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The human connection: First evidence of microplastics in remote high mountain lakes of Sierra Nevada, Spain[☆]

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ABSTRACT

Microplastics have become one of the most serious global threats to animal and human health. While their presence has been documented in all Earth water ecosystems, including remote mountain lakes, the observation that the abundance of microplastics is largely different across nearby lakes has rarely been examined. As part of a citizen science initiative, this study analyzed for the first time the abundance of microplastics in the surface of 35 glacial lakes of Sierra Nevada National Park in Southern Spain with the objective of determining the local factors that control their abundance. First, we described the shape, size, color and nature of microplastics. Second, we tested whether the number of microplastics differed between basins and analyzed environmental and morphometrical features of lakes affecting their abundance. We found that microplastics were common in most lakes, with a maximum abundance of 21.3 particles per liter that akin to some of the most microplastic polluted lakes worldwide. Fragments were the predominant shape (59.7%) followed by fibers (38.8%) and very scarce spheres (1.5%). Microplastics were observed for all size-fractions, but the abundance of particles <45 μm was higher, what advocates for the use of low pore-size filters to prevent underestimation of microplastics. While the mean abundance of microplastics did not differ among basins, their quantity was related to the presence of meadows surrounding the lakes. This result indicates that while atmospheric transport of microsplastics may equally reach all basins, differences in microplastics among nearby-lakes has an anthropic origin caused by mountaineers who find lakes with ample meadows much more attractive to visit relative to barren lakes. The staggering number in these remote lakes, headwaters of rivers that feed drinking reservoirs, is a major concern that warrants further investigation and the strict compliance with waste management laws to reduce the harmful impacts of microplastic contamination.

1. Introduction

Plastics are ubiquitous in most household and man-made products because of their low cost of production, durability and remarkable structural properties. The production of plastics has rapidly grown since their introduction in the 1950s and is expected to increase in the coming decades (Geyer et al., 2017). Plastics fit the profile of poorly reversible pollutants because they persist in the environment for a long time and because there are no immediate plans for shortening their emissions (MacLeod et al., 2021). Although plastics are considered inert particles, the large plastic items such as macroplastics (>2000 μm) become fragmented over time into smaller fractions to form secondary microplastics (MPs) that can be categorize according to size into meso-size plastics (2000-200 μm), micro-size plastics (200-20 μm), or nano-size plastics (20-2 μm) (Bermúdez and Swarzenski, 2021). Primary MPs, however, are designed for commercial use including cosmetics (e.g., microbeds or pellets). Because of its intensive consumption, rapid disposal and low degradation, plastic products are accumulating in every area of the planet. Plastic is the fastest-growing component of urban waste and a major environmental pollutant worldwide (Geyer et al., 2017). Because

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plastic contamination is among the major threats for human development and ecosystem integrity, some authors have referred to the Anthropocene as the Plastic Age (Porta, 2021).

Unfortunately, its widespread use comes with a high environmental and health cost that we have only recently begun to realize. Ingestion of microplastics by biota and humans has been demonstrated, but whether this exposure represents a risk for ecosystem and human health is under debate (Thomas et al., 2021), and much more research is particularly needed at early life exposures (Spripada et al., 2022). The adverse effects on organisms will depend on physical effects related to particle size, shape and concentrations, chemical effects related to hazardous chemicals, properties like hydrophobicity, size, shape, and the presence of microbial biofilms (Bhatt et al., 2021). Both concentration of MPs and chemical leaching affect macroinvertebrate abundance and contribute to ecotoxicological harms in the coral reef, where plastic debris stresses coral through light deprivation, toxin release, and anoxia, giving pathogens a foothold for invasion (Lamb et al., 2018). Besides inhalation and dermal contact, contaminated food, including freshwater and marine species for human consumption, and drinking water are the common sources of macro- and nanoplastics ingestion in humans (Barboza et al., 2018). Systematic exposure of particles smaller than 150 μm can cross the gastrointestinal epithelium in mammalian bodies. Particles of around 10 μm can reach both organs and cellular membranes and pass the blood-brain barrier and placenta (Ragusa et al., 2021).

Since MPs were first documented in the oceans in the early 1970s (Carpenter et al., 1972), MPs contamination has been one active field of research. It is estimated that 11% of the plastic produced every year ends up in aquatic ecosystems (Borrelle et al., 2020). Once in the environment, the weathering process favors the irreversibility of plastic pollution through the fragmentation into micro- and nanoplastics that, at the end, also favor the sinking of buoyant plastics. In oceans, micro- and nanoplastics are expected to reach the floor, but a substantial amount of them remain in the water column through its incorporation to biological cycles. For instance, MPs with a diameter below 10 μm and those that are fiber-shaped will be resuspended throughout the water column due to water forces leading to very long residence times (MacLeod et al., 2021). While the role of oceans in accumulating MPs has been long recognized (Jambeck et al., 2015), we now know that MPs from water as well as soils can be resuspended back to the atmosphere as they continue to cycle through earth's systems (Brahney et al., 2021).

Because freshwater environments are the most direct means of transporting MPs from land to sea, rivers and lakes have also received increasing attention and, particularly since the last decade. In addition, the more recent evidence that MPs can travel long distances via atmospheric transportation (Allen et al., 2019) came hand in hand with the astonishing discovery that no place on Earth is free of MPs (e.g., Bergmann et al., 2019). Thus, a vast amount of research has reported the presence of MPs in different urban and country lakes from all continents, including remote areas in Artic (Bergmann et al., 2019; Lusher et al., 2015; Ross et al., 2021) and Antarctic (Aves et al., 2022; Kelly et al., 2020). However, although much effort has been placed in determining contamination by MPs in densely populated and industrial centers, observations reporting MPs in high mountain remote lakes are still sparse (Negrete Velasco et al., 2020).

