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Raman imaging spectroscopic solutions for microplastics advanced analysis: Insights from *Choqueyapu* river basin (La Paz, Bolivia)

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ABSTRACT

Microplastics (MPs) are causing global concern due to their role as vectors of environmental contaminants. Evaluating their impact on environmental compartments, particularly in sediment and freshwaters, remains challenging due to difficulties in gathering chemical and morphological data. In fact, the analytical process can vary depending on the matrix considered, the non-homogeneous characteristics of MPs, and the targeted size range. Sample treatment is crucial for sediments and waters, requiring a balance between matrix removal and preservation of the MPs. Consequently, MPs often remain embedded in significant amounts of the original matrices, compromising their characterisation. In this regard, Raman spectroscopy shows promise for their comprehensive molecular analysis. However, overcoming the drawbacks associated with fluorescence from organic matter, feldspar, or clays requires considerable effort. Effective signal acquisition necessitates fine-tuning parameters, including background reduction and signal-to-noise ratio amplification. Moreover, data handling involved in chemical scanning large surfaces at high resolution is a challenging task. To overcome these drawbacks, chemometrics have demonstrated high efficacy in processing and extracting targeted information. The application of chemometrics could be relevant in environmental studies due to the large number of samples, the complexity of signal acquisition, and the dataset volumes managed. As such, this study proposes spectroscopic analytical solutions, augmented by chemical imaging and algorithmic processing, for advanced MPs analysis. A spectroscopic working approach was devised and tested through a real case study conducted in the *Choqueyapu* River basin (La Paz, Bolivia). This methodology allowed the morphological, molecular and quantitative identification of over 44 particles/L and 91 MPs particles/kg, in water and sediment, respectively, consisting of PE, PET, PP, PS and PMMA. MP abundance varied significantly across studied areas, spanning 2 to 4 orders of magnitude. PET fibres predominated in freshwaters, while *Lipari Sector* sediments were hotspots for PE and PS fragments.

1. Introduction

The production of petroleum-based plastics has experienced exponential growth since its introduction on an industrial scale in the 1950s. This growth has been driven by factors such as population level and consumption trends, alongside its high versatility and low production costs. Global demand of plastics has surged, reaching 400 Mt in 2022 [1] and is projected to peak at 590 Mt by 2050 [2]. Their degradation

products, known as microplastics (MPs), are found in all environmental compartments, including in high levels of the trophic chain. For this reason, they have garnered significant scientific interest as contaminants of emerging concern (CECs) due to their potential impact on human and ecosystem health [3]. Recent studies have provided evidence that weathered MPs also pose a risk due to structural alterations leading to a more porous, hydrophobic, and chemically active surface. Furthermore, they can release their additives into the environment, in addition to

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adsorbing organic and inorganic pollutants [4–7], and even microorganisms [8]. Therefore, MPs may act as vectors for the spread of contaminants from the surrounding environment. Their potential vectorial capacity depends on various factors, including the contaminant nature, type of plastic, shape, size, and the interaction between plastic and contaminant or synergistic effects of variables present in plastic-contaminant-environment systems. Thus, assessing the presence of MPs is the first task to evaluate their potential impact on the different ecosystems, and to determine the necessity for implementing legislative regulation (production, discharge levels, presence in the drinking water supply, among others)—either preventive or corrective—to ensure public safety and health.

However, despite significant efforts made in recent years to develop protocols for studying MPs in environmental compartments, collecting chemical and morphological quantitative information remains a challenge [9,10]. In fact, the isolation of MPs from environmental matrices without altering them is being investigated for many reasons. This results in a huge variety of sampling methodologies, pre-treatment methods, as well as quantification and characterisation approaches that need to be aligned [11,12]. Specifically, sampling protocols differ according to the matrix considered, and must necessarily take into account the non-homogeneous character of the sample. For sediments and surface waters, the treatment of the sample matrix usually includes flotation, oxidation, and filtration as critical steps to find a compromise between the matrix removal and the potential modification or loss of the MPs present in the sample [13–15]. Depending on the type, location and characteristics of the samples, the commonly applied treatment steps should be tuned (namely, number of cycles, oxidation agent, type and concentrations levels of organic matter or inorganic compounds, digestion times, MPs size of interest, and so on). As a result, in most cases, MP particles remain embedded in significant amounts of the original matrices and thus, the characterisation and quantification information that can be extracted may be highly compromised.

In this regard, a wide array of identification/characterization techniques may be employed, depending on the isolation efficacy and the aims pursued [11,16]. Among the preferred options, a stereomicroscope can be used to assess the plastic appearance of the particles following a well-established criterion [12,17,18]. However, the identification protocol relies on visual selection, which is open to bias and misjudgement [16]. For that reason, it is recommended only for screening purposes or in combination with spectroscopic techniques for a specific molecular identification. Analysis by Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) (possibly coupled with Energy Dispersive Spectroscopy (EDS)) offer a good resolution (nanoscale) and elemental data, but often involve the sample metallization to enhance the image contrast and its electrical conductivity, thus, the posterior study for other purposes is constrained. Besides, as in the previous case, the use of molecular characterisation techniques is required to specifically determine the plastic type. In contrast, among the most commonly used methods, Pyrolysis Gas Chromatography-Mass Spectrometry (Py-GC-MS) and Thermogravimetric Analysis-Mass Spectrometry (TGA-MS) allow effective identification, and also determination of mass. However, their destructive nature makes impossible to obtain further data (morphological, pollutant, or additive content) [19]. These techniques suffer from limitations related to high weights [20] and are not yet recommended for samples with high impurities [11]. On the other hand, vibrational spectroscopic techniques offer a solution to the aforementioned limitations, with the option of acquiring microchemical information and comprehensive matching libraries. Among the most used, micro-Fourier Transform Infrared spectroscopy (micro-FTIR) allows chemical imaging analysis and can reach MPs sizes of 5–10 μm . However, the analysis requires contact with the sample, and the handicaps related to the thickness and the irregular shape of the particles that disturb the signal collection are difficult to avoid [11,21]. Complementary to micro-FTIR, micro-Raman spectroscopy is a technique that have seen recent uses in MPs identification [22,23] allows the

