

Article

# Microplastic and Fibre Contamination in a Remote Mountain Lake in Switzerland

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**Abstract:** The contamination of aquatic environments by microplastics has been largely documented in the last years, especially in oceans, rivers, and lakes, but their occurrence in remote mountain lakes has been scarcely considered. This work aims to investigate the presence and abundance of microplastics and fibres in a remote, alpine, and uninhabited lake in Switzerland (Sassolo). In this study, the water column as well as the sediments were analysed. The isolation of microplastics and fibres from the samples of the sediment was achieved with a digestion process using  $H_2O_2$  and a density separation technique with NaI. Classification of microparticles (from 5 mm to 125  $\mu$ m) was first developed with an optical microscope. Infrared spectroscopy was then used to identify and characterize the chemical nature of the microplastics and fibres. On average, 2.6 microplastics and 4.4 fibres per litre were identified in the water column. On the other hand, the results of the sediment samples revealed significant fibre concentrations compared to plastic microparticles (514 fibres and 33 microplastics per kilogram). The most abundant types of microplastic identified in the samples were composed of polyethylene (PE) and polypropylene (PP). Microplastic and fibre sources were not determined, but it is likely that the number of human activities in this area as well as aerial deposition are contributing to contaminate this remote environment with microplastics and fibres.

**Keywords:** microplastics; fibres; infrared spectroscopy; remote mountain lake; water column; sediments

# 1. Introduction

Nowadays, plastics are widely used in a vast list of household products and are incorporated in numerous other objects due to their relatively low production cost and their attractive physicochemical properties. Consequently, plastic production increased to over 350 million tons [1] last year. In addition, the uncontrolled production and usage of plastics, together with the fact that plastics are materials resistant to degradation, have ultimately led to their accumulation in the environment [2]. Once plastics reach aquatic environments, their fate depends on many factors and they can be found in different shapes and chemical compositions in water columns and sediments [3,4]. Plastics have been found in diverse sizes in aquatic environments, and have been categorised in recent years as macroplastics (>20 mm), mesoplastics (5–20 mm), and microplastics (plastic particles with sizes between 5 mm and 1  $\mu$ m) [5]. Microplastics can be classified as primary (created in these small sizes, like pellets, sequins or microbeads, which are used in cosmetics or decorative products) or secondary (as a result of the



breakdown of bigger plastic materials), depending on their origin, and exhibit different colours, shapes, and sizes.

Plastics are considered as biochemically inert, but their ingestion may cause in some species false satiety [6], blockage of the digestive system [7], and depletion of energy for their egestion [8]. In addition, the surface area to volume ratio combined with the hydrophobicity nature of microplastics make these particles hazardous. The additives added to plastics, unreacted residual monomers that are not chemically attached to the polymer, hazardous elements, and hydrophobic organic contaminants that are adsorbed, are susceptible to leach from the plastic polymer matrix [9,10], increasing the uncertainty of risk.

In addition to the contamination of microplastics, the issue of other microscopic anthropogenic litter that come from natural modified materials, notably fibres like cotton, viscose, or wool [11] is also of concern. All natural and synthetic fibres should be taken into account, notwithstanding the natural origin of these fibres, because of the important quantity of fibres that are released in the environment (more than 1900 fibres per garment per wash [12] or more than 0.3% of the unwashed garment mass [13]). Not to mention that some species are exposed [14] to these microfibres. Furthermore, textile fibres, regardless of their nature, contain additives and dyes, which are in some cases hazardous to wildlife and humans [15–17].

Microplastics were documented first in the oceans back in the early 1970s [4,18], but the ecological impact of these microplastics has made the scientific community more active over the last years. An elevated number of marine species has been reported to be affected by plastic contamination [19] like zooplankton, benthic species, fishes, and predators by trophic transfer [20–24]. Seabirds [6,25–28] are between the most studied species [29] and even plankton species have also been found to be affected by this contamination [20,30]. However, since these species are the foundation of the marine food web, any threat to plankton species can cause serious consequences to marine life [31].