From the comparison of a rather large number of field studies, the observation that numbers of MPs can differ in several orders of magnitude is startling. Also surprising is the large variability that plastic contamination can register for one given site. For example, Uurasjärvi et al. (2020) reported MP abundances from less than one to 155 MPs/m^3 in Kallavesi Lake, Finland. More work needs to be done to resolve the local or regional factors responsible for the spatial variability in the abundance of MPs within and between surrounding lakes. Sierra Nevada in southern Spain, with numerous close-distance lakes within a relatively narrow mountain range, is an ideal location to test for the local factors underlying plastic contamination.

To examine some of these gaps of knowledge we designed a citizen science campaign to simultaneously sample a total of 35 remote lakes located in five major adjacent basins of Sierra Nevada. First, we described MPs shape, size, color and nature according to stereomicroscopic observations. Second, we reported the abundance of MPs and tested the hypothesis that MPs abundance differed among catchment basins. This goal was based on the premise that lakes located in more disturbed basins with ski resorts or affected by prevailing winds, would be more contaminated by MPs. Finally, we analyzed the different environmental and topographic features that best explained differences in the abundance of MPs among lakes. Our overarching goal was to diagnose and understand the factors behind contamination by MPs, and to provide relevant information that may contribute to mitigate the damaging impacts of MPs.

2. Materials and methods

2.1. Area of study

The Sierra Nevada mountainous massif is located in the western part of the Mediterranean basin (Fig. 1). It covers an area of 179,000 ha and includes mountain peaks above the tree line such as the Mulhacen, which is the highest peak in the Iberian Peninsula (3479 m), and among the three highest peaks in Europe (Diaz-Hernandez and Herrera-Martinez, 2021). Sierra Nevada was declared National Park in 1999 and is considered one of the most important hotspots of biodiversity in the Mediterranean region (Cañadas et al., 2014), with a wide variety of vascular plants, many of which are endemic to this area (Diaz-Hernandez and Herrera-Martinez, 2021). This biodiversity is strongly influenced by the flow of water, as microclimates are generated around valley areas with presence of lakes, while the rest of the areas remain more arid.

These lakes originated at the end of the Quaternary period, and today there are areas of periglacial dynamics at altitude over 2500 m. asl, which leads to the current geographical relief. The resulting hydrological network of this environment is very extensive, with numerous streams, rivers and lakes, which are covered with snow from November to June. On the contrary, the water flow increases significantly in the spring season due to the ice melting caused by rising temperatures, which can last until the month of August in some years (Diaz-Hernandez and Herrera-Martinez, 2021).

The Sierra Nevada lakes generally have a pseudo-elliptical shape, with very different depths and volumes of water. Maximum lake depth ranges from below 1 m to over 10 m, and more than 75% of these lakes have a volume under 5000 m^3, with exceptions such as Laguna Larga (50,000 m^3) or Laguna de Vacares (23,800 m^3) (Diaz-Hernandez & Herrera-Martinez, 2021).

With regards to the influence of anthropic activity, Sierra Nevada is characterized by intense winter tourism associated with the ski resort (Dílar valley), one of the most important in Spain and southern Europe. In addition, the human influence in summer is also high, when the 'borreguiles' (local name for grassland meadows surrounding the lakes) are devoid of snow and become the ideal camping ground for many mountaineers. However, meadows of lakes are critical biodiversity hotspots that can be altered by the presence of pollutants such as MPs present in textiles, kitchen utensils or other leisure items that may accompany tourists.

The intrinsic variability of climate in Sierra Nevada is a consequence of its particular geographical location as the most southern mountain in Eurasia that runs parallel to the Mediterranean Sea with elevations over 3000 m. Thus, seasonal climate regime corresponds with a semiarid Mediterranean region with a strong influence of the west to east trajectories of the Atlantic cyclonic fronts. The analysis of air mass back-trajectories in Sierra Nevada has shown that winds carrying aerosols can back-traced to far distances sources such as Sahara desert as a major source of natural dust, and Europe as a principal source of anthropogenic pollutants (Pérez-Martínez et al., 2020).

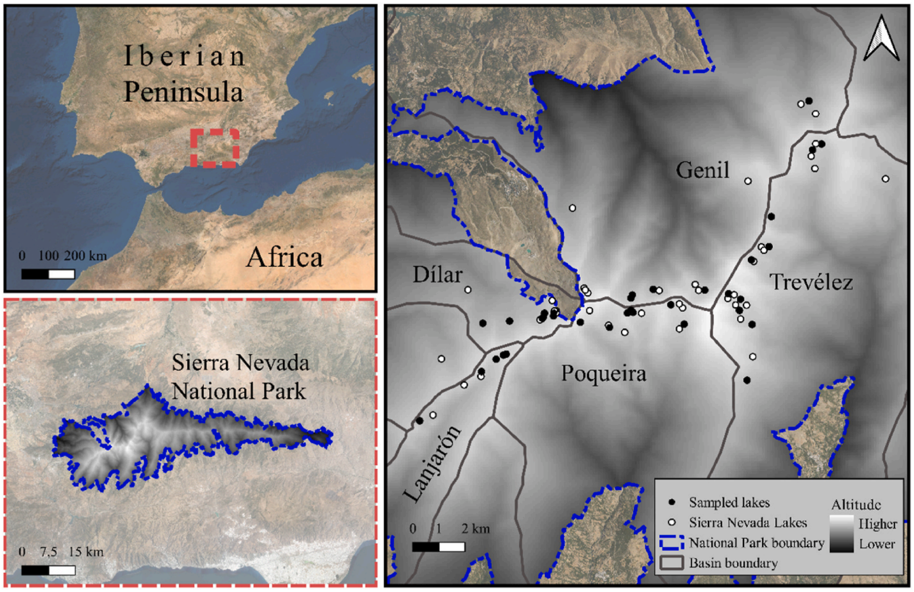

Fig. 1. Map of the high mountain lakes in the five basins studied in Sierra Nevada, Spain.

