Plastic debris in lakes and reservoirs

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Abstract

Plastic debris is thought to be widespread in freshwater ecosystems globally. We sample surface waters of 38 lakes and reservoirs, distributed across gradients of geographical position and limnological attributes, with the aim to identify factors associated with an increased observation of plastics. We find plastic debris in all studied lakes and reservoirs, suggesting that these ecosystems play a key role in the plastic pollution cycle. Our results indicate that two types of lakes are particularly vulnerable to plastic contamination; lakes and reservoirs in densely populated and urbanized areas; and large lakes and reservoirs with elevated deposition areas, long water retention times, and high levels of anthropogenic influence. Plastic concentrations vary widely among lakes; in the most polluted concentrations reach or even exceed those reported in the subtropical oceanic gyres, marine areas collecting large amounts of debris[1]. Our findings highlight the importance of including lakes and reservoirs when addressing plastic pollution, in the context of pollution management and for the continued provision of lake ecosystem services.

Main

Plastic pollution is among the major challenges of our time[2,3]. Marine environments are considered to be the final receptors and sinks of plastic debris[4], with most research focusing on the impacts of plastics in these systems. However, the spotlight on marine ecosystems has overshadowed the role of freshwaters, particularly lakes and reservoirs, as key components in global plastic pathways. Freshwaters may accumulate plastics at rates similar to, or higher than those in marine systems[5–7]. Nonetheless, global research on plastic debris in freshwaters has been hindered by two main challenges. First, studies have focused on a limited number of freshwater systems in restricted geographical regions[8]. Second, a lack of standardised sampling methods has prevented direct quantitative comparisons across studies[9]. So far, results from plastic research in lakes and reservoirs have been compared and synthesised through meta-analyses and reviews, which have acknowledged that comparability is limited due to the different methods used across studies[10,11]. No single study has yet been extended to a global scale, nor has there been any attempt to identify and quantify the drivers of plastic pollution in lentic systems at this scale.

We addressed these fundamental gaps by conducting a globally coordinated study aimed at (1) assessing the occurrence and abundance of plastic debris in surface waters of freshwater lakes and reservoirs, and (2) identifying natural and anthropogenic landscape factors associated with the occurrence of plastic debris. Specifically, we evaluated the 'signature' (i.e., abundance and types) of plastics in lakes and reservoirs as a function of potential sources of contamination, and of the hydro-morphological and watershed features that can affect plastic distribution. We performed standardised sampling and analysis of plastics (>250 μm) in surface waters from 38 lakes and reservoirs (hereafter lakes) in 23 countries, covering a wide range of hydro-morphological and anthropogenic impact features.

The study sites, albeit concentrated in the northern hemisphere, are spread out geographically and encompass a wide gradient of lake features and catchment characteristics (Extended Data Fig. 1). As a result, the sample of study sites is representative of global lake variability in several key characteristics[12] (Extended Data Fig. 1). In the absence of any concerted effort or feasible method to obtain a globally representative sample of >100 million lakes, this gradient approach was considered to be the most suitable for this coordinated international effort to study microplastics in lentic systems. We included lakes spanning 0.04 to 32,600 km^2 in size and 1 to 1,470 m in depth, with population densities of 0 to 3,411 inhabitants km^{-2} and urban land cover of 0 to 98% in their watersheds. As we performed a snapshot sampling, the temporal and spatial variation of plastic abundance was not included. However, snapshot sampling events such as this provide valuable information, covering environmental gradients across space[13]. All samples were collected by horizontal trawls of a plankton net perpendicular to the lake outflow, following the same protocol. The concentrated sample was subsequently treated with hydrogen peroxide (concentration 15% for 24 h at 60°C) to reduce adhered substances and organisms on the plastic particles. A total of 9,425 plastic particles were identified and classified based on shape, colour, and size. Polymer composition was identified on a subset of 2,295 (~25%) particles using micro-Raman spectroscopy following Kedzierski *et al.*[14] (see Methods). We related the occurrence, abundance, and features of plastics to variables describing hydro-morphology (e.g., area, depth, shoreline length, and residence time) and anthropogenic impact (e.g., land cover, presence of wastewater treatment facilities, and population density) affecting each lake. We used a geographical information system to delineate the watershed of each lake and derive information about human impact. Regression tree and redundancy analyses (RDA) were used to identify the predictors of concentration and features of plastics.

Plastic debris occurred in all 38 lakes; however, the plastic signature differed greatly among systems[15]. Concentration of plastics spanned four orders of magnitude, from 10^{-3} to 10^{1} particles m^{-3} (mean ± standard error, S.E. = 1.82 ± 0.37 particles m^{-3}; median = 0.85 particles m^{-3}; Fig. 1a-b). Most of the study sites (55%, 21 lakes) had concentrations below 1 particle m^{-3}, whereas 14 (37%) had concentrations between 1 and 5 particles m^{-3}, and three (8%) had concentrations higher than 5 particles m^{-3}.