Microplastics and fibres enter into the marine environment via the runoff of inland waters because important volumes of sewage are discharged to surface waters without or with very little treatment, especially in developing countries. Furthermore, developed countries also influence, on a smaller scale, marine contamination with microscale litter because the highly contaminated effluents of these cities have been shown not to be sufficiently purified by treatment plants [32,33]. Households are acting as significant sources of contamination due to the presence of microbeads in certain personal care products [34–36] like toothpaste and skin cleaners, and the detachment of fibres from textiles by washing [37]. Moreover, the microscale litter in oceans is mainly generated as a result of weathering fragmentation of bigger debris that are already in marine ecosystems, or most likely in beaches [31]. Therefore, anthropogenic litter tends to accumulate in the oceans [38,39] and it is now considered exceptional not to find microscopic anthropogenic litter in marine environments.

The situation in freshwaters have also been documented and reviewed [40,41]. Some important rivers in Europe have been studied to assess the contamination of microplastics, for instance, the Rhin [42,43], the Seine [44], the Danube [45], and the Rhône [46]. The contamination in lakes around the world have also been assessed. Particularly in Switzerland, Faure et al. carried out a study to evaluate the contamination of the six biggest lakes of the country [47]. They found microplastics and textile fibres in the surface waters and beach sediments of Lake Geneva, Lake Constance, Lake Maggiore, Lake Neuchatel, Lake Zurich, and Lake Brienz. Recently, another study carried out in Lake Geneva by Oceaneye showed that the average contamination of microplastics >300  $\mu$ m in Lake Geneva was in the same order of magnitude than in the North Atlantic sea and the average of the ocean [48]. Furthermore, Sighicelli et al. found microplastics in three subalpine lakes in Italy [49]. This study analysed microplastics >300  $\mu$ m with manta trawls, and focused on the sediment of the beaches, without taking into account the microplastics that have sunk in the lakes. All these studies have in common that the lakes are situated in densely populated, highly productive areas, and therefore, strongly influenced by anthropogenic activities that contaminate the freshwaters.

The question then arises whether the microplastic and fibre contamination have even reached all freshwater systems, like remote lakes. Free et al. found high levels of microplastics (20,264 particles  $km^{-2}$ ) at the surface of a remote mountain lake in Mongolia (Lake Havsgol) [50]. It should be noted here that this lake is surrounded by inhabitants and tourist camps and the resulting sewage enter the lake without treatment. This issue probably explains at least the major level of contamination of this lake. This is why the broader question regarding the general microplastic contamination of remote mountain lakes or with scarce anthropogenic activities is of high interest.

Considering the state-of-the-art, it appears that there is still a lack of knowledge on the extent of microplastic contamination in remote lakes (water column and lake sediment) and that smaller sizes should be considered. This study aimed to investigate the presence and abundance of microplastics in a Swiss alpine lake situated in an uninhabited area by sampling the water and the sediments of the lake at different places and depths. For this purpose, sampling was carried out at the alpine lake of Sassolo the 6 June 2019 by ODYSSEUS 3.1. Lake Sassolo is located in the Sambuco Valley in the territory of the Lavizarra Municipality, in the Canton of Ticino, at 2074 m above sea level (46°28'35″ N 8°35'35″ E) (Figure 1). This lake is fed by a small stream from Lake Superiore and is a tributary of Lake Sambuco. The surface of this lake is near 0.05 km<sup>2</sup> and has a maximum depth of 34 m. Due to its elevation, during the winter season, this lake can present an ice sheet of about 3 m thickness. The towns of Airolo (1175 m above sea level) and Lavizarra (763 m above sea level) are the closest towns (less than 2000 inhabitants each) to the lake and are located inside a 10 km radius from the lake.

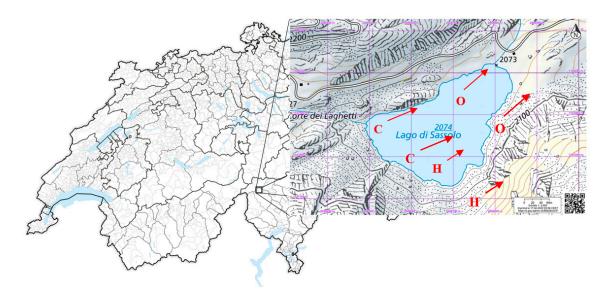


Figure 1. Location of Lake Sassolo and the sampling sites (C: cliff, H: main hole, and O: outlet).

