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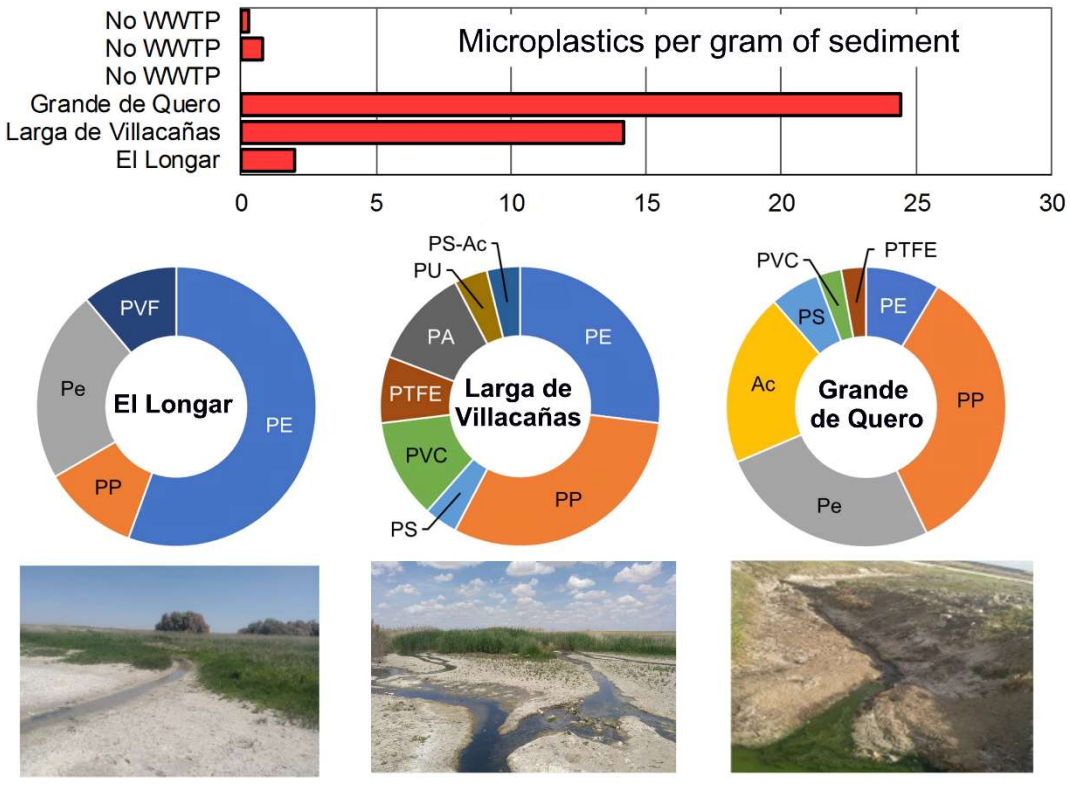

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Microplastics in sediments of artificially recharged lagoons: Case study in a Biosphere Reserve

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Abstract

We studied the occurrence of microplastics in sediments of artificially and non-artificially recharged lagoons from the network of endorheic wetlands called "La Mancha Húmeda", declared Biosphere Reserve by UNESCO. The particles sampled in this study covered the 25 μm-5 mm range. Films were the dominant microplastic typology in non-artificially recharged lagoons, while fibres and fragments were more abundant in those receiving wastewater. The concentration of microplastics in sediments reached up to 24.4 ± 5.2 microplastics/g, while plastic litter counts yielded < 1 particle/g in non-wastewater receiving lagoons. Eleven types of plastic were identified using Micro-Fourier Transform Infrared Spectroscopy (micro-FTIR), the most abundant being the polyolefins polyethylene and polypropylene, and polyester and acrylic fibres. The statistical analysis of FTIR spectra confirmed the similarity between samples taken from recharged lagoons and wastewater treatment plant effluents. Overall, our results showed that endorheic lagoons are very sensitive to the accumulation of persistent pollutants, which include microplastics. The recharge of lagoons with wastewater effluents to maintain water levels, even if correctly treated according to current standards, is not a sustainable practice. Due to the closed character of endorheic basins, the continuous input of wastewater led to the accumulation of microplastics in sediments of wastewater receiving lagoons up to 40 times over non-recharged lagoons.

Keywords: Microplastics, Sediments, Wetlands, FTIR, Wastewater, OPLS-DA

1. Introduction

Worldwide plastic production in 2018 amounted to 359 million tonnes according to PlasticsEurope (PlasticsEurope, 2019). From the same source, in Europe (EU plus Norway and Switzerland) 29.1 million tonnes were collected as post-consumer waste through official schemes, equivalent to 47 % of the amount of plastics produced in the same countries; still 25 % plastic post-consumer waste was sent to landfill and an undefined amount ended up in the environment. The origin of plastic waste disseminated into environmental compartments is diverse. Plastic debris reach the environment due to inadequate disposal practices including open landfills, wastewater discharges, or wind transport of airborne fragments (van Emmerik et al., 2019). It is accepted that most plastic waste ends up in oceans with estimated input in the order of 10 million tonnes every year (Jambeck et al., 2015). According to the 2016 report by the World Economic Forum, plastics in world's oceans will outweigh fish by 2050 if the projections for plastics production follows the estimated current trends (World Economic Forum, 2106). Concerning other compartments, it is well-known the presence of plastic wastes in terrestrial and freshwater environments (Blettler et al., 2018; Guo et al., 2020). Atmospheric fallout has also been recognized as a source of microplastics, particularly for fibres and urban environments (Dris et al., 2016).

Microplastics are a new class of persistent pollutants defined as plastic particles with size < 5 mm with a lower boundary of 1 μm below which they are considered nanoplastics (Gago et al., 2016; Gigault et al., 2018). Some microplastics result from the degradation of bigger particles including textiles or tire wearing, while other have been specifically designed in small sizes for uses in cosmetics or blast cleaning (Godoy et al., 2019). Microplastics, and supposedly their nanosized fragments, are ubiquitous pollutants, found in all possible environments (Farady, 2019; Peeken et al., 2018; Zhang et al., 2020c). The effect of nanoplastics is essentially unknown, but their potential toxicity has already been demonstrated (González-Pleiter et al., 2019). Concerning inland ecosystems, there are three major sources of microplastic pollution: atmospheric deposition, including untreated stormwater collection and runoff, the discharge of wastewater

treatment plants (WWTP) to freshwater environments, and the use of sewage sludge as fertilizer in agricultural soils (Edo et al., 2020; Klein and Fischer, 2019). The sources and distribution of microplastics through atmospheric transport are still poorly known. On the contrary, there is a growing body of evidence about the role of WWTP in spreading microplastics (Carr et al., 2016; Lares et al., 2018). Even if many microplastics are removed with sludge, WWTP effluents still contain sufficient amount of microplastics to pose an environmental threat (Edo et al., 2020).

Once released into the environment, microplastics may interact with biota causing potential toxic effects (de Souza et al., 2018). Microplastics have been associated with chemical toxicity, either due to their capacity to act as vehicle for other pollutants or to the release of substances included in their formulation as additives (Wang et al., 2018). Microplastics have been found in a plethora or organisms, mainly from marine environments. However, acute exposure tests with different species, including their sensitive early life stages, did not result in significant toxic effects even at the highest environmental concentration (Beiras et al., 2018). Toxic concentrations in standard tests are typically several orders of magnitude above concentrations found in polluted environments, like wastewater effluents (Edo et al., 2020). However, few data are available concerning chronic exposures and sub-lethal effects (Jaikumar et al., 2019). There is an important research gap on the accumulation of microplastics within web chains, which includes humans. It has been estimated that the ingestion of microplastics via food may range from tens to tens of thousands of particles per year (Smith et al., 2018). The fragmentation of microplastics is known to give rise to particles < 1 μm, usually classified as nanoplastics (Gigault et al., 2018). The exposure to nanoplastics may result in their accumulation in tissues and synergistic effects in the interaction with other toxicants (Lee et al., 2019). Nanoplastics have been associated with different effects such as reduced growth or alterations in reproductive patterns (Zhang et al., 2020b).

One key characteristic of wetlands is their capacity to act as sinks for some nutrients and to remove pollutants from agricultural runoff (Tournebize et al., 2017). Lagoons systems, engineered as constructed wetlands, are in wide use as a low-cost method to purify wastewater from small communities (Wu et al., 2015). Additionally, in some lagoons, authorities allow direct discharge of effluents from WWTP to cope with low water levels originated by aquifer overexploitation for intensive agriculture. Little is known, however, on the fate of many pollutants including regulated chemicals and contaminants of emerging concern, that accumulate in lagoons (Gorito et al., 2017). For example, the use of reclaimed water alters the fate of nutrients through modification of natural cycles of drying-flooding in semiarid sites (Corrales-González et al., 2019).

In this work, we studied the presence of microplastics in lagoons representative from the extensive network of natural wetlands called "La Mancha Húmeda", declared Biosphere Reserve by UNESCO. We studied lagoons that receive wastewater inputs to maintain water level and compared them with non-artificially recharged lagoons. The amount and type of microplastics found in sediments were monitored and the results were compared with other studies.

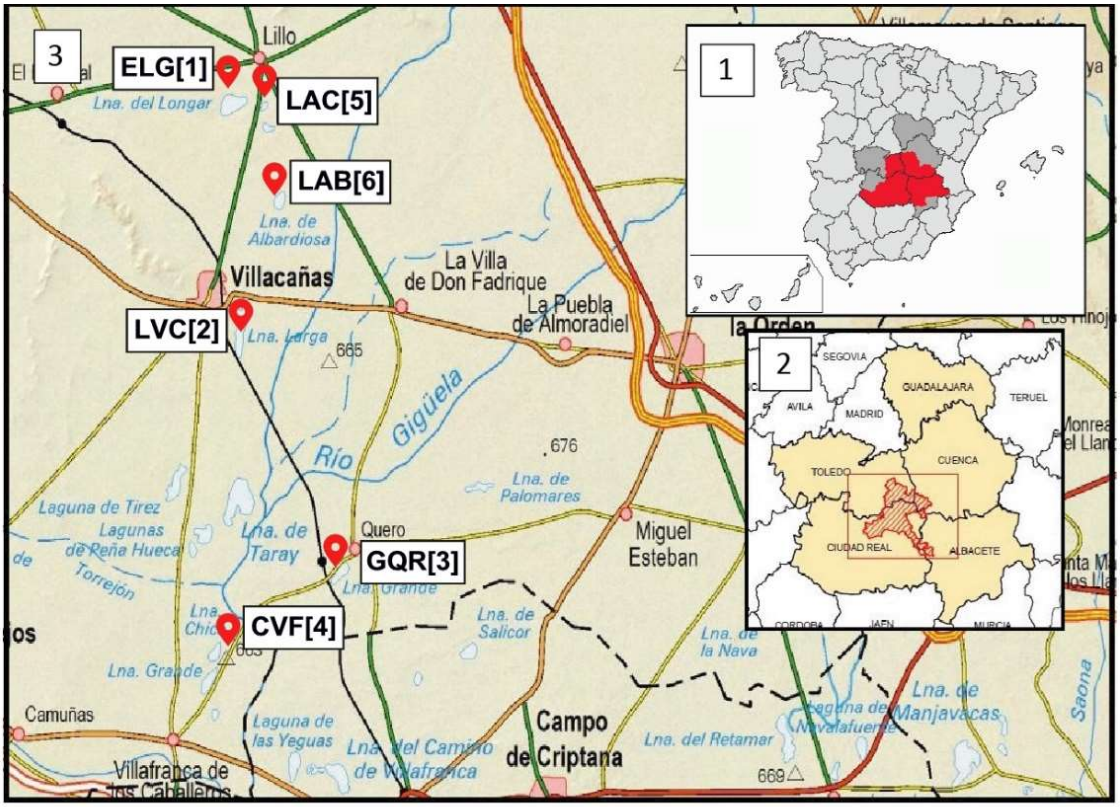
2. Materials and Methods

2.1. Location

The lagoons selected for this study are located in Toledo province, in the wetland area called "La Mancha Húmeda" (Castilla-La Mancha, Spain) (Fig. 1). It is an area of approximately 4000 km^2, including transition zones, which plays an important role for biodiversity protection, aquifer recharge, sediment retention, flooding control and carbon sink, among others. Six lagoons were selected and sampled. Three of them, namely El Longar, ELG[1], Larga de Villacañas, LVC[2], and Laguna Grande de Quero, GQR[3] receive wastewater. (Tables S1 and S2, Supplementary Material, SM, show information related to discharges, water inputs and land use). The other three, Laguna Chica de Villafranca, CVF[4], Laguna del Altillo Chica (LAC[5]) and La Albardiosa, LAB[6], only receive water by rainfall and runoff and were used as control. Additional information related to the sampled lagoons is in the table included with Fig. 1 and in Fig. S1 (SM).

