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High-Mountain Contamination: Microplastic Occurrence and Risk Assessments in Fish from Nero Lake, Italy

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Abstract

Microplastic (MP) pollution is an emerging environmental concern, yet its occurrence in remote high-mountain ecosystems remains poorly understood. This study investigated MP contamination in fish from Nero Lake, an alpine lake in northwestern Italy. Between 2023 and 2024, a total of 33 specimens of *Salmo trutta*, *Phoxinus lumaireul*, and *Salvelinus fontinalis* were analyzed. MPs were detected in 84% of specimens in 2023 and in 93% in 2024. Filaments were the predominant particle type, while polyethylene, polypropylene, and polyethylene terephthalate were the most common polymers. In 2024, polyamide was also detected and showed the highest Polymer Hazard Index (PHI = 12.22), indicating a high hazard risk (Grade III) and elevated toxicological potential. Contamination Factor values exceeded 10 in *S. trutta*, and Pollution Load Index values frequently surpassed 1, both suggesting established contamination. However, the limited number of specimens, particularly for *P. lumaireul* and *S. fontinalis*, reduces statistical power and increases the risk of Type II errors. Although no significant interspecific differences in MP counts were observed, results should be interpreted with caution. Larger sample sizes are recommended but remain difficult to obtain in alpine environments. These findings highlight the vulnerability of remote lakes to both local and long-range MP pollution sources.

Keywords: alien species; emerging contaminants; high-mountain lakes; Polymer Hazard Index

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1. Introduction

Plastic pollution is widely recognized as a major global environmental threat, with several studies reporting its global increase and negative impacts on a wide range of organisms [1]. Among the various forms of plastic debris, microplastics (MPs), particles smaller than 5 mm, are of particular concern due to their pervasive presence, even in remote

and seemingly pristine environments such as high-mountain lakes [2]. These particles can reach alpine regions through atmospheric transport and deposition, tourism, and the fragmentation of improperly managed plastic waste [3].

One of the major concerns regarding MP pollution is its potential impact on aquatic organisms, particularly fish. Fish can ingest MPs either directly from predation of particles resembling prey species or indirectly through contaminated ones, potentially leading to physiological and ecological consequences. In some cases, translocation from skin to other tissues is possible [4]. Ingested MPs have been found to cause intestinal blockages, oxidative stress, inflammatory responses, and disruptions in feeding behavior, potentially affecting fish health [4].

While MP contamination in fish has been widely reported in rivers and lowland lakes [5], much less is known about high-mountain lakes, particularly those in the Alps [3]. These lakes, typically located above 1500–2000 m depending on latitude, are shaped largely by past glaciation and are usually oligotrophic, with cold, clear, and nutrient-poor waters [6,7]. Their ecosystems are relatively simple, dominated by cold-adapted species, and highly sensitive to environmental change [8]. Although often regarded as pristine due to their remoteness, alpine lakes are increasingly influenced by atmospheric deposition, climate warming, and other human pressures that can disrupt ecological balance and biodiversity [9]. This knowledge gap is critical, as high-mountain lakes represent natural laboratories for examining long-term contaminant exposure, as previously demonstrated for both persistent and emerging pollutants [10]. Moreover, their unique conditions, such as low temperatures, intense UV radiation, and limited direct anthropogenic input, may strongly affect the dynamics of MP transport and accumulation compared with lowland environments [3].

In spite of their widespread occurrence today, fish are not native to alpine lakes and were historically absent from these ecosystems [11]. The earliest introductions in the Alps occurred in the late sixteenth century, but most introductions took place from the 1960s onward in response to the rising popularity of recreational angling [12]. Salmonids such as *Salmo trutta*, *Oncorhynchus mykiss*, and *Salvelinus fontinalis* are now widespread in Italian alpine lakes [11,12].

These salmonid introductions have often led to collateral introductions of other species, particularly cyprinids like minnows (*Phoxinus* spp.), which were used as live bait [12]. The presence of these non-native species offers an opportunity to study environmental contamination. They are usually opportunistic feeders [11] and can serve as bioindicators of MP pollution due to their tendency to bioaccumulate contaminants through trophic interactions [13]. While their introduction has often disrupted native biodiversity, they can serve as tools for environmental monitoring. In alpine lakes, where sampling can be logistically challenging, they provide a practical means of monitoring and evaluating the ecological impacts of MPs. This approach has emerged from other monitoring studies in which alien species such as *Silurus glanis* and *Carassius gibelio* have been shown to bioaccumulate contaminants that reflect the habitat use and trophic ecology [14].

Furthermore, as management often prioritize the removal or control of invasive species, combining the removal of non-native species with the collection of data on MP contamination could represent a dual-purpose strategy, contributing simultaneously to biodiversity conservation and advancing ecological monitoring efforts.

However, these benefits must be carefully balanced against ecological risk. Using alien species as bioindicators could unintentionally facilitate their spread, so it is important that monitoring programs focus on well-established populations to minimize the ecological cost [14]. In addition, the distribution of alien species such as *S. fontinalis* and *S. trutta* can facilitate cross-regional comparisons, providing insights into contamination across diverse

ecosystems. This integrated approach highlights a novel paradigm: invasive species, which are traditionally regarded only as ecological threats, can be repurposed as tools in environmental chemistry and ecological monitoring.

