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## **Sources of microplastics pollution in the marine environment: importance of wastewater treatment plant and coastal landfill**

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This study investigated the role of a municipal wastewater treatment plant (WWTP) effluent and an abandoned coastal landfill as pathways for microplastics (MPs) input into the marine environment. MPs were first analyzed in raw sewage influent, sludge and effluent samples, and their fate was studied along a distance gradient from the WWTP in three matrices: surface water, sediments and wild mussels. All suspected MPs were characterized according to their polymer nature using micro-Raman spectroscopy. The investigated WWTP had an estimated daily discharge of 227 million MPs. MPs were found in all matrices with a decreasing **abundance from the effluent**. Strong MPs abundances (higher than those found near the WWTP effluent) were observed in the vicinity of the coastal landfill suggesting its importance as a MPs entry route into the marine coastal environment. Our study supports the idea that blue mussels are a promising sentinel species for MPs (**< 200  $\mu$ m**).

**Keywords:** Microplastics; Wastewater Treatment Plant; coastal landfill; mussels; micro-Raman

## 1. Introduction

The recent increase of microplastics (MPs) occurrence into the aquatic environment is raising concern about their potential harm on diverse organisms (Gall and Thompson, 2015). These plastics of a size below 5 mm (GESAMP, 2015) can be divided into either primary microplastics (directly produced MPs) or secondary microplastics (resulting from macroplastics fragmentation) (GESAMP, 2015; Koelmans et al., 2014). Their entry routes into the marine environment mainly come from land-based origin: accidental loss or mishandling plastics, dumping plastic wastes and mismanaged landfills i.e an absence of a fence surrounding the landfill with no appropriate synthetic material for covering wastes (reviewed by Duis and Coors, 2016). Wastewater treatment plants (WWTP) add up to these entry routes and is considered as an important MPs pathway into the aquatic environments (Talvitie et al., 2015). These WWTPs may collect both industrial and domestic water containing MPs used in various products (Zbyszewski et al., 2014; Eerkes-Medrano et al., 2015). Several factors influence the MPs quantities being discharged by the WWTPs such as the volume of treated water, the flow rate, the filtration processes and sewer systems (Mason et al., 2016; Ziajahromi et al., 2017). As indicated in previous studies, most of the MPs can be effectively removed by secondary and tertiary treatment (Lares et al., 2018; Murphy et al., 2016), but it doesn't exclude that a large number of MPs is still being released in the aquatic systems (Browne et al., 2011; Mathalon and Hill, 2014; Murphy et al., 2016) and most specifically into river catchments (Roex et al., 2013). Removal efficiencies of microplastics in WWTPs range generally between 72–99.4% (Gatidou et al., 2019; Sun et al., 2019) suggesting that most particles are either accumulated during the sedimentation process or the grease removal (Magni et al., 2019, Murphy et al., 2016). **These rates remain limited by current detection techniques.**

Both fibers and fragments are observed in the WWTPs water (Magnusson and Norén, 2014). Their dispersion into the aquatic environment leads to their ingestion by aquatic species like bivalves (Browne et al., 2008, Railo et al., 2018). Mussels, *Mytilus* spp. are considered as suitable animals for monitoring chemical pollutants in aquatic **environments** (Green et al., 2017). They are sessile animals capable of giving precise spatial information, easily collected where they form mussel's beds in shallow waters and contain enough tissue amounts for laboratory analysis. They are filter-feeders (feed mainly on phytoplankton) which increase their probability

to ingest MPs and integrating them in the food chain (Wright et al., 2013). MPs ingestion might lead to several physiological issues for bivalves such as strong inflammatory responses in *Mytilus sp.* (Avio et al., 2015) or adverse consequences in *Mytilus edulis* digestive glands (Von Moos et al., 2012). Mussels are important prey source for many fish and they are highly consumed by humans making it critical to evaluate the amount of MPs ingestion.

Even though several studies evaluated WWTPs discharge into the aquatic systems, most of them focused on treatment plants discharging solely in the freshwater systems (Gies et al., 2018; Lares et al., 2018; Mintenig et al., 2017; Dris et al., 2015; Murphy et al., 2016; Wolff et al., 2019). A few assessed WWTPs discharges in the marine environment (Talvitie et al., 2015, 2017; Ziajahromi et al., 2017; Leslie et al., 2017). **To our knowledge, no study has evaluated microplastics input, dispersion and accumulation in coastal marine environment via wastewater treatment plant outlet in three different matrices.** Also, no study has evaluated the role of coastal landfills in MPs entry into the coastal marine environment making its relevance not well-known.

The aims of this research were (1) to assess the **contribution** of a major tertiary WWTP located in Le Havre (France) in MPs discharge into the marine coastal environment, (2) to follow the released MPs in the marine environment along a distance gradient from the discharge pipe in three matrices (surface water, bottom sediments and mussels), and (3) to evaluate the role of an abandoned coastal landfill as a source of MPs entry into the marine coastal environment.

## **2. Material and Methods**

### **2.1. Study zone and sampling sites:**

The sampling area was located in Le Havre, Northern France. The study zone investigated was divided into three areas: The Edelweiss Wastewater Treatment Plant (WWTP), Le Havre harbor and Sainte-Adresse coastal zone. The WWTP is located inside Le Havre harbor and receives the effluent of 20 municipalities (serving a population of 415 000 habitants and a volume of 80 000 m<sup>3</sup> per day). This tertiary WWTP was constructed in 2011 and is capable of treating collected

water in five different steps before discharging it into the harbor's basin. Le Havre harbor is located at the mouth of the Seine estuary, in the Eastern English Channel. It is the second biggest French harbor in tonnage and receives 59% of containers traffic (Report GPMH, 2015). The Sainte-Adresse's site is located in the vicinity of the Dollemard coastal landfill. This landfill was constructed in 1960 and received tons of household, industrial and special wastes. This landfill was closed in 2000, yet the huge amount of dumped garbage led to its collapse on the bottom of the cliff resulting a 750 m waste distribution along the coast. During high tide, the waves collect the wastes sitting on the coast promoting their entry into nearby coastal waters (Report SCE, 2011).

In the WWTP, water samples from raw influent and effluent were collected as well as a sample of sewage sludge. In the marine environment, samples of water, sediments and mussels were collected in four sites (1 to 4) in the harbor, and one site (site 5) in the vicinity of the coastal landfill (Fig. 1). The sites were located from the WWTP outlet with respective distances of 50 m, 1.1 km, 1.6 km, 5.3 km and 10.3 km. These sampling sites were selected in order to follow MPs dispersion and concentration in the three analyzed matrices.

## 2.2. Sampling Methods:

The sampling was done between the 3<sup>rd</sup> and the 4<sup>th</sup> April 2018. Inside the WWTP, two samples of 500 mL raw influent and a sample of 100 g of sewage sludge were taken for analysis on the 3<sup>rd</sup> of April at 3:00 pm. Since we wanted to sample all sites the same day to have the same environmental conditions, we chose to sample a large volume of water (one to two m<sup>3</sup>) representative of the analyzed water body rather than making replicates. The first ten centimeters of the water column was pumped and filtered on stainless steel sieves of different mesh sizes of 500 µm, 200 µm, 80 µm and 20 µm. The filtering system consisted of a water pump (Bilge pump, SEAFLO®) connected to a flowmeter (Turbine Digital Pro K24) that calculate the exact volume being filtered throughout the sieves. Before collecting the water samples, the pump and the pipe were rinsed with the seawater then with Milli-Q water. The 20 µm sieve was the fastest to get clogged so it was analyzed separately. Each sieve was rinsed with Milli-Q water inside clean glass bottles (previously washed with Milli-Q water) covered in aluminum foil and conserved at 4°C until analysis.

One kilogram (kg) of sediment samples was taken with a stainless-steel sediment core sampler from all sites except in site 4 where it was a rocky substrate. They were conserved inside aluminum foil then stored at -10°C until analysis.

Wild mussels (*Mytilus sp.*) specimens were collected in all sites (1 to 5). Around 20 individuals were sampled near the water filtration location. Each individual was immediately embedded in aluminum foil then frozen at -10°C until analysis.

Water temperature, salinity, dissolved oxygen and turbidity were measured at each site with a multiparameter probe (Mutiparameter HI 982, HANNA instruments).

