

Peer Review File

Manuscript Title: Plastic debris in lakes and reservoirs

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

This submitted manuscript is a study of microplastics in 38 lakes sampled around the world. The study uses standard protocols to sample so that the data is comparable. Moreover, all samples are processed in the same laboratory to avoid variability among labs. The paper is well-written, and the study seems to be well-done, and thus if revision is granted I only have minor suggestions.

My only concern is regarding the novelty of the manuscript relevant to its suitability for Nature, and I wonder whether it would be better suited for a different journal – like ES&T or another very good journal in the field.

Some methodological questions that should be cleared up in a revision:

Did the authors spectroscopy-correct the data?

What was used to confirm something was plastic – i.e., percent match, and did it have to be a recognizable particle? What did authors do about dyes for example? Since they used Raman it would be good to have a bit more info on this.

How did they blank-correct? i.e., by total count, relevant to polymer type, etc...

For the RDA, what data was provided in the matrix? It looks like polymer and morphology are there, but separately when that doesn't really make sense in the same vector. i.e., a fragment can also be PE. I would suggest doing only one or the other or combined for that axis. My experience is that trends are more relevant to shape, so maybe focus there only, especially if spectroscopy is from a subsample.

So each site has three replicates? It says, 'the three trawl transects...' but it's not clear if these are replicates? Were they towed side by side, or one after the other, etc..? Were they meant to be reps or capture spatial variability. Also, in your results, do you talk about the within lake variability. I think people would be very interested in this from a method development standpoint for field sampling.

Some line-by-line comments:

In line 72 – I'm not sure this is true anymore, i.e., "until now". San Francisco Bay and the Great Lakes have both had concentrations reported to be above those in the "garbage patches".

Line 197 – this is of course up to the authors, but I would not create two categories of microplastics.

I would just use one, and then show the size distribution in the SI.

Line 213 – I'm not sure I agree that primary microplastics are more largely avoidable than secondary microplastic – if broken down single-use products are in the mix. I do agree for fibers and tire wear particles. Maybe reconsider that statement?

Line 216 – size is also relevant to effect, and maybe moreso than shape or color – do authors mention that? See Mehinto et al., 2022, Thornton-Hampton et al., 2022, or work from Koelmans and colleagues.

Line 228 – if black was most common, did authors note if any were tire rubber? Did they measure that at all? Would be interesting to know 9.

Line 322 – I suggest looking at the work led by Koelmans and/or Mehinto starting around 2019. There are numbers for comparison. Work on risk is no longer in its infancy I don't think.

Referee #2 (Remarks to the Author):

A. Summary of key results: Environmental micro plastic pollution is an issue with lots of momentum and is one of the newer additions, along with PFAS. Micro-plastics are yet another example of humanities signature defacing our environment. The long-term implications are frightening. Although I am not surprised to hear plastic particles were found in all lakes, I am shocked at the plastic particle density in Lake Tahoe (even though it has been reported previously). As the authors point out, the prevalence of microplastics in lakes was described years ago, and the literature is growing.

B. Originality and significance: The unique and important contribution of this manuscript is the coordinated sampling approach scattered around the globe. At present, there is no way to obtain a globally representative sample of the >100M lakes. Instead, the authors have taken a gradient approach to sampling, with gradients being size of the lake and its catchment characteristics. This is an appropriate and necessary next step in the science of lake microplastics. The results shown in Fig. 2A are interesting (but see suggestions for that figure below), as well as the results about concentrations in different systems. It immediately makes me wonder how microplastics may be similar to or different from conservative tracers, such as chloride – both in origin and in how they are processed. I am convinced this could be an important contribution to the literature, and I sense that we are at the beginnings of a new coordinated international effort to study microplastics in lakes. The impact of this work will be in the global patterns and global messaging regarding the unfortunate and indelible signature of humanity on lakes. This reminds me of the wakeup call for the community (IPCC) to include lakes in global carbon budgets.