Table 1
Geomorphological and environmental characterization of the studied lakes. All chemical and biological parameters were measured in water samples at the time microplastics were sampled.

Basin	Lake	Altitude	Catchment area	Lake area	Lake surrounding meadow area	Type of basin	Chlorophyll *a*	Total Phosphorus
		m.asl	ha	ha	ha	Surface inlets (I)/ outlets (O)	μg/L	μg/L
Dílar	Lagunillo Grande de la Virgen	2953	25.1	0.08	0.15	I/O	0.5	13.2
	Lagunillo de la Virgen que drena aguas abajo de las Yeguas	2947	21.2	0.01	0.07	O	0.4	8.7
	Lagunillo Misterioso	2693	7.5	0.05	0.04	I/O	b.d.l.	4.5
	Lagunillo Medio de la Ermita	2872	71.3	0.02	0.13	I/O	0.6	4.8
	Lagunillo Alto de la Ermita	2872	71.3	0.01	0.005	–	1.3	32.1
	Charca de la Ermita	2874	1.0	0.002	0.01	I/O	3.6	8.6
	Laguna del Carnero	2685	18.0	0.02	0.27	I/O	1.7	45.0
Genil	Laguna de la Gabata	2785	35.6	0.10	0.18	I/O	0.1	6.4
	Lagunillo Bajo del Valdeinfierno	2870	2.8	0.02	0.07	I/O	1.1	9.1
	Lagunillo Alto del Valdeinfierno	2890	6.1	0.02	0.00	I/O	0.3	17.1
	Laguna de la Mosca	2897	39.7	0.48	0.37	I/O	1.0	8.6
	Lagunillo de los Lavaderos de la Reina	2619	1.1	0.02	0.10	–	3.4	18.3
	Laguna de los Lavaderos de la Reina	2636	12.9	0.04	0.12	–	1.7	13.3
Lanjarón	Laguna del Caballo	2847	10.5	0.48	0.16	–	0.7	9.9
	Charcas de Tajos Altos	2921	3.0	0.01	0.01	–	n.a.	n.a
	Lagunillo de Charca Pala	2937	7.0	0.05	0.06	I/O	2.9	29.3
	Lagunillo de Lanjarón	2980	7.8	0.02	0.12	I/O	0.4	11.2
	Laguna de Lanjarón	2985	35.8	0.29	0.19	I/O	0.9	20.9
Poqueira	Laguna de Aguas Verdes	3061	12.8	0.19	0.62	I/O	0.7	11.1
	Lagunillo Bajo del Púlpito	2881	96.2	0.07	0.22	I/O	0.6	6.6
	Laguna del Majano	2907	72.2	0.07	0.83	I/O	2.2	6.0
	Laguna de la Caldera	3030	23.5	2.10	0.00	–	1.1	3.0
	Lagunillo Alto de Rio Seco	3042	4.7	0.06	0.02	–	0.5	22.1
	Laguna Baja de Río Seco	2998	13.5	0.01	0.00	–	2.1	22.4
	Laguna de Río Seco	3029	9.9	0.46	0.73	I/O	0.8	25.5
Trevélez	Laguna Altera	3066	11.4	0.01	0.00	–	1.6	5.9
	Laguna de Tajos Coloraos	3024	27.4	0.34	0.63	I	0.9	8.1
	Laguna del Borreguil	2984	50.9	0.18	0.57	I/O	2.7	2.5
	Laguna Hondera	2898	154.6	0.57	4.05	I/O	0.2	3.2
	Laguna de Peñón Negro	2808	28.2	0.4	0.06	O	1.7	108.7
	Laguna Baja de las Calderetas	2882	50.4	0.23	0.58	–	26.7	62
	Laguna de Vacares	2999	9.9	1.10	0.00	–	1.3	4.7
	Lagunillo Alto del Goterón	2880	18.4	0.01	0.00	–	2.2	12.9
	Lagunillo de Juntillas	2930	39.9	0.06	0.23	I/O	1.6	10.8
	Laguna de Juntillas	2933	6.3	0.11	0.62	I/O	0.5	7.9

n.a., not available; –, not applicable; b.d.l., below detection limit.
Lake altitude is from Diaz-Hernandez and Herrera-Martinez (2021).

2.2. Citizen science campaign

This work was carried out in the framework of the “74 High Mountain Glacial-Lake Oases” Citizen Science initiative (see further information at https://lagunasdesierranevada.com). The engagement of citizens, mainly mountaineers, has contributed to strengthen long-term monitoring program of lakes by gathering scientific data that would be unapproachable due to the limited personnel resources of the scientific teams (Villar-Argaiz et al., 2022). On the third weekend of July 2020, five multidisciplinary groups, each comprised of five to seven citizen volunteers, one park ranger and one researcher (a total of 40 participants), sampled 35 lakes located in five catchment basins of Sierra Nevada (Fig. 1). Lakes were distributed across a wide environmental gradient that ensured differences in catchment features (basin size, altitude, lake size, presence of surrounding meadows, etc.), as well as in the disturbance intensity. Data shown in Table 1 includes key physical, chemical and biological variables collected during the field expedition using well-defined and comparable sampling techniques (Villar-Argaiz et al., 2022). During sampling, participants noted and classified any plastic waste visible to the naked eye within and around the lake. To estimate meadow area surrounding the lakes, we used the freely available Very High Resolution images from Google Earth Pro (Gorelick et al., 2017). The meadow area was calculated using the ruler icon after subtracting the meadow-plus-lake area from the lake area.