In our study, a size range comprised between 125 and 5000  $\mu$ m, and a microscale particle classification based both on the nature of the microparticles and shape were used. On one hand, we considered plastic microparticles (fragments, film, pellets, beads ...), and on the other hand, mineral and organic microparticles that were also extracted from sample analysis. A distinction was made for fibres that can be natural or synthetic, and which constitute a particular type of microparticles. Indeed, synthetic microfibers have emerged as a relatively new area of study such as the shedding of microfibers from synthetic fabrics during conventional clothes washing. Like many other types of microparticles, microfibers have been found in oceans, rivers, agricultural soils, marine and freshwater systems.

## 2. Materials and Methods

# 2.1. Water Sampling and Sample Treatment

Two samples of water were collected from Lake Sassolo with glass jars of 5 L volume. The first sample was collected in the surface waters of the main hole (Figure 2) that was dug to dive. The second sample was collected at the outlet of the lake. The samples were transported to the laboratory for sample treatment and analysis. For this, the samples were passed under gravity through a 63 µm stainless steel mesh of 100 mm diameter (ATECHNIK GmbH, Leinburg, Germany). The retained particles in the sieve were transferred directly to an aluminium oxide (Al<sub>2</sub>O<sub>3</sub>) filter (Anodisc<sup>™</sup> 47, 0.2 µm, GE Healthcare Life Sciences Whatman<sup>™</sup>, Buckinghamshire, United Kingdom). Since the water sample contained a limited amount of suspended matter, no digestion process was considered. On the other hand, since the outlet sample contained a significant concentration of microparticles (suspected mostly as organic matter), a digestion treatment was considered to reduce the amount of organic microparticles, and improve the visual identification of microplastics and fibres. Therefore, the sample was transferred from the steel mesh to a beaker with 150 mL of ultrapure water (Milli Q water, Millipore, Schaffhaussen, Switzerland, with  $R > 18 M\Omega \cdot cm$ ). A volume of 45 mL of a 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> 30% wt, REACTOLAB SA, Servion, Switzerland) solution was added to digest the organic matter. The sample was left at 60 °C and visually checked every day to monitor the off-gassing and temperature. This sample still had low release of bubbles on the fifth day, which means that the reaction was not completed. Nevertheless, to prevent degradation (discoloration and reduction of size) of microplastics [51], the filtration of the sample was done after five days with one 63 µm stainless steel mesh, in order to remove the digested matter. Finally, the sample was transferred to one aluminium oxide filter using ultrapure water.



**Figure 2.** View of sampling conditions at the main hole at alpine Lake Sassolo (June 2019) Photo made by: Odysseus 3.1.

#### 2.2. Sediment Sampling and Sample Treatment

Three samples of 1 L were collected from the sediments of the lake by gently scraping the sediments with clean plastic containers, in order to minimise the contact of the sample with the watertight gloves.

The containers were immediately closed afterward to avoid contamination. Two samples were collected in the vicinity of the main hole (at 8 and 33 m depths, respectively) and one near the cliff (at 14 m depth), see Figure 1. Once in the laboratory, the wet sediments were first transferred to glass beakers with a metallic spoon and were dried at 60 °C. A sample weight of 50 g of dried sediments was passed under gravity through three (ATECHNIK GmbH, Leinburg, Germany) stainless steel meshes of 500 µm,  $250 \,\mu\text{m}$ , and  $125 \,\mu\text{m}$  pore sizes, respectively. The fractions of the sample (>500  $\mu\text{m}$ , 250–500  $\mu\text{m}$ , and  $125-250 \ \mu m$ ) were transferred from the steel meshes with ultrapure water to a set of glass beakers (of 1 L) and left to dry at 60 °C until the water was completely evaporated. The weight of the sample was checked several times until obtaining a stable value. Around 200 mL of a 30% wt hydrogen peroxide  $(H_2O_2)$  solution was added to the beakers, in order to digest the organic matter, and left at 60 °C and visually checked every day to monitor the off-gassing and oxidation reaction. Then after off-gassing, samples were filtered with a 63  $\mu$ m stainless steel mesh to remove the digested matter, and then retransferred to the beakers. Each sample was left to dry at 60 °C to proceed with a density separation technique. For that purpose, a volume of 200 mL of a NaI (99+% pure anhydrous, ACROS ORGANICS, Budapest, Hungary) solution (density >1.70 g/mL) was added to the dried sample and left for one day. The buoyant layer (circa 50 mL) was recovered with a glass pipette and filtered with an aluminium oxide filter. Ultrapure water was added to avoid NaI crystallisation at the surface of the filter.