The six selected lagoons are part of a wide set of endorheic water bodies located in the Biosphere Reserve "La Mancha Húmeda". The landscape is flat, with predominance of agricultural lands spotted by endorheic lagoons fed by runoff and aquifer upwellings. Untouched lagoons are seasonal, with elevated salinity, even five times higher than seawater. These wetlands have a high ecological value, acting as breeding ground for migratory and aquatic birds. They are also colonised by a large number of endemic or endangered halophilic and aquatic plants (Cirujano and Medina, 2014). It is a highly protected area declared UNESCO Biosphere Reserve in 1981. Parts of it are listed under several protection figures including the Ramsar List of Wetlands of International Importance, one Specially Protected Bird Area, and two National Parks, among others. However, all the area is highly menaced by aquifer over-exploitation for irrigation, which led groundwater level so low that natural replenishment by rainfall is insufficient. In this context, some lagoons receive treated wastewater in part in an attempt to maintain some water level and also because they are the natural sink for nearby communities. It is important to note that they are endorheic lagoons, connected with the underground aquifer, but without visible outlet.

On sampling, LAC[5] and LAB[6] were completely dried with a considerable number of macroplastics of agricultural origin, most of them easily recognized as



El Longar: ELG[1]

Altillo Chica: LAC[5]

La Albardiosa: LAB[6]

Larga de Villacañas: LVC[2]

Laguna Chica: CVF[4]

Laguna Grande: GQR[3]

ID	Name and location	Surface (ha)	Datum	Zone	UTM X	UTM Y	Discharge
ELG[1]	El Longar (Lillo)	96	ETRS89	30	472249.85	4394837.59	WWTP Lillo
LVC[2]	Laguna Larga (Villacañas)	84	ETRS89	30	472669.74	4383902.02	WWTP Villacañas
GQR[3]	Laguna Grande (Quero)	72	ETRS89	30	478114.83	4372345.71	Untreated discharge
CVF[4]	Laguna Chica (Villafranca de los Caballeros)	37	ETRS89	30	471333.61	4368219.27	-
LAC[5]	Altillo Chica (Lillo)	15	ETRS89	30	473969.83	4394805.72	-
LAB[6]	La Albardiosa (Lillo)	32	ETRS89	30	474763.98	4390292.34	-

Figure 1. Map and main characteristics of the study zone and aerial view of sampling points. 1. Spain map with "La Mancha" natural zone red marked. 2. Castilla-La Mancha administrative region. 3. Location of sampling points and aerial view of the six lagoons.

the usual green plant protectors. GQR[3] is surrounded by Quero village (1006 inhab.). Despite been upstream to the WWTP, there was a wastewater drainage coming from a collector without evidence of any treatments. A green filter made of macrophytes existed at the water discharge to LVC[2]. This lagoon showed a high amount of macrolitter inside and around the lagoon and also presented bad smell with high amount of black sediments, different from the rest of sampled locations. ELG[1] was almost dried except for the contribution of a WWTP discharge and showed many debris along the sediment line. CVF[4] was visually the most unaffected lagoon, surrounded by canes and without macrolitter. Fig. S1 (SM) shows pictures of the sampling points and some of the evidences of anthropogenic pollution.

2.2. Sampling

Sediment samples were collected using 1 L high density polyethylene (HDPE) bottles. Sediments were collected with a stainless-steel sediment collector cleaned with ultrapure water between samples. All sampling material was covered with aluminium foils to prevent particle deposition. Except for HDPE bottles in field sampling, plastic material was avoided, and only glassware was used in laboratory manipulation. To prevent cross contamination, all materials were carefully cleaned with ultrapure water and clothes worn by manipulators were controlled during sampling avoiding synthetic textiles and using cotton in bright colours whenever possible. To ensure absence of cross-contamination HDPE bottles were cleaned several times with ultrapure water and the resulting liquid examined for possible rests of plastic material. Both bottles and lids were added to a reference micro-FTIR database to check for possible coincidences. We didn't detect any plastic debris from these bottles and lids during validation or in any of the samples.

Sampling was performed in May 2019, in a sunny day without wind (< 10 km/h). Three different areas separated at least by 2 m were chosen close to the entry of the stream feeding each lagoon. For each sampling zone, a surface of 1 m x 1 m was selected and sediment from the first 2 cm was collected directly into the bottles. The minimum volume recovered was 500 mL. During sampling, an additional bottle was kept opened close to the sampling point as control for air deposition or contamination. All samples were covered with aluminium foil, capped, and stored in the freezer to avoid microbial growth. Once in laboratory, samples were dried under vacuum at 60 °C to remove water without affecting the plastics present in the sample and then frozen until subsequent analysis.

The microplastics obtained from sediments were compared with samples taken from wet deposition and from the effluent of a WWTP. Wet deposition samples were obtained in the rain events that took place during the month of July in nearby area. This was the first rain event that took place after lagoon sampling. For it, glass recipients were kept opened during rainfall with an additional one set close to the sampling point but protected from rain as contamination control. Samples from treated wastewater were collected in Spring 2019 from the effluent of the secondary settler of a WWTP located in the same region. Details can be found elsewhere (Edo et al., 2020).

2.3. Recovery of microplastics

For microplastic extraction, dried sediment samples (5 g) were treated with 25 mL of H_2O_2 (33 % w/v) to remove organic matter and left in oven (60 °C) for 20-24 h. This procedure, selected after different trials, removed enough organic matter to make microplastic counting feasible (Edo et al., 2020; Helcoski et al., 2020). A sodium chloride hypersaline solution (1.2 g mL^{-1}) was used to separate plastics by density. Several authors proposed NaCl solution as cheap and safe method for separating materials from sediments preferred over other salts like $ZnCl_2$ or NaI (Bayo et al., 2020; Cannas et al., 2017; Masura et al., 2015). Samples were magnetically stirred for 15 min and stored overnight at 4 °C to complete density separation.

The supernatant was filtered through 25 μm stainless-steel mesh and dried at 60 °C. The 25 μm lower boundary was chosen in view of the spatial resolution of micro-FTIR spectroscopy, which is limited to 10−20 μm (Araujo et al., 2018). The rest of the sediment was also dried and evaluated in order to count the particles that could have been settled with the sediment due to their higher density or attached to other particles. These represented between 10-30% of the total number of suspected anthropogenic litter. Therefore, both supernatant and sediment, without any loss of particles during the process, were inspected. All particles > 5 mm discarded.

Processed samples were kept in glass Petri dishes until analyses. Throughout sample handling, clean 25 μm stainless-steel meshes were kept in open Petri dishes near the samples to control possible contamination during laboratory procedures. The same process was performed with rainfall samples and with minor modifications with the samples from the secondary WWTP effluent. Additional details can be found elsewhere (Edo et al., 2020).

2.4. Analytical procedure

Particle counting was performed with a Euromex-Edublue stereomicroscope fitted with ImageFocus 4 camera software. ImageJ software was used to measure particles. Polymer identification was performed by Micro-Fourier Transform Infrared Spectroscopy (micro-FTIR) using a Perkin-Elmer Spotlight 200 Spectrum Two apparatus equipped with an MCT detector. This equipment uses Fourier-Transformed infrared spectroscopy (mid-infrared region) to obtain spectra that are compared with existing databases. Particles were placed individually with a zircon

microneedle over potassium bromide (KBr) discs. The equipment operated in transmission mode with 8 cm^{-1} resolution and spectral range 4000-550 cm^{-1}. The amount of microplastics per gram of sediment in the first 2 cm of sediment was calculated by multiplying the counting of microparticles with the percentages of microparticles identified as microplastics using micro-FTIR. Control samples both from field and laboratory were examined under the stereomicroscope and micro-FTIR and compared with the clothes worn by the personnel. Particles and fibers similar in colour and shape with those in controls were subtracted from the counting.

The spectra from lagoon samples were compared with materials previously collected from wet deposition and with material recovered from another WWTP in the same region. All samples were equally treated with 33% H_2O_2 to remove organic matter present and avoid microbial growth, cleaned with ultrapure water, dried and stored until FTIR analysis. A total of 445 spectrums were studied with five different models. 190 spectrums from lagoons, 172 from WWTP and 83 from rain deposition. The spectra were randomly selected and in those from lagoons we ensured the same proportion in all of them. A group to group comparison was performed to assess the regions of the spectrum responsible for the difference among groups.

2.5. Statistics

Statistical methods were used to compare the FTIR spectra of microparticles recovered samples from lagoons with those from rainfall and wastewater. FTIR spectra for all samples were obtained under the same conditions and procedures. The identification of polymer type was performed with OMNIC 9 software obtained from Thermo Scientific. A minimum percentage of 60 % was selected as matching as stated elsewhere (Liu et al., 2019). Matching system uses Pearson correlation to compare recorded spectra with databases.

The obtained infrared spectra were processed with the multivariate tool SIMCA 15 (Sartorius Stedim Data Analytics, Umeå, Sweden). All spectra were smoothed and normalized with both rubberband baseline correction and standard normal variate (SNV) methods, respectively. These methods allow a better comparison of the spectra while minimizing the differences in the light dispersion produced by the various particle sizes.

To discriminate between groups (lagoons-wet deposition-wastewater) and to highlight differences among them, orthogonal partial least squares-discrimination analysis (OPLS-DA) was performed. This method uses PCA methodologies to reduce the dimension of the data set allowing better correlations (Silva et al., 2017). Model fitting was assessed using R^2Y and Q^2 parameters. Hoteling's T^2 test was performed for hypothesis testing to recognise any possible outliers (Bylesjö et al., 2006).