### 2.3. Microplastics analysis

#### 2.3.1. Contamination prevention

In order to prevent contamination, several precautions were followed. Cotton lab coats were worn throughout the experiments, and all steps (filtration, measurements and digestion) were realized under a laminar flow hood. All laboratory materials used were made of glass: bottles, petri dishes, Erlenmeyers, filtration system; and they were covered immediately after manipulation. All surfaces and equipment were rinsed before usage with filtered ethanol 70% and MilliQ water. Whereas for the solutions: Ethanol 70%, KOH 10%, ZnCl<sub>2</sub>, H<sub>2</sub>O<sub>2</sub> 30%; they were filtered 3 times on GF/A filters (Whatman, France) to eliminate any unwanted particles or fibers. Controls were made with every step in order to track the contamination during the work.

#### 2.3.2. Water Samples

All water samples have undergone density separation (DS) using a solution of zinc chloride (ZnCl<sub>2</sub>) of a proper density of 1.8 g/cm<sup>3</sup>. This solution was chosen due to its high efficacy in retrieving microplastics (Rodrigues et al., 2018). Before adding ZnCl<sub>2</sub> to our samples, a series of density separation trials were tested: different volumes of filtered ZnCl<sub>2</sub> (50 mL, 100 mL, and

150 mL) were added to different separating funnels containing a solution of 600 mL of water and 5 g of sediments with seventeen different polymers of different densities (in order to replicate a solution similar to our samples). Samples were left 24 hours; they were shaken twice: once every 4 hours then left to rest for 16 hours to ensure the formation of two separate phases where the supernatant consisted of particles with a density  $< 1.8 \text{ g/cm}^3$ . The density separation efficacy was calculated following the formula (Phuong et al. 2017):

$$\%Efficacy = \left( \frac{\text{Number of MPs after DS}}{\text{Number of MPs before DS}} \right) \times 100$$

The percentage obtained was  $82.7 \pm 0.5\%$  (above 80 %) and the volume of 50 mL was chosen.

### 2.3.3. Sludge samples

Sludge samples were put in aluminum foil and dried at 60°C. After testing several solutions (hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> 30%), hydroxide potassium (KOH 10%) and sodium hypochlorite NaClO) and encountering filtration difficulties or a small sample analysis (<1 g), visual observation was chosen. Three dried subsamples of 3 g were put inside clean Petri dishes and observed under a stereomicroscope (Murphy et al., 2016). Microplastics (including fibers, fragments and particles) were put on a microscopic slide for further counting and analysis (color, length and polymers type).

### 2.3.4. Sediment samples

Wet sediments collected were defrosted at room temperature. For each site, 3 subsamples of 100 g were taken and oven dried at 60°C for 24 hrs. From each subsample, 10 g of dried sediments (Mathalon and Hill, 2014; Liebezeit and Dubaish, 2012; Harrison et al., 2012) was weighed and placed inside an Erlenmeyer pre-rinsed with Milli-Q water. Firstly, 40 mL of 30% hydrogen peroxide H<sub>2</sub>O<sub>2</sub> (Nuelle et al., 2014; Avio et al., 2015) was added to the Erlenmeyer that was placed on a heating stirrer plate for 24 hour at 40°C with a rotation per minute of 100 rpm in order to remove organic debris. Afterwards, a density separation solution was used. Briefly, the solution containing the sediments were put inside a separating funnel and a volume of 40 mL of

ZnCl<sub>2</sub> (1.8 g/cm<sup>3</sup>) was added (Liebezeit and Dubaish, 2012; Imhof et al., 2013; Nuelle et al., 2014); the mix was then agitated for 5 minutes and let sit for 24 hours. The supernatant containing the MPs was filtered on GF/A filters and stored inside clean glass Petri dishes.

### 2.3.5. Mussels samples

Mussels were thawed 4 hours prior to digestion. Ten mussels were taken from each site: the total length, width (cm), total and net weight (g) was taken for each individual. Their condition index (CI) was calculated as follow (AFNOR, 1985):

$$CI = \left( \frac{\text{Net Weight (g)}}{\text{Total weight (g)}} \right) \times 100$$

This index is a good indicator for the physiological condition (tissue growth, energy reserves) of mussels (Orban et al., 2002).

Mussels were digested using a solution of potassium hydroxide (KOH) 10% (m/v, ChimiePlus, France) was used in order to eliminate any organic material (Dehaut et al., 2016; Kühn et al., 2017). The correct KOH 10% volume was chosen after testing different volumes (150 mL, 200 mL, and 250 mL) added inside Erlenmeyers containing mussels. Then, these Erlenmeyers were transferred to a heating magnetic stirrer at 60°C for 24 hours. After digestion, the digestat of each individual was filtered on GF/A filter and the digestion efficiency was calculated following the equation:

$$\%DE = 100 - \left[ \left[ \frac{(DFW - IFW)}{TW} \right] \times 100 \right]$$

DE: Digestion efficiency

DW: Dry filter weight after digestion (g)

IFW: Initial filter weight (g)

TW: Mussel total weight (g)



The volume with the highest digestion efficiency was chosen for all wild mussels individuals. A volume of 250 mL of KOH% was chosen for the samples (%DE=  $99.82 \pm 0.3\%$ )

## 2.4. Microplastics observation and identification:

### 2.4.1. Visual observation

After digestion, filters were observed under 120x magnification using Leica M165 C Stereomicroscope and images of suspected MP particles were taken with a Leica M170 HD camera and LAS (Leica application suite) software. To prevent double-counting of MPs, filters were methodically examined from the highest edge to the lowest along the first column, lowest to highest edge along the second column and so on. All suspected MPs particles were counted, categorized by type (films, fragments, microbeads or fibers) and color (dark, red, yellow, blue, white or green). The following criteria were taken into consideration while counting as mentioned by Hidalgo-Ruz et al. 2012: (1) absence of cellular or organic structures; (2) a homogenous thickness across the particles; and, (3) homogenous colors. Specific criteria were taken for counting synthetic fibers: consistent in thickness with no taper toward the ends and are clear and homogeneously colored (red, blue, green and black) (Song et al., 2015). Measurements were done on the suspected items at their longest dimension using “Piximètre” software version 5.9 and they were divided into five different size classes:  $<20\text{ }\mu\text{m}$ ;  $20 - 80\text{ }\mu\text{m}$ ;  $80 - 200\text{ }\mu\text{m}$ ;  $200 - 500\text{ }\mu\text{m}$ ;  $>500\text{ }\mu\text{m}$ .

### 2.4.2. Raman spectroscopy

Micro-Raman Xplora Plus (HORIBA Scientific®, France) was used in order to identify the polymer nature of suspected plastics. The machine is equipped with an automated plate protected by a door that can be shut during analysis so the filter placed inside would not be affected by any airborne contamination.

Due to the time consumption that takes when analyzing filters under micro-Raman, five filters of mussels containing the highest number of potential MPs were chosen and analyzed. All sludge and sediments items, and subsamples of water items were also taken for Raman analysis. For identification, two lasers with a wavelength of 532 nm and 785 nm and a range of 200-3400  $\text{cm}^{-1}$  were used with x10 and x100 objectives (Olympus). Filters were either analyzed manually or using automated ParticleFinder module for LabSpec 6. Each particle spectrum is compared to a polymer database identification software (KnowItAll, BioRad®) and a personal library made with standard polymers obtained from Goodfellow (France). Pigments spectra (Copper phthalocyanine, Hostasol Green, etc...) obtained were identified using an option called “Mixture Analysis” existing in “KnowItAll software” that is capable to decompose a mixed spectrum into two spectra consisting of the dye and its associated polymer.

## 2.5. Statistical analysis

All statistical comparison tests were done using SPSS software (IBM SPSS STATISTICS 20). Microplastics found in sediments and mussels were reported per gram of sample and in unit volume (per L) for the MPs in water samples. After a Shapiro-Wilk test, mussel's and water data did not follow a normal distribution and non-parametric tests (Kruskal-Wallis and Mann-Whitney U tests) were used to test for difference of ingested MPs between all sites (significance level: 0.05). The mussels' condition index and microplastics found in sediments followed a normal distribution and one way analysis of variance (ANOVA) test with a post-hoc Tukey test was used to determine if there was significant difference between sites ( $p < 0.05$  significance level). Spearman's rank correlation was used to test any correlation between the number of ingested MPs and the mussels' condition index.

Non-metric Multidimensional Scaling (n-MDS) was performed on a Bray-Curtis distance matrix created from transformed data (square root). This test was used as an ordination method alongside ANOSIM and SIMPER tests to compare the contribution of each observed polymer in the similarity and dissimilarity between water, sediments and mussels of all sites. These tests were done using PRIMER v7.0 (Primer-E Ltd., Plymouth Marine Laboratory, UK).