#### 2.3. Characterisation of Microplastics

The whole surface of all filters (samples of water and sediments) was first observed using an optical microscope (OLYMPUS BX-40, Tokyo, Japan) to achieve a qualitative analysis. All microscale objects (organic and mineral microparticles, fragments, films, pellets, fibres, etc.) in the water samples with at least one dimension higher than 125  $\mu$ m were considered for further chemical identification. This limit (instead of the physical limit of the 63  $\mu$ m steel mesh used) allowed us to achieve a good microparticle separation and characterisation, especially for the sediment. Due to the final number of microparticles in the 63–125  $\mu$ m range of sizes, no systematic observation and analysis by Fourier-Transform Infrared FTIR was possible and only a few selected microparticles suspected to be microplastics were analysed based on microscope observations.

All fibres and microparticles (plastic and non-plastic) in the water samples were systematically analysed in transmittance mode with FTIR spectroscopy (Spotlight 200i FTIR microscopy system, PerkinElmer, Beaconsfield, United Kingdom). For the sediment samples, all the microparticles were observed and in most cases tested by FTIR, except when mineral microparticles were suspected (no transmittance signal). The obtained spectra were subjected to a library search using the Attenuated Total Reflectance (ATR) Perkin Elmer polymer library (with over one hundred spectra). A spectral range was defined for FTIR analysis between 4000 to  $1250 \text{ cm}^{-1}$  because of the absorption of the Al<sub>2</sub>O<sub>3</sub> filter. The use of the entire overlap region (4000 to  $600 \text{ cm}^{-1}$ ) decreased the degree of correlation. Microparticles and fibres having spectra with a search score higher than 75% of coincidence with a plastic polymer were considered as microplastics. Microscale objects were divided into three groups: organic and mineral microparticles, natural and synthetic fibre microparticles, and plastic microparticles (including fragments, films, pellets, foam, etc.).

#### 2.4. Contamination Mitigation

To reduce the incidence of microplastic and fibre contamination, a number of actions were taken. In the sampling, synthetic materials could not be avoided due to the diving, meteorological, and site conditions, as seen in Figure 2. The samples were collected preventing the contact of several plastic materials. All the sediment samples were rapidly stored in glass containers once in the laboratory. The use of plastic materials was reduced as much as possible in the laboratory, although they could not be avoided entirely. The filtration unit, the washing bottle, and the Petri dishes were made of plastic materials. Only cotton clothing was worn in the laboratory, even under a cotton lab coat. The steel

meshes were washed each time with soap and a natural bristle brush, rinsed with ultrapure water, and covered with aluminium foil. The filtration unit was also washed and rinsed with ultrapure water prior each use. Only ultrapure water was used to transfer the samples. The Petri dishes and filters were examined before use. The spatula and forceps were rinsed with ethanol and examined before each use. In addition, all work surfaces were wiped down with paper towel and ethanol before starting work. Atmospheric deposition was reduced by covering the material and the samples with aluminium foil or by working in the extractor hood at all times.