3. Results

3.1. Abundance and morphology of microparticles

Fig. 2 shows the results of sampling for microparticles in the 25 μm - 5 mm range. Fig. 2A shows the histogram with microplastic sizes for all samples. Fig. 2B shows the typology and concentration in microparticles per gram of dry sediment in all sampled lagoons. The term microparticle refers here to fragments, filaments, films or fibres with possible anthropogenic origin either separated with flotation using the hypersaline solution or identified in the sediment from hypersaline flotation. Microparticles with clear natural origin, such as mineral particles or vegetal fragments, were not included. Therefore, and in what follows, the term *microparticles* refer to *suspected small anthropogenic litter*. Fig. 2B refers to concentration of microparticles (microplastics and non-microplastic fraction). The maximum concentration of microparticles was found in GQR[3] with 36.3 ± 7.7 particles/g followed by LVC[2] with of 28.9 ± 7.0 particles/g. The samples from the other four lagoons contained much less microparticles, with < 10 particles/g of suspected anthropogenic litter, the lower figures recorded in samples from CVF[4] with 2.9 ± 1.2 particles/g (Fig 2B). According to typology the majority of microparticles were fibres followed by fragments. Fibres represented between 50-65 % of microparticles in all lagoons except LAB[6], in which fragments (53 %) were the predominant typology. Films and filaments were in all cases less abundant, with occurrence < 10 % among all recovered microparticles. Using micro-FTIR, microparticles could be classified as microplastics and a non-microplastic fraction as shown in Fig. 2C, while Fig. 2D shows the typology distribution of microparticles identified as microplastics. Size distribution was calculated from projected area diameter recorded from microscopic measurements (Fig. 2A). The median of size distribution for all microparticles was 86.4 μm (first and third quartiles 49.4 and 140.4 μm, respectively).

3.2. Microplastics in sediments

A subsample of 190 microparticles was analysed by means of micro-FTIR, which represented 15 % (Table S3, SM) of the total number of microparticles suspected to be anthropogenic. The use of a subsample was due to the impossibility of sampling the full population. In this case, the subsampling of the full population represented a theoretical accuracy of 6.3 %, derived as shown elsewhere (Kedzierski et al., 2019). The total number of microparticles identified as microplastic was 76 representing 40 % of the potentially anthropogenic microparticles. The maximum number of microplastics were found in the subsample from GQR[3] (35 out of 52, or 67 %). As indicated before, this lagoon does not receive authorized discharge from any WWTP, but there is at least one obvious emission of untreated wastewater. An important number of microplastics

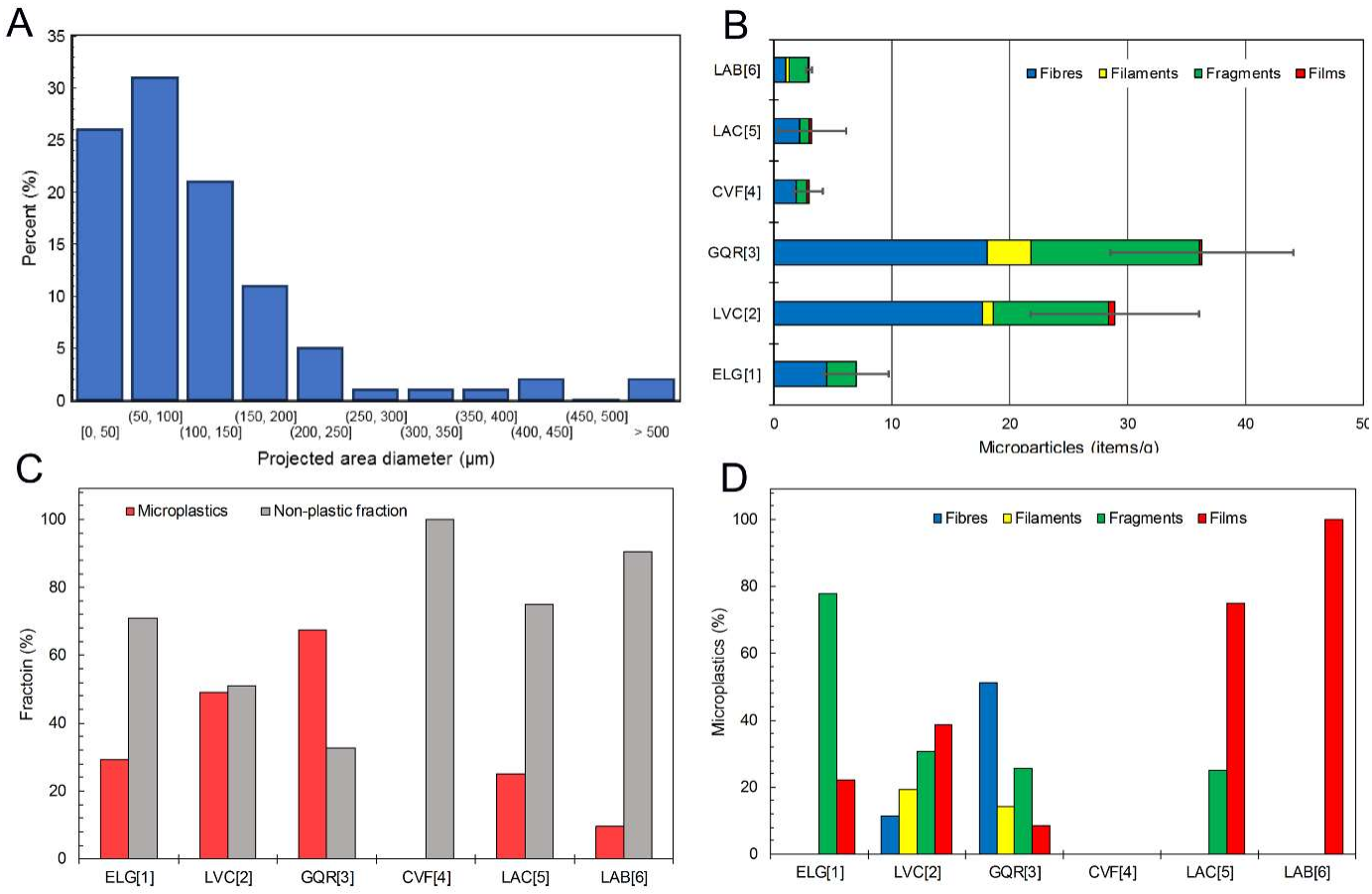


Figure 2. Projected area diameter histogram for microplastics identified in all samples (A). Microparticles (including microplastic and non-microplastic fraction) per gram of sediment according to typology (B). Fraction of microplastics (and non-microplastics) in all sampled lagoons according to micro-FTIR analyses (C). Fraction of microplastics within each typology as identified by micro-FTIR (D). (Legends refer to lagoons: El Longar: ELG[1]; Larga de Villacañas: LVC[2]; Laguna Grande de Quero: GQR[3]; Laguna Chica de Villafranca: CVF[4]; Laguna del Altillo Chica: LAC[5]; La Albardiosa: LAB[6].)

were also found in ELG[1] and LVC[2], which are lagoons receiving treated wastewater from Lillo and Villacañas WWTP respectively. In ELG[1] 9 out of 31 (29 %) microparticles were identified as microplastic, whereas the same figures from LVC[2] were 26 out of 53 (49 %). Microplastics were much less abundant in the three lagoons that do not receive wastewater. Only 4 and 2 microplastics were found in LAC[5] and LAB[6] respectively, whereas none of the 15 microparticles analysed from CVF[4] were plastics. Table S3 (SM) summarizes the results from micro-FTIR analyses.

In all cases, the most frequent material found in samples was cellulose, both natural, with probable origin in vegetal tissues, and anthropogenic, as part of textile fabrics or other manufactured items. These microparticles, listed in Table S3 (SM) as "anthropogenic" mainly consisted of fibres (> 80 %) identified as cotton/cellulose that could be classified as anthropogenic litter because of their non-natural colours. Particles or fibres of natural materials like wool or cellulose derivatives may evidence anthropogenic origin due to their non-natural colour or the presence of other industrial additives. They comprise a category of anthropogenic litter including natural materials that underwent industrial processing and bear artificial additives like dyes, light stabilizers, and other chemicals used as part of their composition of for their manufacturing (González-Pleiter et al., 2020).

Most microparticles identified as microplastics (92 %) were found in the three lagoons receiving wastewater, namely ELG[1], LVC[2], GQR[3]. Noticeably, the highest amount (35 out of 52 microparticles analysed) corresponded to the samples taken from GRR[3], a lagoon suffering from non-treated wastewater discharge. Within the plastic fraction, 11 different types of polymers were found, namely polyethylene (PE), polypropylene (PP), polyester fibres (Pe), acrylic fibres (Ac), polystyrene (PS), polyvinyl chloride (PVC), polyvinyl fluoride (PVF), polytetrafluoroethylene (PTFE), polyamide (PA), polyurethane (PU), and polystyrene-acrylic blend (PS-Ac). Only six microplastic particles were found in lagoons without wastewater discharge: LAC[5] with three PE films and one PP fragment, and LAB[6] with two films of PE and PVC. Polymer variety was higher in LVC[2] and GQR[3], with eight different polymers found in each lagoon. Fig. 3 shows the distribution of identified polymers in the three lagoons receiving wastewater.

The most frequently found polymers, which accounted for 77 % of the total number of microplastics were the polyolefins PE and PP, polyester (Pe), and acrylic (Ac). Figs. S2 and S3 show infrared spectra from representative materials found in samples.

Images of representative microplastics are shown in Fig. S4 (SM). Significant differences in typology were also observed in samples from different lagoons. Films were the main microplastic shape found in non-artificially recharged lagoons LAC[5] and LAB[6], whereas in those receiving wastewater discharges fibres were the dominant typology (Fig. 2B). The presence of fibres in wastewater, mainly originated in domestic wastewater machines has been reported elsewhere and is a tracer of anthropogenic pollution (Napper and Thompson, 2016). In GQR[3], fibres accounted for almost 50 % of microplastics.

3.3. Discrimination among spectra from different sources: lagoons, wet deposition and wastewater effluent

445 FTIR spectra were used to feed five different models. 190 (76 microplastics) corresponded to samples spectra taken from different lagoons, 172 (77 microplastics) from WWTP effluent, and 83 (35 microplastics) were microparticles recovered from wet deposition. Wet deposition plastics essentially corresponded to fragments (60 %) and fibres (35 %). The median value for the size of these plastics was 360 μm for length, 39 μm for width, and 133 μm for projected area diameter. The more abundant polymers obtained from wet deposition were polyester and acrylic fibres (19 % and 4 % respectively), while cotton-cellulose fibres accounted for 26 % of the total number of items. Details concerning WWTP effluent are available elsewhere (Edo et al., 2020). Briefly, the set used for this study mainly consisted of fibres (49 %) and fragments (43 %). Among them, the more abundant were polyester fibres (17 %) followed by PE (9 %), PP (6 %) and acrylic fibres (5 %).Besides, 28 % of the total number of items were identified as cotton-cellulose. The median size of these particles was 181 μm. Accordingly, the three sets were comparable both in size and composition. Some spectra considered as outliers were removed during pre-screening. For each model, several group-to-group comparisons were performed, and contribution plots were used to identify the bands associated with the main differences.