The results for the latter three lakes are remarkable because they show that plastic concentrations in lentic systems can even exceed those detected in some of the ocean's most impacted locations. The greatest concentrations in our study (i.e., Lake Lugano with 11.5 particles m^{-3}, Lake Maggiore with 8.2 particles m^{-3}, and Lake Tahoe with 5.4 particles m^{-3}) are higher than those observed in the subtropical oceanic gyres, which are currently considered some of the greatest plastic accumulation zones in the world[1]. Maximum concentrations detected in ocean gyres using a similar sampling protocol to this study (trawl nets with a 333 µm mesh) were 1.62 particles m^{-3} in the North Atlantic subtropical gyre[16]. It should be noted that we focused on particles larger than 250 µm; if we included plastics with lower size limits, the concentrations would have been greater than the maximum observed here. Previous studies have identified even higher plastic concentration in some lakes (e.g., Lake Poyang, China: 5,000–34,000 particles m^{-3} [17]). However, samples with such high concentrations are usually collected using a grab method. While this grab method has the advantage of capturing micro- and nano-scale plastics, the small sample volume may result in higher variability in plastic concentrations, and therefore, unrepresentative characterisation of the diversity of plastics compared to methods similar to ours, where nets were used to filter an average of 140 m^3 of lake water per site[18].

The variability among the three replicates (i.e., trawls) collected within each lake was generally low (mean S.E. value = 0.47 particles m^{-3}), especially for lakes with a low average plastic

concentration (mean S.E. value = 0.14 particles m^{-3}, see Extended Data Fig. 2). Small scale (among-replicate) variation in plastic distribution may arise from hydrodynamic processes[19], lake morphology, and topography[20]. Within-lake variability may cause uncertainty when estimating the overall plastic concentration, but the substantial lakewater volume filtered at each site enabled us to obtain reliable average concentrations and capture spatial differences of interest.

Plastic signatures differed depending on the morphometric characteristics of lakes. As shown by cluster analysis (Extended Data Fig. 3), we observed a higher percentage of fibres (mean ± S.E. = 77 ± 0.6%), mainly black or blue and composed of polyester (mean ± S.E. = 39 ± 1.6%), in lakes with comparatively small surface area, volume, maximum depth, and shoreline length. In large, deep lakes with more extensive shorelines, the plastic signature was dominated by fragments (mean ± S.E. = 53 ± 0.9%), mainly transparent and white, with a polymer composition of polypropylene (mean ± S.E. = 35 ± 1.5%) and polyethylene (mean ± S.E. = 31 ± 1.8%). The difference between the two clusters of lakes was statistically significant for surface area, volume, maximum depth, and shoreline length (Wilcoxon test p-values all <0.01).

Most plastics from all sites were classified as microplastics (<5 mm, 93.8%). Only 4.7% were mesoplastics (5-10 mm), and we observed very few macroplastics (>10 mm, 1.5%). More than 90% of the plastic particles belonged to two shape categories (Fig. 1c), fibres (49.4%) and fragments (41.0%). Fibres, which were widespread, dominated mainly where the total plastic concentration was low (<1 particle m^{-3}). We found textile fibres even in lakes and reservoirs located in remote areas with limited human presence, such as Avery Lake in Michigan, USA. While atmospheric deposition may be a relatively important source of fibres for pristine systems[21], inputs from tributaries are likely to be more important for lakes and reservoirs with a greater human presence in their catchments. It is well established that fibres from textile materials are a major source of plastic contamination[22]; more than 700,000 fibres can be

released into the water system from the washing of 6 kg of laundry[23]. Moreover, recreational activities such as swimming can increase direct input of fibres into aquatic systems[24].

The fact that fibres and fragments were the most abundant types of microplastics is consistent with field evidence suggesting that secondary microplastics (i.e., particles that result from the fragmentation of larger plastic items) are common in the aquatic environment[25]. Pellets and spheres, whose shape suggests a primary origin, accounted for less than 1% of plastics (Fig. 1c). Indeed, primary microplastics, which are produced either for indirect use as precursors (nurdles or virgin resin pellets) or for direct use, such as in cosmetics, scrubs and abrasives, are generally less abundant in aquatic systems and are expected to decrease in concentration, at least in some countries, as a result of regulatory measures on single-use plastics[26]. The relevance of secondary plastic pollution emphasises the need to focus mitigation on preventing plastics from entering waterways or removing them before degradation occurs[27].