Although MP contamination has been widely quantified, the potential risks posed to human and environmental health remain largely unexplored, highlighting a critical knowledge gap. This study compares MP contamination in fish from two sampling years in Nero Lake, a high-mountain alpine lake located in Piedmont (northwestern Italy). Over a two-year period, MP abundance, shape, color, and polymer composition in fish were analyzed, and polymer-specific hazard indices were assessed. The most common indices used are the Polymer Hazard Index (PHI) [15,16], applied together with the Contamination Factor (CF) and Pollution Load Index (PLI) [17]. PHI classifies polymers according to their chemical composition and associated hazards, thereby providing insights into their potential ecological and human health impacts. Originally developed for sediment contamination studies, CF and PLI have been adapted to evaluate plastic pollution [17]. Specifically, CF quantifies the degree of contamination by comparing the pollutant concentration to a baseline or background value. In contrast, PLI integrates multiple CF values to provide an overall assessment of the system's pollution status. Values of PLI above 1 indicate deterioration in environmental quality. Indeed, the PLI reports how many times the concentration of MPs exceeds background concentrations, representing the overall risk.

Although our analysis focused on the gastrointestinal tract of fish, which is generally not consumed, assessing MP contamination risks remains essential from an ecological point of view. Indeed, MP can serve as carriers for chemical contaminants and pathogenic microorganisms [18], posing risks not only to fish health but also to the entire aquatic ecosystem.

2. Materials and Methods

2.1. Study Area

Nero Lake is a high-mountain lake (2020 m a.s.l.) situated in the upper Susa Valley (44°54′03.89″ N 6°47′40.81″ E), within the municipality of Cesana Torinese (Turin, Piedmont, Italy) (Figure 1). It is incorporated within the protected area "Cima Fournier—Lago Nero" (SCI IT1110058), part of the European Natura 2000 network, established to safeguard habitats and biodiversity. The lake covers an area of approximately 1.65 hectares, has a perimeter of 718 m, and reaches a maximum depth of 6 m. To the south, it borders a relatively large wetland, while smaller wet areas, irregularly distributed along the shoreline, host plant species of conservation interest, thereby enhancing the ecological value of the ecosystem [19]. In the immediate vicinity, there is also a seasonal alpine pasture known as "Lago Nero".

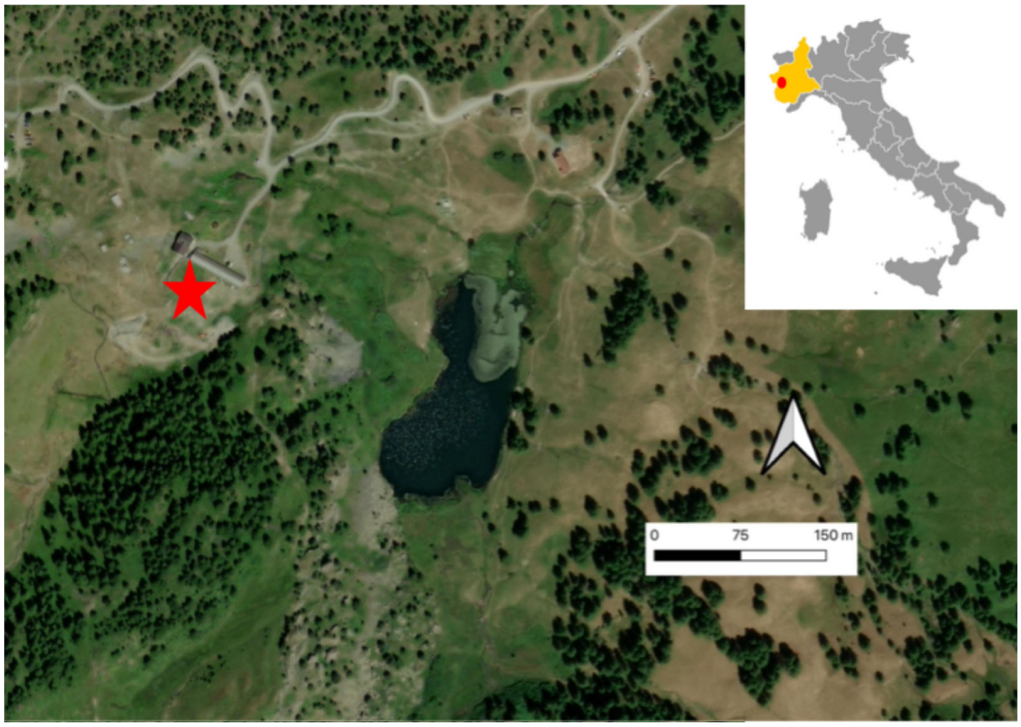


Figure 1. Location of the sampling site (Red star: alpine pasture "Lago Nero").