characterisation of MP chemical structure through their unique fingerprints, with spatial resolutions down to 1 μm . However, this technique suffers from the fluorescence from (micro)biological, organic, and inorganic compounds, which may hamper the signal collection of the particles of interest. In this case, selecting appropriate acquisition parameters (laser wavelength, laser power, magnification of the objective lens, measurement times and acquisitions, range or photobleaching) is crucial to minimize fluorescence and improve the signal noise (S/N) ratio, without causing photothermal degradation of the sample or detector saturation [24]. Thus, while Raman spectroscopy offers the most promising characteristics for comprehensive MP characterisation, it often requires considerable efforts to address the drawbacks associated with the technique and the challenging samples. Additionally, the complexity of raw data pre-processing and treatment related to spectral scanning of large sample surfaces or high volumes of particles, at high resolution, is not trivial [25,26]. To overcome the intrinsic handicaps of the technique, chemometric methods demonstrated their power as tools to process and extract information of interest, including pattern searching, clustering, classification, and others [23,27,28]. This is especially relevant for Raman-based MPs applications in environmental studies, due to the number of samples involved, the complexity of acquiring valid signals in the matrices and dataset volumes managed [29,30]. Here, we propose Raman spectroscopic solutions, augmented by chemical imaging and algorithm processing, for the advanced analysis of MPs. For that purpose, a spectroscopic working approach was designed to face the challenges of the morphological, molecular and quantitative identification through a real case study, involving surface water and river sediment compartments of an unexplored and highly contaminated region, the *Choqueyapu* River Basin (La Paz, Bolivia). Recently, Latin America has raised awareness about the state of their natural resources [31] due to limited access to waste collection and wastewater treatment plants. This situation probably increases the occurrence of MPs in the environment, making them twice as risky for ecosystem and human safety concerns [32]. The financial limitations for conducting scientific research further hinders the development of specific environmental studies to gain visibility and attract the needed economical and research means. Most research focuses on beaches [33,34], superficial water [35–39], and sediment [40–42], from freshwater ecosystems. To our knowledge, only one investigation has been conducted in Bolivia, and it aimed to assess the problematic environmental issues of Lake *Titicaca* [43], although it only provided visual characterisation (size and shape) of the investigated MP particles.

2. Materials and methods

2.1. Sampling sites

The sampling campaign was conducted along the *Choqueyapu* River Basin of La Paz during the autumn season of 2022, corresponding to dry period of the year. According to the possible impact sources of MPs, six sampling areas (Fig. 1) were selected to determine the influence of the different human activities in each region [44]. In detail, sampling areas 1 (*Orkojahuira* river), 2 (*Irpavi* river) and 3 (junction of *Orkojahuira* and *Irpavi* rivers) correspond to urban areas (394,2 hab./km²) [45] along the course of the main river, which are also directly exposed to industries (mainly, textile, agricultural and mining) [46]. At sampling areas 2 and 3, the main river received the contribution of effluent groups from the natural protected area surrounding La Paz and crosses a part of the urban nucleus. Sampling area 5 (junction of *Achocalla* and *Irpavi* rivers) is located at the end of the course of the river in the *Mecapaca* municipality (27,7 hab./km²) [45] and thus, could be a potential indicator of the total MPs impact suffered by the entire urban area. In contrast, sampling area 4 (*Achocalla* river) is related to a mostly agricultural zone with low population (94 hab./km²) [45], and sampling area 6 (*Hampaturi* water reservoir) corresponds to a naturally protected zona, unexposed to human activity.