Recording the shape and other characteristics of plastics helps not only to identify the possible sources of pollution, but also to characterise the impact of plastic pollution. Different shape-dependent impacts are reported in the literature[28,29]. For instance, up to 10-fold greater adverse effects of fibres compared to beads have been observed on the freshwater zooplankton *Ceriodaphnia dubia* Richard, 1894, with reduced reproductive output at fibre concentrations higher than environmental levels[30]. Particle size is even more critical in influencing both the toxicokinetics and toxicodynamics of plastics, highlighting the importance of considering size when evaluating the potential risks associated with microplastic exposure[31]. Colour can also affect the toxicity of plastics, and selective feeding on different colours of microplastics has been observed in fish and other organisms because plastics can be mistaken for food of similar coloration[32]. Manual colour assignment during sample analysis can be difficult due to weathering of particles, and different colour perception among researchers[33]. Nonetheless, it is still recommended to record particle colour during visual assessments. Whilst source derivation

is not likely possible based on colour alone, recording colour may help to identify broad trends, such as ingestion preference. In our study, the most common colour was black (30%), followed by transparent (24%), blue (18%), and white (13%). The remaining colours were present in lower abundances (<5%). We found a considerable number of bluish plastics, contrasting with the very low number of reddish ones (i.e., red, orange). Similarly, Martí et al.[34], analysing marine plastic items collected in the five subtropical gyres and semi-enclosed regions, reported that white, transparent/translucent, black/grey, and blue particles were particularly common (31%, 16%, 12%, and 11% of the total, respectively), with a very low number of red particles. A proposed explanation for the difference in colour prevalence, apart from different proportions in the waste stream cycle, is that blue is a camouflage colour in aquatic systems (i.e., crypsis mechanisms). Non-blue items (e.g., red items) would have a higher probability of detection and ingestion by visual predators resulting in a progressive enrichment in blue plastic debris[34].

Polyester (PES), polypropylene (PP), and polyethylene (PE) constituted the majority of polymers identified, with a mean percentage (± S.E.) of 30.4 ± 3.3%, 20.3 ± 2.9% and 15.7 ± 2.9%, respectively (Fig. 1e). This is not surprising as PE and PP account for more than half of global plastic production (36% and 21%, respectively), while PES, most of which is polyethylene terephthalate (PET), accounts for 70% of all polyester, polyamide, and acrylic fibres production[35]. The dominance of these polymers is in agreement with previous observations in marine[36] and freshwater ecosystems[37,38] and likely reflects their use in short life-cycle and mass produced products.

Population density and surface area of lakes were the most important predictors of plastic signature, as highlighted by our regression tree analysis (Fig. 2a). The tree with the lowest cross-validated relative error had two splits and three terminal nodes. The first split

differentiated lentic systems with surface area greater than 213 km^2 (terminal node 1). Lakes with lower surface area were not split further, and the mean plastic concentration for these systems was 4.1 particles m^{-3}. Sites with higher surface area were then split based on watershed population density, giving terminal node 2 (lakes > 25 inhabitants km^{-2}, mean plastic concentration = 1.6 particles m^{-3}) and terminal node 3 (lakes < 25 inhabitants km^{-2}, mean plastic concentration = 0.4 particles m^{-3}).

The positive association between urban-related watershed attributes and abundance of plastic debris has been widely observed[39,40]. In studies where this correlation has not been identified, it has been hypothesised that the study design encompassed a limited number of sites or included sites representing only population density extremes[5,41]. Lakes were divided based on surface area, highlighting that high concentrations of plastic were found in sites with larger surface area. There may be several explanations for this observation. Larger lakes and reservoirs are usually associated with larger watersheds, greater water inflows, greater shoreline length, and more shoreline development[42], which implies a larger deposition area and a greater number of point and nonpoint (including atmospheric deposition) sources of contamination. Large lakes are particularly exposed to anthropogenic stressors, as cities and other urban developments are usually widespread on the shores of large lakes, and they may receive inputs from larger and more polluted rivers[42]. In addition, larger lakes have longer residence times than smaller, shallower systems[43]. For instance, in Lake Tahoe, which has a hydraulic residence time of approximately 650 years[44], we recorded one of the highest concentrations of plastics (i.e., 5.4 particles m^{-3}). Lakes have the potential to act as "traps" and accumulate substantial amounts of plastic debris over time[45]. Additional research is warranted that addresses how the landscape position of lakes and lake characteristics affect microplastic abundance, their fate within lakes due to sedimentation, or transport within from nearshore to offshore habitats, and out of the ecosystem through outflows.

Concentration and features of plastics varied based on environmental factors representing the impact of human activity and morphometric lake characteristics. Based on redundancy analysis, these environmental variables explained 55.9% of the variation in plastic concentration across sampling sites. Lakes with lower plastic concentration were clustered, while lakes with high plastic concentration were more spread out in ordination space (scaling 1, Extended Data Fig. 4), suggesting plastic features were more similar in less polluted lakes. There were two categories of lakes with high plastic concentration (scaling 2, Fig. 2b). The first group was characterised by a high number of wastewater treatment plants and high surface area, depth, volume and shoreline length, and a high abundance of fragments. The second group was characterised by high human impact (human footprint, population density, and urban land cover), and high abundance of fibres. The remaining sites with lower plastic concentrations (<1 particle m^{-3}) had negative relationships with measured anthropogenic variables and seemed weakly related to presence of cropland, which in turn was negatively related to urban land cover and population density.

Our analysis indicated that two types of lakes are particularly vulnerable to plastic contamination: (1) those located within highly urbanised and populated watersheds, and (2) those with high surface area, where we found high surface concentrations of plastic fragments, due to their elevated drainage area and long retention time. This result is particularly relevant because the elevated concentration of plastics in lakes with large water volumes implies high plastic loading, which could impact their ecosystem services locally, regionally and globally[42].