2.2. Fish Sampling and Processing

Fish samplings were conducted over two years (summer 2023 and 2024) following the protocol described by Volta et al. [20] for fish sampling in lakes. This method includes a single sampling session (21 July and 27 July, respectively) using both benthic (30 × 1.5 m) and mesopelagic (27.5 × 6 m) gillnets, which were set in the evening and retrieved the following morning. The nets consisted of 12 panels, each 2.5 m long, with mesh sizes varying from 5 to 55 mm. At Nero Lake, a total of six nets (i.e., four benthic and two mesopelagic) were positioned according to the bathymetric profile of the lake. All sampled fish were measured for total length and weighed, then transported to the laboratory in refrigerated boxes. In the laboratory, specimens were necropsied on pre-cleaned surfaces. The fish were dissected using a stainless-steel dissecting kit, and the entire gastrointestinal tract was removed, wrapped in aluminum foil and stored at −20 °C until analysis.

2.3. Microplastics Characterization

The gastrointestinal tracts of the fish samples were digested using a saturated solution of KOH and NaOH, followed by sonication at 40 Hz for 20 min at 30 °C. The resulting mixture was then vacuum-filtered through a 6 µm Millipore® (Sigma-Aldrich, St. Louis, MO, USA) fiber filter. These filters were oven-dried at 35 °C until fully dehydrated. Using a stereomicroscope (Nikon P-DSL32; NS-Elements D.4.60, 64-bit, Tokyo, Japan) at 10–80× magnification, the filters were examined, and target particles were analyzed. All potential target particles were then analyzed in reflection mode with FT-IR spectroscopy (Nicolet iN10, ThermoFisher Scientific, Waltham, MA, USA). Spectral matches were assessed by comparing the obtained spectra to both standard and weathered microplastic libraries (OMNIC™ Picta™ software `v1.7.192`, ThermoFisher Scientific, Waltham, MA, USA) as well as to internal reference libraries from the Bioscience Research Centre (Fonteblanda, GR, Italy). The detection limit for particle size was 10 µm, and spectral matches were considered from 80%.

2.4. Quality Assurance and Quality Control

The QA/QC approach followed in this study is summarized according to the main criteria reported in the literature [21]. The Bioscience Research Centre (BsRC) is an ACCREDIA-certified laboratory (accreditation n. 01403) for conducting MP analyses (test method: IO 004.31) in various matrices, including biota, and ensures that the laboratory environment, equipment, and procedures are appropriate to minimize sample contamination and improve the quality of the analytical process. Sample processing was conducted under air-controlled conditions to minimize airborne microplastic contamination, using a clean chamber equipped with a HEPA-filtered laminar-flow fume hood and a glove box (Iteco engineering, mod. SGS20-13599, serial number 103421), which guarantees the absence of contamination from air and other external sources. All samples were processed entirely in the glove box. The materials, equipment, laboratory surfaces, and gloves were carefully cleaned after the analysis of each sample. The water and reagents used to rinse and extract the samples were pre-filtered through 0.45 µm cellulose acetate filters (Millipore®, Darmstadt, Germany). The absence of airborne contaminants was monitored by exposing blank fiber filter disks (n = 5) inside the glove box and by including blank samples (n = 5) processed through the entire workflow as negative controls. Blanks prepared by treating the extraction solutions as samples confirmed the absence of contamination. In addition, positive controls with targeted MP particles (n = 3) were subjected to the same treatment as the tested samples, achieving a 100% recovery rate. The instrument (µFT-IR; Nicolet iN10 MX, ThermoFisher Scientific, Waltham, MA, USA) was qualified annually by a Thermo Fischer® technician, and calibration (pass/fail) was verified every six months by a BsRC-qualified researcher using a reference polystyrene standard (Thermo®, Instrument Qualification Kit). Before daily analyses, certified plastic particles (PP, PE, PVC, PET) were analyzed and used as internal standards to ensure instrument performance across different polymers before proceeding with the chemical identification of particles from tested samples.

2.5. Risk Assessment of MP Pollution

2.5.1. Polymer Hazard Index (PHI)

The Polymer Hazard Index (PHI) was calculated using the following formula:

$$\mathrm{PHI} = \Sigma \mathrm{Pn} \times \mathrm{Sn}$$

where Sn represents the hazard score assigned to each polymer type and Pn denotes the proportion of each polymer detected in the samples [15]. Following the methodology outlined by Ranjani et al. [16], the calculated PHI values were used to classify the risk levels and hazard categories of plastic debris across all analyzed samples.

2.5.2. Contamination Factor (CF) and Pollution Load Index (PLI)

The Contamination Factor (CF) and Pollution Load Index (PLI) are calculated using the following equations [16,22]:

$$\mathrm{CFi} = \frac{\mathrm{Ci}}{\mathrm{C0}}$$

$$\mathrm{PLI} = \sqrt[2]{\mathrm{CFi}}$$

where Ci is the concentration of MPs found, and C0 is the reference or background concentration. Since background values were unavailable, the lowest concentrations observed in biota samples were used [23]. CF values are categorized into four levels (e.g., below 1 indicates low contamination, values between 1 and 3 moderate contaminations, values from 3 to 6 considerable contamination, and values above 6 very high contamination) [24,25]. A site is considered contaminated if the PLI is greater than 1 [17].