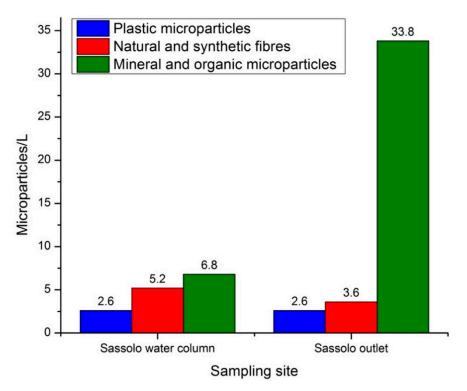
### 3. Results

#### 3.1. Water Column

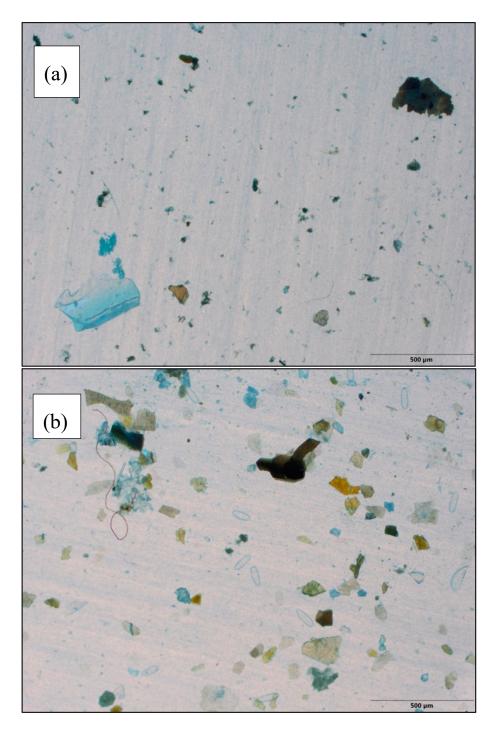
A total of 275 microparticles (including fibres) from both samples (10 L) had a length superior to 125  $\mu$ m in at least one of their dimensions. A majority of these microparticles were of mineral origin, and to a lesser extent composed of organic matter in the case of the main hole sample.

Despite the remoteness of this lake, both water samples contained plastic microparticles and fibres. Many plastic microparticles were recognised during the visual analysis of the samples using the optical microscope, thanks to their shapes (pellets, fragments, films) and colour. It should be noted that smaller microparticles of plastic were also identified, with sizes down to 63  $\mu$ m, but they were not considered in the results since a full analysis was not made.

As shown in Figure 3, 47% of the microparticles (6.8 particles/L) found in the sample of the main hole of the lake (water column) did not have the characteristic to be considered as plastic or fibre and the Infrared (IR) spectrum did not correspond to a plastic spectrum. They were then considered of mineral or organic origin. In the sample of the lake outlet, this percentage increased up to 84% of the microparticles corresponding to 33.8 particles/L. As shown in Figure 4, among the non-plastic microparticles, we found gilded flakes, green particles, and transparent particles.



**Figure 3.** Distribution and microparticle concentrations (microparticles/L) found in the sample of the main hole water column (H) and at the outlet of the lake (O).



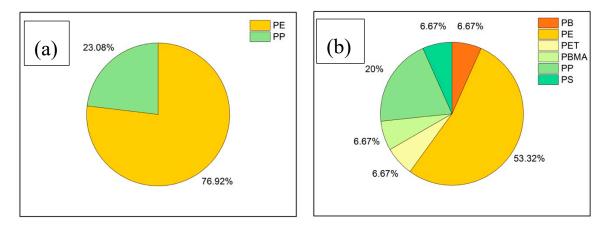
**Figure 4.** (a) Particles deposited on  $Al_2O_3$  filters, observed by optical microscopy in the sample of the lake water column (H) and (b) in the sample of the outlet of the lake (O).

Plastic microparticles were identified in both samples. Around 18% of the microparticles (excluding fibres) of the water column were identified as microplastics (2.6 microplastics/L) after FTIR analysis (Figure 3). On the other hand, 6% of the microparticles (2.6 microplastics/L) were identified as microplastics in the sample of the lake outlet after FTIR analysis. Most of these plastic microparticles were transparent and irregular (like fragments) and only one plastic microparticle had a similar shape of a pellet.

Fibres were easily recognised in the samples. In the sample of the main hole, 36% of the microparticles were fibres (5.2 fibres/L) and in the outlet sample, 9% of the microparticles (3.6 fibres/L)

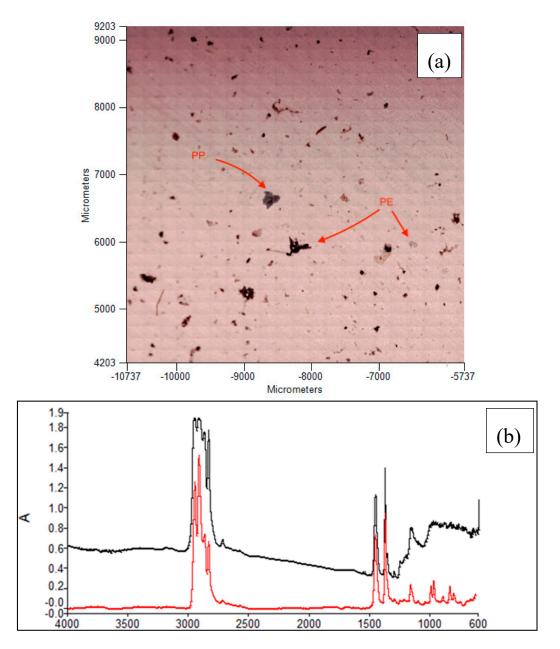
were identified as fibres. However, due to their elongated forms and reduced thickness, most of these fibres were not successfully identified with FTIR. The spectra of some fibres indicated the presence of cellulose and only two fibres in the sample of the outlet of the lake were clearly identified as synthetic fibres (one of polypropylene (PP) and the other of polystyrene (PS)), hence suggesting that 11% of the fibres were synthetic (0.4 synthetic fibre/L).