The spectra were statistically compared using OPLS-DA after baseline correction. This method uses multivariate PCA to represent potentially correlated variables with linearly uncorrelated principal components. Model 1 used all spectra taken from lagoons, WWTP and wet deposition and was split into two Model 1-C with samples from non-artificially recharged lagoons (CVF[4], LAC[5] and LAB[6]) and Model 1-R with spectra from lagoons receiving wastewater (ELG[1], LVC[2] and GQR[3]). Model 2 used only the microparticles positively identified as microplastics and also comprised two Models 2-C and 2-R with the same background as Model 1. Model 3 compared non-plastic materials and Models 4 and 5 compared specific microplastics in different sets (polyester and acrylic respectively). None of the models used any Y orthogonal component. Table 1 shows all details including the parameters R^2X, R^2Y and Q^2. In order to avoid model overfitting, the final number of components was based on the auto-fitting cross-validation setting as suggested by OPLS-DA software. The number of model components prioritised class discrimination of each dataset.

The results from all models suggested the presence of intrinsic properties in FTIR spectra that allowed the discrimination among the three data groups. In all the analyses performed, the data points representing polymers from different origins grouped closely with certain overlapping between them.

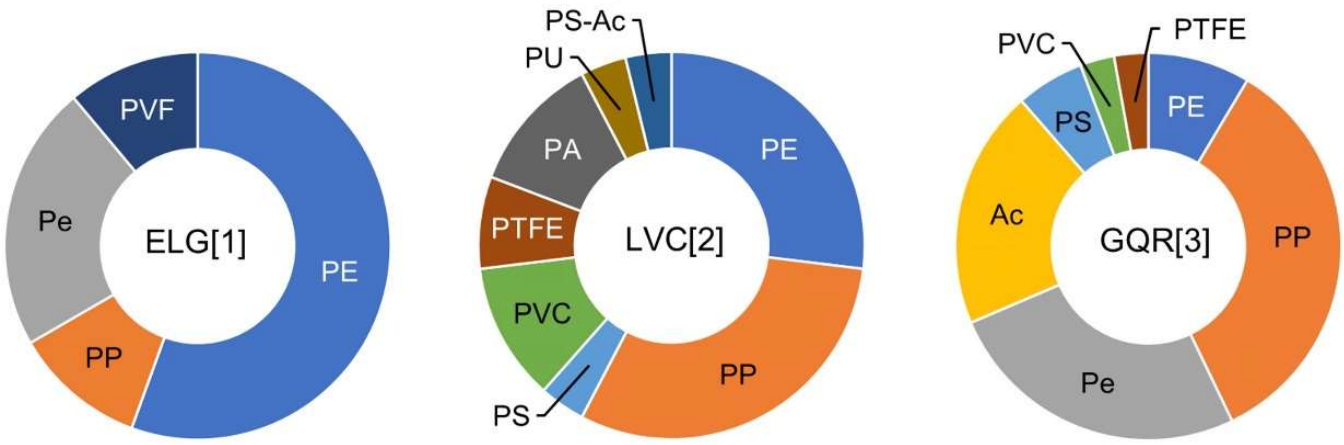


Figure 3. Polymer distribution per sample in lagoons ELG[1], LVC[2] and GQR[3]. (PE: polyethylene; PP: polypropylene; Pe: polyester fibres; Ac: acrylic fibres (Ac); PS: polystyrene (PS); PVC: polyvinyl chloride; PVF: polyvinyl fluoride; PTFE: polytetrafluoroethylene (PTFE); PA: polyamide; PU: polyurethane; PS-Ac: polystyrene-acrylic blend.)

Table 1. Parameters obtained from the different models calculated with orthogonal partial least squares-discrimination analysis OPLS-DA.

Id.	Description	Number of spectra	Descriptive Components	Orthogonal Components (x)	R^2X	R^2Y	Q^2
1	All microparticles in all lagoons, WWTP and wet deposition	428	2	9	0.80	0.67	0.50
1-C	All microparticles in lagoons CVF[4], LAC[5] and LAB[6], WWTP and wet deposition	296	2	5	0.72	0.39	0.21
1-R	All microparticles in lagoons ELG[1], LVC[2] and GQR[3], WWTP and wet deposition	378	2	10	0.82	0.70	0.54
2	Microplastics in all lagoons, WWTP and wet deposition	178	2	3	0.61	0.33	0.10
2-C	Microplastics in lagoons CVF[4], LAC[5] and LAB[6], WWTP and wet deposition	131	2	5	0.72	0.53	0.20
2-R	Microplastics in lagoons ELG[1], LVC[2] and GQR[3], WWTP and wet deposition	178	2	6	0.74	0.49	0.19
3	All non-plastics microparticles in all lagoons, WWTP and wet deposition	251	2	3	0.68	0.33	0.20
4	Polyesters in all lagoons, WWTP and wet deposition	54	3	4	0.75	0.69	0.12
5	Acrylics in all lagoons, WWTP and wet deposition	19	3	1	0.74	0.63	0.06

OPLS-DA analysis was quantitatively assessed using the explained variation (R^2) of each principal component. In this work, the most appropriated models resulted from to the use of all materials at once. The best model for diagnosing differences between groups was Model 1-R with both the best explanation (R^2 = 82 %) and the highest predictability (Q^2 = 54 %). In general, predictability was < 20 % in all cases except for Models 1 and 1-R. The removal of the non-plastic spectra reduced the explanation and predictability of the remaining models (2, 3, 4, and 5) meaning that the only groups exhibiting significant differences appeared when comparing microparticles in lagoons undergoing wastewater discharge with particles from WWTP effluent and wet deposition. Visually, the spectra from lagoons slightly overlapped with the other two groups. Nonetheless, they appeared always closer to WWTP effluent than to wet deposition samples. Fig. 4 shows the S-Plot of the model 1-R for all the materials in lagoons ELG[1], LVC[2] and GQR[3]. S-plots for the rest of the models are given in Fig. S5 and S6 (SM).

The differences between samples were highlighted by performing group comparisons from spectra with different sources, which allowed identifying the contributions of each group to the FTIR spectra. Fig. 5 shows the differences in spectral regions among samples from lagoons, WWTP effluent and wet deposition as obtained from the application of Models 1 (including all microparticles) and 2 (only microplastics). In Model 1-C non-artificially recharged lagoons were differentiated by an intense carbonyl vibration (C=O), a C-O stretching, and C=C bending vibrations present in the ~1700 cm^{-1}, 1300-1100 and 900-700 cm^{-1} regions, respectively (Fleming and Williams, 2020). The materials in recharged lagoons (Model 1-R) displayed intense vibrations in the 3600-3000 cm^{-1} region. They would correspond to O-H, N-H, and aromatic C-H stretching and were found in lagoons at the same level as in WWTP effluent and wet deposition. FTIR in recharged lagoons showed vibrations in the 2500-2000 cm^{-1} intermediate region, which would correspond to vibrations in double and triple bonds that were no present in WWTP effluent or wet deposition samples. There were also differences in carbonyl region in samples from lagoons receiving wastewater discharge (R). The samples in lagoons showed an important absence of peaks close to ~1500 cm^{-1}, especially in recharged lagoons because of the difference with wet deposition and WWTP samples. Samples from WWTP effluent and wet deposition showed bands in the 580-560 cm^{-1}, region that could be attributed to C-X bonds. The results for Model 2 (Fig. 5, lower panels), that only considered microplastics,

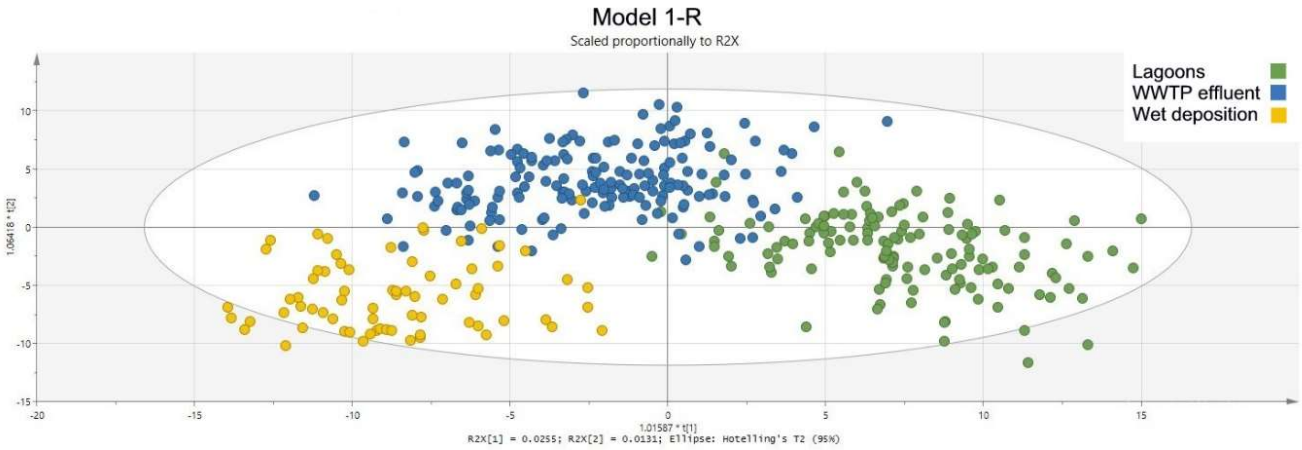


Figure 4. Scatter-Plot for model 1-R (details in Table 1) with data inside Hoteling's bubble (T^2 test).