2.6. Statistical Analysis

To assess MP abundance in fish, we analyzed MP count in relation to species, body size metrics (length and weight), and collection year (2023 and 2024). Normality of MP count, fish length, and weight was tested using the Shapiro–Wilk test. As all variables deviated from normality ($p < 0.05$), non-parametric statistical methods were applied throughout.

Differences in MP count among fish species were assessed using the Kruskal–Wallis rank sum test. Differences between years (2023 vs. 2024) were evaluated using the Wilcoxon rank-sum test. Relationships between MP count and fish body length and weight were analyzed separately for each species using Spearman's rank correlation. All analyses were conducted in R (version R 4.5.1) [26] and significance was set at $p < 0.05$.

3. Results

3.1. Sampling

A total of 19 fish were collected in 2023 and 14 in 2024. In 2023, the samples were predominantly composed of brown trout (*Salmo trutta*, n = 18), with a single specimen of minnow (*Phoxinus lumaireul*). In contrast, the 2024 sampling session included six specimens of *S. trutta*, five of *P. lumaireul*, and three brook char (*Salvelinus fontinalis*). All the biometric data (i.e., weight and length) of each specimen are provided in Table S1.

3.2. Microplastics Occurrence and Abundance

In 2023, 84% of the fish specimens (16 out of 19) were found to have MPs in their gastrointestinal tracts (GIT). In 2024, 93% of the specimens were contaminated (13 out of 14). Table 1 presents species-specific contamination rates, mean biometric data, and MPs contamination rates.

Table 1. Mean biometric values and MP contamination rates by species and year (MPs: microplastics, GIT: gastrointestinal tract).

Year	Species	Weight (g)	Length (cm)	N° Specimens	Specimens with MPs in GIT	Contamination Rate
2023	*P. lumaireul*	1.84	6.5	1	0	0
2023	*S. trutta*	196.84 ± 200.11	24.47 ± 7.73	18	16	88.89%
2024	*P. lumaireul*	3.21 ± 1.80	6.98 ± 1.16	5	4	80%
2024	*S. trutta*	609.83 ± 224.19	38.18 ± 3.91	6	6	100%
2024	*S. fontinalis*	121.33 ± 54.37	22.17 ± 3.59	3	3	100%

The Shapiro–Wilk test indicated non-normal distributions for MP count, length, and weight. A Kruskal–Wallis test also revealed no significant differences in MP count among species ($\chi^2 = 4.08$, df = 2, $p > 0.05$). Similarly, a Wilcoxon rank-sum test found no significant differences in MP count between the years 2023 and 2024 ($p > 0.05$). It is important to note that these findings are considered within the context of limited sampling, particularly in the case of *Phoxinus lumaireul* and *Salvelinus fontinalis*, for which the sample sizes were especially limited. The reduced representation of these species has been demonstrated to weaken the sensitivity of the statistical tests.

Spearman's rank correlation analyses revealed a statistically significant positive correlation between MP count and both body length (ρ: 0.44, *p*-value: 0.033) and body weight (ρ: 0.43, *p*-value: 0.038) in *S. trutta* (Figure 2). No correlations were analyzed for *P. lumaireul* or *S. fontinalis* due to the limited sample sizes.

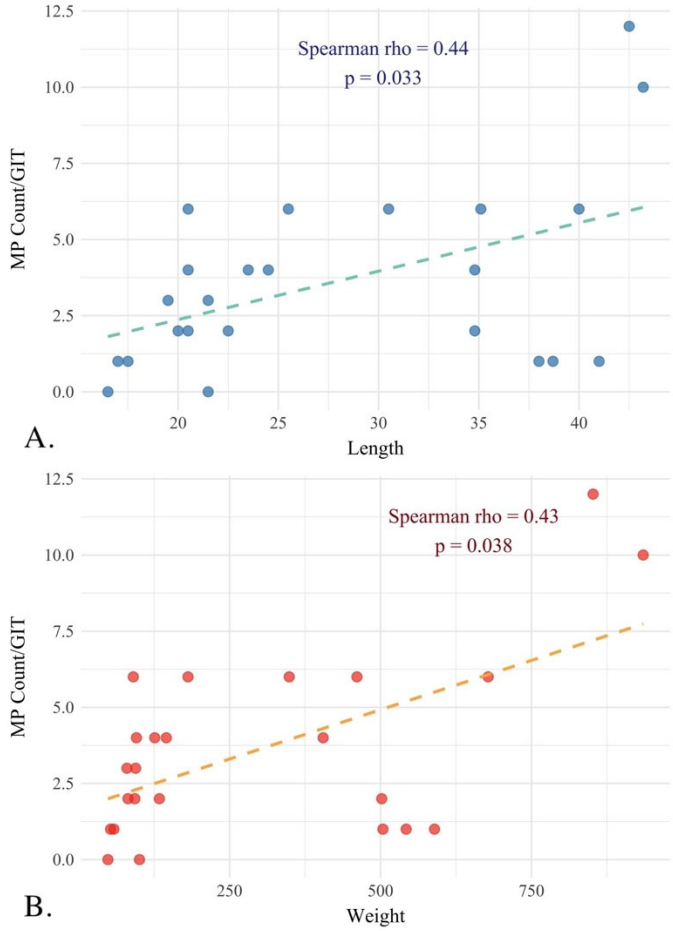


Figure 2. Spearman correlations between MP count and (**A**) body length, and (**B**) body weight in *S. trutta*. Each dot represents an individual fish and the dotted lines indicate the Spearman correlation trend. The correlation coefficients and p-values are displayed in each panel.