### 3. Results

#### 3.1. Contamination control

Blank samples only showed the presence of fibers with an average of  $1.71 \pm 1.5$  during the dissection and digestion of mussels (representing the average found for a batch of 9 mussels representing a total one hour constant lab work under the laminar flow hood),  $0.5 \pm 1$  for water samples density separation and  $0.25 \pm 0.43$  for sediment samples,  $\text{H}_2\text{O}_2$  digestion and density separation (also representing an hour of laboratory work). Fibers on background blanks were dominated by black (66.7% of total) and clear (33.7%). The absence of fragments, films, foams and beads indicates that the contamination was only limited to airborne fibers. The average numbers of fibers found in the control blanks were similar to other studies (Horton et al., 2017; Bråte et al., 2018). These blanks are important in any microplastics study in order to evaluate the number of procedural contamination every batch of samples is undergoing and, hence, correcting the results.

#### 3.2. Wastewater Treatment Plant and microplastics dispersion

The amount of suspected MPs items in the raw sewage influent was 244 items/L; dominated by fragments with 183 fragments/L. This number decreased to 2.84 items/L in the effluent leading to an estimated MPs retention of 98.83% by the WWTP. Synthetic fibers constituted 25% and 40% of suspected MPs found in the influent and the effluent water sample, respectively. Blue fragments and black fibers dominated both the influent and the effluent. The collected sewage sludge had an average of  $16.13 \pm 1.2$  suspected MPs/g with a dominance of fibers ( $12.46 \pm 1.08$  fibers/g). Red fibers, and blue and green fragments dominated the sludge samples.

Supplementary Table 1 shows the environmental parameters measured during sampling. Temperature ranged between 8°C in site 3 and 9.1°C in site 1. Turbidity ranged between 4.23 N.T.U in site 2 and 17.6 N.T.U in site 1; it reached a higher value in site 5 (121 N.T.U). The number of suspected MPs in the surface water decreased with the increasing distance from the

outlet flow of the WWTP (Fig. 2). The number of suspected MPs decreased by 96% at site 4 (5.3 km from effluent) when comparing to the amount of MPs initially emitted from the plant (2.844 items/L to 0.099 items/L). Yet in site 5, near the landfill, the amount of MPs was alarmingly high reaching 6 items/L (double amounts of MPs discharged from the WWTP). Particles (fragments, films or beads) dominated sites 2 and 5 whereas fibers dominated in sites 1, 3 and 4 (1; 0.27 and 0.073 fibers/L, respectively). Site 5 had the presence of secondary microplastics (fragmented shapes) but also primary microplastics (polystyrene raw microbeads represented 22% of identified particles). Blue fragments (45%) and black fibers (51%) dominated the water samples.

### 3.2. Microplastics in sediments

Microplastics analyzed were expressed in number of plastics found in 1 g of dried sediments. Although there was no significant difference in MPs concentration among the analyzed sites ( $p > 0.05$ ), the highest concentration was recorded in site 2 ( $1.04 \pm 0.07$  MPs/g) and the lowest in site 5 ( $0.41 \pm 0.36$  MPs/g) (Fig. 3). Fragments dominated the type of MPs found in all sampling sites representing 70.5%, 84%, 86.9, and 77.7% at sites 1, 2, 3 and 5, respectively. Site 1 had the highest number of fibers with an average of 0.2 fibers/g. Films were not observed in all sediment samples. Blue color dominated all fragments (69%) whereas blue and green colors constituted 71.4% of observed fibers.

### 3.3. Microplastics in mussels

For all sites, mussels collected had a shell length ranging between 1.4 and 7.72 cm with an average of  $5.6 \pm 1.6$  cm. The net weight ranged between 0.5 and 20.4 g with an average of  $6.52 \pm 4.2$  g. There were significant differences in mussels' wet weight between sites ( $p < 0.05$ ): mussels from site 5 were significantly smaller than those from the others sites. To avoid disparity between different sized mussels, the number of ingested MPs was converted per gram of soft tissue (wet weight) and not per individual (Bråte et al., 2018). 93% of collected mussels had ingested suspected MPs. Mussels from site 5 ingested a significantly higher number of suspected MPs ( $2.75 \pm 3.08$  items/g) compared to the others sites ( $p < 0.05$ ) except for site 4 (Fig. 4). Mussels from site 2 had the lowest number of ingested MPs ( $0.41 \pm 0.33$  items/g). Fragments dominated mussels in all sites (52% in sites 1, 3 and 4; 72% in site 5) except in site 2 where fragments and fibers were equally distributed (~40%). Among the investigated fragments, blue,

red and transparent fragments (~20% for each color) dominated. Whereas for the fibers, both blue and black colors represented 80% of the observed fibers. There was significant difference between the mussels' condition index (CI) ( $p < 0.05$ ) with site 1 being significantly different from site 5. Yet, there was no significant correlation (Spearman's correlation,  $r = -0.084$ ;  $p > 0.05$ ) between the CI index and the ingested items.

### 3.4. Items size distribution in the three different matrices

Suspected MPs were categorized into five different size classes as shown in Fig. 5. Inside the WWTP (Fig. 5 A), two MPs size classes (20 - 80  $\mu\text{m}$  and 80 - 200  $\mu\text{m}$ ) dominated in the raw sewage influent samples and were equally distributed (30%) whereas the other size classes: < 20  $\mu\text{m}$  and 200 - 500  $\mu\text{m}$  represented a respective 14% and 17.5% of the sample. The effluent water sample was dominated by small MPs from the size class (20 - 80  $\mu\text{m}$ ) representing 42% of the sample. Two size classes: 80 - 200  $\mu\text{m}$  and 200-500  $\mu\text{m}$  were equally distributed in the effluent; each one represented a percentage of 20.8%. Whereas in the sludge, the size class 200-500  $\mu\text{m}$  dominated with 56.25% of the suspected MPs followed by size classes 80-200  $\mu\text{m}$  and > 500  $\mu\text{m}$  (18.75% each). Smaller items were less abundant in the sewage sludge with only 6.25% items in the size class of 20-80  $\mu\text{m}$  with no observed items in the size class <20  $\mu\text{m}$ .

The size class 20 - 80  $\mu\text{m}$  was the dominant size class in all water samples (Fig. 5 A). The size class 200 - 500  $\mu\text{m}$  had a higher dominance in site 2 (37.7%) in comparison to other sites. All sites had the presence of < 20  $\mu\text{m}$  size class with an abundance less than or equal to 5%.

For the sediments, the dominant MPs size class in all sites was the 20 - 80  $\mu\text{m}$  representing between 40% at site 3 and 56% at sites 3 and 5 (Fig. 5 B). Plastics with a size class > 500  $\mu\text{m}$  consisted mainly of fibers with an abundance ranging between 12 % (sites 2 and 3) and 22% (sites 1 and 5).

Ingested items of a size < 200  $\mu\text{m}$  were the most abundant in mussels representing 78.5%, 84.4%, 72.2%, 63.1%, 61.9% at sites 1, 2, 3, 4 and 5, respectively (Fig. 5 C). Among them, suspected MPs of size class 20 - 80  $\mu\text{m}$  were the most abundant in all sites with an average of 41% of the ingested items.

### 3.5. Polymers identification

In our study, approximately 19% of all items were identified with the Raman spectroscopy: half of mussels' filters and all items found in the sludge and sediments samples were analyzed. For the water, an average of 27 MPs was identified per site. Of the 398 items analyzed, 344 (86.4%) were positively identified as a known polymer. Among the non-polymers items, only 2% were identified as cellulose-based fibers. The rest were non identifiable (for example if a sample is too thin, Raman tends to detect the underlying substrate instead of the sample K  ppler et al., 2015).

In the WWTP, 10 types of polymers were identified with a dominance of polystyrene (PS) and polyethylene (PE) in the influent, sludge and effluent samples (Fig. 6 A). PS was dominant in the influent (37.5%) and the sludge (25%) whereas PE (39.7%) dominated the effluent sample. The other polymers only represented a limited proportion ( $\sim 3 - 17\%$ ) of total MPs in the wastewater.

In the surface water samples, 11 types of polymers were identified with polystyrene (PS = 40%), polyamide (PA = 18%) and polyethylene terephthalate (PET = 14%) the most detected polymers. Polypropylene (PP) and PET were mainly detected in site 5 and PA in sites 2, 3 and 5.