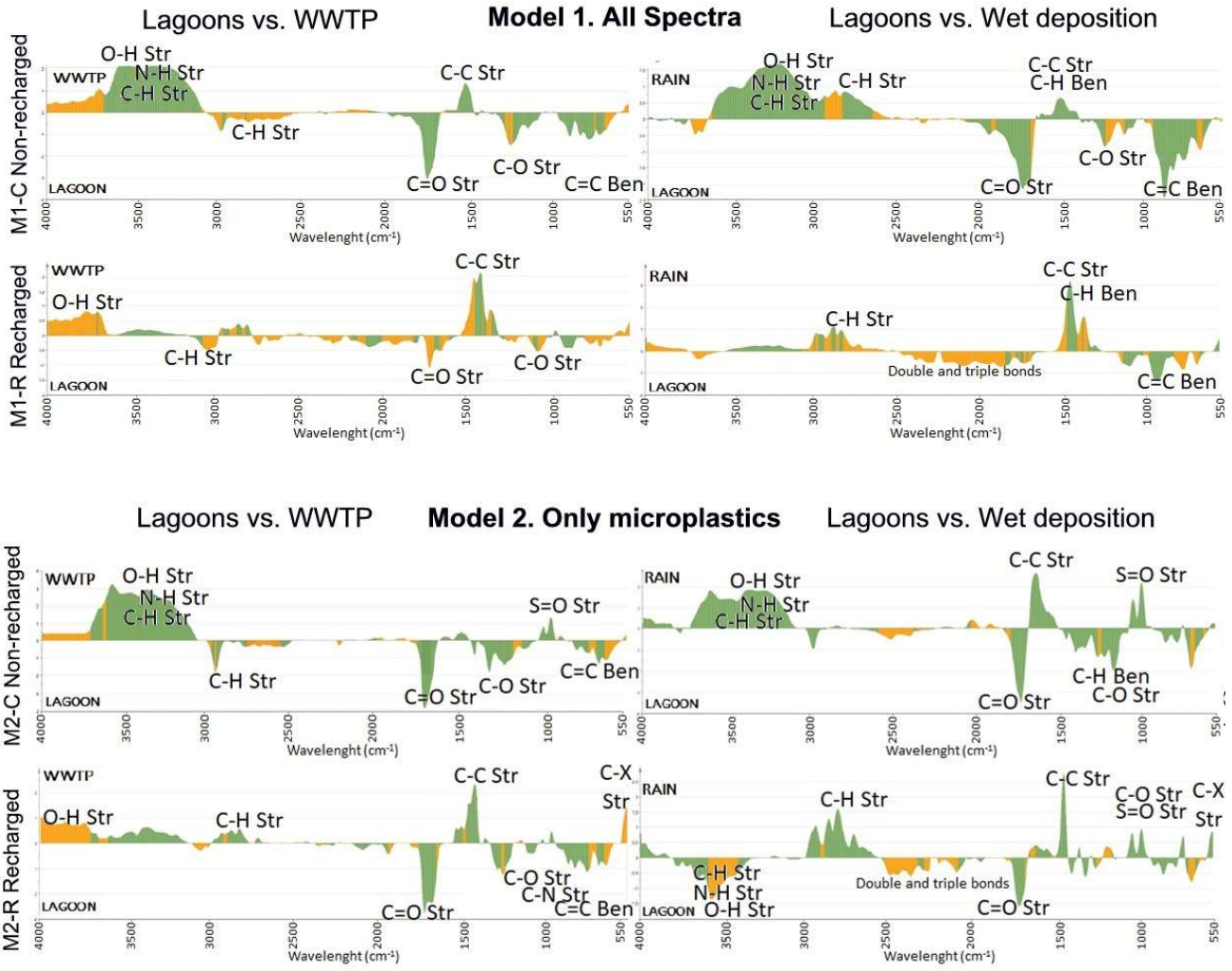


Figure 5. Contribution plot for comparing samples from different source. Inside each model the upper bands are non-artificially recharged lagoons (C) and the lower samples from recharged lagoons (R). Left panels: Lagoons vs. WWTP effluent; right panel Lagoons vs. Wet deposition. Str: Stretching vibration. Ben: Bending vibration. Orange represent variables outside three std. dev. range. All parameters are normalised (SNV).

yielded similar results. Both in control and wastewater-receiving lagoons, differences in the stretching vibration of C=O (~1700 cm^{-1}) and C-O (~1160 cm^{-1}) were clearly identified. Control lagoons showed a lack of bands in the characteristic C-H, N-H, O-H vibration region (3600-3000 cm^{-1}) that were present in recharged lagoons. On the contrary, these vibrations were abundant when comparing the recharged lagoons against wet deposition samples. The aliphatic C-H chains close to 2900 cm^{-1} were present only in non-artificially recharged lagoons (Fleming and Williams, 2020). Other bonds absent in plastics from the lagoons

were the bands at 1500 cm^{1} or close to the 1000 cm^{-1} (possibly S=O or C-O stretching) that were present in particles from WWTP effluent and wet deposition. The band from halogenated carbons (~550 cm^{-1}) was also found in samples from WWTP effluent and wet deposition in contrast to lagoon samples.

4. Discussion

For a long time, the wetlands in La Mancha were threatened by the risk of disappearance. Until the middle of 20th century these natural spaces remained almost intact providing water for agriculture and shelter for different animals, especially birds. After the 50's, and due to the increasing demand for agricultural land, an important part of these wetlands was put in production under intensive agricultural schemes. Irrigated areas increased from historical 200-300 km^2 to 1300-1400 km^2 in the early nineties (Fornés et al., 2000). Most wetlands became eventually polluted with pesticides, industrial chemicals or untreated wastewater, and sometimes, even total desiccation happened because of aquifer overexploitation (Álvarez-Cobelas et al., 2010). The area was declared Biosphere Reserve by UNESCO in 1981 in view of its high ecological value. Long after that, the ecological situation of many lagoons and aquifers is poor. Several protection figures and preservation plans showed limited success due to the disregard of the administrations involved. In this context, some wetlands, formerly temporary, became permanent due to the continuous supply of wastewater inflows from nearby WWTP. This artificial recharge offered an apparent solution against the desiccation caused by the overexploitation of groundwater reservoirs. Consequently, recharged lagoons suffer from a continuous supply of pollutants, including microplastics that accumulate due to their endorheic character.

GQR[3] showed the highest concentration of microplastics with 24.4 ± 5.2 particles/g followed by LVC[2] with 14.2 ± 3.5 particles/g. Much lower levels were found in ELG[1], despite this lagoon receives a discharge of treated wastewater, and non-artificially recharged lagoons, in which the concentration of microplastics was < 1 particle/g in CVF[4], LAC[5] and LAB[6]. Table 2 shows these values put in context with other data from literature.

Previous works have established the occurrence of microplastics in sediments of rivers, lakes and marine ecosystems. Scheurer and Bigalke reported concentrations up to 593 microparticles of plastic per kilogram of sediment in Swiss floodplain areas (Scheurer and Bigalke, 2018). Fuller and Gautam studied contaminated soils in Australia and found higher values that reached 67.5 g/kg (results in particle number not given) (Fuller and Gautam, 2016). Regarding wetlands, the study of Ziajahromi et al. revealed up to 595 microplastic particles/kg in an Australian constructed wetland arranged to treat storm runoff waters (Ziajahromi et al., 2020). Towsend et al. reported somewhat lower figures for sediments of a set of 20 urban wetlands near Melboune, Australia (Townsend et al., 2019). River sediments were reported to contain different loads of microplastics with higher values in the thousands of particles per kilogram range

Table 2. Microplastics in sediments. Our data in the context of other author's findings.

Sampling point	Size range	Concentration of microplastics	Reference
Laguna Grande (Quero, Spain) - GQR[3]	25 μm - 5 mm	24.4 ± 5.2 particles/g	This work
Laguna Larga (Villacañas, Spain) - LVC[2]	25 μm - 5 mm	14.2 ± 3.5 particles/g	This work
El Longar (Lillo, Spain) - ELG[1]	25 μm - 5 mm	2.0 ± 0.8 particles/g	This work
Swiss foodplain soils (29 sites)	125 μm - 5 mm	593 particles/kg 55.5 mg/kg	(Scheurer and Bigalke, 2018)
Soils from an industrial area in Australia (17 samples)	~30 μm - 5 mm	300-67500 mg/kg	(Fuller and Gautam, 2016)
Constructed wetland in Australia	> 25 μm	595 ± 120 particles/kg (inlet) 320 ± 42 particles/kg (outlet)	(Ziajahromi et al., 2020)
Sediments from 20 urban wetlands in Australia	35 μm - 1 mm	2-147 particles/kg (average 47 particles/kg)	(Townsend et al., 2019)
Urban section of Qin River, Guangxi, China.	25 μm - 5 mm	up to 97 particles/kg	(Zhang et al., 2020a)
River shore sediments in the Rhine-Main area, Germany	63 μm - 5 mm	21.8-932 mg/kg 228−3763 particles/kg	(Klein et al., 2015)
Sediments in Changjiang Estuary, China	46.8 μm - 5 mm	20-340 particles/kg	(Peng et al., 2017)

(Klein et al., 2015; Peng et al., 2017; Zhang et al., 2020a). Our results showed that endorheic lagoons receiving wastewater, even if treated according to current standards may result in high concentration of microplastics in sediments, at least one order of magnitude higher that the highest values reported elsewhere. Assuming the usual values for the density of dry sediments, the extrapolation of our data to the microplastics per unit surface would yield values over 10^4 microplastics/m^2 (Verstraeten and Poesen, 2001). It has to be considered that our samples were taken in points near the inlet of wastewater discharges in wastewater-receiving lagoons. It is reasonable to assume that non-flooded areas would have concentrations of microplastics closer to non-wastewater receiving lagoons. Also, it is conceivable that a large number of microplastics < 25 μm may exist, which are generally outside the capacity of current identification techniques. Overall, we demonstrated that microplastic concentration may reach very high values. Higher than those reported before for any kind of sediment elsewhere. It is important to note that the concentration of pollutants in a given area is the balance between inflow and outflow and in this case, the lagoons are endorheic and do not discharge to any other stream or water body. On the contrary, microplastics accumulate in sediments and their concentration is expected to continuously increase with time.

The ecological risk of microplastics in sediments is difficult to assess. There are knowledge gaps that include a lack of standardized quantification methods and scattered data for the concentration of microplastics in most environmental compartments. Peng et al. found an average abundance of microplastics in river sediments of 802 particles/kg and suggested that their chemical composition may result in environmental risk associated to the presence of phenoxy resins, produced from bisphenol A and usually cured with isocyanates (Peng et al., 2018). Other studies suggest that microplastics act as a vector for other pollutants like metals (Akhbarizadeh et al., 2017). Recent studies indicate that long-term exposure to microplastics may impact sediment biota even at environmentally relevant concentrations by affecting sublethal endpoints such as energy reserves (Bour et al., 2018).

Our work demonstrated the presence of at least 11 different types of microplastics in the sediments of lagoons receiving wastewater discharges. The predominant polymers were those in most common use like PE and PP, which account for 90 % of polymers in materials used in daily routine (PlasticsEurope, 2019). A minor fraction of the polymers identified in this work corresponded to those with higher density like PVC, PU or PVF that tend to sink and tend to appear in sediment samplings (Huang et al., 2020; Sun et al., 2019). Besides, many fibres were found, essentially polyester and acrylic fibres, which are typical residues from domestic washing machines (Napper and Thompson, 2016). The occurrence of fibres in wastewater has been reported elsewhere (Bayo et al., 2019; Zambrano et al., 2019). An additional cause for concern is that natural fibres, like cotton or wool, when industrially processed, contain potentially harmful additives that may end up in the environment (Cesa et al., 2017). These chemicals include dyes, fire retardants, softening additives and many others and constitute a source of anthropogenic pollution somehow comparable to microplastics. Fragments, also usual in WWTP effluents, are common in the sediments from wastewater-fed lagoons and wetlands (Townsend et al., 2019; Zhang and Liu, 2018). The presence of relatively high amount of films in LVC[2], might be influenced by the green filter located immediately before sampling points. Laminated plastics are common in the construction of these filtering systems and their occurrence in downstream ecosystems has sometimes been reported (Ziajahromi et al., 2020). Microplastics found in non-artificially recharged lagoons were mostly dominated by films, probably materials generated elsewhere and transported by wind (Zhang et al., 2019).

In this work we use OPLS-DA to compare microparticles/microplastics from three different sources. The rationale was to assess the origin of the anthropogenic pollutants found in the sediments of recharged lagoons. Several studies highlighted the importance of atmospheric transport and wet or dry deposition in the spreading of anthropogenic materials to different environments (Klein and Fischer, 2019; Wright et al., 2019). Moreover, the presence of wastewater discharges in some lagoons would explain the similarities between collected microplastics with samples taken from other WWTP. The real situation is somewhat more complex due to the ageing of plastic materials deposited in natural environments during prolonged periods. Photolytic, photo-oxidative and thermo-oxidative reactions are responsible of accelerating polymer degradation and modify FTIR spectra with an increase in oxygenated moieties including those containing carbonyl, carboxyl of hydroxyl groups (Andrady, 2017; Prata et al., 2020). Another effect complicating the analysis is the colonization of debris materials by different organisms when disposed in a biotic medium for prolonged periods (Arias-Andres et al., 2018). The plastic fraction from lagoons receiving wastewater showed bands corresponding to O-H, N-H and C-H bonds similar to those found in wastewater samples and absent from control lagoons and wet deposition samples. Wet deposition samples differ from recharged lagoons in specific vibrations in the C-H region. Non-artificially recharged lagoons clearly differed from the rest of samples because of the absence of vibrations in the 3600-3000 cm^{-1} region, which probably indicates a different origin for microplastics found in non-artificially recharged lagoons. Because of abundance and composition, the most probable origin of microplastics in lagoons receiving wastewater is wastewater discharge itself.