C. Data and methodology: The unique approach and dataset, coupled with the straight-forward analysis and interpretation, are the highlights of this manuscript.

D. Appropriate use of statistics and treatment of uncertainties: The statistics are simple and appropriate. There is little (or none) inclusion of uncertainties.

E. Conclusions: Given the relatively small sample size (38 lakes), it is difficult to make broad generalizations about drivers of lake microplastics. What is reported in Figures 1 and 2A seem robust.

F. Suggested improvements: My main criticism of the manuscript has to do with the writing. It needs an organizational approach that allows the significance of the findings to shine. I had to work too hard to extract the important points buried in paragraphs. Many examples are listed below.

G. References: Fine

H. Clarity and context (mostly details)

Line 139: The use of “worldwide” is an overstatement here. It will be challenging to find a succinct way to state that 38 lakes were sampled, and these lakes are scattered across the globe. This point is repeated, but more accurately, at the end of the paragraph.

Line 146: I appreciate the need to emphasize the uniqueness of the paper; however, the topic sentence of this paragraph comes across as more hyperbole than informative. Setup of the paragraph’s content would be more appropriate, e.g., the design of the of the sampling effort, which considers lake characteristics, unified sampling protocols, and high-level explanation of the analyses.

Line 163: At this point, the reader will understand that it is an international dataset. The sentence could be more succinct by removing, “Our cross-national dataset showed that.”

Line 175: One of the most important points of the paper, which is the high concentration of plastics in lakes, is buried in this paragraph. While this may be a matter of preference, I think it would be more impactful to separate the implications from the findings. Line 169 would be a natural place to split the paragraph.

Line 186: This topic sentence could be stronger by making it more about the findings than the methods.

Line 197: Similarly to comments above, the real news is buried in the paragraph. A topic sentence that includes the news (e.g., the nature of the fibers suggest a link between their source and their pathways to lakes).

Lines 216 and 221: Use that precious space in the topic sentence for stating something other than the obvious. The statements, “Shape is also relevant...” and “Colour is...also relevant...” while true, are too general for supporting the contributions of the manuscript.

Line 235: Presumably, the camouflage nature of blue applies to aquatic systems. Example, “...blue is a common camouflage colour in [lakes]...”

Line 261: Remove redundancy by deleting “possible.”

Line 246: This is a very long paragraph, and I am now seeing a pattern in the presentation of results/discussion in which the first half of the paragraph is results (with methods sprinkled in) and the second half is discussion. In this paragraph, there is a natural split on line 255.

Line 272: The metaphor of lakes as “conveyor belts” does not quite make sense to me. Streams and rivers seem like conveyor belts, but lakes seem more like storage and processing sites.

Line 277: Again, a very long paragraph, which could be split at either/both lines 283 and 291. The topic sentence is a method. See previous suggestions for stronger topic sentences.

Line 291: Regarding point 2 for the vulnerability of lakes, I am not convinced there are data indicating human exploitation. While most ecologists would agree it’s likely true, such a statement needs to be supported with evidence.

Line 303: Similar to previous points about topic sentences – is the news really that all lakes have plastic? I think it is more that lakes may be “sentinels of plastic pollution,” and this issue demands additional study.

Finally, the last 3 paragraphs of text could be combined into a shorter and punchier series of points about why microplastics in lakes deserves more attention.

Figures

Figure 1: Larger text in all panels, please. In “a” the dot sizes all look the same to me.

Figure 2: For (a) I find the combination of text in the colored boxes and yes/no answers to be a confusing combination. To route my brain through high population density and high lake area, I had to answer “no” twice. That’s weird. Why not split each of those boxes and eliminate the yes/no? For example, one box as “Population <24.6 inhabitants km⁻²” and a second box as “Population >24.6 inhabitants km⁻².”

Methods

Line 524: So what was the value for n?