Sediments samples had eight different polymer types (Fig. 6 B). A copolymer ethylene-propylene was observed in site 1; it was the first copolymer identified in this study. PS was the most abundant polymer (38%) and observed in all sites.

Nine types of microplastics polymers were ingested by mussels (Fig. 6 C) with PS (33%), acrylonitrile butadiene styrene (ABS = 19%) and PP (16%) the most abundant polymers.

Polystyrene (PS) and acrylonitrile butadiene styrene (ABS) tend to be found in blue or green colored MPs with a size below 200  $\mu\text{m}$ . Polypropylene (PP) and polyethylene (PE) were found either in transparent or in multicolor shades (green, blue and red colors) with a size class greater than 200  $\mu\text{m}$ . All PET found were of fibers whereas those in PA were both fibers and films. There was no specific relationship between colors and size for the other polymers.

Comparison of the polymers composition identified in the different matrices (water, sediments and mussels) indicated a high dissimilarity between groups (ANOSIM,  $R=0.857$ , significance

level = 0.1%). Non-metric multidimensional scaling (n-MDS) showed the dimensional distance separating these groups (a, b, c, d and e) (Fig. 7). Mussels and sediments of all sites (except sediments from site 5) were regrouped in the same category, indicating a similarity in polymers composition between mussels and sediments. Water samples (except site 5) formed two more or less similar groups (SIMPER test indicated a similarity of 57%). At site 5, polymer composition of water and sediment samples were very different from the other sampling sites (65% of dissimilarity). They are characterized by the high abundance of PET and PS.

## 4. Discussion

### 4.1. Wastewater treatment plant as a MPs source in the marine environment

Many recent studies have highlighted the crucial role of municipal wastewater treatment plants as potential pathways of microplastics entry into the aquatic environment. The detected amounts of suspected MPs items in the raw sewage influent (244 items/L) and treated wastewater effluent (2.84 items/L) were in the range of those reported in other WWTPs in Europe, USA and Australia : between 1- 3160 particles/L and 0.0007-125 particles/L for raw and treated wastewater, respectively (reviewed by Sun et al., 2019). According to the results of the present study, Le Havre WWTP had a MPs removal efficiency of 98.83%. Removal efficiencies of microplastics in WWTPs range generally between 72–99.4% (Gatidou et al., 2019; Sun et al., 2019). Variations between studies are mainly caused by different types of wastewaters and treatments used in studied WWTPs with different sampling size limitations, preparation and identification methods (Lares et al., 2018; Gatidou et al., 2019). The dominance of fibers and fragments in WWTP effluent is consistent with other studies (Mason et al., 2016; Michielssen et al., 2016; Sutton et al., 2016), yet we found a higher proportion of fragments. Generally, fibers were the most abundant type of microplastics in the wastewater (Leslie et al., 2017; Lares et al., 2018). **Le Havre harbor has a strong presence of industrial activities (petrochemicals, refineries) and is also the leading harbor in containers traffic. Several accredited industries in Le Havre collect both solid wastes (plastics, groceries, metal, glass, ashes for example) and liquid wastes (bilges water, hydrocarbon mud, wastewater, slops) resulting from cargo and ships' self-generated wastes. These collected wastes by Le Havre industries could end up in the treated water of the WWTP and potentially be an important fragment source in the influent.**

The concentration of suspected MPs items in the sludge samples ( $16.13 \pm 1.25$  MPs/g of dried weight) is in the average of what has been observed in other WWTPs such as in Glasgow ( $19.67 \pm 4.51$  MPs /g; Murphy et al., 2016). Microplastics concentration in sludge generally varies between 1 to 170 MPs/g (Gatidou et al., 2019). Normally, the detected amounts of MPs found in the sludge are in orders of magnitude higher than those found in treated wastewaters (Talvitie et al., 2017; Gatidou et al., 2019) suggesting the high potential of WWTPs in retaining microplastics from raw wastewater. Even though the number of MPs retained in the sludge might seem high, but when converting the number of sludge found per g to per  $\text{m}^3$  (the studied WWTP generates about 800 g of sludge per  $\text{m}^3$  of treated raw influent) a concentration of about 13 000 MPs per  $\text{m}^3$  of raw wastewater treated are retained in the sludge. Compared to the number of MPs found in influent (244 000 MPs per  $\text{m}^3$ ), a large number of MPs seems to have been either removed during the pretreatment processes, during grease and grit removal steps or during the secondary/tertiary treatments. For example, Murphy et al. (2016) observed a high proportion of microplastics' removal during grit and grease removal (44.6%). The contribution of the different processes on MPs removal is still limited. However, recent experiments using a lab-scale sequencing batch biological WWTP showed that 52% of microbeads were accumulated in the activated sludge (Kalčíková et al., 2017). Inside the Edelweiss WWTP, activated sludge is collected, dehydrated, incinerated and transformed to a non-harmful valuable mineral product. In case of agricultural usage, this obtained product will be free of plastic (MPs will be incinerated and won't cause any potential harm to the soil). Yet, WWTP incinerate their sludge without taking into consideration the plastics existing in their samples; plastics combustion can lead to the formation of two by-products: airborne particulate emission (soot) and solid residue ash (black carbonaceous color) (Reviewed by Verma et al., 2016). Soot has a high potential of health and environmental risks (particulate bound heavy metals and dioxins (Valavanidid et al., 2008)). Incinerating sludge should be reconsidered due to the plastics presence and its toxicity when burned. We found differences in suspected MPs size classes in the raw influent, effluent and sludge. Large sized MPs ( $> 200 \mu\text{m}$ ) were observed in the sludge indicating their retention during treatment and explaining their low presence in the effluent. This is generally the case where larger and heavier particles are more easily retained (e.g. during sedimentation), whereas smaller particles remain in suspension (Murphy et al., 2016; Magni et al., 2019). The dominance of smaller particles ( $20 - 80 \mu\text{m}$ ) in the effluent may also have resulted from particles



**degradation during treatment.** As reviewed by Sun et al. 2019, polystyrene (PS = 27%), polyethylene (PE = 24%), polyamide (PA = 12%), polyethylene terephthalate (PET) and polypropylene (PP) (11% each) were the most detected polymers in WWTPs. Polystyrene (PS) are used in packaging (Marsh and Bugusu, 2007) such as polystyrene foam used for coffee cups, trays, takeaway food containers, eating utensils (Thompsett et al., 1995, Issam et al., 2009). The PET and PA are all widely used in synthetic clothes; while PE are used in food packaging, films, plastics bottles and beauty care products (Lares et al., 2018; Ziajahromi et al., 2017). **Some of the polymers found in our samples can be traced to maritime activities: for example, PUR is mainly used as rigid and flexible foams, coatings, adhesives, and binders (Zhang et al. 2015).**

#### 4.2. Release, dispersion and concentration of microplastics in the marine coastal environment

Low concentrations of MPs (e.g. < 0.1 MP/L) in **the** effluent may contribute significantly to MPs pollution in the receiving environment due to the large volumes that are continuously discharged by WWTPs (Mason et al., 2016; Mathalon and Hill, 2014; Talvitie et al., 2015, 2017; Michielssen et al., 2016; Carr et al., 2016; Ziajahromi et al., 2017). With an average discharge volume of 80 000 m<sup>3</sup>/day, the daily amount of MPs released in the marine environment by Le Havre WWTP would be approximately 227 x 10<sup>6</sup> MPs/day. Correcting this number with our Raman identification data results in an estimated daily discharge of 210 x 10<sup>6</sup> MPs/day. The daily discharge of microplastics by WWTPs varies according to the number of serving habitants, the volume of treated raw influent and also depending on the seasons or environmental conditions. For example, Talvitie et al. (2017) estimated that a Finnish WWTP treating a volume of 270 000 m<sup>3</sup>/day may discharge MPs with values as high as 1.7×10<sup>6</sup> and 1.4×10<sup>8</sup> particles/day in different days of the week.