3.3. Microplastic Size, Shape, Color, and Polyer Types

The mean particle size increased from 286.43 ± 102.77 µm in 2023 to 309.25 ± 157.47 µm in 2024.

Figure 3 summarizes MP characteristics across years and species. Microplastic shapes varied from filaments and fragments, with an equal presence in 2023 and a slight difference in 2024 (39% fragments and 61% filaments). In 2023, *S. trutta* was the only species found with MPs and showed an approximately equal distribution of filaments and fragments. In 2024, filamentous MPs were slightly more prevalent across all species. Color composition in 2023, based solely on *S. trutta*, was limited to white (35%), blue (29%), and black (37%) particles. In contrast, 2024 samples exhibited greater diversity, including green particles (20%), and a more balanced distribution among the other colors among species.

Regarding polymer type, polypropylene (PP) and polyethylene (PE) were the dominant polymers in *S. trutta* in 2023 (34.6% both), followed by polyethylene terephthalate (PET, 30.8%). A different trend was observed in 2024, with an equal distribution of PP, PE, and polyamide (PA, 26.2%), with a decrease in PET (21.3%).

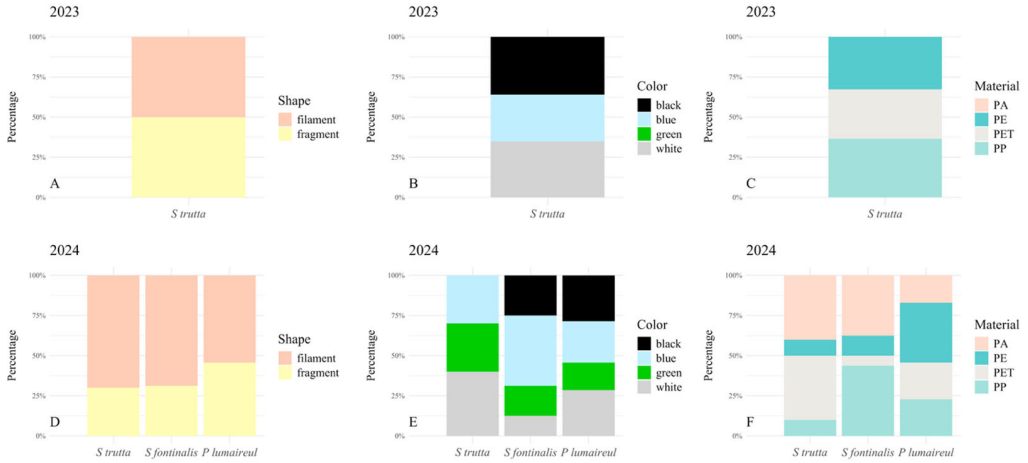


Figure 3. Proportional breakdown (%) of microplastic characteristics (shape, color, and polymer type) in fish species across 2023 and 2024. Panels (**A**–**C**) show MPs in *S. trutta* (2023), and panels (**D**–**F**) show MPs in *S. trutta*, *S. fontinalis*, and *P. lumaireul* (2024).

3.4. Risk Assessment

The risk assessed in our study using the Polymer Hazard Index (Figure 4) ranged from 0.26 to 12.22, encompassing multiple hazard categories across different polymer types, from Grade I (<1) to Grade III (10–100). The highest index value was associated with PA (PHI = 12.22), while lower values were observed for PE (PHI = 3.63 in 2023 and 2.86 in 2024), PP (PHI = 0.37 in 2023 and 0.26 in 2024), and PET (PHI = 1.2 in 2023 and 0.84 in 2024).