Referee #3 (Remarks to the Author):

· Key results: Please summarise what you consider to be the outstanding features of the work. This is a very interesting paper wherein 38 lakes and reservoirs were sampled at their outflows with a standardized procedure to collect and characterize plastic particles less than 250 µm in diameter. Studies of plastics in oceans have already been conducted, but lakes and reservoirs also play an important role in the plastic pollution cycle. The results indicate that lakes in densely populated and

urbanized areas and very large lakes/reservoirs are more likely to have higher concentrations of plastic fragments. While the results are not surprising, this work shines a light on the important problem of plastic accumulation in lakes and the ecosystem impacts that such pollution can cause.

· Validity: Does the manuscript have flaws which should prohibit its publication? If so, please provide details.

See concerns as detailed in the comments that follow. There is no major overarching fundamental flaw in the work that would prevent publication.

· Originality and significance: If the conclusions are not original, please provide relevant references.

On a more subjective note, do you feel that the results presented are of immediate interest to many people in your own discipline, and/or to people from several disciplines?

I believe that the results would be of interest to a general audience.

· Data & methodology: Please comment on the validity of the approach, quality of the data and quality of presentation. Please note that we expect our reviewers to review all data, including any extended data and supplementary information. Is the reporting of data and methodology sufficiently detailed and transparent to enable reproducing the results?

There is one major drawback of the study in terms of its study design, and the authors may be able to address it. The authors do not describe how the 38 lakes/reservoirs were selected, so there could be some selection bias in this sample such that the conclusions reached for these lakes/reservoirs cannot be applied to the population of lakes/reservoirs throughout the world. They note that there is overrepresentation in their sample of lakes/reservoirs in the northern hemisphere and Europe.

Furthermore, the wide range of variability in the features of these 38 lakes/reservoirs (see lines 147-149) makes me question whether or not this sample size is large enough to be representative of all lakes and whether or not the analysis of this set of lakes is skewed towards those with extreme features. Are the features of the sampled lakes listed on lines 464 to 468 representative of all lakes?

In terms of reproducibility, the authors state that the data and code are available at

https://osf.io/du3sc/?view_only=858cbb89d75c4ec2a530c234401cb798.

I checked this website, and the code requires a dataset called Lakes final dataset REV.csv, but this file is not in the folder given by the authors, so I cannot confirm that the figures generated by the R script match what is in the paper. Furthermore, the authors state that they use the R package RamanMP (line 541) to analyze the polymer spectra, but this code is not provided.

· Appropriate use of statistics and treatment of uncertainties: All error bars should be defined in the corresponding figure legends; please comment if that's not the case. Please include in your report a specific comment on the appropriateness of any statistical tests, and the accuracy of the description of any error bars and probability values.

How was the regression tree fit and selected? In the R code provided, the regression tree was fit to all of the data once, and there was no testing and training set and no leave-one-out-cross-validation. How small was the error of the reported tree in Figure 2? Were there any other possible trees with similar error but different variables chosen and different splits?

In the extrapolation of the total number of plastics to the entire lake (see lines 297-301), no uncertainty metric is presented along with the estimate of 817 billion pieces of plastic. The authors do acknowledge that they are ignoring spatial variability and plastics in sediments, but given that the samples that they took were at the outflows of the lakes, and the fact that the concentration of plastics is likely to be greater here, this is likely to be a gross overestimate, and it is misleading.

One line 522, please be specific in terms of what proportion you are estimating. I assume that it is the proportion of plastics with a particular chemical identity.

On line 524, what is N set to be equal to? The authors state that it is the global population size.

The description of Variance Inflation Factor on line 572 is incorrect. The VIF is used to remove variables that are highly correlated to the remaining variables in the candidate set, not to “further remove the autocorrelation of independent variables,” which is a contradictory statement in and of itself.

· Conclusions: Do you find that the conclusions and data interpretation are robust, valid and reliable?