The results of this study reveal the presence of MPs in all examined matrices (water, sediments and mussels) and in all sites, indicating their extensive dispersion in the coastal marine environment. To our knowledge, this is the first study that assessed MPs dispersion in the coastal marine environment along a gradient from the WWTP effluent **in three different matrices**. Our results showed that the number of MPs in the surface water decreased with the increasing distance from the WWTP outflow. The number of MPs decreased by 96% at site 4 (5.3 km from the outflow) when comparing to the amount of MPs in the effluent. **Such spatial gradient of decrease in MPs was not observed for sediments and mussels samples. There was no relationship**

between MPs concentration found in the water and concentrations in sediments or mussels samples. The reason for this discrepancy between surrounding water and sediments or mussels samples may be related to several factors including wear conditions, water circulation, dispersion velocity and MP polymers density that may affect MPs sink to the water column and seabed. In addition, the concentration of MPs in surface water is directly affected by the amount of MPs found in the effluent which varies temporally (diurnal or daily variations) (Talvitie et al., 2017). When comparing different matrices or compartments, a more adapted sampling strategy should take into account the time lag necessary for MPs to sink in the water column, to be ingested by mussels and to be sedimented at the bottom. So, the concentration of MPs measured in the surface water near a source of MPs does not necessarily reflect their bioavailability to organisms living in the water column or in the bottom. Railo et al. (2018) showed no significant difference in MPs concentration between mussels located 30 meters or 700 meters from a WWTP discharge pipe. Another study demonstrated that bivalves located downstream and upstream the WWTP accumulated more particles than those located directly near the effluent (Domogalla-Urbansky et al., 2018). The heterogeneity of the MPs distribution and of its concentration levels in different matrices are likely reflecting a complex interplay between the distance from the pollution sources, hydrodynamic features such as currents, gyres, fronts and shape of the coast line (Schmidt et al., 2018). In open aquatic environment, far from direct sources of MPs, a correlation was found between microplastic levels in the water and in the mussels. This is the case in open coastal waters along the China coasts where Qu et al. (2018) found a positive and quantitative correlation of MPs in mussels and in their surrounding waters.

In comparison with the MPs concentrations reported for other water bodies nearby the discharge of WWTP effluents, the concentrations of the present study were considerably higher. For example, MPs concentrations of 0.001 - 0.002 MPs/L were reported for seawater collected 20 - 200 meters from the WWTP effluent outlet in Sweden (Magnusson and Norén, 2014). In the Seine River (near Paris) MPs concentrations of 0.013 - 0.1 MPs/L were recorded downstream the Seine-Centre wastewater treatment plant (Dris et al., 2015). In the bay of Brest (France), near an important military harbor, the mean MPs concentration in surface water was  $0.00024 \pm 0.00035$  MPs/L (Frère et al., 2017). In sediments, as for water, the MPs concentrations were also higher than those recorded in the Southern North Sea in the harbor of Nieuwpoort  $166.7 \pm 92.1$  MP/kg DW (Claessens et al., 2011) or in the bay of Brest  $0.97 \pm 2.08$  MPs/kg DW (Frère et al., 2017),

but in the range of those recorded in Mediterranean coastal sediments (Alomar et al., 2016) or in the Venetian Lagoon (Vianello et al., 2013). Concerning mussels, the number of MPs ingested ranged between 0.4 - 1 MPs/g w.w and was similar to those found by Li et al. (2018) in the UK coastal water (0.7–2.9 items/g w.w) but higher than those recorded by Van Cauwenbergh et al. (2015) in Belgian, Dutch and French North Sea coasts ( $0.2 \pm 0.3$  items/g w.w) or in the French Atlantic coast by Phuong et al. (2018) ( $0.23 \pm 0.20$  items/g d.w).

The high number of MPs in surface water, sediments and mussels found in Le Havre harbor can be linked to the continuous discharge of the WWTP in this semi-enclosed basin. Harbor's geometry might contribute to a low flushing rate (Claessens et al. 2011); its morphology (the basin) along with a great amount of daily discharged MPs will result to a high accumulation of MPs and raises their chances of ending at the bottom. Small water bodies have higher MPs concentration than that in open sea (Luo et al., 2019) explaining the great abundance of MPs found inside the harbor.

Several studies showed that fibers were the most dominant in sediments (Alomar et al., 2016; Cannas et al., 2017; Lots et al., 2017; Blair et al., 2018) or mussels (Catarino et al., 2017; Bråte et al., 2018; De Witte et al., 2014) whereas in our study, fragments were the most dominant as observed in the sediments of the Venetian Lagoon (Vianello et al., 2013). The dominance of fragments over other types of MPs in sediments and mussels samples suggests an additional source of their entry other than the WWTP where textile-derived fibers are dominant (Frère et al., 2017; Gatidou et al., 2019). The main source of MPs fragments in the harbor may be related to anthropogenic activities and to the breakdown of larger plastic debris (Cole et al., 2011; Vianello et al., 2013). The shape and color characteristics of fragments found indicate that they are mainly secondary microplastics resulting from the decomposition of larger plastics. The MPs polymers composition identified in the sediments and mussels' samples were different from those identified from the effluent or surface water samples (Fig. 6) suggesting that there are other sources of MPs inputs into the harbor. Le Havre is a highly industrialized city and a leading chemical industrial platform in Europe. It is the 1<sup>st</sup> French petrochemical complex, with 24.3 tons of crude oil processed per year. Many refineries and plastic companies (about 200 companies) are located in and around the harbor.

#### 4.3. Coastal landfill as important source and route for MPs in the coastal environment

This study revealed strong MPs abundances in the coastal environment near the Sainte Adresse's abandoned coastal landfill (site 5). To our knowledge, no study to date has evaluated MPs concentrations in coastal areas near landfills. The concentrations found in the water and mussels samples are 2 to 4 times higher than those found in Le Havre Harbor. However lower MPs concentration in sediments was observed in site 5. This can be explained by the difference in sediments composition between sites. Site 5 consists of sandy sediments whereas for the other sites (inside the harbor), the sediments are sandy-muddy to muddy. Strand et al. (2013) demonstrated that there is a strong relationship between MPs abundance and both organic (%TOC) and fine fraction ( $< 63 \mu\text{m}$ ) content in sediments, supporting the hypothesis that MPs will accumulate in depositional areas. In the Lagoon of Venice, Vianello et al. (2013) detected the lowest microplastic concentrations in the outer Lagoon, where water currents are higher ( $> 1 \text{ m/s}$ ). Consequently, the highest concentrations were encountered in the inner Lagoon which is characterized by lower hydrodynamics and a higher fine particle ( $< 63 \mu\text{m}$ ) fraction in the sediment.

Our results highlight the significant role that coastal landfills play in MPs entry into the marine coastal environment. Many environmental factors and microbial degradation (Zettler et al., 2013; He et al., 2019) may lead to the fragmentation of plastics to microplastics, and small plastic debris would be carried out by the discharge of leachate. Microplastics were identified in the leachate from both active and closed landfills (He et al., 2019). Microplastics polymer composition found at site 5 near the landfill was different from other sampling sites although this cannot provide conclusive evidence of these microplastics origins. This site had also a dominance of polystyrene (PS) ( $\sim 25 - 42\%$ ) with a high abundance of PS microbeads (22%) in the water sample. PS is generally more prone to weathering by UV radiation than other plastics (Alimi et al., 2018). The landfill could potentially carry more PS plastic into the environment than the WWTP discharge. Although fragmentation and release of microplastics is a long-term process, our results confirm that landfill isn't the final sink of plastics, but a potential source of microplastics (He et al., 2019). This can pose serious environmental problems since microplastic is not listed as pollutant in the any country's regulations of landfill.

#### 4.4. Mussels as sentinel species for MPs pollution

Mussels from all sites have mainly ingested small sized MPs (< 200 µm). This was similar to other studies that demonstrated that MPs below 300 µm were the most found in bivalves (Leslie et al., 2017; Naji et al., 2018; Phuong et al., 2018). Although we found that small sized MPs dominated the water and sediments samples, it is currently unknown whether their abundance in mussels are due to mussels size preferences in regards to prey, or because the majority of MPs in the environment were typically in this size range. In laboratory exposure experiments, Browne et al., (2008) previously demonstrated that mussels are capable of ingesting plastic particles between 3 and 10 µm in size that they filter from the water phase. Several studies showed that bivalves are capable to ingest different type of polymers and the most common plastic inside their soft tissues differed from one study to another (Avio et al., 2017; Phuong et al., 2018; Digka et al., 2018; Bråte et al., 2018). Polystyrene (PS) was the most common polymer found in our mussels, followed by acrylonitrile butadiene styrene (ABS) and polypropylene (PP). They are the most common plastics used worldwide whether in packaging or other household materials. **The comparison of ingested polymers by mussels indicated a high similarity with sediments (69% of average similarity – SIMPER test).** Mussels, such as the blue mussel *Mytilus edulis* is often selected as model species as they inhabit a wide geographic range, are sedentary, and filter large volumes of water. Our study supports the idea that the blue mussels are a promising sentinel species for small MPs (< 1 mm) in the marine environment (Bråte et al., 2018; Li et al., 2019). However, if they are suggested to be used as a standard bioindicator for MPs contamination in the marine environment (OSPAR, 2015), it is necessary to resolve a standardized method for mussel sampling and MPs extraction and identification in order to ameliorate the comparability between MPs data studies.

