawater of the three sites studies; instead, in sediments, these fibers together with silk-like particles are in lower abundance. Silk-like particles in sediments and seawater can be sea silk, which is produced by mollusks such as oysters to allow anchoring to the substrates (Corami et al., 2020b).

NNE (N-(2-ethoxy phenyl)-N-(2-ethyl phenyl)-ethanediamide), also known with its brand name Tinuvin® 312), is more abundant in sediment samples (48% in SE; 44% in PB and 27% in SS) than in seawater. This additive is a light stabilizer for various plastics and other organic substrates, including unsaturated polyesters and PVC. Polyester was identified in sediments and seawater of this study, while PVC was never observed, although PVC manufacturing plants were present in Porto Marghera. However, this additive can be a proxy of the presence of PVC in the Venice Lagoon; this hypothesis is supported by the presence in sediments of another additive: Calcium Zinc PVC stabilizer, which is a heat stabilizer additive for PVC plastics. PVC containing 20–50% plasticizer is considered flexible PVC employed in the building and packaging industry.

Poly-(n-methyl) acrylamide was observed in seawater and sediments of PB and SE. It is a material employed in polymer and copolymer synthesis; it is frequently employed in the marine industry, especially in coatings for antifouling in the marine environment, coating for waterproofing, and corrosion protection, primarily. It has excellent wetting properties and readily forms films, making it suitable as a coating or an additive to coatings.

The Kruskall Wallis test was also applied to APFs observed in water and sediment, respectively. The differences were statistically significant ($p < 0.05$). The identification of additives and plasticizers can be helpful to understand the pathways and sources of SMPs.

4. Conclusions

The oleo-extraction procedure harmonized and optimized, and the purification procedure developed allow an optimal assessment of SMPs and APFs in sediments and water of the Venice Lagoon. An innovative aspect of the pretreatment method is that the extraction procedure allows retrieving polymers in a wide density range, from PE to fluorinated plastics and APFs as well. Another innovative aspect is that the oleo-extraction and the purification procedures developed in this study do not induce further degradation/denaturation of polymers. The purification procedure allows for more accurate identification of polymers and other components of micro-litter with high matches. Plastic particles were identified when the match percentage was ≥65%, but some polymers (e.g., PE) were > 95%. Increasing the match percentage allows a more precise polymer identification and consequently a thorough quantification without over- and underestimations of the abundance of SMPs and APFs in sediments and seawater.

The method is robust, SMPs and APFs are quantified and identified, and results are repeatable and reliable.

The differences observed in the distribution and abundance of SMPs in the sediments and waters of the three sites investigated are statistically significant; SMPs' distribution may be site-specific, related to the usage and sources of polymers in the lagoon in the drainage basis.

Differences in abundance and distribution of APFs in sediment and seawater were statistically significant. Other components of micro-litter, i.e., some additives and plasticizers identified in this study, were specifically related to a particular polymer; hence, these compounds may be good proxies of the presence of polymers in the environment and help to comprehend not only sources but also pathways and destinies of SMPs and micro-litter in the environment.

The results obtained in this study are preliminary and are part of a more thorough study in which diffuse and point sources, transportation, and seasonal variations will be investigated in further detail.

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