Lines 234-237: The authors suggest that red plastic may have been less frequently observed in their sample because they are more visible and were likely ingested. Another reason that red plastic may have been less frequently observed is simply that it occurs less frequently in the waste stream cycle and was already present in lower proportions than other colors.

Lines 255-256: The statement “The effect of human population density on the total concentration of plastic is self-evident.” is too strongly worded. One must remember that this sample is likely to be strongly biased.

Line 303-304: The word “affect” should be changed to “are linked to”. The authors have evidence from their sample indicating a relationship, but to say that one things

effects another ignores the uncertainty in the sample and the data.

· Suggested improvements: Please list additional experiments or data that could help strengthening the work in a revision.

None to suggest.

· References: Does this manuscript reference previous literature appropriately? If not, what references should be included or excluded?

As far as I can tell, yes.

· Clarity and context: Is the abstract clear, accessible? Are abstract, introduction and conclusions appropriate?

· Inflammatory material: Does the manuscript contain any language that is inappropriate or potentially libelous?

No.

Minor Comments:

1. Abstract, Page 3, line 119: ... likely due to of their elevated ... → ... likely due to their elevated ...
2. Line 284: I believe that the reference of Fig. 2c should be Fig. 2b.
3. Figure 1a: The dots are all the same size, but the captions says that they are sized differently.
4. Figure 1b: The word “boxplot” is spelled as both “boxplot” and “box plot” in the caption.
5. Figure 1c: When printed, the text is too small to read, and when I zoom in on the pdf, the resolution of the figure is so poor that some of the words are not legible.
6. The sentence on line 492 is repeated verbatim on line 495.

Author Rebuttals to Initial Comments:

Referee #1:

This submitted manuscript is a study of microplastics in 38 lakes sampled around the world. The study uses standard protocols to sample so that the data is comparable. Moreover, all samples are processed in the same laboratory to avoid variability among labs. The paper is well-written, and the study seems to be well-done, and thus if revision is granted I only have minor suggestions.

My only concern is regarding the novelty of the manuscript relevant to its suitability for Nature, and I wonder whether it would be better suited for a different journal – like ES&T or another very good journal in the field.

Reply: We would like to thank the Reviewer for the comments on our manuscript. Before this study, our understanding of the prevalence of microplastic in freshwaters was mostly based on compilations of individual, non-standardized, and locally contingent studies, often dismissed on methodological grounds, failing to capture global attention to this important topic. Our study is unique as it presents the first coordinated, standardised dataset of the prevalence of microplastics in lakes and reservoirs globally. The implications of our findings will be key to setting the basis for a new international network on global microplastic pollution in freshwaters. All the samples have been analysed by the same laboratory and this, to the best of our knowledge, has not been completed to date. We obtained fully standardised data, and we were able to draw unprecedentedly robust conclusions. To date, only meta-analyses and reviews have compared microplastics in freshwaters on a global scale, and each of these studies strongly emphasised the lack of reliable, standardised, and consistent analytical methods (e.g., Guo et al., 2021; Li et al., 2020). Consequently, a truly comparative study is still missing in the field. Our effort, taking advantage of a collaborative network existing among limnologists, created valuable results that can contribute towards a global understanding of microplastics and human impacts on lakes and reservoirs, and also shows an avenue for future studies, as the dataset can be easily supplemented with more lakes in the future. Indeed, this paper not only provides a dataset, but it also demonstrates that the sampling protocol can be successfully used by people anywhere to obtain standardised results. In addition, this dataset can serve as a basis for several follow up studies to better analyse spatial and temporal trends.

We have also highlighted the drivers of plastic pollution taking into consideration the morphometric features of lakes and watersheds. As indicated by Talbot and Chang (2021), few previous studies have addressed the role of physical watershed characteristics on plastic pollution. We believe that our results fill key knowledge gaps by identifying drivers of anthropogenic pollution to lakes and reservoirs and emphasises that plastic debris is accumulating in lakes/reservoirs and not transporting plastics to the oceans. Our findings also showed that even in remote areas away from direct human pressure, no lake can be considered to be truly 'pristine' from plastic pollution, highlighting the relevance of addressing plastic pollution in lentic systems.