## 5. Conclusion

The investigated WWTP had microplastics retention efficiency of 98.83% yielding to daily discharges of 227 million MPs predominantly composed of fibers and fragments. These MPs were found in all examined matrices (water, sediments and mussels) and in all sites, indicating their extensive dispersion in the coastal marine environment. Polystyrene (PS), polyethylene

(PE), polypropylene (PP) and acrylonitrile butadiene styrene (ABS) were the most common polymers identified by Raman spectroscopy. The number, size and type of MPs in the water, sediments and mussels differed but a similarity in polymer composition was found between mussels and sediments samples. Our study supports the idea that the blue mussels are a promising sentinel species for small MPs (< 200 µm) in the marine environment.

To our knowledge, this study is the first that assessed MPs dispersion in the coastal marine environment along a distance gradient from the WWTP outflow in three matrices. Our results showed that the number of MPs in the surface water decreased with the increasing distance from the WWTP outflow. It revealed strong MPs abundances (2 to 4 times higher than those found near the WWTP outlet) in the vicinity of the abandoned coastal landfill suggesting that it contributes more than the WWTP effluent as routes of MPs entry into the marine coastal environment. This study was limited by the number of water samples taken even though high volumes of water were filtered. Yet, it gives an idea about the MPs being released by the WWTP and coastal landfill. However, more researches should concentrate on microplastic sources and their temporal variations in relation with different factors such as environmental conditions (seasons, precipitation, wind, water current, tide,...) and factors associated with the operation of the WWTP (the volume of treated raw influent, timing of effluent discharge,...).

Such knowledge could help controlling different MPs sources in order to prevent related MPs pollution and, should be, therefore, more comprehensively investigated in the future.

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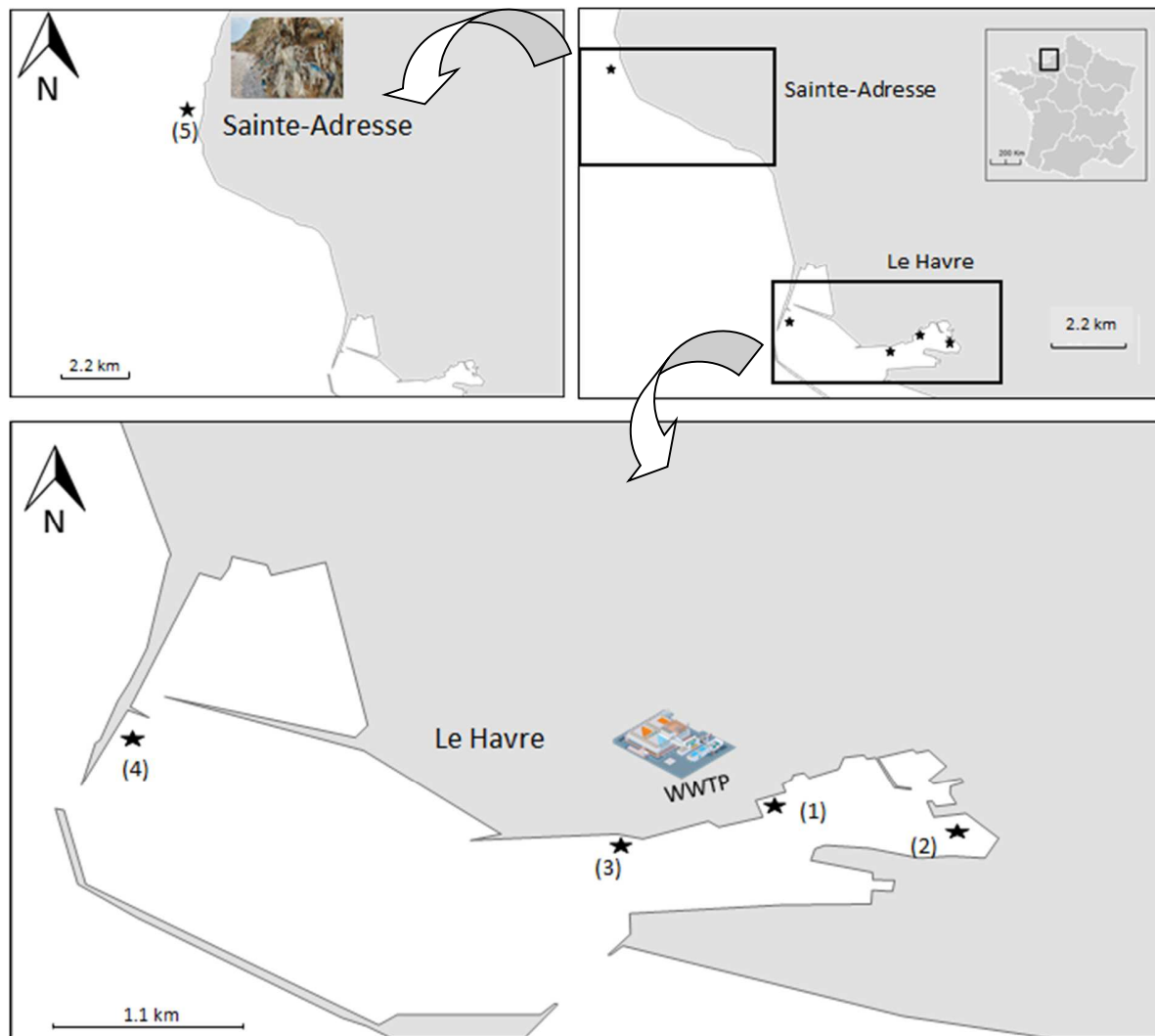


Figure 1: Localization of the WWTP (Le Havre wastewater treatment plant) and the sampling sites: 1 (near the effluent of the WWTP), (2) upstream and (3) & (4) downstream the effluent, (5) Sainte-Adresse's site (near the coastal landfill).

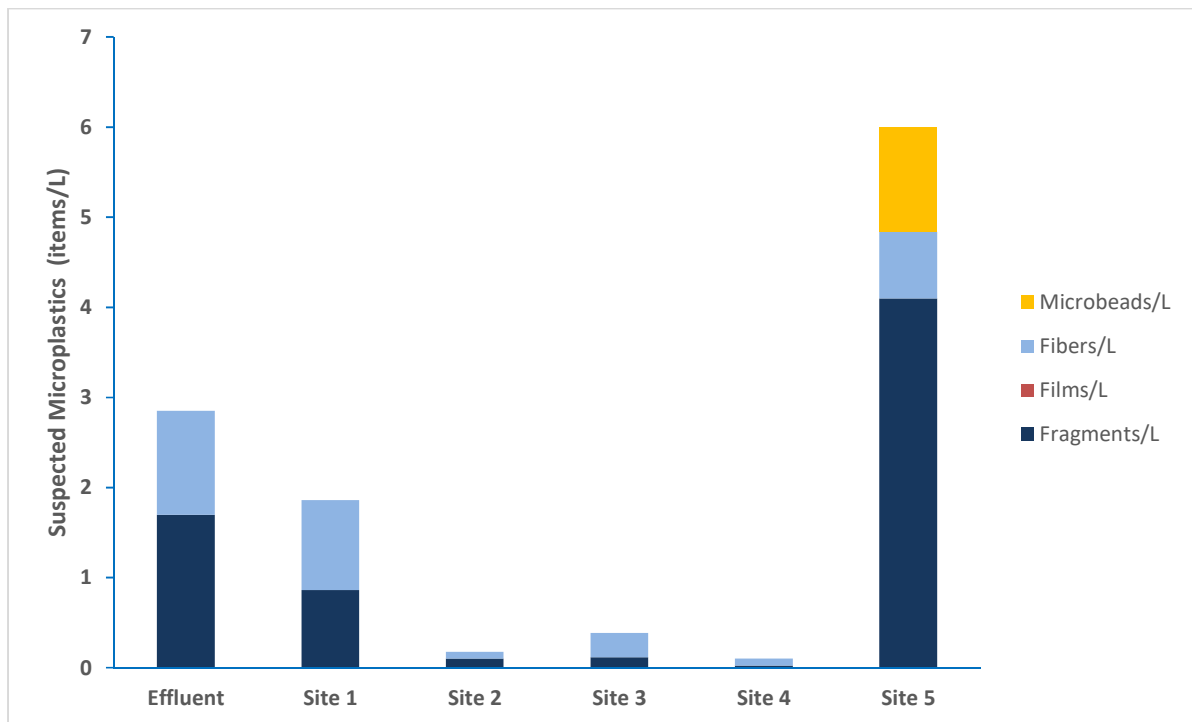


Figure 2: Suspected microplastics concentration (items/L) by shape categories from the effluent to the different surface water sampling sites (site 1 being the closest to the effluent to site 5 being the farthest but close the landfill; see figure 1).