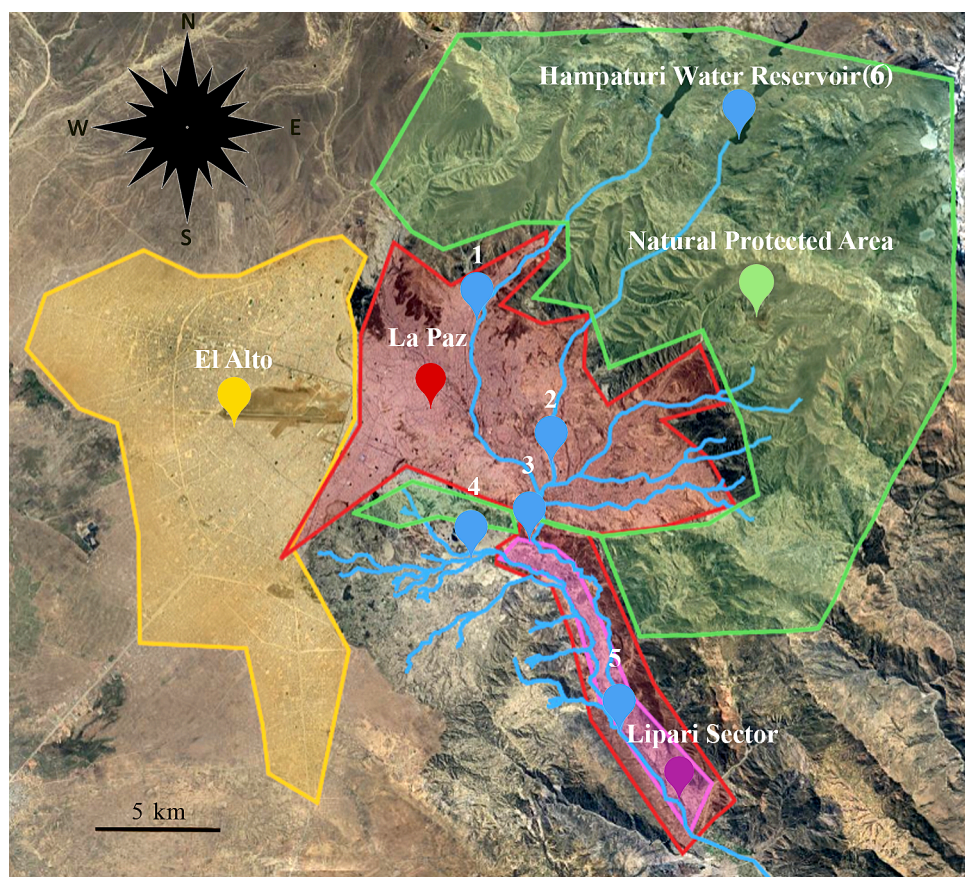


Fig. 1. Map of Choqueyapu river basin (La Paz, Bolivia) [44]: Topographic and hydrographic information. The sampling areas for the MPs study are highlighted in colour according to the region activity, specifically, the La Paz and the Alto urbans nucleus in red and yellow, respectively, a natural protected reserve in green, and the residential Lipari Sector in purple. Sampling sites are shown as blue points; 1) $16^{\circ}28'43.3''S / 68^{\circ}06'54.3''W$, 2) $16^{\circ}31'57.2''S / 68^{\circ}05'17.8''W$, 3) $16^{\circ}33'11.8''S / 68^{\circ}05'34.0''W$, 4) $16^{\circ}33'56.1''S / 68^{\circ}07'01.6''W$, 5) $16^{\circ}37'39.4''S / 68^{\circ}03'47.4''W$ and 6) $16^{\circ}24'59.3''S / 68^{\circ}01'13.9''W$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Preventive measures to avoid MP contamination

Potential MP sources were established along both sampling and treatment processes in an attempt to evaluate probable contaminations, and different preventive measures were implemented. Accordingly, all material employed during sampling and sample treatment was made of glass or metal, previously washed with pre-filtered ($<20 \mu\text{m}$) Milli-Q water, dried at 100°C , and observed with a microscope. All the filters used ($20 \mu\text{m}$, $240 \mu\text{m}$ and 1 mm) were made of 304 stainless steel. The sodium iodide solution (NaI) was also filtered on a $1.7 \mu\text{m}$ glass fibre mesh, before and after use. Furthermore, all sample manipulations were performed under a fume hood, and blank filters were placed next to the working area to be verified by microscope. In the same way, procedural blanks were also generated and processed for both type of samples. These did not show any MP contamination.

2.3. Sample collection

Due to the low level of the river water and the amount of suspended solids, water samples were taken at approximately 10 cm depth from the surface. Glass bottles, each equipped with a mesh filter of 1 mm in its mouth to prevent collection of large particles, were used to take three samples of 5 L at each sampling area. Then, the samples were further filtered using a $240 \mu\text{m}$ mesh filter to isolate the MP fraction of interest ($240\text{--}1000 \mu\text{m}$) and the filters were saved in a glass Petri dish and stored at 4°C until treatment. In parallel, three sediment samples were collected at each sampling area at 3 cm of depth from the river bed using cylindrical metallic boxes ($\varnothing 8 \text{ cm}$, 2.7 cm height). Once in the lab, the

sediment in the container was dried in an incubator chamber for 48 h at 40°C to transcript the results by dry mass. The dry sediments were also stored at 4°C until treatment in the metallic boxes. Accordingly, the results were referred as particles/L and particles/kg, for water and dry sediments samples, respectively. The quantification data were unified and reported as a single representative sample for the entire area due to the variability of MPs observed among the set of samples collected at the same location.

2.4. Sample treatment

Each filter obtained after filtration of the water samples was treated with 50 mL of hydrogen peroxide solution (H_2O_2 , 30%) in a borosilicate beaker to digest the organic matter content [47]. After 2 h , the filter was thoroughly washed with fresh peroxide solution, the washing liquid was added to the beaker and the cleaned filter was stored in a glass Petri dish for later reuse. The sample suspension in the beaker was left to react for another 24 h at 55°C and then vacuum filtered using the filter stored in the Petri dish to minimize MP loss and avoid further contamination. In most cases, these filters showed significant amounts of inorganic matter that can interfere in the analysis with fluorescence phenomena [48] and that cannot be reduced with more digestion steps. Subsequently, the sample was thoroughly cleaned with Milli-Q water within the vacuum system, the filter transferred to a new glass Petri dish that was kept in a glass desiccator at 4°C until the analysis.