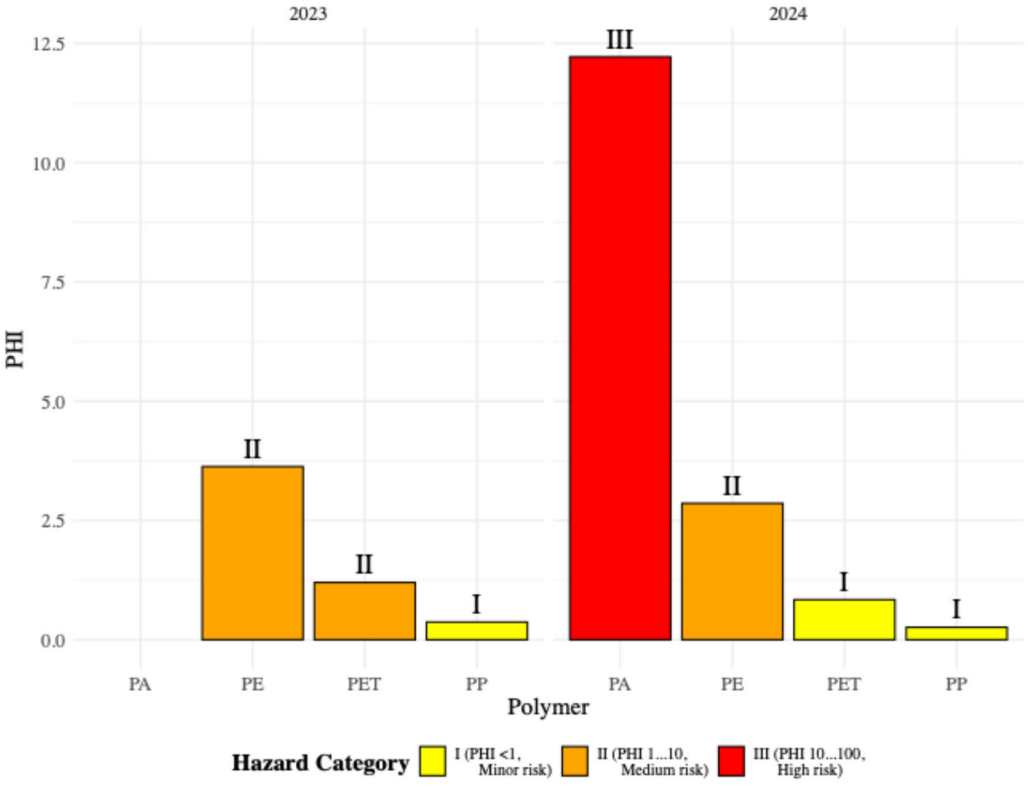


Figure 4. Polymer Hazard Index (PHI) used for the potential health risks associated with plastic particles.

Polymer-specific risk was assessed using the Contamination Factor (CF) and Pollution Load Index (PLI) (Figure 5, Table S2). In *S. trutta* (2023), CF values varied, with several individuals exhibiting CF above 3, indicative of high MP accumulation. PLI values ranged from slightly below to slightly above the threshold of 1. In 2024, *S. trutta* exhibited markedly

elevated CF values, with some individuals exceeding CF values of 10. PLI values were more variable. In *P. lumaireul*, CF values ranged from 1 to 4, and PLI values generally met or exceeded 1, indicating moderate contamination. *S. fontinalis* exhibited comparable CF values (1–4) and PLI values between 1 and 2, suggesting the presence of MPs but lower accumulation compared to *S. trutta*.