2.3. Microplastics sampling

Water from each lake was sampled for MPs using a 250 ml-jar attached to a 4 m steel-pole. The scooped-up samples were randomly collected around the lake into a large bucket. After water collection, microplastic sampling was carried out by filtration following two procedures. First, two 1 L subsamples from each site were in situ filtered through precombusted (24 h at 550 °C) glass fiber filters (GF/B Whatman, 1.0 μm pore size) by using glass filtration systems. Second, one 10 L sample from each site was filtered through 50 μm-pore size Nytal filters. The use of Nytal filters allowed filtration of larger volumes of water while expediting the process of filtration.

To account for the possibility of MPs contamination during sample collection and manipulation procedural blanks were taken following recommendations by González-Pleiter et al. (2020). For this purpose, Petri dishes containing GF/B and Nytal filters (three blank replicates for each type of filter) remained open during sampling. We found an average concentration of 4.7 MPs per GF/B filter and 9.3 MPs per Nytal filter, similar to other locations (e.g., Wang et al., 2017). Only MPs similar in composition to those found in samples were subtracted for microplastic counting validation. All filters (samples and blanks) were folded with a metal clamp and carefully inserted into aluminum foils to prevent from the incidence of sunlight. The filters were then transported to the laboratory for sample treatment and analyses.

2.4. Microplastics analysis and identification

Before examining the filters, the organic matter was removed following the methodology described by Masura et al. (2015). For that purpose, 5 ml of hydrogen peroxide (15%) and 5 ml of Fe^{2+} solution prepared from $FeSO_4 \cdot 7H_2O$ were applied to each filter. After 15 min, filters were washed with Milli-Q water and left to dry for 24 h.

The filters were then examined at 8 × to 56 × magnification under a stereomicroscope (Olympus SZ×7), equipped with a digital camera and Image J software. While the entire surface of the GF/B filter was examined for MPs, only one fourth of Nytal filter was examined due to their much larger size (mean size of 9.2 × 8.5 cm).

For each sample, MPs were counted and sorted into categories based on their shape, color, nature (natural or synthetic) and size. The nature of the fibers was visually identified by using the structure features proposed by Prata et al. (2020), whereas six size categories were considered according to Simon-Sánchez et al. (2019): <50 μm, 50–100 μm, 100–200 μm, 200–300 μm, 300–500 μm, and >500 μm.

2.5. Statistical analysis

To test for differences in MPs concentration among valleys and among major size fractions, we first check for normal distribution (Shapiro Wilk’s *W* test) and homoscedasticity (Levene’s test) to ensure that the assumptions of parametric tests were met. Because MPs concentration was not normally distributed and could not be transformed to fit a normal distribution, differences in abundance among size fractions and among valleys were tested using non-parametric Kruskal-Wallis tests. When differences were significant, paired size differences among size fractions were determined with a Wilcoxon signed rank test.

Finally, we analyzed the relationship between geographical and environmental factors and MPs abundance by fitting generalized linear models. We first assumed a Poisson distribution for the model construction, and the global model containing altitude, lake size, surrounding meadow area and type of basin (surface inlets and outlets) was analyzed using lme4 package (Barton, 2012). Because overdispersion was observed in all models, we chose negative binomial models to fit the data (Lindén and Mäntyniemi, 2011). Negative binomial has a number of advantages over quasi-Poisson models as they give more weight to smaller counts (Ver Hoef and Boveng, 2007) and AIC can be directly calculated from the likelihoods (Lindén and Mäntyniemi, 2011). To avoid overfitting, the negative binomial model was followed by a forward and backward stepwise model, and the model with the lowest AIC value was selected when the ΔAIC (difference in AIC with model with minimum AIC) was >2 (Burnham and Anderson, 2002). All statistical analyses were made in R Software version 3.1.3.

3. Results and discussion

3.1. Microplastics shape, size, color and nature

Fragments were the dominant shape of MPs with almost 60% of all particles (Fig. 2A). Because irregular fragments are usually regarded as secondary MPs, the large number of fragments indicates that most MPs found in the lakes derived from the degradation of larger macroplastics (Eunomia, 2018). Previous studies have shown that fragments, similar to other coarse aerosols, can travel from distant locations by air (Free et al., 2014; Wang et al., 2018; Negrete Velasco et al., 2020; Felismino et al., 2021), although other sources of plastic contamination cannot be ruled out in lakes of Sierra Nevada.

Fibers were the second-most abundant MPs with ~39% of all particles. The presence of fibers has been previously associated with leisure activities (Alfonso et al., 2020a, 2020b; Felismino et al., 2021). Nowadays, microfibers are considered the most abundant type of MPs in the environment which, similarly to fragments, are easily distributed worldwide reaching remote areas after dry or wet deposition events (Ryan et al., 2020). Indeed, the abundance of microfiber MPs presents a contamination challenge due to their high persistence in the water column, and the high probability of ingestion by aquatic organisms (Welden and Cowie, 2016).