As seen in Figure 5, the most abundant type of plastic (considering plastic microparticles and fibres) in both samples was PE, with 76.9% (2 microplastics/L) and 53.3% (0.6 microplastic/L) of the microplastics in the water column and outlet of the lake samples, respectively. The second most abundant plastic was PP with 23.1% and 20% (0.6 microplastic/L in each sample). The sample of the outlet also contained various types of plastics like poly (1-butene) (PB), poly (butyl methacrylate) (PBMA), polyethylene terephthalate (PET), and PS (0.2 microplastic/L from each type of polymer). The composition of microplastics in the sample of the outlet of the lake was found to be more heterogeneous than in the sample of the water column.



**Figure 5.** Chemical distribution of microplastics in (**a**) the water column (H) and (**b**) at the outlet of the lake (O).

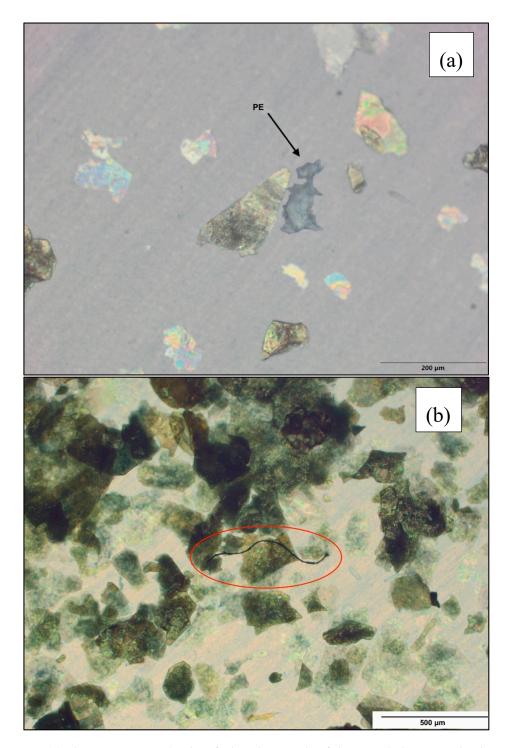
An image of the surface of an aluminium oxide filter of the lake water column sample is shown in Figure 6, in which three plastic microparticles were detected. The spectra shown in this figure illustrate the similitudes of the spectrum of the PP microplastic with the PP reference spectrum. The degree of correlation of this spectrum was 97%, having used the defined spectral range (between 4000 to  $1250 \text{ cm}^{-1}$ ).



**Figure 6.** (a) Image of microparticles using FTIR microscopy from the lake sample. Red arrows indicate the microplastics and (b) spectrum (absorbance versus wavelength in  $cm^{-1}$ ) of a PP microplastic (black) and PP reference spectra of the library (red).

# 3.2. Sediments

Despite the remoteness of Lake Sassolo, two of the three sediment samples contained plastic microparticles: the 8 m depth sample of the main hole and the 14 m depth sample of the cliff. Plastic microparticles were recognised in the visual analysis of the samples, thanks to their shape and different colour from the sediments, as seen in Figure 7. Plastic microparticles were found in the samples in different sizes, shapes, and colours. The concentration of plastic microparticles varied importantly among the different sample sites and depths. The sample of the cliff (14 m depth) presented the highest concentration of plastic microparticles (60 microplastics/kg), whereas in the samples of the main hole, 40 and 0 plastic microparticles per kilogram of sediments were detected at 8 and 33 m depths, respectively.