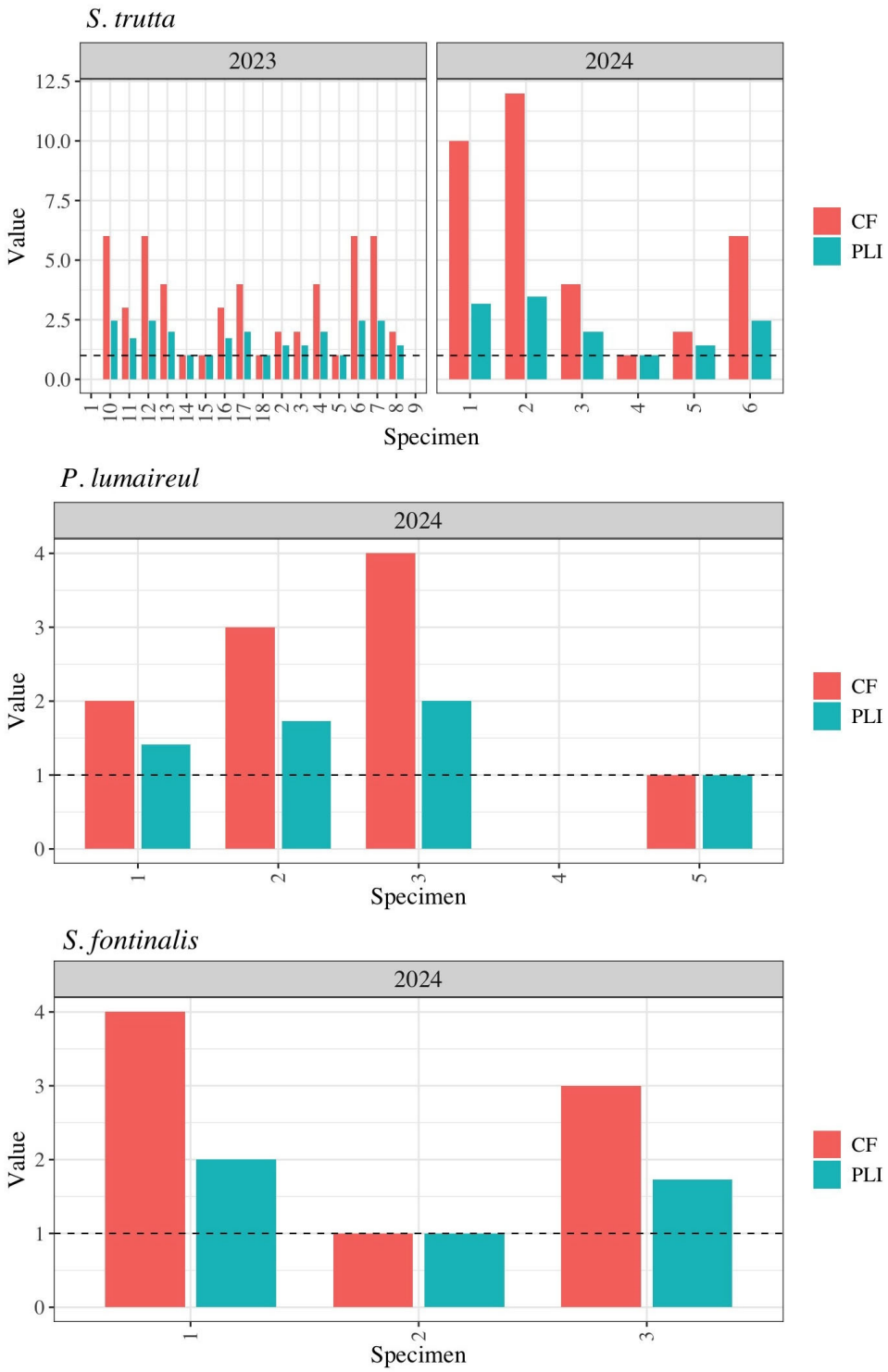


Figure 5. Contamination Factor (CF) and Pollution Load Index (PLI) are shown for the following species, from top to bottom: *Salmo trutta*, *Phoxinus lumaireul*, and *Salvelinus fontinalis*. Dotted lines represent the reference value of 1; PLI values above 1 indicate deterioration in environmental quality.

4. Discussion

4.1. Abundance and Characteristics of Plastic Particles

The present study reveals a high prevalence of MP contamination in the gastrointestinal tracts (GIT) of fish from the alpine Nero Lake across two years. While sampling protocols remained consistent, the observed differences in MP counts between 2023 and 2024 may reflect natural interannual variability or chronic environmental MP inputs. However, due to limited data, these differences should be interpreted as observations rather than evidence of a temporal trend. This difference may also be linked to seasonal tourism, atmospheric deposition, or snowmelt-driven MP runoff, all of which are plausible MP sources in alpine environments, as reported in previous studies [13]. However, it is important to note that these sources were not directly measured in this study, and their role remains speculative. *Salmo trutta* exhibited high levels of MP contamination across both years. Larger specimens showed higher MP loads, as confirmed by the positive size-related correlation. This pattern may be related to long exposure time and trophic transfer of MPs from prey. As a species frequently introduced for recreational angling in Alpine regions [27], *S. trutta* may serve both as a sentinel organism and a potential vector for MP transfer within aquatic food webs and to human consumers. In contrast, *S. fontinalis*, another introduced species, was only recorded in 2024 and displayed 100% MP contamination in all specimens. Despite the small sample size, this finding is concerning. Given its opportunistic diet and potentially different feeding strategies when coexisting with other non-native salmonids, its benthic foraging behavior may have increased its exposure to resuspended MPs within lake sediments [28]. Its presence also reflects anthropogenic influence on fish stocking practices, which not only impacts native biodiversity but may also amplify MP exposure risk in alpine ecosystems. Moreover, *P. lumaireul* showed a lower contamination rate; however, the small and uneven sample sizes across species significantly limit the statistical power of interspecific comparisons. Only one specimen of *P. lumeireul* was collected in 2023 and five in 2024, while *S. fontinalis* was only recorded in 2024 with three specimens. Such limited sampling constrains the reliability of statistical analyses and increases the risk of Type II errors (false negatives), where true differences may exist but go undetected. Although no significant differences in MP counts across species were observed, these findings should be interpreted with caution, as the study may be underpowered to detect real interspecific variation. Larger sample sizes are generally recommended to achieve more robust and reliable results; however, obtaining more than 50 individuals per species, considered an appropriate sample size threshold in MP research [29], is challenging in alpine lakes due to the ecological constraints and logistical difficulties inherent to such environments.

Beyond abundance, qualitative changes in MP characteristics between years suggest evolving pollution sources. In 2023, MPs isolated from *S. trutta* were predominantly blue, black, and white, colors previously reported in other alpine freshwater systems [30]. In 2024, a broader range of particle colors was detected, likely reflecting shifts in local pollution sources. Colored filaments (e.g., red, green, blue) are often associated with synthetic textiles, ropes, or fishing gear, while white particles typically originate from packaging [3]. It is important to note that visual characteristics such as color are not sufficient to suppose the origin of MPs. Polymer analyses provide more reliable information on the chemical composition, which can better indicate potential sources. The proximity of Nero Lake to tourist trails and vehicle access points (i.e., the alpine pasture) suggests a strong anthropogenic footprint. Technical clothing worn by hikers, for instance, is a known contributor to microfiber pollution in high-altitude environments [31]. This is also supported by the high presence of filaments over the years, which are usually indicative of secondary MPs [32]. Other particle types observed (i.e., fragments) are likely secondary MPs

as well, resulting from the degradation of larger plastic items, whereas spheres (if present) would be classified as primary MPs originating from manufactured microplastic products.