With regards to the size, small particles under 200 μm accounted for >60% of all MPs in this study (Fig. 2B). This finding is consistent with the predominance of fragments in this study which, under the weathering caused by exposure to UV radiation, high temperatures and other meteorological agents, progressively break down into smaller pieces. Also, these results are consistent with Wang et al. (2018) who found that fragments constituted 94.8% of all MPs, out of which 45.9% ranged between 20 and 100 μm. Remarkably, particles >500 μm accounted for over 15% of all MPs, which suggest the possibility of recent contamination related to leisure or mountaineer activities including the field sampling research carried out during this study. Indeed, it has been shown that the rate of particle release from clothing fibers is influenced

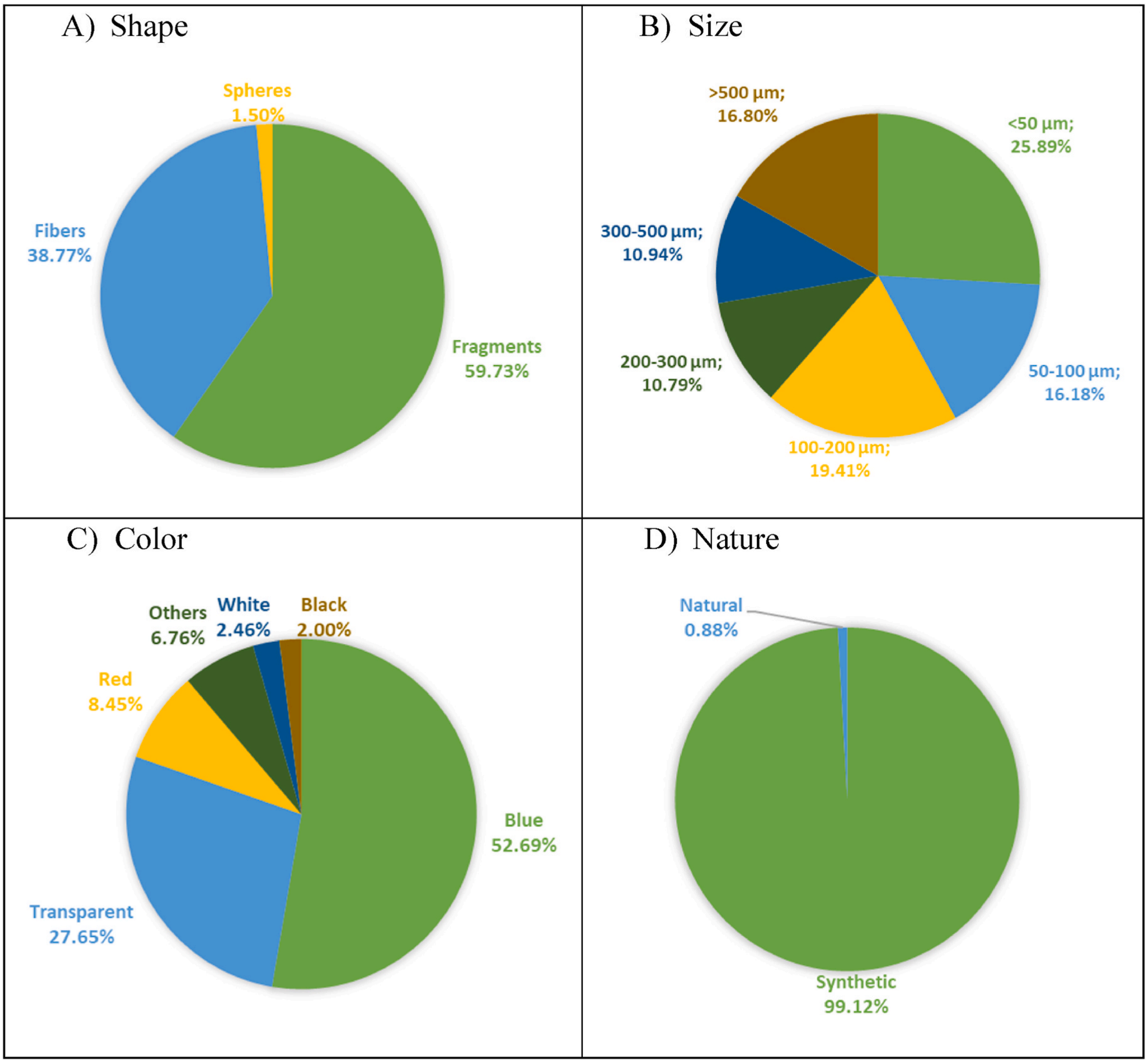

Fig. 2. Descriptive statistics of the main parameters studied in microplastics: (A) shape, (B) size, (C) color, and (D) nature. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

by several factors related to properties of clothing and environmental conditions, being the intensity of movement a dominant factor (Licina et al., 2019). This suggest that, although long-range atmospheric transport cannot be disregarded, plastic sources from mountaineers and occasional visitors may provide a relevant source of plastic waste.

With regards to the color of MPs more than 50% of the particles were blue (Fig. 2C). This observation is in accordance with Alfonso et al. (2020a, 2020b), who made a correlation between the presence of blue particles and the indigo blue dye often used by textile and other industries. This pigment, together with others such as Reactive Black 5 or Copper Phthalocyanine, is frequently used to dye plastics, and specifically by the textile industry to dye clothes.

How the predominance of blue fragments could impact food webs and ecosystems is still highly unknown and merits further research. However, an extensive study carried by Martí et al. (2020) showed that blue is dominant color in ocean MPs as it usually goes unnoticed by birds or fish, which tend to ingest MPs of more vivid colors. The authors also argue that blue pigments may be more resistant to UV degradation compared to other colors. It is worth noting that almost 30% of the MPs found in Sierra Nevada lakes were transparent. Other worldwide studies reporting similar results attribute transparent MPs to the contamination by single-use plastics, such as bags, cutlery or bottles which are commonly used in leisure activities (Peng et al., 2018; Yang et al., 2021).