We stressed these novelty aspects in the revisions to the text (see lines 178-183, 193-199). Moreover, we provided a more precise characterisation of our study sites as compared to lakes worldwide to show how our dataset is representative of lakes globally (see Extended Data Fig. 1).

Some methodological questions that should be cleared up in a revision:

Did the authors spectroscopy-correct the data? What was used to confirm something was plastic – i.e., percent match, and did it have to be a recognizable particle? What did authors do about dyes for example? Since they used Raman it would be good to have a bit more info on this.

Reply: We added more details to explain the procedure adopted for the identification of microplastics using Raman spectroscopy in the method section (see lines 558-561, 591-600). In particular, we described the sequential steps that we adopted to visually identify the suspected plastics, verify the visual classification by Raman spectroscopy, and estimate the percentage of occurrence of the different polymers. We also clarified the procedure adopted when the dye was detected and how we handled the so-called ‘anthropogenic’ fibres (see lines 597-600).

How did they blank-correct? i.e., by total count, relevant to polymer type, etc...

Reply: We randomly removed particles that matched the shape, colour, and polymeric composition of the blank particles. We added this information in lines 626-629.

For the RDA, what data was provided in the matrix? It looks like polymer and morphology are there, but separately when that doesn’t really make sense in the same vector. i.e., a fragment can also be PE. I would suggest doing only one or the other or combined for that axis. My experience is that trends are more relevant to shape, so maybe focus there only, especially if spectroscopy is from a subsample.

Reply: The matrix used for the RDA contained information about the abundance of various polymers, as well as the shapes and colours of microplastics in each lake. We considered shape and polymeric composition to be the most relevant information for analysing the contamination in the lakes and understanding the relationships between variables. Our RDA analysis revealed a correlation between microplastic fragments and PE and PP polymers, while fibres were primarily made of polyester. However, we agree with the referee’s comment that it can be more useful to focus only on the shape feature and we re-performed the RDA analysis including only shape as a factor (see Fig. 1 and Extended Data Fig. 4). This has not qualitatively changed the distribution of the lakes in the ordination space because the variables shape and polymeric composition were highly correlated. We have also changed the description in the results and the method section accordingly (see lines 347-354, 671-673).

So each site has three replicates? It says, ‘the three trawl transects...’ but it’s not clear if these are replicates? Were they towed side by side, or one after the other, etc..? Were they meant to be reps or capture spatial variability. Also, in your results, do you talk about the within lake variability. I think people would be very interested in this from a method development standpoint for field sampling.

Reply: As the reviewer correctly pointed out, we collected three replicates for each lake. The replicates were collected to cover a larger area and obtain greater spatial representativeness. We agree with the referee that among-replicate variability is relevant information. Therefore, we added this information at lines 240-247. In addition, we added quantitative information about the variability among the replicates in the Extended Data Figure 2.

In line 72 – I’m not sure this is true anymore, i.e., “until now”. San Francisco Bay and the Great Lakes have both had concentrations reported to be above those in the “garbage patches”.

Reply: We agree that the sentence can be misleading, and we have removed “until now”.

Line 197 – this is of course up to the authors, but I would not create two categories of microplastics. I would just use one, and then show the size distribution in the SI.

Reply: We agree with the referee’s comment, and we included only one category of microplastics (see lines 564-566). We decided to adopt the size category that is more commonly used (i.e., particles smaller than 5 mm), which is also adopted at the policy level (see for instance ECHA, 2019). The size distribution for the different shapes is reported in Fig. 1d. We added the overall size distribution for the different lakes in the Extended Data Figure 5.