The polymer composition was mainly composed of polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), three of the most common polymers found in aquatic environments worldwide [33], as well as in alpine lakes [34] and even in residual snow from high-mountain ecosystems [35]. These polymers, while generally considered with low chemical toxicity, can act as carriers for contaminants, thereby increasing their ecological impact [36]. Interestingly, polyamide (PA) was found only in 2024. PA is commonly derived from fishing lines, toothbrushes, and textiles [37] and has been documented as an atmospheric fallout particle in French regions [38]. It is not uncommon to find this polymer in remote ecosystems as it was detected in Artic regions [39] and in high altitude lakes in Himalaya [31]. Its presence in our fish samples suggests a chronic and possible increase in the input of higher-risk polymers into the Nero Lake system. Moreover, PA has been linked to neurotoxic effects in fish species, such as *Carassius carassius* [40], raising further concerns about physiological impacts.

The presence of MPs across multiple species, trophic levels, and years highlights the pervasiveness of MP pollution in alpine freshwater ecosystems. These findings underscore the vulnerability of high-altitude lakes, which receive inputs not only from local anthropogenic activities but also from long-range atmospheric transport.

4.2. Risk Assessment of Plastic Particles

Even though no standardized framework exists for microplastics risk assessment, several studies have applied risk index-based hazard grading to estimate potential harm based on polymeric composition [15,16,41]. In our study, the plastic pollution risk index ranged from 0.26 to 12.22, corresponding to hazard categories from Grade I (values < 1) to Grade III (10–100). Polyamide exhibited the highest hazard score and was identified as potentially harmful to human health, whereas other polymers posed comparatively minor risks.

These values were lower than those reported for commercial species, which reached PHI values up to 8403.78 [42], and those documented in marine fish muscles and gastrointestinal tracts (0.17–4569.52) [41] or Antarctic species (4.8–1480.2) [43]. Despite being lower, our results may be concerning given the physical behavior of microplastics, which can influence the environmental fate and potential long-term contamination.

To further evaluate ecological risk, we applied the Contamination Factor (CF) and Pollution Load Index (PLI). CF values, which in our study quantify contamination relative to a background level, ranged from 0 to 12. Values above 6 are typically considered indicative of severe contamination in aquatic ecosystems [41,44], while PLI values greater than 1 reflect pollution levels exceeding background conditions [45].

In 2023, *Salmo trutta* exhibited CF values frequently above 3 and PLI values near 1, suggesting a moderate but established microplastic contamination. By 2024, CF values in *S. trutta* increased, and PLI values became more variable but often exceeded 1. This species showed the highest CF values, likely reflecting its relatively large body size and broader diet breadth, which may increase exposure through both direct ingestion and trophic transfer. In contrast, *Phoxinus lumaireul* and *Salvelinus fontinalis* displayed lower CF values, indicative of moderate contamination, although their PLI values frequently exceeded 1, suggesting persistent exposure even in smaller-bodied, less predatory species.

5. Conclusions

This study provides the assessments of microplastic contamination and associated risks in fish inhabiting a high-altitude alpine lake in northwestern Italy. Over two years,

microplastics were detected in multiple species, with *S. trutta* consistently exhibiting the highest levels of contamination and risk indicators. Although overall hazard scores were lower than those reported for marine and commercial species, the detection of polymers such as polyamide, which is associated with higher toxicity, raises concern. The combined evidence from the Polymer Hazard Index, Contamination Factor, and Pollution Load Index highlights a scenario of moderate but established contamination in Nero Lake, likely influenced by both local anthropogenic activities and long-range atmospheric inputs.

The high prevalence of MP contamination observed in fish from Nero Lake across both years underscores that alpine lakes, despite their remoteness, are not exempt from global plastic pollution. While an increase in contamination was noted between 2023 and 2024, this should not be interpreted as a definitive trend, given the weak statistical significance and the limited temporal analysis. Instead, it may reflect interannual variability or chronic MP exposure. Given the ecological vulnerability of high-altitude ecosystems and their recreational importance, further monitoring is needed to assess long-term trends and better understand the implications for food webs and human health.

This study represents an initial contribution to characterizing microplastic occurrence and associated risk in Nero Lake. The two-year sampling period offers valuable temporal insight, but longer-term studies are needed to capture broader variability. Sample sizes were constrained by the naturally low fish abundance in alpine systems, which limits the power of interspecific comparisons. Moreover, while polymer types were identified, factors such as chemical additives and nanoplastic fractions were beyond the present scope but represent important avenues for future ecological and toxicological research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ecologies6040064/s1, Table S1: Biometric characteristics (length, weight, and sex) and microplastic (MP) abundance (count per gastrointestinal tract, GIT) of fish species collected from Nero Lake in 2023 and 2024; Table S2: Values of Contamination Factor (CF) and Pollution Load Index (PLI) for each specimen.

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Data Availability Statement: The original contributions presented in this study are included in the article and supplementary materials. Further inquiries can be directed to the corresponding authors.

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