Likewise, almost all the particles identified in this study were synthetic (99.12%) (Fig. 2D). This result exceeds observations by Lu et al. (2021) who, after the examination of 131 studies, concluded that ~60% of MP reported in natural waters are synthetic. This type of MPs can derive from textile, tyre, paint degradation or fragmentation of all kind of plastic waste. According to different studies, 180,000–250,000 fibers are released every year from textiles and paints (Eunomia, 2018), whereas the wearing of tyres causes the release of more than 1 million particles per year (Baensch-Baltruschat et al., 2020) which are easily long-range transported by air further polluting worldwide ecosystems.

3.2. Microplastic concentration

The abundance of MPs in this study using GF/B filters ranged from 0.3 to 21.3 MPs/L, with a mean concentration that varied from 4.7 ± 3.1 in Dílar to 9.3 ± 7.1 MPs/L in Trevélez (Fig. 3A). In contrast, MPs in Nytal filters ranged from 0 to 13.9 MPs/L in Poqueira, with a mean concentration that varied from 0 in Lanjarón to 2.9 ± 5.1 MPs/L in Poqueira (Fig. 3A). Fig. 3B shows the fraction of MPs <50 µm (as % of total MPs) in GF/B vs. Nytal filters. While MPs <50 µm in size represented between 22 and 50% of the total number of plastic particles in the GF/B, these were below 2% in the Nytal filters (Fig. 3B). The comparison between MPs in these two filters suggests that Nytal filters

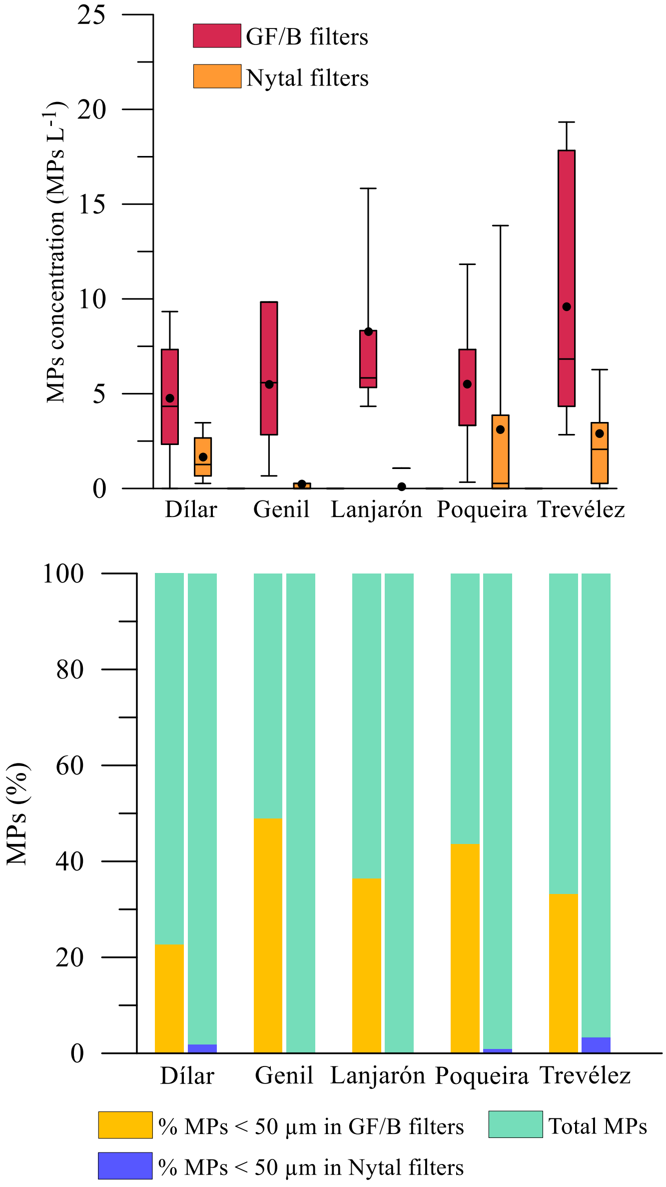

Fig. 3. (A) Box-Plot of MP concentration (MPs L^{-1}) in lakes from five basins of Sierra Nevada National Park. Full lines and points inside the box represent the median and mean value, respectively. The boxes represent the upper and lower quartiles, while vertical lines indicate the 10th and 90th percentiles. (B) Percentage of microplastics (MPs) classified according to the type of filter (GF/B on the left column and Nytal filters on the right column for each basin) in lakes from five basins of Sierra Nevada National Park.

underestimated the <50 µm MPs fraction compared to GF/B filters, and therefore may not be a very suitable means to retain and detect the widespread abundance of the smaller size fractions of plastics in aquatic ecosystems. Despite these differences, the use of Nytal filters is very common in studies of MPs in water, as planktonic nets are mainly composed of this material, and large volumes of water can be filtered over a shorter period of time (Palermo et al., 2020). Therefore, the use of two widely different pore size filters in this study reinforces the idea that studies using larger pore size can strongly underestimate MPs abundance in natural waters, and is consistent with the observation of decreased abundance with increasing size of MPs (Urban-Malinga et al., 2020). As a consequence, this study will particularly focus on the data obtained for GF/B filters which, by having lower pore sizes, provide with an unbiased estimation of the contamination by MPs.

Table 2 reports the concentration of MPs for several mountain, urban and rural lakes in Europe, Asia and America. Based on these data, and contrary to what we might have expected, MPs concentrations were not lower in mountain or rural versus urban lakes. In fact, our assessment of MPs in Sierra Nevada lakes showed that MPs densities were considerably higher than those found in other remote lakes (e.g., Negrete Velasco et al., 2020; Pastorino et al., 2021). However, a very striking result of this study is that MPs densities falls within values observed for many urban lakes in China, which is the world's largest consumer of plastic and one of the places where most plastic waste accumulates due to the mismanagement (Jambeck et al., 2015). The authors of these studies claim that the high pollution is related to the location of the lakes near highly populated areas (Wang et al., 2017, 2018).