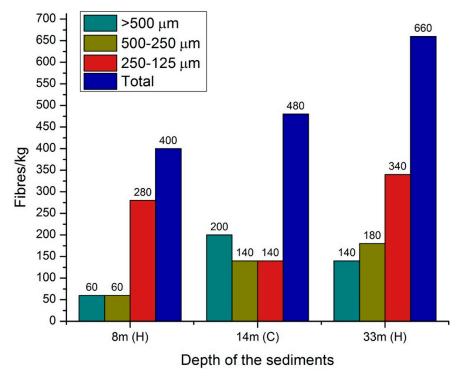


**Figure 7.** (a) Plastic microparticle identified in the sample of the main hole at 8 m depth in the 250–125  $\mu$ m fraction and (b) blue fibre found in the sample of the main hole at 33 m depth in the 250–125  $\mu$ m the fraction.

Only two types of plastic were found in the samples: PE and PP, which is in good agreement with the chemical microplastic composition in the water column. In the 8 m depth sample of the main hole, the only type of plastic found was PE. This polymer comprised one third of the detected plastic microparticles (20 microplastics/kg) in the 14 m sample and the remaining (40 microplastics/kg) was composed of PP.

Despite the fact that no plastic microparticles were identified in the sample of the main hole at the 33 m depth, this sample presented the highest number of fibres. Fibres were simpler to recognise in the

samples than plastic microparticles (as seen in Figure 7b) and were also more abundant in all samples and fractions. Figure 8 indicates that the concentration of fibres increases with the sediment depth. Furthermore, the most important concentrations of fibres (340 fibres/kg) were found in the smallest fraction (125–250  $\mu$ m) in the 33 m depth sample. The 8 m depth also had a significant concentration of fibres (280 fibres/kg) in the smallest fraction. Nevertheless, the determination of the fibres' chemical composition was not possible to perform in either case.



**Figure 8.** Number of fibres per kilogram in each size fraction of the sediment samples in Lake Sassolo indicating a significant contamination. On average, the number of fibres was equal to 514 fibres/kg of sediment.

# 4. Discussion

As shown in Figure 3, the concentration of microplastics (excluding fibres) in the water samples was found to be similar (2.6 microplastics/L). However, as shown in Figure 5, there was a difference in the microplastic composition, which could be due to sample heterogeneity. The sources of microplastics and fibres are unknown, but the number of human activities in the area related to hiking, pasturing, and diving activities could contribute to the microplastic and fibre contamination. On the other hand, it is also possible that these microparticles enter the lake due to wind transport processes [52,53]. Allen et al. (2019) reported microplastic deposition (wet and dry) in a remote mountain location, in which fragments were the most abundant shape of microplastics. They suspected that the source of microplastics could be at least regional (>100 km). For their part, Gonzáles-Pleiter et al. (2020) found fibres in a remote polar fresh water lake and suspected that atmospheric deposition from local and distant sources was the origin of the microfibres [53]. Other studies have proven or modelled the transport of microplastics by winds [54,55] and indicate that the concentration of microplastics could vary from one season to another, depending on the regional human activity and the atmospheric conditions in the region.

Despite no digestion being carried out for the main hole sample, the visual identification of plastic microparticles was straightforward, partly due to the threshold set of 125  $\mu$ m of length. The content of mineral and organic microparticles in the water samples was considered not abundant enough to apply a density separation technique, so as to avoid the loss of microplastics in the sample or to increase the risk of contamination.

Since no one has achieved a unique and standardised operation protocol for sampling or extracting microplastics [56] in sediments, we used the method that gave us the best results in the analysis of other types of samples and in the trials (results not presented). In the trials, we began the analysis of 100 g of sediment and we also used a 63  $\mu$ m steel sieve. However, the quantity of the sample turned out to be unfavourable in the density separation phase, forcing us to reduce the sample weight by half. Moreover, the 63–125  $\mu$ m fraction (used only in the trials) was impossible to analyse optically and to identify chemically, due to the thick layer of sediment that was recovered after the density separation step. This is why we also decided to raise the water threshold.

Hydrogen peroxide  $(H_2O_2)$  (30%) was chosen to digest natural organic matter over other digestion techniques (with strong acids or bases) that could be more aggressive or less convenient [57]. This oxidant was also used in the digestion of compost and less charged samples (waste water and freshwater samples), showing good results in the degradation of organic matter and did not affect the visual identification.

Concerning the separation technique, the use of a significant high density solution is important to correctly separate the most of the microplastics from the sediment particles, since there could be an important loss of microplastics due to the different densities of plastics [58]. For that purpose, a NaI solution was preferred over a NaCl saturated solution. Furthermore, we also realised that the stirring of the sample during the separation process did not really help the microparticles to float or sink. Instead, the microparticles already sedimented were resuspended, even with a minimal movement. We also considered that the aeration of the NaI solution would reduce the solution density and bubbles could trap microparticles, making them float.