Line 213 – I’m not sure I agree that primary microplastics are more largely avoidable than secondary microplastic – if broken down single-use products are in the mix. I do agree for fibers and tire wear particles. Maybe reconsider that statement?

Reply: We agree with the referee’s comment that the sentence was not clear. We have changed the sentence to better highlight that primary microplastics are less frequently found in aquatic systems and that their concentration is expected to decrease as a result of policies that are banning, in some countries, single-use plastics (see lines 275-276).

Line 216 – size is also relevant to effect, and maybe moreso than shape or color – do authors mention that? See Mehinto et al., 2022, Thornton-Hampton et al., 2022, or work from Koelmans and colleagues.

Reply: We agree with the referee’s comment, and we have changed the text accordingly (see lines 284-286). We have also included the references suggested.

Line 228 – if black was most common, did authors note if any were tire rubber? Did they measure that at all? Would be interesting to know.

Reply: We measured 537 Raman spectra of black particles, and we were not able to identify particles amenable to tire rubber (considering polymeric composition and shape). Moreover, the majority of black particles were fibres. Presumably, the main reason that can explain the lack of tire rubber is the dimensional range we have adopted ($>250\text{ }\mu\text{m}$). Indeed, the size distribution of collected tire rubber ranges between $5\text{ }\mu\text{m}$ and $220\text{ }\mu\text{m}$ with a maximum abundance at $70\text{--}80\text{ }\mu\text{m}$ (Kreider et al., 2010).

Line 322 – I suggest looking at the work led by Koelmans and/or Mehinto starting around 2019. There are numbers for comparison. Work on risk is no longer in its infancy I don't think.

Reply: We agree with the referee's comment, and we changed the text accordingly (lines 376-380), also including the references provided.

Referee #2:

Summary of key results: Environmental micro plastic pollution is an issue with lots of momentum and is one of the newer additions, along with PFAS. Micro-plastics are yet another example of humanities signature defacing our environment. The long-term implications are frightening. Although I am not surprised to hear plastic particles were found in all lakes, I am shocked at the plastic particle density in Lake Tahoe (even though it has been reported previously). As the authors point out, the prevalence of microplastics in lakes was described years ago, and the literature is growing.

Originality and significance: The unique and important contribution of this manuscript is the coordinated sampling approach scattered around the globe. At present, there is no way to obtain a globally representative sample of the $>100\text{M}$ lakes. Instead, the authors have taken a gradient approach to sampling, with gradients being size of the lake and its catchment characteristics. This is an appropriate and necessary next step in the science of lake microplastics. The results shown in Fig. 2A are interesting (but see suggestions for that figure below), as well as the results about concentrations in different systems. It immediately makes me wonder how microplastics may be similar to or different from conservative tracers, such as chloride – both in origin and in how they are processed. I am convinced this could be an important contribution to the literature, and I sense that we are at the beginnings of a new coordinated international effort to study microplastics in lakes. The impact of this work will be in the global patterns and global messaging regarding the unfortunate and indelible signature of humanity on lakes. This reminds me of the wakeup call for the community (IPCC) to include lakes in global carbon budgets.

Reply: We would like to thank the reviewer for the valuable feedback on the paper. The referee's sentence effectively encapsulates the main message we aimed to convey with this paper and we incorporated it in the text (see lines 391-393).

Data and methodology: The unique approach and dataset, coupled with the straight-forward analysis and interpretation, are the highlights of this manuscript.

Appropriate use of statistics and treatment of uncertainties: The statistics are simple and appropriate. There is little (or none) inclusion of uncertainties.

Reply: We added uncertainty values to all the data reported in the result section (e.g., see lines 250, 253, 254, 306-307, 592 etc.).

Conclusions: Given the relatively small sample size (38 lakes), it is difficult to make broad generalizations about drivers of lake microplastics. What is reported in Figures 1 and 2A seem robust.

Reply: We acknowledge that the small sample size hinders the ability to make wider generalisations. Therefore, we have revised the text and removed overly broad statements. Our intent is not to make broad generalisations across the lakes.