The isolation of MPs present in sediments samples started with flotation [47] using a custom-made glass phase-separator device, adapted from Nakajima et al. [49] (Fig. 1S). To this end, the whole

sediment sample contained in the metallic box was transferred to the beaker of the device (Fig. 1Sa). Then, a concentrated NaI solution, (≥ 1.6 g/L) was added up to a few centimetres above the sliding platform, and the suspension was manually stirred for 2 min (Fig. 1Sb). After decantation, the sliding platform was moved horizontally to isolate the upper part of the suspension containing the light solid phase of interest (Fig. 1Sc). Finally, the supernatant was vacuum filtered using a 240 μm mesh filter. The flotation step was repeated twice, and the supernatants were filtered using the same filter to increase the recovery of MPs. The filter was cleaned with Milli-Q water within the vacuum system to eliminate NaI traces and avoid secondary exothermic reactions during the oxidation step. Then it was treated with the peroxide solution and stored as described before for water samples.

2.5. Methodology of analysis

2.5.1. Instrumentation

A Renishaw InVia Raman spectrophotometer with a Peltier-cooled Charge Coupled Device (CCD) detector and coupled to a DMLM Leica microscope with an XYZ position controller was used to identify the MPs particles and conduct the molecular and morphological characterisation. The instrument is equipped with objectives of 5 \times , 20 \times , 50 \times , and 100 \times , and 532, 633 and 785 nm lasers of adjustable power (very low; 0.0000005 %–0.0001 %, low; 0.05 %–1 %, moderate; 5 % and 10 %, high; 50 % and 100 %). A daily instrumental calibration was performed using the 520.5 cm^{-1} band of a silicon chip. Data acquisition and treatment were conducted by the Renishaw Wire package 3.2 version and Origin 2018 software. The MP signals were assigned by comparison with our internal database (Table S1). The operation conditions used to improve the signal-to-noise (S/N) ratio and avoid the MPs thermal damage, as well as to minimize the fluorescent and shielding effects are further detailed along the tuning of the analysis approach.

2.5.2. Spectroscopic approach: measurement parameters and data analysis

A non-destructive analysis strategy is proposed to optimize the processing of the filters produced after sampling and sample treatment. The workflow (Fig. 2) consists of two parallel pathways corresponding to the level of expected complexity in the identification of the MP particles (namely, *high* and *low* amount of residual particulates).

Samples with low amount of matrix residues were firstly inspected at 5 \times magnification to faster record the filter area and to select the particles of interest. Pre-established experimental parameters were employed to rapidly identify the polymer composition from the rest of the particles: 574 cm^{-1} to 1689 cm^{-1} spectral range; 785 nm laser; ≈ 7.9 μm laser spot size; 50 % laser power; 2 s exposure time; and 2 accumulations. Such laser power combined with the use of 5 \times objective and short acquisition times was previously tested, and proved to be efficient for obtaining good S/N ratios in MPs analysis without damaging them, at least in size higher than 50 μm . The impacts of strong fluorescence from chromophore compounds (e.g., feldspar, clays) were minimized by the photobleaching-induced method [48,50] (over exposure to the laser) to avoid the masking of the main Raman signals. Once the particle was identified as plastic, the spectroscopic signals were improved by a fine-tuning of the measuring parameters. Then, the polymers were quantified by simple counting and MP morphology was registered by capturing a photograph.

In case of significant matrix residues on the filter, an automated imaging Raman analysis approach was applied to acquire one mapping per sample. For this, the previous measurement parameters were crucial to minimize the time consumed to scan the whole surface of the filter at 5 \times magnification (1 acq/step of 240 μm), and to facilitate the morphological characterization. Besides, the 785 nm excitation laser effectively helped to reduce the characteristic curvature of the baseline caused by fluorescence phenomena due to residual environmental matrix. Once the whole filter surface was completely recorded, the spectroscopic raw data set was automatically pre-processed using classical

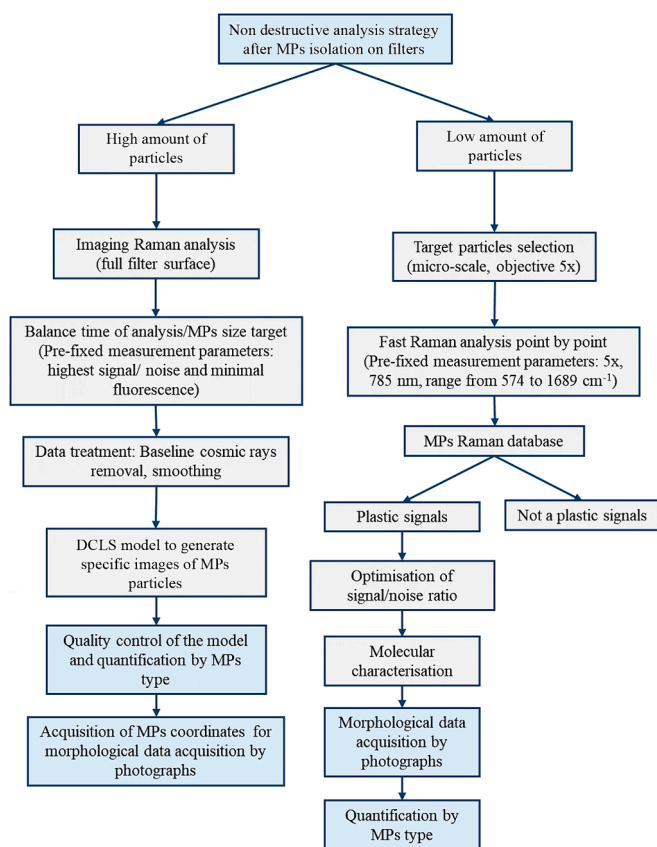


Fig. 2. Workflow designed for applying the analysis strategy for molecular and morphological characterization, and quantification of microplastics from environmental samples, specifically water and sediment matrices.