So, how do MPs reach Sierra Nevada remote high mountain lakes? Since human activity is responsible for MPs contamination, one would expect higher abundance of MPs in areas with high intensity of human activities. While lakes are located in unpopulated basins at a relatively long distance from the town of Granada (~50 km), a large part of Dílar basin holds one of the most touristic ski resorts in southern Europe with more than a million visitors on average each year (STATISTA, 2020). Although mass tourism related to ski practitioners could plausibly be a major origin of plastic pollution in Sierra Nevada, failure to find significant differences in MPs among basins (all Kruskal-Wallis's test, $p > 0.05$; Fig. 3A) indicate that plastic contamination should likely be attributed to other factors beyond the ski activities prevailing at a particular valley.

However, the high elevation of Sierra Nevada lakes located at a mean altitude of 2900 m. asl in small glacial basins with very limited human activities, points to a crucial role of atmospheric transport of MPs contamination compared to other low-land lakes. This is supported by recent studies claiming that MPs can travel via atmospheric transport on a global scale (Allen et al., 2019; Brahney et al., 2020). Once in the troposphere, MPs is just another type of aerosol that can be transported vast distances and contribute to the flux of MPs into lake waters via dry or wet deposition (Xiong et al., 2022). Thus, Bellasi et al. (2020) suggested that, among other factors, wind direction can be associated with plastic contamination in lakes. Specifically, Fischer et al. (2016) showed that the predominant factor in the distribution of MPs in the Bolsena and Chiusi lakes was the direction and strength of the wind. The wind in Sierra Nevada usually has a northerly or northeasterly component with speeds between 10 and 15 km/h at an altitude of 1500 m, and between 20 and 30 km/h at an altitude of 3000 m (AEMET, 2021). Such wind patterns over the metropolitan area of Granada may carry suspended MPs and other polluting particles, and disproportionally affect lakes located in the northern basins of Sierra Nevada. Also, the atmospheric transport of MPs into the lakes could be associated with a higher frequency and intensity of dust export events from southern Saharan winds as these find in Sierra Nevada the first mountain barrier in the continent due to its altitude and southernmost location on Europe (Cabrerizo et al., 2016; González-Olalla et al., 2018). However, the lack of differences in the abundance of MPs among basins located at either face of the mountain range (Figs. 1 and 3) suggest that atmospheric transport may evenly reach all basins and lakes. This particular result combined with the strikingly different abundance of MPs between surrounding lakes (from barely no MPs to 21.3 MPs/L in the most polluted lakes) demonstrates that, in addition to atmospheric transport, other less-intuitive sources of plastic contamination are likely.

Some evidence suggests that natural factors contribute to the distribution and abundance of MPs including the geomorphology of lakes (Bellasi et al., 2020; Cera et al., 2020). For example, it has been shown that the type of basin where the lake is located (open vs. close, small vs. large) has a strong effect on the residence time of the water (Morales-Baquero et al., 1999), which in turn may affect the density of floating particles such as MPs. Thus, lakes with long residence times such as Lake Hovsgol in Mongolia hold considerable density of MPs despite its remote location far from urban areas and anthropogenic activities (Free et al., 2014). In this work, we analyzed the relevance of the type of basin as

Table 2
Microplastic concentration in some urban and rural lakes around the world.

Location	Altitude	Lake category	MPs (particles/L)	Extraction method	Pore filter size (μm)	Reference
Sierra Nevada lakes	2615–3069	Mountain	6.97	Direct filtration	1.0	Present study
Lake Sassolo	2074	Mountain	2.6	Direct filtration	63	Negrete Velasco et al. (2020)
Dimon Lake	1872	Mountain	0.33	Direct filtration	50	Pastorino et al., 2021
Sassolo lake, Switzerland	2074	Rural	2.60–4.40	Direct filtration	63	Negrete-Velasco et al. (2020)
Baikal lake, Russia	451	Rural	1.06	Sieving	n.a.	Karnaukhov et al. (2020)
Wuliangsuhai lake, Mongolia	1019	Rural	3.12–11.25	Vacuum filtration	0.45	Mao et al. (2020)
Hovsgol lake, Mongolia	1645	Rural	0.29	Sieving	n.a.	Karnaukhov et al. (2020)
Patagonia lakes, USA	1234	Rural	0.0009	Direct sieving	8.0	Alfonso et al. (2020a)
La Salada lake, Argentina	48	Rural	0.14	Direct sieving	8.0	Alfonso et al. (2020b)
Chiusi lake, Italy	251	Urban	0.0025	Density separation	5.0	Fischer et al. (2016)
Bolsena lake, Italy	305	Urban	0.0031	Density separation	5.0	Fischer et al. (2016)
Kallavesi lake, Finland	82	Urban	0.00027–0.15	Direct filtration	20	Uurasjärvi et al. (2020)
Swiss lakes, Switzerland	n.a.	Urban	11.00–61.00	Direct filtration	300	Faure et al. (2015)
Donting lake, China	33	Urban	0.90–2.80	Direct filtration	0.45	Wang et al. (2018)
Hong lake, China	12	Urban	1.25–4.65	Direct filtration	0.45	Wang et al. (2018)
Wuhan urban lakes, China	10	Urban	1.66–8.92	Direct filtration	0.45	Wang et al. (2017)
Poyang lake, China	16	Urban	5.00–34.00	Direct filtration	0.45	Yuan et al. (2019)
Taihu lake, China	3	Urban	3.40–25.80	Direct filtration	100	Su et al. (2016)
Three Gorges Reservoir, China	181	Urban	4.70	Density separation	48	Di and Wang (2017)
Simcoe lake, Canada	219	Urban	0.0004–0.001	Direct sieving	300	Felismino et al. (2021)

n.a., not available.
Lake category: Rural, lake close to a village less than 10,000 inhabitants (distance <10 km); Urban, lake located near to a town of more than 100,000 inhabitants (distance <50 km); Mountain, lake located in a remote area with no population or lightly populated basin (sensu Dusaucy et al., 2021).