Given the relatively high concentration of plastic debris, particularly in large lakes and reservoirs, lakes may be 'sentinels of plastic pollution' because they act as collectors and integrators of different sources of plastics from the watersheds and atmosphere, and 'active pipes' as they may retain, process and transport plastics through watersheds to the oceans. We find that plastic concentrations in freshwater ecosystems can be higher than those in marine

ecosystems, which are generally considered final receptors of plastic debris. This underpins the relevance of lakes as key components in the global 'plastic cycle'. Optimising management policies to mitigate plastic pollution in upstream freshwater lakes is therefore essential to prevent plastics from entering waterways[27], and ending up in marine systems.

Some of the lakes most contaminated with plastic debris, including lakes Maggiore (IT), Lugano (CH-IT), Tahoe (USA), and Neagh (UK), are important sources of drinking water for local populations and support important recreational based economies. The proportion of plastic debris that ends up in the water supply is unknown, but we suggest that the potential contamination of microplastics in drinking water should become a global management and research priority.

In addition to contaminating the water supply for human needs, plastic pollution has detrimental effects on aquatic organisms and ecosystem functioning. Detecting the concentration of plastics is possible through the methods employed in this study but understanding their fate and ecological impacts remains an important and novel area of research. For instance, plastics at the surface of aquatic systems can aid the release of methane and other greenhouse gases, demonstrating that the effects of plastics can span across ecosystem boundaries[46,47]. Plastics can go beyond the hydrosphere and interact with the atmosphere, biosphere and lithosphere, potentially affecting biogeochemical cycles through mechanisms that still need to be understood. These multiple and potentially synergistic effects call for a holistic assessment of plastic pollution in lentic systems.

Furthermore, our findings indicate that microplastics occur even in lakes that are not subjected to direct anthropogenic impacts. Microplastics, therefore, add a new stressor to these lakes and the organisms that live within them, which already face a range of pressures, including climate change[48], salinization[49], increased nutrient deposition, and nearshore filamentous algal blooms[50], to name a few. Therefore, even in remote areas away from direct human pressure,

no lake can be considered to be truly 'pristine' with respect to plastic pollution. These results demonstrate the global reach of plastic pollution and serve as yet another reminder of the unfortunate and indelible signature of humanity on lakes.