algorithms to enhance the quality of the spectra, and then, the MPs identification by chemical imaging was obtained by application of a modified Direct Classical Least Squares (DCLS) method. This included cosmic ray removal (nearest neighbour and width of feature: scaling factors of 10 and 50 % for noise level and spectrum height, respectively), smoothing (Savitzky-Golay; interval: 7, polynomial order = 2), and baseline correction (least squares polynomial fit, $n = 2$) operations [30,51]. Given that each scanning map contained between 6211 and 7524 spectra, where the signals of residues may hamper their direct identification, DCLS was applied as a pre-screening tool for identifying targeted MPs. DCLS is a straightforward manner to decompose mixtures of signals. It obeys the linear relationship between the signal and the concentration for all the molecular species in a mixture, assuming that the different components are known beforehand. That is why DCLS is used for direct quantification of spectral imaging data [52,53]. To correctly use the algorithm, the spectra of the pure compounds expected in the sample should be acquired under the same conditions and be incorporated into the model. The algorithm is designed to check the concordance of each spectral component with the sample signals through a comprehensive deconvolution process, accounting factors such as shifts and intensity ratios. The automatic selection is processed in accordance with the programmed coincidence restrictions [54]. However, there are many situations in which having an aprioristic knowledge of the number of components is not feasible and then, the addition of a lack of fit component avoids false positives by matching exclusion. Once the model is applied, the recognized spectra are included in the mapping generation showing the locations (x,y) of each compound in the sample surface. Thus, DCLS can still be a powerful tool used as a screening method, offering significant benefits for spectroscopic identification of target molecular species [55] in these severely complicated samples. Accordingly, the spectra of the most common

plastic *i.e.*: polyethylene, polypropylene, polystyrene, polycarbonate, polymethylmethacrylate, polyvinyl Chloride, polyethylene terephthalate and polyamide (PE, PP, PS, PC, PMMA, PVC, PET and PA respectively) were acquired on standard samples under the pre-established parameters to be included as prior components and build the automatic MPs recognition model. The data were processed under a 70 % of correlation coefficient, and the identified MPs were molecularly represented in a single image by different colours. Furthermore, the morphology of the plastic particles was assessed by using the advantage of the XY parameters knowledge, and then registered by a photograph that allowed manually quantification.

The usefulness of the proposed workflow was tested through the participation into the international Quasimeme interlaboratory exercise led by EuroQcharm [56]. In this, the most common plastics were spiked (>50 μm) into different environmental matrices and provided to the participants to fully test the experimental and analytical procedures. The result report corroborated that the methodology used here provided values close to 100 % ($Z = 2$) of MPs characterization and quantification.

3. Results and discussion

The MPs found in each sampling area were classified in accordance with the environmental matrix. For filters produced from water samples, the spectroscopic analytical approach followed the pathway designed for *high* amount of matrix residues based on Raman imaging analysis (Fig. 2). On the contrary, sediments samples were processed following the parallel strategy designed for *low* amounts of residual particles. In this line, the molecular and morphological characterisation are following detailed, together with the quantification data for both kind of environmental matrices.

3.1. MP analysis and quantification: occurrence in water compartment

The designed working strategy allowed a complete molecular and morphological characterisation of the recovered MPs using Raman imaging, making it possible to overcome the challenges related to the nature and amount of the residual matrix particles present on the surface analysed. Indeed, the selected operating conditions and the pre-processing steps were key to solving the technique drawbacks in water and sediment samples, obtaining a good compromise between time invested and spectral quality. DCLS allows accurate spectral screening search of the MPs of interest, and did not show false positives. In addition, the model was capable of discarding the non-target particles as lack of fit components, mainly identified as hydrolysed feldspar, related to the suspension solids dragged by the water flow.

As general findings of the water samples set, PE, PP, PET, PS, and PMMA were the five types of polymers found across the *Choqueyapu*

river basin (excluding the natural reserve sampling area), with total concentrations of 6.8, 5.4, 28.6, 2.4, and 0.2 particles/L, respectively (Table 1). These are the first results observed in river waters in Bolivia, together with a previous investigation on MPs presence in the freshwaters of the Lake *Titicaca* [43]. In this case, the most predominant colours were white and colourless and, MP shapes correspond to pellets, fragments, and fibres from 300 μm to 2 mm, reaching 4 mm of length in the case of PET fibres that are the most prevalent in all cases followed by the fragments (Table 1).