well as other lake geomorphological features potentially influencing plastic contamination (altitude, surrounding meadow area, type of basin and lake size). Our major finding was that MPs concentration was only related to the area of the meadow surrounding the lake (Table 3). This result suggests a tentative and important link between abundance of MP particles and visitor activity, since lakes surrounded by green meadows are much more attractive and visited by picnickers, hikers, and mountaineers who regularly spend their nighttime in tents or bivouac. We postulate that, similar to observations in Lake Baikal (Karnaukhov et al., 2020), MPs contamination reach surface waters from tourist and mountaineer activities in the vicinity of the areas sampled. In fact, we found that total coverture of meadows surrounding the lake was higher in lakes where waste plastic was visually detected in this study. These differences proved highly significant (Mann Whitney *U* Test, $p < 0.001$) with median percentage meadows surrounding the lake nearly twice higher in sites where plastic waste was found (median values: 100 vs. 57; Fig. 4). Therefore, differences in MPs contamination in Sierra Nevada lakes is most likely associated with mountain materials, textiles, cosmetics, and other leisure items carried out by visitors, as it has been shown for other lakes (Schwabl et al., 2019). Although our data cannot prove the causal relation between visitors and number of MPs, it is highly noticeable that lakes with 10 or more MPs per liter (e.g., Lagunillo de los Lavaderos de la Reina, Laguna Hondera, Lagunillo de Lanjarón) are located alongside the most popular trails including the “Los Tres Miles” a multi-day trek covering all the major 3000 peaks in Sierra Nevada mountain range (Hartley, 2017).

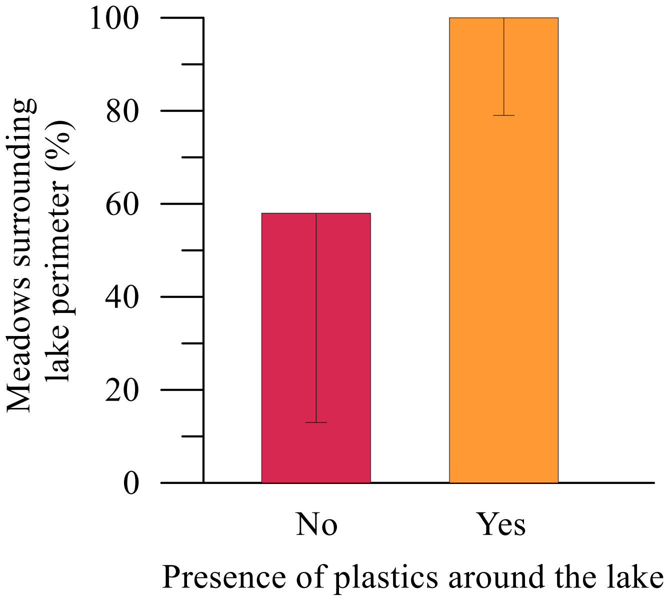

Fig. 4. Meadow coverture surrounding lake-perimeter in lakes where no plastics were visually observed around the lake versus lakes with plastics around the lake. Values represent the median, and error bars displayed in the decreasing Y-axis direction represent the standard deviation.

Table 3
Generalized linear model results of altitude, lake size, lake surrounding meadow area and type of basin (open vs. close lake) as predictors of microplastic concentration. Only models with all versus significant predictors are shown (see statistical analysis for further explanation). Significant results ($p < 0.05$) are shown in bold.

Response variable	Predictors	Estimated coefficient	Standard error	*z*-value	*p*-value	AIC
Microplastic concentration	Intercept	−0.498	1.877	−0.265	0.791	217.41
	Altitude	0.001	0.001	1.181	0.238	
	Surrounding meadow area	0.402	0.180	2.231	**0.026**	
	Lake size	−0.147	0.381	−0.387	0.699	
	Type of basin	0.263	0.347	0.758	0.448	
Microplastic concentration	Intercept	1.760	0.145	12.162	<0.001	213.12
	Surrounding meadow area	0.374	0.178	2.105	**0.035**	

4. Conclusion

So far, most studies have centered in how MPs are carried out by waterways into the ocean, whereas remote mountain lakes have been traditionally considered more pristine sites due to their limited access and distances from major population and industrial centers. This, as well as other recent studies, contribute to emphasize the global extend of plastic pollution, and raise the warning derived from the fact that no mountain is high enough to escape from their impacts, whether they fall down from the sky or, as this study also suggests, intensively accumulate due to human outdoor and leisure activities. The astonishing abundance of MPs found in lakes of Sierra Nevada deserves further investigation, and can serve as a base to improve regulations regarding waste management in order to effectively reduce plastic pollution in natural preserve areas. The ubiquity of microplastics in all environments argues for global policies in favor of the use of degradable bioplastics. Bioplastics may not only reduce microplastic pollution but effectively contributed to a circular economy that contribute to return carbon into the environment.

Author contribution statement

MVA and NO design the sampling campaign and carried it out. VG and ARG analyzed microplastic samples. MC and MAM-L supervised the separation, identification and characterization of microplastics. All authors contributed to the writting, supervision and revision of the paper.

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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