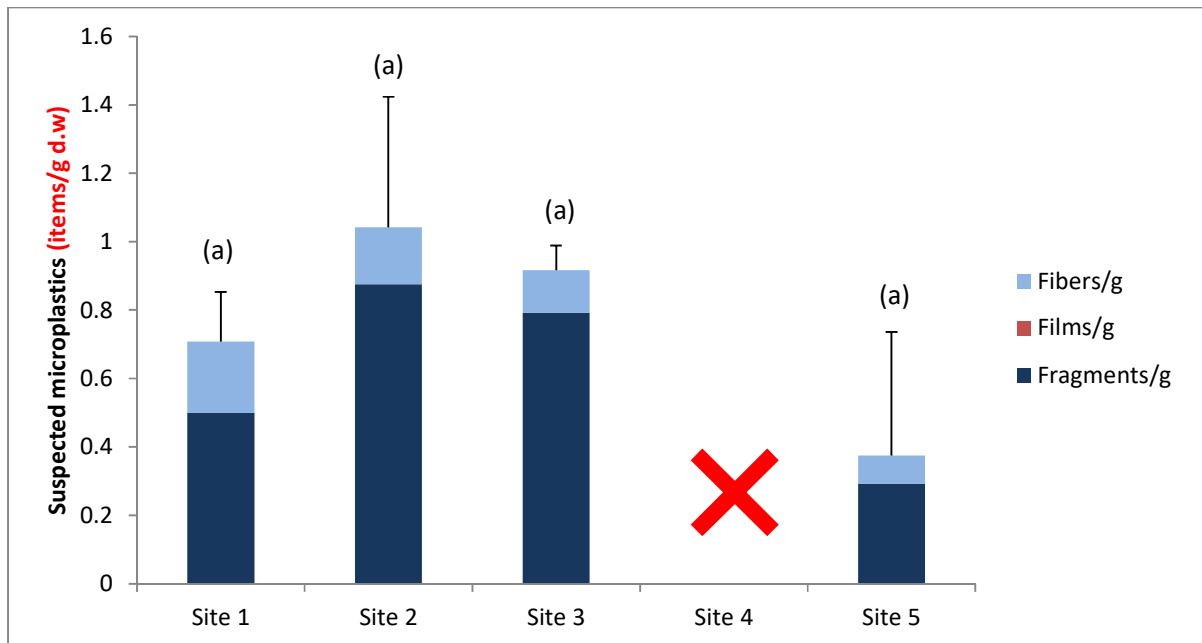


Figure 3: Average ( $\pm$ SD) number of suspected microplastics (items/g of dried sediments) by shape categories in sediments from different sampling sites except for site 4 where sediments could not be sampled (rocky substrate) (Statistics: letters indicate significant differences between sites;  $p < 0.05$ .)

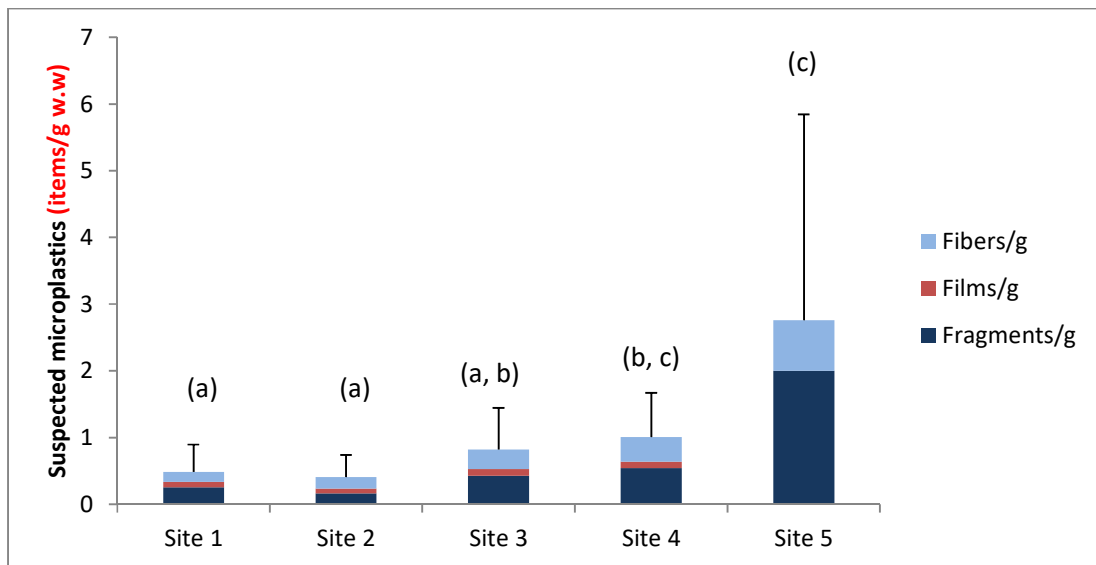


Figure 4: Average ( $\pm$ SD) number of suspected microplastics (items/g of mussels' wet weight) by shape categories in mussels collected in all the investigated sites (statistics: letters indicate significant differences between sites;  $p < 0.05$ ).