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Figure

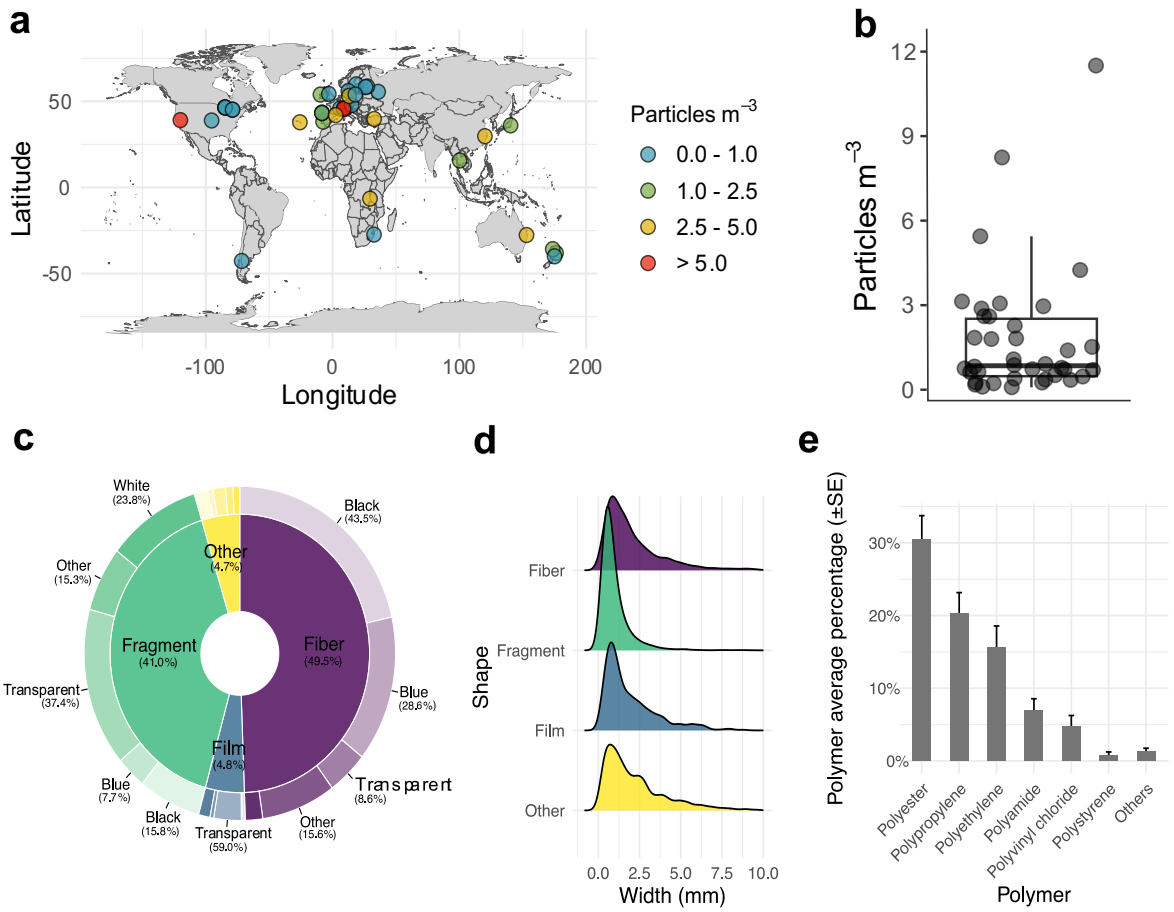

Fig. 1: Concentration and features of plastics identified in the 38 lakes and reservoirs.

a, Map showing distribution and concentration of plastics in the 38 lakes and reservoirs included in the study. The dots are coloured based on the concentration of plastics (particles m^{-3}) detected. **b**, Boxplot showing the concentration of plastic (particles m^{-3}) in the 38 lakes and reservoirs. Boxplot statistics: the lower and upper hinges correspond to the first and third quartiles. The upper (lower) whisker extends from the hinge to the largest (smallest) value no further than 1.5 x IQR (Inter Quartile Range) from the hinge. Data beyond the end of the whiskers are outlying points and are plotted with triangular shape. **c**, Donut pie chart with percentage abundances of the different shapes and relative colours for all the plastic particles analysed. **d**, Width distribution (from 0 to 10 mm) of the different particle shapes. **e,** Average percentage ± standard error (S.E.) of polymer composition for all the plastic particles analysed.

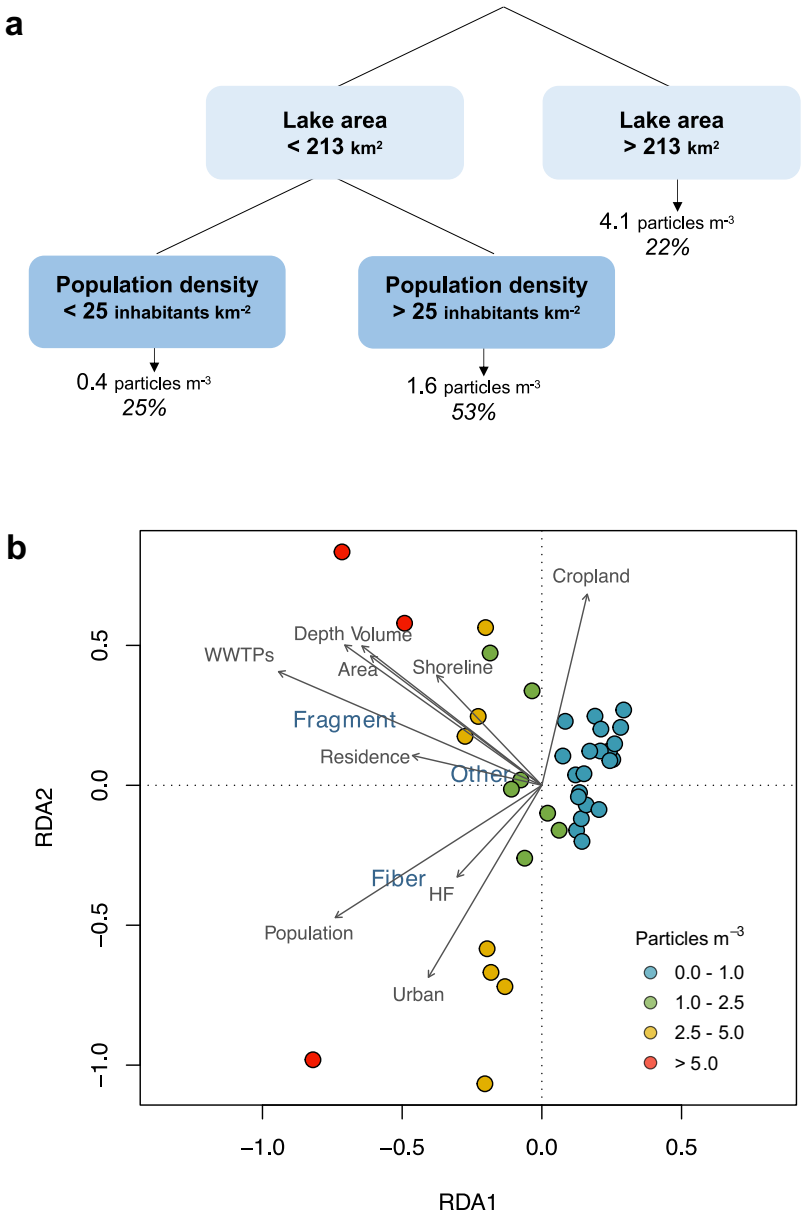

Fig. 2: Relationship of plastic concentration and features with environmental and anthropogenic drivers.

a, Results of regression tree analysis based on the total concentration of plastics in the studied lakes, with three terminal nodes. **b**, Redundancy analysis between plastic concentration in lakes, features of plastics, and environmental and anthropogenic drivers potentially able to explain the distribution and characteristics of plastic contamination (scaling 2). The dots are coloured based on the concentration of plastics (particles m^{-3}) detected.