The MPs presence in each area is shown in Fig. 3 through imaging mappings and the corresponding MPs spectra. To sum up, the quantification, shape and size data are gathered in Table 1, accompanied by photographs of the most predominant morphologies (Fig. 4). The highest concentration of MPs particles was detected at point 3 (16.8 particles/L), situated directly downstream from the most densely populated urban area, where points 1 and 2 converge (8.8 and 6.2 particles/L, respectively). The significant presence of PET fibres found aligns with the prevalent human activities established in the city of La Paz [46]. Specifically, the data suggested that MP sources include wastewater from the textile industry, recognized as one of the main contributors to global PET production [57], and possibly synthetic (polyester) textiles discharged during the home washing process, although no polyamide MPs were detected. In contrast, sampling area 4 showed evidence of minor MP pollution, (4.2 particles/L), consistent with its isolated location, far from densely populated urban areas. In comparison with areas 1, 2 and 3, the PET content was less than half and a higher contribution of PP to the total MP contamination level was determined by the evident impact of agricultural activities, mostly likely due to open-air cultivation of crops and fruits [58] commonly supported by thermal protective covers. On the other hand, intermediate plastic pollution was encountered at sampling area 5 as receptor of the previous effluents coming from the city and the field, not being observed any accumulative effect in the water compartment.

These data suggest that many of them were secondary MPs generated from the degradation of household plastic products and garbage due to hydrodynamic and mechanical actions within the river [59]. This observation is supported by the minimal number of particles found as pellets, typically associated with either direct discharge from companies that pelletize or use virgin pellets as raw material, as well as from a strong and prolonged erosive process [11]. Thus, the spread of MP pollution through the water compartment could be effectively minimized in adjacent areas not yet impacted by implementing recycling or incineration plants, along with appropriate water treatment facilities equipped with simple primary reactors, strategically placed throughout the *Choqueyapu* river basin.

Table 1

Data summary of number, shape and size of microplastic particles found within each set of water samples collected alongside the *Choqueyapu* river basin.

		PE (particles/L)	PP (particles/L)	PET (particles/L)	PS (particles/L)	PMMA (particles/L)	Total MPs (particles/L)
Sampling site	P1	1.8	1.2	5.0	0.8	0	8.8
	P2	1.0	0.6	4.2	0.4	0	6.2
	P3	2.2	1.0	13.0	0.6	0	16.8
	P4	0.2	1.4	2.6	0	0	4.2
	P5	1.6	1.2	3.8	0.6	0.2	7.4
	P6	0	0	0	0	0	0
Shape	Pellets	0.4	–	–	–	–	0.4
	Fragments	6.4	5.4	–	2.4	0.2	14.4
	Fibers	–	–	28.6	–	–	28.6
Size	Pellets	300 μm	–	–	–	–	300 μm
	Fragments	300 μm –2 mm	300 μm –2 mm	–	300 μm –2 mm	≈3 mm	300 μm –3 mm
	Fibers	–	–	300 μm –4 mm	–	–	300 μm –4 mm

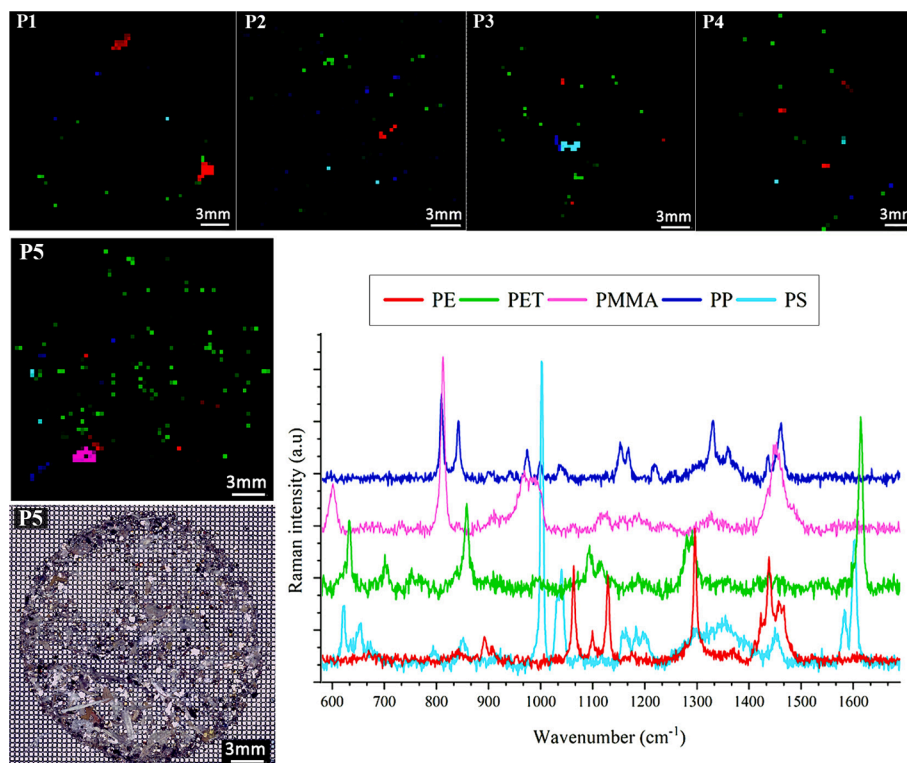


Fig. 3. Raman imaging analysis of filters produced for the most representative water sample collected at each sampling area (P1-P5) are shown. The microplastic particles were identified and classified by colours according to their molecular composition, *i.e.*: PE, PET, PMMA, PP and PS in red, green, pink, deep blue and cyan blue, respectively. Additionally, a series of the MPs corresponding treated spectra and a mosaic image of the filter surface from P5 were included to illustrate the data concordance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