This work demonstrated that wastewater discharges in inland water bodies, particularly in endorheic lagoons, result in the accumulation of organic pollutants in high amounts. The occurrence of microplastics have never been reported in La Mancha wetlands, but the impact associated to wastewater recharge has been associated to changes in nutrient cycles due to the alteration of natural drying-flooding cycles (Corrales-González et al., 2019). Overall, our work showed that current wastewater treatment is not enough to avoid the accumulation of microplastic pollutants in the sensitive environment of endorheic lagoons. It is to be stressed that these lagoons do not discharge to other external bodies of water and, therefore, pollutants may accumulate in large amounts. The data available indicate the need to establish criteria concerning the quality of wastewater used to recharge lagoons and to decide if this is a sustainable practice compatible with preserving the natural status and biodiversity of protected areas.

5. Conclusions

This work studied the presence of microplastics in six lagoons from the extensive network of wetlands called "La Mancha Húmeda", declared Biosphere Reserve by UNESCO. It was found that lagoons receiving wastewater effluents displayed very high concentration of microplastics in sediments with concentrations reaching the order of tens of microplastics (25 μm - 5 mm) per gram,

In contrast to lagoons receiving wastewater, non-artificially recharged lagoons, that kept their natural drying and flooding cycle, showed much less microplastics, with films being the dominant shape. In lagoons receiving wastewater, fibres were the dominant typology, which can be attributed to domestic wastewater discharges.

Chemical analyses performed by micro-FTIR showed that the main materials in anthropogenic microlitter were the polyolefins polyethylene and polypropylene, and polyester and acrylic fibres. Up to 11 different polymer types were found in lagoons receiving wastewater. Statistical analysis of FTIR spectra confirmed similarity with samples taken from WWTP effluent rather than from wet deposition samples.

Our results showed that wastewater recharge is not a suitable practice to maintain water levels in endorheic lagoons as it leads to the accumulation of microplastics in very high amounts. This is due to the closed or terminal character of endorheic basins.

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References

Akhbarizadeh R, Moore F, Keshavarzi B, Moeinpour A. Microplastics and potentially toxic elements in coastal sediments of Iran's main oil terminal (Khark Island). Environmental Pollution 2017; 220: 720-731.

Álvarez-Cobelas M, Sánchez-Carrillo S, Cirujano S, Angeler DG. A story of the wetland water quality deterioration: Salinization, pollution, eutrophication and siltation. In: Sánchez-Carrillo S, Angeler D, editors. Ecology of Threatened Semi-Arid Wetlands. Springer, New York, 2010.

Andrady AL. The plastic in microplastics: A review. Marine Pollution Bulletin 2017; 119: 12-22.

Araujo CF, Nolasco MM, Ribeiro AMP, Ribeiro-Claro PJA. Identification of microplastics using Raman spectroscopy: Latest developments and future prospects. Water Research 2018; 142: 426-440.

Arias-Andres M, Kettner MT, Miki T, Grossart H-P. Microplastics: New substrates for heterotrophic activity contribute to altering organic matter cycles in aquatic ecosystems. Science of The Total Environment 2018; 635: 1152-1159.

Bayo J, Olmos S, López-Castellanos J. Microplastics in an urban wastewater treatment plant: The influence of physicochemical parameters and environmental factors. Chemosphere 2020; 238: 124593.

Bayo J, Rojo D, Olmos S. Abundance, morphology and chemical composition of microplastics in sand and sediments from a protected coastal area: The Mar Menor lagoon (SE Spain). Environmental Pollution 2019; 252: 1357-1366.

Beiras R, Bellas J, Cachot J, Cormier B, Cousin X, Engwall M, et al. Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. Journal of Hazardous Materials 2018; 360: 452-460.

Blettler MCM, Abrial E, Khan FR, Sivri N, Espinola LA. Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. Water Research 2018; 143: 416-424.

Bour A, Haarr A, Keiter S, Hylland K. Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. Environmental Pollution 2018; 236: 652-660.

Bylesjö M, Rantalainen M, Cloarec O, Nicholson JK, Holmes E, Trygg J. OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification. Journal of Chemometrics: A Journal of the Chemometrics Society 2006; 20: 341-351.

Cannas S, Fastelli P, Guerranti C, Renzi M. Plastic litter in sediments from the coasts of south Tuscany (Tyrrhenian Sea). Marine Pollution Bulletin 2017; 119: 372-375.

Carr SA, Liu J, Tesoro AG. Transport and fate of microplastic particles in wastewater treatment plants. Water Research 2016; 91: 174-182.

Cesa FS, Turra A, Baruque-Ramos J. Synthetic fibers as microplastics in the marine environment: A review from textile perspective with a focus on domestic washings. Science of The Total Environment 2017; 598: 1116-1129.

Cirujano S, Medina L. Plantas acuáticas de las lagunas y humedales de Castilla-La Mancha: Editorial Liber Factory, 2014.

Corrales-González M, Rochera C, Picazo A, Camacho A. Effect of wastewater management on phosphorus content

and sedimentary fractionation in Mediterranean saline lakes. Science of The Total Environment 2019; 668: 350-361.
de Souza AA, Kloas W, Zarfl C, Hempel S, Rillig MC. Microplastics as an emerging threat to terrestrial ecosystems. Global Change Biology 2018; 24: 1405-1416.
Dris R, Gasperi J, Saad M, Mirande C, Tassin B. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Marine Pollution Bulletin 2016; 104: 290-293.
Edo C, González-Pleiter M, Leganés F, Fernández-Piñas F, Rosal R. Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge. Environmental Pollution 2020; 259: 113837.
Farady SE. Microplastics as a new, ubiquitous pollutant: Strategies to anticipate management and advise seafood consumers. Marine Policy 2019; 104: 103-107.
Fleming I, Williams DH. Spectroscopic Methods in Organic Chemistry: Springer Int. Pub., 2020.
Fornés J, Rodríguez JA, Hernández N, Llamas MR. Possible solutions to avoid conflicts between water resources development and wetland conservation in the "La Mancha Húmeda" biosphere reserve (Spain). Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere 2000; 25: 623-627.
Fuller S, Gautam A. A procedure for measuring microplastics using pressurized fluid extraction. Environmental Science & Technology 2016; 50: 5774-5780.
Gago J, Galgani F, Maes T, Thompson RC. Microplastics in Seawater: Recommendations from the Marine Strategy Framework Directive Implementation Process. Frontiers in Marine Science 2016; 3.
Gigault J, Halle At, Baudrimont M, Pascal P-Y, Gauffre F, Phi T-L, et al. Current opinion: What is a nanoplastic? Environmental Pollution 2018; 235: 1030-1034.
Godoy V, Martín-Lara MA, Calero M, Blázquez G. Physical-chemical characterization of microplastics present in some exfoliating products from Spain. Marine Pollution Bulletin 2019; 139: 91-99.
González-Pleiter M, Tamayo-Belda M, Pulido-Reyes G, Amariei G, Leganés F, Rosal R, et al. Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments. Environmental Science: Nano 2019; 6: 1382-1392.
González-Pleiter M, Velázquez D, Edo C, Carretero O, Gago J, Barón-Sola Á, et al. Fibers spreading worldwide: Microplastics and other anthropogenic litter in an Arctic freshwater lake. Science of The Total Environment 2020; 722, 137904.
Gorito AM, Ribeiro AR, Almeida CMR, Silva AMT. A review on the application of constructed wetlands for the removal of priority substances and contaminants of emerging concern listed in recently launched EU legislation. Environmental Pollution 2017; 227: 428-443.
Guo J-J, Huang X-P, Xiang L, Wang Y-Z, Li Y-W, Li H, et al. Source, migration and toxicology of microplastics in soil. Environment International 2020; 137: 105263.
Helcoski R, Yonkos LT, Sanchez A, Baldwin AH. Wetland soil microplastics are negatively related to vegetation cover and stem density. Environmental Pollution 2020; 256: 113391.
Huang J-S, Koongolla JB, Li H-X, Lin L, Pan Y-F, Liu S, et al. Microplastic accumulation in fish from Zhanjiang mangrove wetland, South China. Science of The Total Environment 2020; 708: 134839.
Jaikumar G, Brun NR, Vijver MG, Bosker T. Reproductive toxicity of primary and secondary microplastics to three cladocerans during chronic exposure. Environmental Pollution 2019; 249: 638-646.
Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, et al. Plastic waste inputs from land into the ocean. Science 2015; 347: 768-771.
Kedzierski M, Villain J, Falcou-Préfol M, Kerros ME, Henry M, Pedrotti ML, et al. Microplastics in Mediterranean Sea: A protocol to robustly assess contamination characteristics. PLoS One 2019; 14: e0212088.
Klein M, Fischer EK. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Science of The Total Environment 2019; 685: 96-103.
Klein S, Worch E, Knepper TP. Occurrence and spatial distribution of microplastics in river shore sediments of the Rhine-Main area in Germany. Environmental Science & Technology 2015; 49: 6070-6076.
Lares M, Ncibi MC, Sillanpää M, Sillanpää M. Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Research 2018; 133: 236-246.
Lee WS, Cho H-J, Kim E, Huh YH, Kim H-J, Kim B, et al. Bioaccumulation of polystyrene nanoplastics and their effect on the toxicity of Au ions in zebrafish embryos. Nanoscale 2019; 11: 3173-3185.
Liu K, Wang X, Wei N, Song Z, Li D. Accurate quantification and transport estimation of suspended atmospheric microplastics in megacities: Implications for human health. Environment International 2019; 132: 105127.
Masura J, Baker J, Foster G, Arthur C. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. . NOAA Technical Memorandum NOS-OR&R-48., Silver Spring, 2015.
Napper IE, Thompson RC. Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. Marine Pollution Bulletin 2016; 112: 39-45.
Peeken I, Primpke S, Beyer B, Gütermann J, Katlein C, Krumpen T, et al. Arctic sea ice is an important temporal sink and means of transport for microplastic. Nature Communications 2018; 9: 1505.
Peng G, Xu P, Zhu B, Bai M, Li D. Microplastics in freshwater river sediments in Shanghai, China: A case study of risk assessment in mega-cities. Environmental Pollution 2018; 234: 448-456.
Peng G, Zhu B, Yang D, Su L, Shi H, Li D. Microplastics in sediments of the Changjiang Estuary, China. Environmental Pollution 2017; 225: 283-290.
PlasticsEurope. Plastics – the Facts 2019: An analysis of European plastics production, demand and waste data. PlasticsEurope: Association of Plastics Manufacturers, Brussels, 2019.
Prata JC, Reis V, Paço A, Martins P, Cruz A, da Costa JP, et al. Effects of spatial and seasonal factors on the characteristics and carbonyl index of (micro)plastics in a sandy beach in Aveiro, Portugal. Science of The Total Environment 2020; 709: 135892.
Scheurer M, Bigalke M. Microplastics in Swiss floodplain soils. Environmental Science & Technology 2018; 52: 3591-3598.