Methods

Study sites and sample collection

The samples were collected from 38 lakes and reservoirs located in 23 countries distributed in both hemispheres, but with a higher density in the northern hemisphere. The sampled sites represent a wide assortment of limnological conditions (Supplementary Table 1). Surface area ranged from 0.04 to 32,600 km^2 (median = 19.50 km^2), mean depth from 0.5 to 580 m (median = 9.7 m), and volume from 1.8 x 10^{-5} to 18,980 km^3 (median = 0.18 km^3). The lakes spanned different mixing regimes (i.e., polymictic, 11; monomictic, 12; dimictic, 8; and meromictic, 5) and trophic states (i.e., ultra-oligotrophic, 3; oligotrophic, 10; mesotrophic, 12; eutrophic, 11; and hyper-eutrophic, 2).

During 2020–2021, samples of plastic debris were collected following a standardised protocol. The samples were collected from a boat using horizontal net trawls (mesh sizes ranging from 50 to 300 μm) and three replicates were obtained from each lake. Sampling occurred in the pelagic zone, near the main lake outlet, on a calm day to minimise the risk of missing an unknown portion of the sample area, because rough water may cause nets to rise above or below the water's surface. The three trawl transects were oriented perpendicular to the outflow. The net was placed at the port side and the boat speed was maintained at around 1–1.5 m s^{-1}, following GESAMP[51]. At least 50 m^3 of water was filtered for each trawl and GPS tracks were recorded to estimate the exact volume filtered. In case of net clogging, the trawls were divided into different sub-trawls to allow net cleaning.

Sample analysis

All the samples were analysed at the Laboratory of Freshwater Ecology and Management of the University of Milano-Bicocca (Italy) following a common standardised procedure. The samples were wet-sieved on a 250 μm mesh to align the lower limit size across samples,

because nets with different mesh sizes were used for the sampling work. Then, the samples were treated with 15% H_2O_2 for 24 h at 60°C to eliminate organic matter and organisms adhering to the plastic particles. This procedure was selected to reduce potential damage to plastic particles[52–54]. The samples were then filtered onto 0.45 µm glass microfibre filters (GF/F, 47 mm Ø, Whatman), which were stored in clean glass Petri dishes. The filters were examined under a dissecting microscope (40x, Heerbrugg WILD M3Z) and particles recognized as plastics were transferred to glass slides for the subsequent spectroscopic analysis. Particles were either accepted or rejected as microplastics based on a catalogue of morphological criteria. Visual classification was considered reliable as a first step since we focused on the larger size fraction of microplastics (>250 µm). If the morphological classification was uncertain, Raman spectra were acquired to confirm or reject the hypothesised classification (for more details see 'Micro-spectroscopy analyses' section). Pictures of all plastic particles were taken using a high-resolution camera (Leica ICC50). All particles were counted, and their Feret's diameter was measured using the software ImageJ (version 1.52q). The largest plastic that we found had a Feret's diameter equal to 8.6 cm. Based on their dimensions, plastics were assigned to three different size categories: microplastics (250 µm to 5 mm)[55,56], mesoplastics (5 to 10 mm), and macroplastics (>10 mm; Extended Data Fig. 5). For shape categorization, we adopted a modified version of the classification proposed by Hartmann *et al.*[9]. In particular, plastics were classified as fibres, fragments, films, spheres/pellets, or lines. A lines class was added to identify those plastics that have a shape similar to fibres (longer in one dimension), but have a larger diameter, to differentiate them from fibres derived from textiles (Extended Data Fig. 6). Moreover, plastic particles were classified based on colour (i.e., red, orange, yellow, green, blue, violet, black, white, transparent, or multi-coloured), following a RAL standard colour scale, according to Lusher *et al.*[33].

Micro-spectroscopy analyses

Raman micro-spectroscopy was used to provide reliable data on the total number of plastics identified in each sample, because visual classification alone is insufficient to determine microplastic abundance[33]. The particles for which the result of the visual classification was uncertain were subjected to Raman analysis to decide whether to include or exclude them. Then, to estimate the percent occurrence of the different polymers of plastics collected, Raman micro-spectroscopy was performed on a random subsample of the visually identified microplastic particles, as widely suggested in the literature[57–59]. In particular, a robust procedure was adopted to first determine the minimum number of particles to be studied to reach a certain confidence level in the estimated proportion of different polymers in the sample, following Kedzierski *et al.*[14]. The size of the subsample, *n*, for each lake was derived as follows:

$$n = \frac{\frac{1}{4} + \frac{\varepsilon^2}{\left(u_{1-\frac{\alpha}{2}}\right)^2}}{\frac{\varepsilon^2}{\left(u_{1-\frac{\alpha}{2}}\right)^2} + \frac{1}{4N}}$$

with ε being the desired accuracy; $u_{1-\alpha/2}$ the fractal of order α of the standardised normal law; and N the total number of plastics counted in each sample. We chose a degree of confidence of 95% (i.e., $\alpha = 0.05$; $u_{1-\alpha/2} = 1.96$) and $\varepsilon = 0.1$.