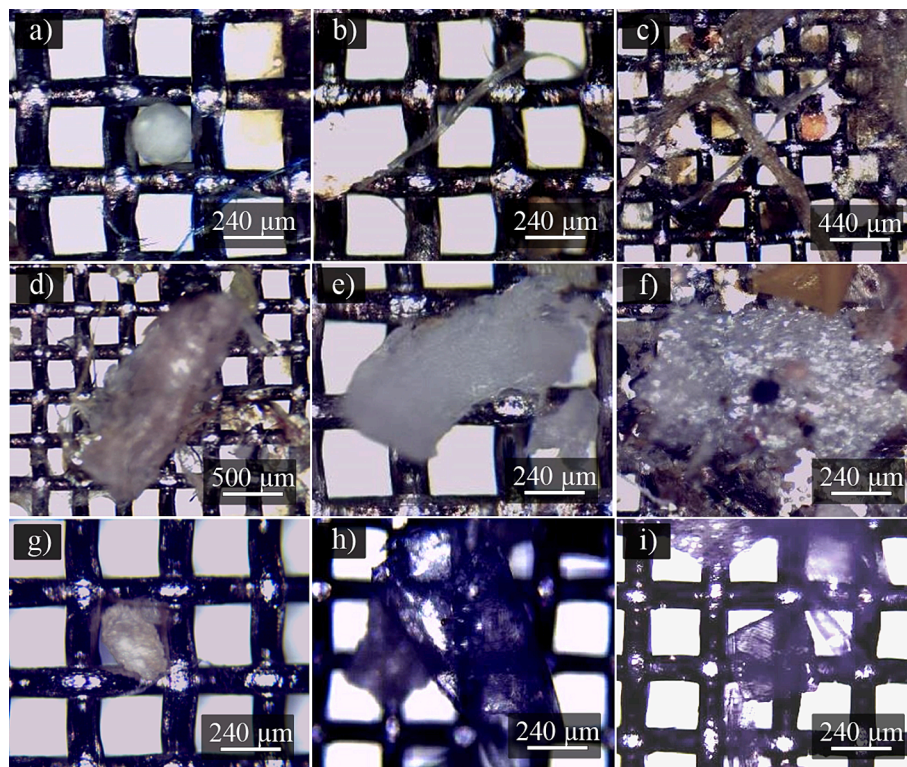


Fig. 4. Images of microplastics collected during the morphological and molecular characterization analysis: a) PE, b) and c) PET, d) PE, e) PP, and f) PMMA found in water samples as pellet, fibres and fragments; g) PE, h) PET, and i) PS in sediments samples as fragments.

3.2. MP analysis and quantification: occurrence in sediment compartment

The point-by-point strategy was effective to obtain high S/N ratios and high-quality spectra, allowed by the modulation of the experimental parameters. The disadvantages related to the diameter of the fibres or the discrimination between signals of the embedded dyes and the MPs matrices were easily handled thanks to the optimization of specific measurement parameters. In this study, the characteristic plastic signals were hindered by the dye during the analysis of the two blue coloured particles. Specifically, phthalocyanine blue is commonly used in the plastic industry for the PE manufacturing [60]. These macrocyclic aromatic compounds are highly active under the laser due to the resonance effect caused by the energy matching of the incident photon and the resonance of the vibrational mode of the aromatic ring [61]. The spectral quality acquired allowed the discrimination of the PE signals in the dye spectrum by the weak peaks at 1295 and 1062 cm^{-1} (Fig. 5, Table S1), assuring its MP identification. These special cases can be implemented in the DCLS model used in case of *high* environmental matrix residues to improve the MPs identification during the automatic spectroscopic screening.

PE, PP, PET and PS were the plastic pollutants found in the sediments along the basin in sampling areas 1–5, with total content levels of 19, 11, 48 and 13 particles/kg, respectively, between two and five times higher than in the case of water. Fibers and fragments were the majoritarian shapes found in similar values, with sizes between 250 μm to 2 mm (Fig. 4), being most of them white or colourless.

As detailed in Table 2, the impact of the MPs for all the sediment collected did not follow the trend of the water compartment. Points 1, 2 and 4 showed similar levels of MPs (about 16 particles/kg) and the lowest levels were observed in point 3 (6 particles/kg). The MP contribution of both previous effluents was primarily spread through action of water, also favoured by the high floatability of PET fibres that do not have time to settle. In contrast, point 5 presented important levels (38 particles/kg) with PE and PS as main contributors due to the harvesting activities of the river upstream [11]. Points 3 and 4 seems to have an accumulative action in the freshwater sediments, they are observed in low amounts in the water matrix probably by the increase of the water flow coming from point 3, that provoke a dilution.