Silva AFT, Sarraguça MC, Ribeiro PR, Santos AO, De Beer T, Lopes JA. Statistical process control of cocrystallization processes: A comparison between OPLS and PLS. International Journal of Pharmaceutics 2017; 520: 29-38.
Smith M, Love DC, Rochman CM, Neff RA. Microplastics in Seafood and the Implications for Human Health. Current Environmental Health Reports 2018; 5: 375-386.
Sun J, Dai X, Wang Q, van Loosdrecht MCM, Ni B-J. Microplastics in wastewater treatment plants: Detection, occurrence and removal. Water Research 2019; 152: 21-37.
Tournebize J, Chaumont C, Mander Ü. Implications for constructed wetlands to mitigate nitrate and pesticide pollution in agricultural drained watersheds. Ecological Engineering 2017; 103: 415-425.
Townsend KR, Lu H-C, Sharley DJ, Pettigrove V. Associations between microplastic pollution and land use in urban wetland sediments. Environmental Science and Pollution Research 2019; 26: 22551-22561.
van Emmerik T, Tramoy R, van Calcar C, Alligant S, Treilles R, Tassin B, et al. Seine plastic debris transport tenfolded during increased river discharge. Frontiers in Marine Science 2019; 6.
Verstraeten G, Poesen J. Variability of dry sediment bulk density between and within retention ponds and its impact on the calculation of sediment yields. Earth Surface Processes and Landforms 2001; 26: 375-394.
Wang F, Wong CS, Chen D, Lu X, Wang F, Zeng EY. Interaction of toxic chemicals with microplastics: A critical review. Water Research 2018; 139: 208-219.
World Economic Forum. The New Plastics Economy — Rethinking the future of plastics. In: World Economic Forum EMFaMC, editor, 2106.
Wright SL, Ulke J, Font A, Chan KLA, Kelly FJ. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. Environment International 2019: 105411.
Wu H, Zhang J, Ngo HH, Guo W, Hu Z, Liang S, et al. A review on the sustainability of constructed wetlands for wastewater treatment: design and operation. Bioresource technology 2015; 175: 594-601.
Zambrano MC, Pawlak JJ, Daystar J, Ankeny M, Cheng JJ, Venditti RA. Microfibers generated from the laundering of cotton, rayon and polyester based fabrics and their aquatic biodegradation. Marine Pollution Bulletin 2019; 142: 394-407.
Zhang GS, Liu YF. The distribution of microplastics in soil aggregate fractions in southwestern China. Science of The Total Environment 2018; 642: 12-20.
Zhang L, Liu J, Xie Y, Zhong S, Yang B, Lu D, et al. Distribution of microplastics in surface water and sediments of Qin river in Beibu Gulf, China. Science of The Total Environment 2020a; 708: 135176.
Zhang W, Liu Z, Tang S, Li D, Jiang Q, Zhang T. Transcriptional response provides insights into the effect of chronic polystyrene nanoplastic exposure on Daphnia pulex. Chemosphere 2020b; 238: 124563.
Zhang Y, Gao T, Kang S, Sillanpää M. Importance of atmospheric transport for microplastics deposited in remote areas. Environmental Pollution 2019; 254: 112953.
Zhang Y, Kang S, Allen S, Allen D, Gao T, Sillanpää M. Atmospheric microplastics: A review on current status and perspectives. Earth-Science Reviews 2020c: 103118.
Ziajahromi S, Drapper D, Hornbuckle A, Rintoul L, Leusch FDL. Microplastic pollution in a stormwater floating treatment wetland: Detection of tyre particles in sediment. Science of The Total Environment 2020; 713: 136356.

SUPPLEMENTARY MATERIAL

Microplastics in sediments of artificially recharged lagoons: Case study in a Biosphere Reserve

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Contents:

Table S1. List of annual authorised discharges to ELG[1] and LVC[2]. Source: Ministry of Agriculture, Food and Environment of Spain.

Location	Origin	Max Vol (m^3)	UTM	Coordinate X	Coordinate Y	Receiving Medium	Inhabitants	Lagoon	River
Lillo	Lillo WWTP	207685	30T	473649	4395945	Cat II	2000-9999 IE	ELG[1]	
Villacañas	Villacañas WWTP	929962	30T	474547	4380114	Cat I	10000 IE	LVC[2]	Riánsares
			30T	472737	4382619				

Cat I. Drinking water production, places destined to recreative activities, suitable for salmonids, special protection zones, protected areas, sensitive zones and groundwaters

Cat II. Suitable for cyprinids, suitable for molluscs farming and public use for certain recreative activities

Table S2. Water inputs, precipitations and use of soil in sampled lagoons.

ID	Water inputs	Average annual precipitation (mm)	Land use in surrounding areas
ELG[1]	Precipitation, runoff and wastewater	360	Pastures, dry farming, woody crops, arable crops, and urban green zones
LVC[2]	Precipitation, runoff and wastewater	500	Bare soils, woody crops, leaf forest, arable crops, and pastures
GQR[3]	Precipitation, runoff and wastewater	393	Arable crops, pastures, salt mines, and other crops
CVF[4]	Precipitation, runoff, Cigüela River and aquifer 20	390	Pastures, arable crops, vineyards, conifers, and other crops
LAC[5]	Precipitation and runoff	393	Dry farming, olive groves, combined crops, and bare soils
LAB[6]	Precipitation and runoff	360	Pastures, arable crops, woody crops, and vineyards

Table S3. Additional information concerning micro-FTIR analyses.

Sampling Point	Total number of sampled microparticles	Analysed	Microplastics	Anthropogenic*	Other non-plastic microparticles	Microplastic (%)	Different polymer types
ELG[1]	139	31	9	3	19	29.0	4
LVC[2]	433	53	26	5	22	49.1	8
GQR[3]	544	52	35	3	14	67.3	8
CVF[4]	44	17	-	1	16	-	-
LAC[5]	48	16	4	5	7	25.0	2
LAB[6]	45	21	2	-	19	9.5	2

* Particles of natural materials like wool or cellulose with evidence of industrial origin

Figure S1. Photographs of sampling points and visual evidences of anthropogenic pollution.

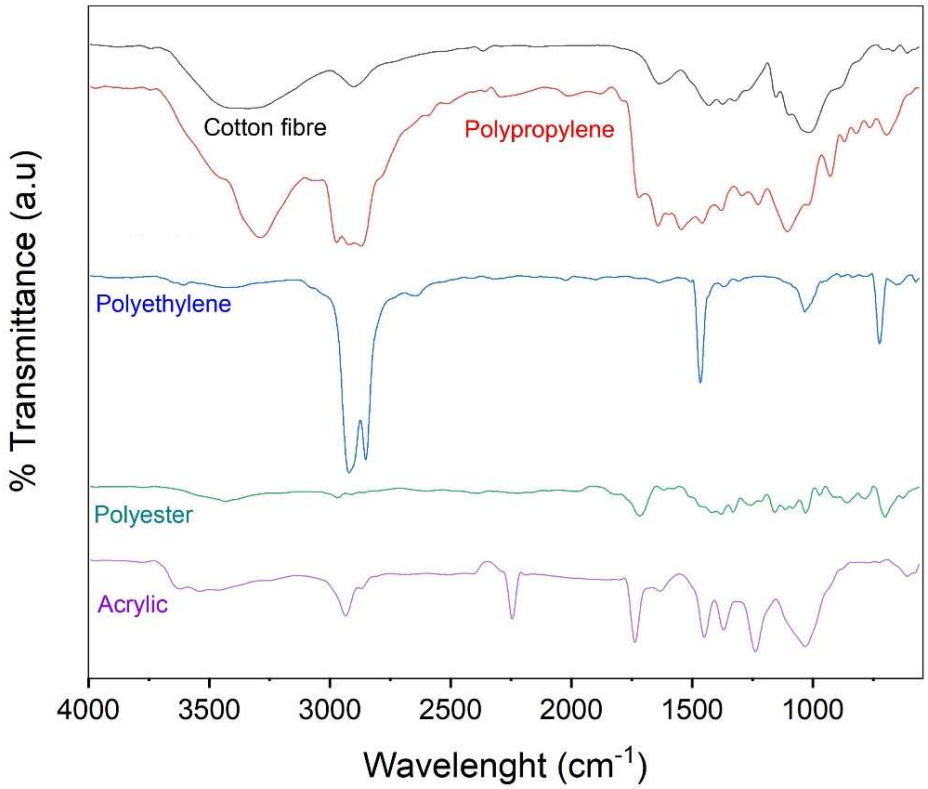

Figure S2. Examples of micro-FTIR spectra for the more abundant polymers in sediment samples.

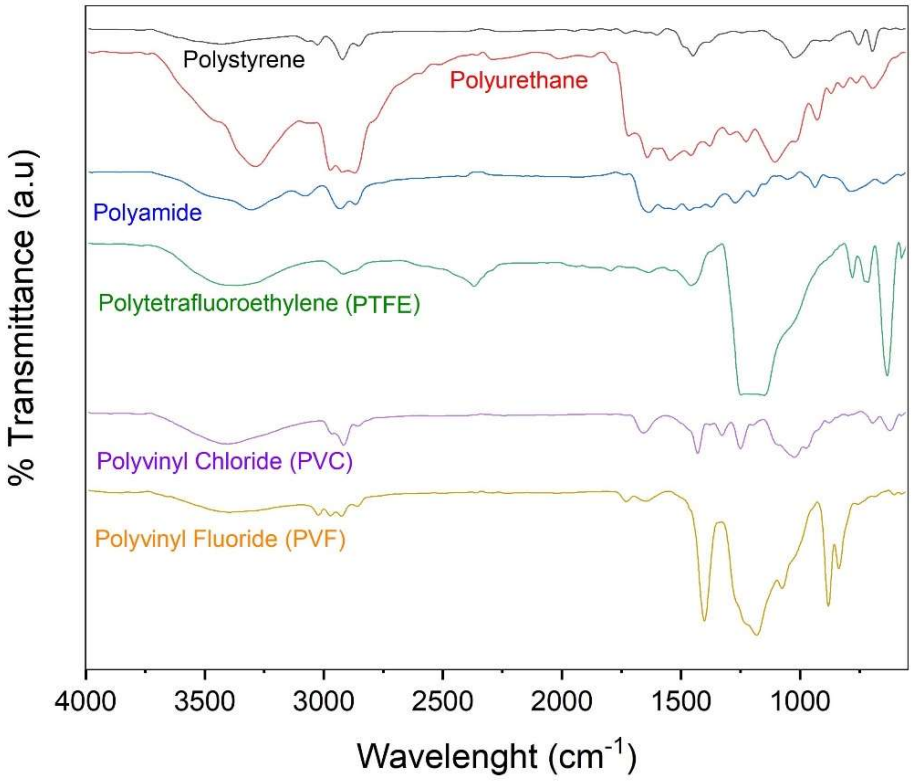

Figure S3. Examples of micro-FTIR spectra for the less abundant polymers in sediment samples.