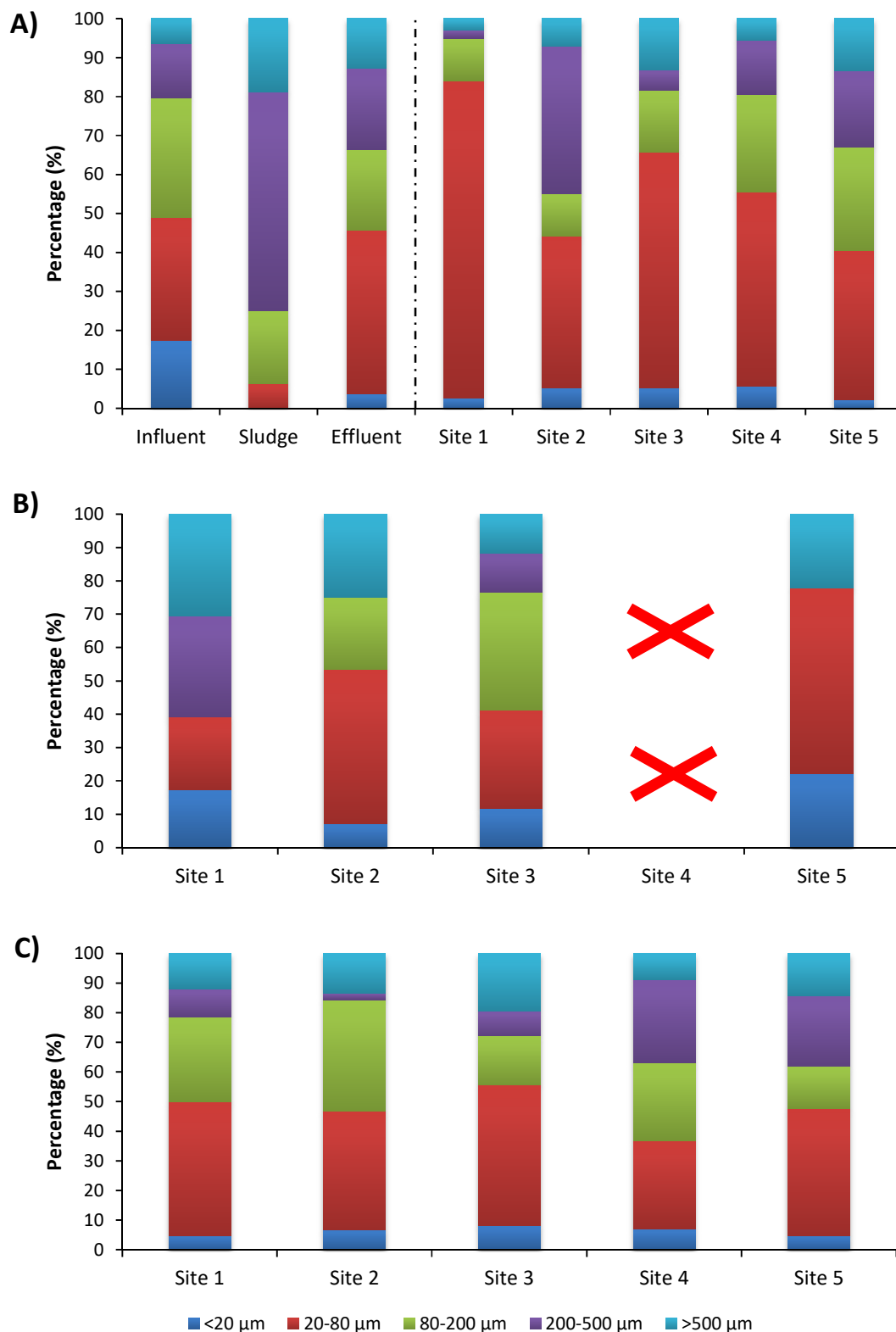


Figure 5: Proportion of size class composition of suspected microplastics in the three analyzed matrices A) WWTP and the surface water, B) sediments and (C) mussels from the different sampling sites (site 1 being the closest to the effluent to site 5 being the farthest near the landfill; see figure 1).

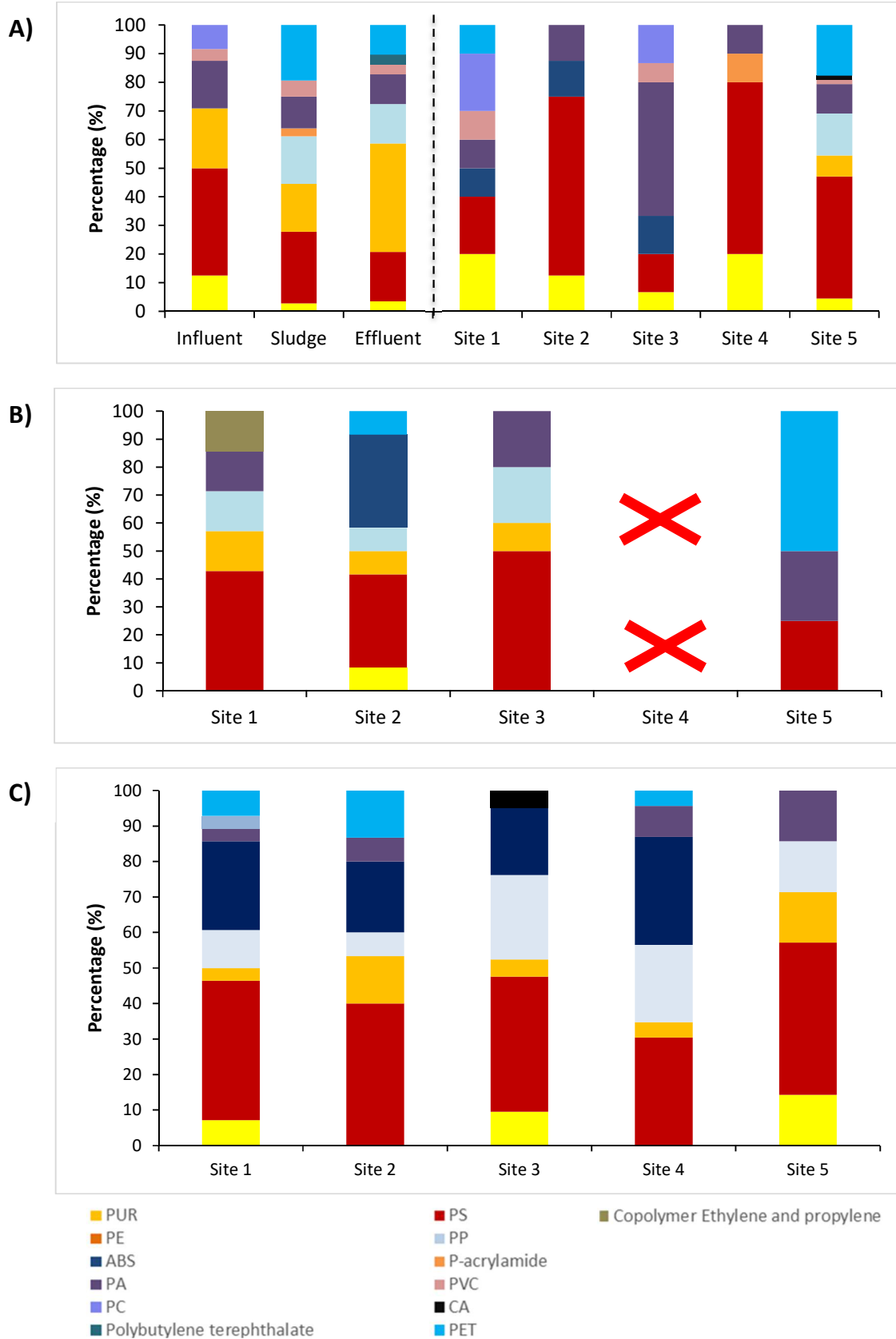


Figure 6: Proportion of different polymers identified by micro-Raman spectroscopy in the three analyzed matrices: A) WWTP and surface water; B) sediments, C) mussels from the different sampling sites (site 1 being the closest to the effluent to site 5 being the farthest near the landfill; see figure 1).

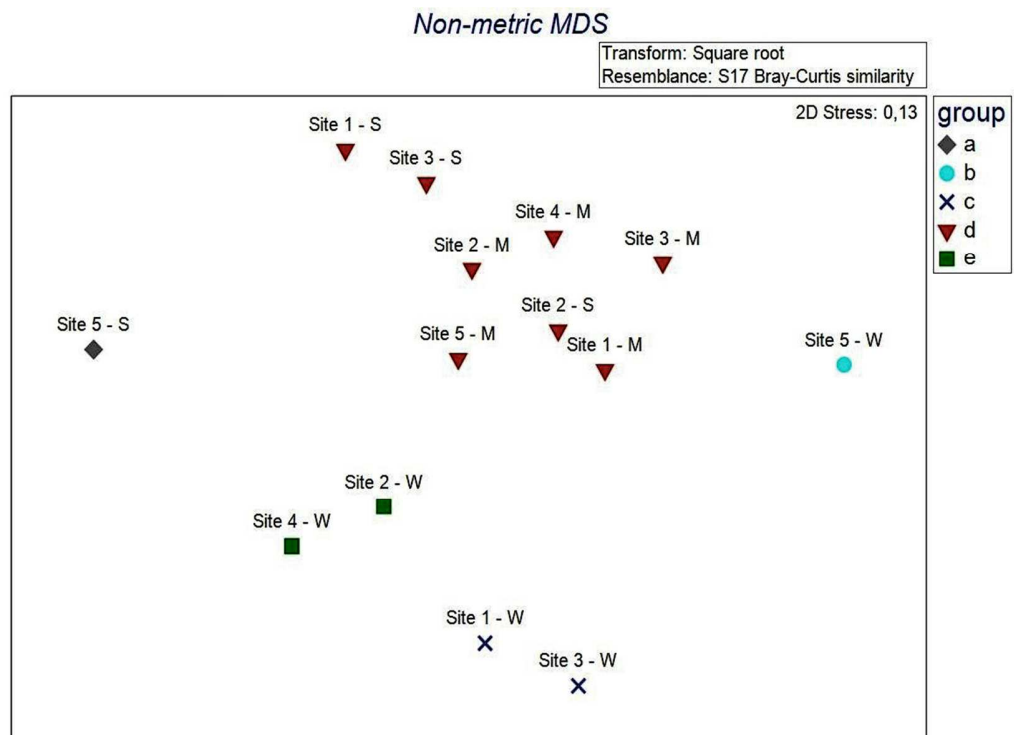


Figure 7: Non-metric multidimensional scaling (n-MDS) of polymer composition for the three compartments (water, sediments and mussels) analyzed in all sites. The represented data regrouped the sampling site with its appropriate collected sample (W: Water, M: Mussels, S: Sediments)