As this data shows, some points are a clear long-term sink for MPs that pose a threat to aquatic ecosystems and human health and can act as a continuous source of MPs release into the environment, as water movements and physical processes may resuspend microplastics,

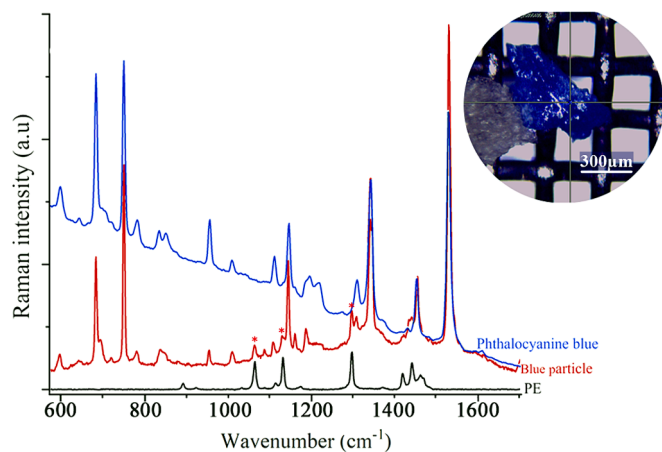


Fig. 5. Raman treated spectra collected from an unknown blue particle and two references samples of polyethylene (PE) and phthalocyanine blue dye, for its molecular characterization through signals matching. The plastic signals identified in the unknown particle are highlighted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

contributing to their dispersion and contamination to adjacent areas not yet impacted. However, the concentrations found in Bolivia for both compartments are still lower than other Latin-American areas studied. In fact, the numbers of MPs were between 8 and 11 times lower than in Mexico [41] and comparable to almost 81 times lower than in some areas of Brazil [37,40].

4. Conclusion

This work evidences the potential of Raman imaging analysis assisted by chemometrics to handle of large volumes of spectral data, involving minimal time consumed for raw data pre-processing operations (spikes peak removal, baseline subtraction and smoothing). It also highlights the opportunities offered in the data treatment to automatically perform spectroscopic screenings for MPs identification, even in extreme cases of high number of particles. Firstly, the specific measurement parameters used together with simple application of nearest neighbour, polynomial fitting and polynomial regression methods were fundamental to minimize the technique handicaps in these environmental matrices, and thereby better distinguish the target signals. Subsequently, the employment of DCLS provided high performance and clearly enhanced the identification accuracy in comparison with other image plotting methods, such as intensity at point or peak position, that do not provide useful imaging data in these types of samples. Moreover, the model used is commonly integrated into spectral treatment software as a direct quantification method for systems comprising a known number of molecular species. Thus, it can easily be used for automatic spectral search without requiring specific expertise in machine learning. This is particularly valuable in MPs monitoring studies, when additional digestion or flotation steps proved to be completely ineffective due to the inorganic nature and inherent low density of a high proportion of non-target particles, while the MP size range of interest is also maintained. In addition, the benefits of the spectroscopic working approach detailed here could be also exploited towards automated routines for conducting monitoring campaigns, facilitating long-term studies that usually involve a huge number of samples. In the same manner, the methodology provided can be fine-tuned (spot, step and time of acquisition) to fulfil different purposes or matrices for the MPs study.

As proof of its usefulness, the workflow successfully overcomes the challenges presented by the *Choqueyapu* river basin study. This was the first monitoring campaign at this site, and no information related to the amount of organic or inorganic matter, nor were concentrations of MPs available to manage specific sampling and treatment methods. Therefore, the spectroscopic analytical strategy here applied was crucial in terms of organizing and limiting the steps that could potentially introduce multiple drawbacks and cause the loss of these valuable samples. As shown, this strategy allowed the extraction of significant information for a better understanding of the impact of MPs in La Paz and provided evidence of high contamination levels, identifying more than 44 particles/L and 91 MPs particles/kg in the water and sediment compartments, respectively. For both cases, no MP contamination was detected in the natural protected zone of the target size range. These findings aligned with the human activities taking place in each area, and emphasized the importance of including various environmental compartments to detect potential risk zones that could pass undetected along the river by individual water or sediments studies, such as in *Lipari Sector*. In this regard, only one water treatment plant currently operates upstream of the La Paz urban core. However, the urban development plan includes the construction of two wastewater treatment facilities near the *Kantutani* and *Lipari Sector*, aimed at boosting food production. Hence, the data provided could support the selection of the most suitable locations as preventive measures to avoid the spread of plastic pollution to less affected areas. In fact, the abundance of the MPs found spans 2 to 4 orders of magnitude across the different regions. Further studies are required to deepen scientific understanding of the basin, and to register its spatial and temporal variations through monitoring campaigns,

Table 2Data summary of number, shape and size of microplastic particles found within each set of dry sediment samples collected alongside the *Choqueyapu* river basin.

		PE (particles/kg)	PP (particles/kg)	PET (particles/kg)	PS (particles/kg)	Total MPs (particles/kg)
Sampling site	P1	0	0	13	0	13
	P2	0	6	12	0	18
	P3	0	0	6	0	6
	P4	0	5	11	0	16
	P5	19	0	6	13	38
	P6	0	0	0	0	0
Shape	Pellets	–	–	–	–	–
	Fragments	19	11	5	13	48
	Fibers	–	–	43	–	43
Size	Pellets	–	–	–	–	–
	Fragments	300 µm–2 mm	400 µm–2 mm	<2 mm	250 µm–2 mm	250 µm–2 mm
	Fibers	–	–	500 µm–2 mm	–	500 µm–2 mm

including unexplored points along the river, as well as additional environmental compartments.

CRediT authorship contribution statement

T. Maupas: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **O. Gomez-Laserna:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation. **U. Uribe-Martinez:** Methodology, Investigation, Formal analysis. **D. Salazar:** Formal analysis. **B. Grassl:** Writing – review & editing. **A. de Diego:** Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.microc.2024.110988>.

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