Misidentification at this stage (after visual analysis and Raman pre-checking) was infrequent (< 3% ± 2% on average among samples). Any particles found to be non-plastic at this stage were removed from the total count, and, when this happened, additional particles equal to the amount removed have been analysed spectroscopically to increase the confidence of the estimate. For textile fibres, anthropogenic fibres (i.e., fibres containing a mixture of dyes and cellulose) were included in the total count in agreement with previously published papers that

highlights their relevance for aquatic toxicity[60]. The category 'anthropogenic' was also assigned to fibres when the dye masked the polymer and no information other than the colourants could be obtained. Also in these cases, to improve the accuracy of the estimate, an equal number of extra particles was examined using Raman micro-spectroscopy.

Raman spectra were acquired using a Horiba Jobin Yvon LabRAM HR Evolution Raman System at the Department of Earth and Environmental Sciences, University of Milano – Bicocca (Italy), characterised with 800 mm of focal distance coupled with an air-cooled 1024×256 px CCD detector. The spectra were obtained by using an attenuated green Nd 532.06 nm laser source (300 mW) with a 50× magnification (Olympus BXFM). The grating was 600 g mm^{-1} and the spectral per pixel resolution was about 1.6 cm^{-1}. Two spectra were acquired for each particle with a spectral interval from 222.86 to 1,899.01 cm^{-1} and from 1,762.24 to 3,177.02 cm^{-1}. Depending on the particles analysed, the acquisition parameters were changed: accumulation ranged between 1–3, integration time between 20–60 s, and power between 0.3–300 mW. Instrument calibration was performed daily based on the auto-calibration process performed by the Raman System Service with respect to the zero line and the silicon standard (520.7 cm^{-1}), according to the ASTM 1840–96 prescription[61,62]. Raman spectra were baseline corrected and processed using the Fityk software[63,64]. Further analyses on polymer spectra were performed in R (4.0.3), using the package 'RamanMP[65]. The final identification of microplastics was based on individual assessment of each spectrum, by identifying the characteristic bands of the suspected polymer in the sample spectrum.

Quality controls (QA/QC) of plastic abundance data

Prior to sampling, the nets were thoroughly cleaned using ultrapure water and then 5 L of ultrapure water was filtered through the net. This volume was collected to account for possible contamination of the sampling equipment. Laboratory-based quality assurance and quality

control (QA/QC) included procedural blanks. To assess potential contamination from laboratory materials or air, laboratory blanks were also collected. Moreover, all laboratory equipment was rinsed three times with ultrapure water. Glassware equipment was used where possible, and all the surfaces were cleaned before use. In addition, the samples were covered with aluminium foil, and cotton laboratory coats were worn. Plastic particles observed on the blanks were subtracted from the total values in environmental samples by randomly removing particles that matched the colour, shape, and polymeric composition of the blank particles (Extended Data Table 1).

Watershed and lake attributes

Sixteen variables were extracted to characterise the level of anthropogenic impact across the different lakes. We delineated the watershed boundary of all lakes (i.e., the land and water areas that drain toward the lake) using ArcMap 10.7 (Spatial Analyst tool) Geographic Information Systems (GIS) software, and the ASTER Global Digital Elevation Model (GDEM) Version 3 (ASTGTM), which provides a spatial resolution of 1 arc second (approximately 30 m horizontal spacing at the equator)[66].

Land cover in each watershed was obtained by clipping the 100 m resolution land cover map provided by the Copernicus Global Land Service[67]. Data on watershed population were obtained from the UN WPP-adjusted population estimates for the year 2020[68]. Information about wastewater treatment plants (WWTPs) was retrieved using the HydroWASTE dataset, a spatially explicit global database of 58,502 WWTPs and their characteristics[20]. Additionally, the mean Global Human Footprint Index was derived for each system. This index is the Human Influence Index (HII) normalised by biome and realm. The HII is a global dataset of 1 km grid cells, created from nine global data layers of human population pressure (population density), human land use and infrastructure (built-up areas, night time lights, and land use/land cover),

and human access (coastlines, roads, railroads, and navigable rivers). A HII value of zero represents the least influenced part of the biome with a value of 100 representing the most influenced[69].

Data analyses

To identify coherent groups of lakes based on the percentage of occurrence of the different plastic features (shape, colour, and polymer composition), we performed K-means clustering. The optimal number of clusters (2) was determined using the Elbow Method[70]. Statistical differences among lake attributes within the two clusters were evaluated using Wilcoxon rank-sum tests.

We evaluated the association among the 16 explanatory variables through Pearson product-moment correlation tests and variables that were highly correlated ($r > 0.85$, $p<0.001$) were removed from subsequent analyses to avoid collinearity. Moreover, we used the Variance Inflation Factor technique to remove further variables that were highly correlated[71].