The content of organic matter in the three sediment samples was considered to be completely removed after one week of reaction, since there was no more or limited off-gassing. In addition, the density separation process allowed the separation of microparticles with a density <1.7 g/mL and the significantly reduction of sediment content. Nevertheless, the visual identification of plastic microparticles was difficult in some fractions due to the amount of sediments still found in the buoyant layer of the NaI solution, as seen in Figure 9. The density of the NaI solution was verified prior to utilisation to assure a smaller loss of the heaviest microplastics in this process (sinking of microplastic with the sediments). NaI solutions were filtered with fibre glass filters for reuse.



**Figure 9.** Buoyant particles recovered from the sample of the main hole (H) at 33 m depth in the  $250-500 \mu$ m fraction after density separation with NaI.

Concerning fibre contamination, Fisher et al. found on average  $75 \pm 30$  and  $168 \pm 85$  fibres/kg in lake shore sediments in Lakes Bolsena and Chiusi, respectively [60]. They also found bigger concentrations of fibres in the smaller fractions (<500 µm). Nevertheless, the concentration of fibres found in our study was more significant (514 fibres/kg), considering that Lake Sassolo is an alpine lake of 0.05 km<sup>2</sup> and Lake Chiusi is a small, shallow lake of 3.9 km<sup>2</sup>.

Overall, two types of plastics were found as the most important plastic contamination: PE and PP. These plastics were the only types of plastic found in the sediments, but they sum up, on average, circa 85% of the concentration of plastic microparticles in the water samples. It is not surprising that PE was found as the most abundant of plastics, considering that this plastic is the most widely used worldwide [61] for applications in clear food wrap, shopping bags, and microbeads in personal care products and cosmetics. Regarding the PP microplastics, they were mostly present as fragments in all samples, but some fibres of this material were also identified. This plastic is used in several daily products, thanks to the toughness, flexibility, lightweight, and heat resistance of the polymer. Some of the applications of this plastic are packaging, household products, fibres, and fabrics. For instance, the vast majority of hiking backpacks have these materials in some of their parts. Ropes and carpets are also made from these materials.

The identification of fibres was not possible because of their reduced sizes and thickness. The flattening of the fibres was not an option, considering that the isolation of the fibres was extremely hard to realise in the majority of samples. In addition, flattening the fibres over the filter should have meant the fracture of the filter and loss of sample. Furthermore, diffuse reflectance technique DRIFT with KBr powder is considered as a useful method for the identification of fibres [62], but this has a certain complexity and the risk of sample loss or contamination increases.

Our study was about a single and challenging sampling event (regarding diving conditions). Further research should be conducted considering the monitoring of meteorological conditions as well as the spatial and temporal variability of plastic particles and fibres in Lake Sassolo (notably in the water column and in the sediment). Moreover, a further analysis of the fibres using different methods such as Raman spectroscopy and an analysis of smaller fractions by adapting protocols should be considered in future studies. Textile contamination is sometimes undervalued because of the non-synthetic nature of this anthropogenic litter. However, considering that fibres were still present in the samples even after a digestion treatment, this indicates that despite their natural origin, they have followed modifications that allow them to be more persistent, increasing their accumulation in the environment.

#### 5. Conclusions

The findings of this study indicate that even a remote mountain lake is not free of microscale anthropogenic contamination. Indeed, microplastics and fibres were found in alpine Lake Sassolo in the water column and the sediment. Average concentrations in the water column were found equal on average to 2.6 microplastics/L and 4.4 fibres/L. With regard to the analysis of sediments, our results indicated an accumulation of microplastics and fibres in the sediment with on average 33 microplastics/kg and 514 fibres/kg.

From a more prospective point of view, our results indicate that focus is also needed on smaller microplastic fractions instead of mainly considering fractions greater than 300  $\mu$ m. Indeed, the amount of microplastics and fibres increases significantly with smaller sizes. Moreover, all fibres should be considered in future studies, not only those made of plastic, since the amount of natural and synthetic fibres entering the natural environments will keep rising and the risk associated with dyes and adsorption of hazardous pollutants is unknown.

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