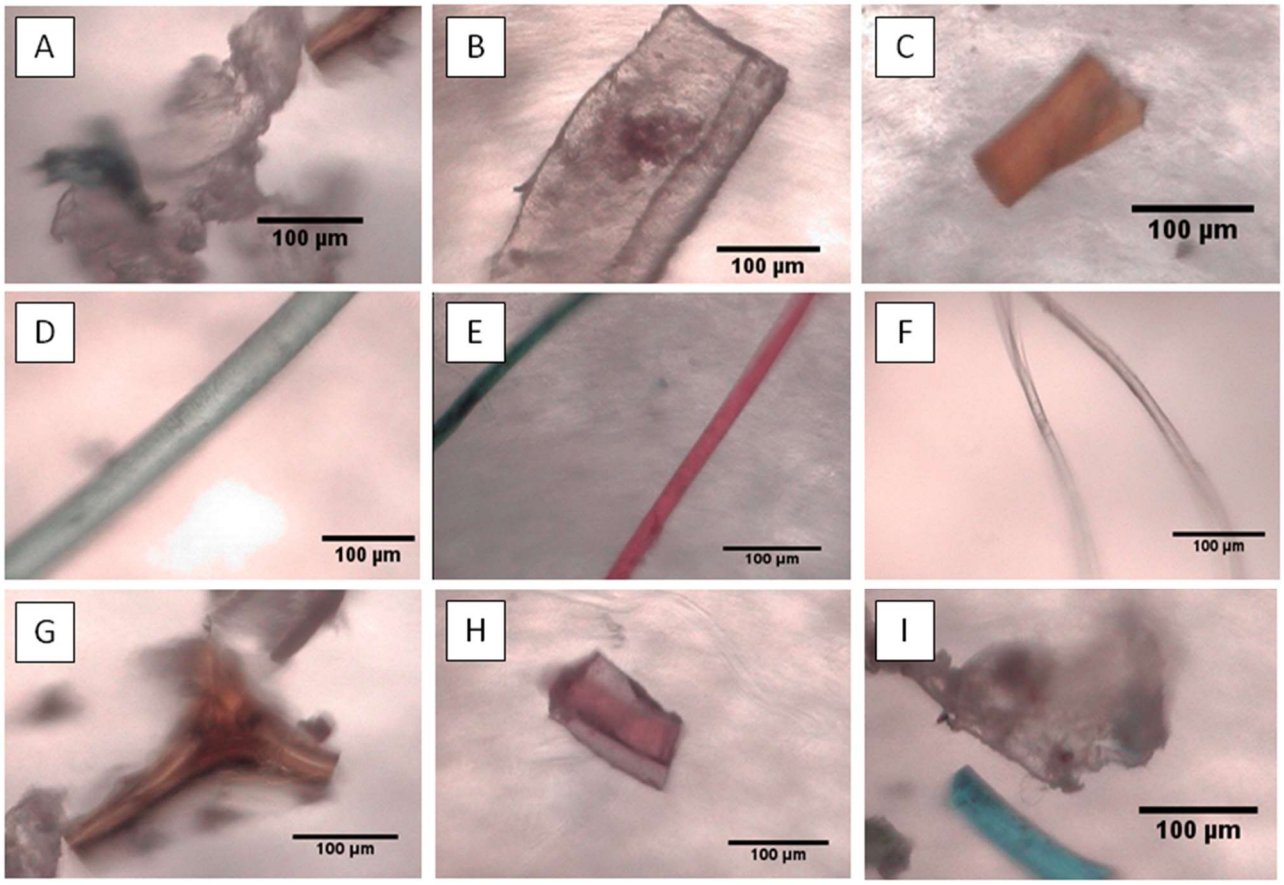

Figure S4. Microplastic particles. A-PFTE film (LVC[2]), B-PE fragment (ELG[1]), C-PP fragment (GQR[3]), D-PP filament (LVC[2]), E-Acrylic fibres (GQR[3]), F-Polyester fibres (GQR[3]), G-PU fragment (LVC[2]), H-PP fragment (GQR[3]) and I-PS film and PP filament (GQR[3]).

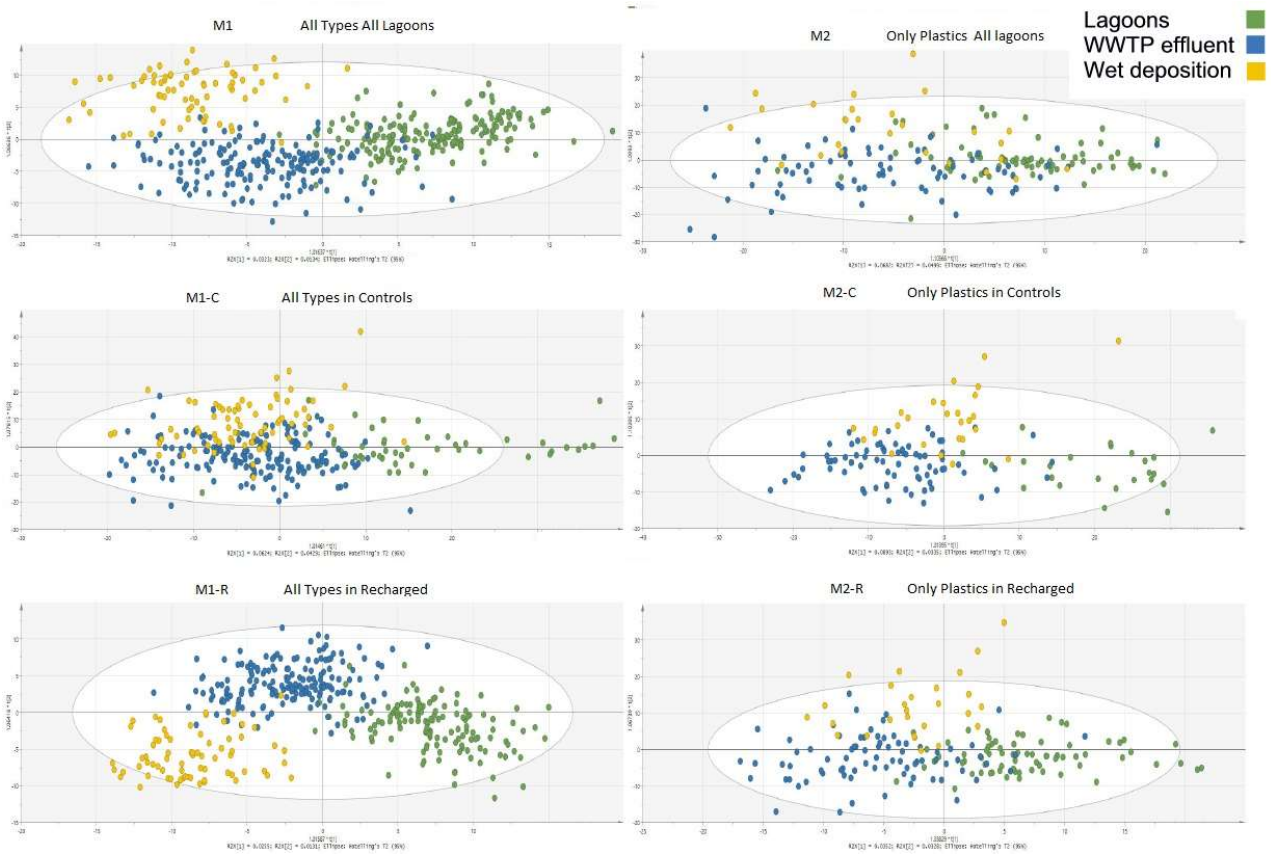

Figure S5. Score scatter plots of OPLS-DA developed Models 1 (1, 1-C and 1-R) and 2 (2, 2-C and 2-R) as indicated in Table 1.

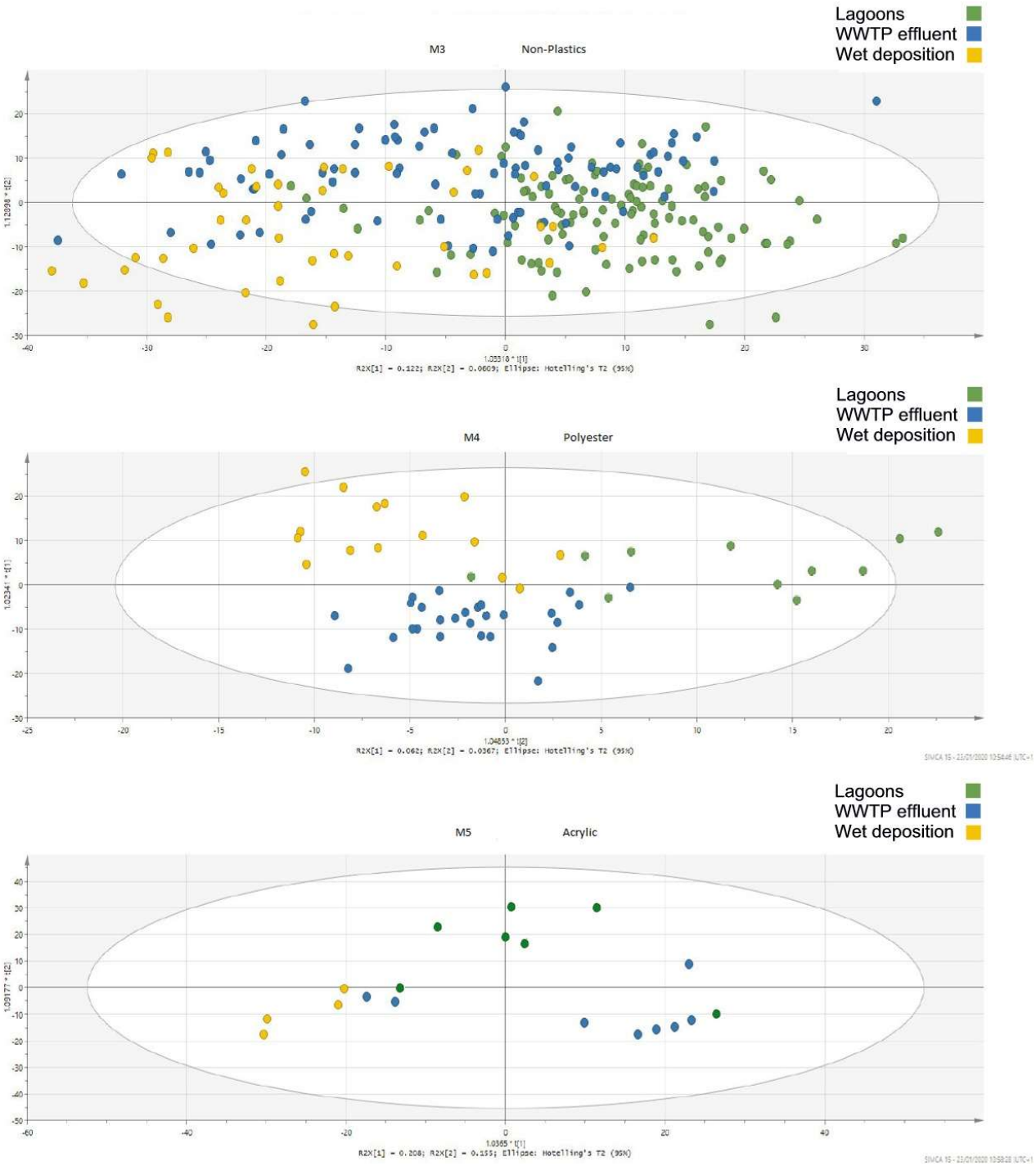

Figure S6. Score scatter plots of OPLS-DA developed Models 3, 4 and 5 as indicated in Table 1.