To determine which candidate explanatory variables were associated with variation in the concentration of plastics, we used univariate regression trees[72]. Regression trees use recursive partitioning algorithms to reveal the structure in the data, by successive binary partitions based on the different predictors, in a way that minimises the sum of squares in the concentration of plastic within each group (node). The algorithm functioned by maximising the between-node sum of squares (minimising the within-node sum of squares) and then repeating the procedure until an overly large regression tree was constructed. The dataset was divided into training data (80%) and testing data (20%), and the mean squared error was calculated. The tree was then pruned to avoid overfitting based on the complexity parameter, which is the amount by which splitting a node improved the relative error[73].

Redundancy Analysis was then performed to explicitly model response variables (i.e., concentration of the shapes of plastics: fibres, fragments, film and others) as a function of explanatory variables. We reported values based on both scaling 1 and 2: scaling 1 shows similarities between objects in the response matrix, while scaling 2 shows the effects of explanatory variables[74].

All statistical analyses were completed in R (version 4.2.2), using the packages 'corrplot'[75], 'factoextra'[76], 'ggplot2'[77], 'rpart'[78], and 'vegan'[79].

Data availability statement

The datasets generated during and/or analysed during the current study are available in the Zenodo repository, 10.5281/zenodo.7824882[15]

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Author contributions

V.N. and B.L. designed the study, and drafted the manuscript; V.N. compiled the data, conducted analyses; V.N., J.A., M.B.A, A.M.A.G., S.A.B., R.B., M.C.A, M.C., C.C., S.C., E.D.E., J.P.D., O.E., Z.E., H.F., S.G., H.P.G., D.P.H., T.D.H., K.K., C.K., A.L.P., M.G.M., M.C.M., S.N., C.O., D.Ö., S.P., F.R., F.S., C.S., U.N.T., P.V., G.A.W., L.Z., B.L. contributed to the discussion and conceptualization of the paper; V.N., J.A., M.B.A, A.M.A.G, K.A., R.B., S.A.B, R.B., M.C, C.C., R.C., J.C., S.C., E.D.E, T.N.D, J.P.D., J.D., O.E., Z.E., H.F., S.G., H.P.G., D.P.H., T.D.H., K.K., G.B.K., B.E.K., C.K., E.M.K., A.L.P., Y.M., M.C.M., S.N., V.O., D.Ö., S.P., A.P., P.M.R., E.I.R., F.R., F.S., C.S., D.S., K.S., K.S.C., U.N.T., M.T., J.T., P.V., G.A.W., N.W., E.Z. collected the samples; M.L.F. provided guidance and support for Raman analyses; J.A., M.C.A, T.D.H., M.G.M., C.O., D.S., B.W. contributed to data analysis; J.A., S.A.B, M.C.A, E.D.E., Z.E., H.F., S.G., D.P.H., E.M.K., A.L.P., Y.M., S.N., C.O., P.V., G.A.W., L.Z. performed language editing; J.A., M.B.A., A.M.A.G, M.C.A, M.C, C.C., R.C., S.C., T.N.D., J.P.D., Z.E., H.F., M.L.F., H.P.G, D.P.H., K.K., K.S.C., E.M.K., F.L., M.G.M., M.C.M., C.O., S.P., S.N., S.S.S.S., P.V., G.A.W., L.Z., B.L. provided constructive reviews to the paper.

Competing interest declaration

The authors declare no competing interests.

Additional information

Supplementary Information is available for this paper.

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Extended data figure/table legends

Extended Table 1. Blank levels for laboratory-based QA/QC, reporting the absolute number of fibres detected in the blank filters used as control for each replicated sample (i.e., trawl).

Extended Data Fig. 1. Comparison of the density distribution of the features of our study lakes and reservoirs (in yellow) to the boxplot of freshwater systems included in the 'HydroLAKES' global dataset. The features being compared are: a) lake area in km^2; b) mean depth in m; c) lake volume in km^3; d) residence time of lakes in years.

Extended Data Fig. 2. Means and standard errors of plastic concentration (particles m^{-3}) resulting from the three trawls collected in each lake. The lakes are ranked in descending order based on their particle concentration, from highest to lowest.

Extended Data Fig. 3. Clusters of lakes based on the percentage occurrence of the different plastic shapes, colours, and polymeric compositions.

Extended Data Fig. 4. Scaling 1 of redundancy analysis between plastic concentration in lakes, features of plastics, and environmental and anthropogenic drivers. The dots are coloured based on the concentration of plastics (particles m^{-3}) detected.

Extended Data Fig. 5. Density plots and histogram of the Feret's diameter (width, mm) of the 9,425 particles identified in the 38 lakes analysed. The median trend is indicated by the vertical blue and dashed line.

Extended Data Fig. 6. Images of different shapes of plastic fragments collected in water samples: a, b, c) fragment; d, e, f) fibre; g, h, i) filament; j, k) film; l) sphere/pellet.