Suggested improvements: My main criticism of the manuscript has to do with the writing. It needs an organizational approach that allows the significance of the findings to shine. I had to work too hard to extract the important points buried in paragraphs. Many examples are listed below.

Reply: We agree with the referee's comment and have made significant changes to the text to highlight the importance of the findings (see below). We believe that this restructuring has substantially improved the manuscript and increased its overall readability.

References: Fine

Clarity and context (mostly details)

Line 139: The use of "worldwide" is an overstatement here. It will be challenging to find a succinct way to state that 38 lakes were sampled, and these lakes are scattered across the globe. This point is repeated, but more accurately, at the end of the paragraph.

Reply: We agree with the referee's comment, and we have removed the word "worldwide" as suggested (see lines 193-195). As the reviewer correctly pointed out, the geographical scale is adequately addressed in other sections of the manuscript.

Line 146: I appreciate the need to emphasize the uniqueness of the paper; however, the topic sentence of this paragraph comes across as more hyperbole than informative. Setup of the paragraph's content would be more appropriate, e.g., the design of the sampling effort, which considers lake characteristics, unified sampling protocols, and high-level explanation of the analyses.

Reply: We appreciate the referee's comment. We addressed it by revising the opening sentences to better introduce the paragraph's content as suggested (see lines 193-199). We made the text

more informative, and we eliminated the topic sentence, which was more hyperbolic than informative as indicated by the Reviewer.

Line 163: At this point, the reader will understand that it is an international dataset. The sentence could be more succinct by removing, “Our cross-national dataset showed that.”

Reply: We agree with the Reviewer’s comment, and we removed the first part of the sentence as suggested.

Line 175: One of the most important points of the paper, which is the high concentration of plastics in lakes, is buried in this paragraph. While this may be a matter of preference, I think it would be more impactful to separate the implications from the findings. Line 169 would be a natural place to split the paragraph.

Reply: We agree with the Reviewer’s comment. To increase impact, we have emphasised the highest plastic concentrations that we found (see lines 223-228). We have also split the paragraph as suggested (see line 223). In addition, we have underscored these results and their implications in the manuscript's discussion and concluding section.

Line 186: This topic sentence could be stronger by making it more about the findings than the methods.

Reply: We acknowledge and agree with the Referee's comment, and we have revised the paragraph in question. Specifically, we have shifted the focus from methodological aspects to the main findings of our study (see lines 248-256).

Line 197: Similarly to comments above, the real news is buried in the paragraph. A topic sentence that includes the news (e.g., the nature of the fibers suggest a link between their source and their pathways to lakes).

Reply: We agree with the Referee’s comment, and we have changed the paragraph adding a topic sentence as suggested (see lines 261-265).

Lines 216 and 221: Use that precious space in the topic sentence for stating something other than the obvious. The statements, “Shape is also relevant...” and “Colour is...also relevant...” while true, are too general for supporting the contributions of the manuscript.

Reply: We agree with the Referee's comment that the sentences were repetitive and did not contribute significantly to the manuscript's content. To address the comment, we have reworked the paragraph to provide a more relevant and insightful discussion of our results. Additionally, we have included appropriate references from the literature to explain the importance of reporting colour, shape, and size information (see lines 279-289).

Line 235: Presumably, the camouflage nature of blue applies to aquatic systems. Example, "...blue is a common camouflage colour in [lakes]..."

Reply: We agree with the Referee's comment, and we have changed the sentence accordingly (see lines 300-302).

Line 261: Remove redundancy by deleting "possible."

Reply: We agree with the Referee's comment, and as recommended, we have removed the word 'possible' from the sentence (see lines 327).

Line 246: This is a very long paragraph, and I am now seeing a pattern in the presentation of results/discussion in which the first half of the paragraph is results (with methods sprinkled in) and the second half is discussion. In this paragraph, there is a natural split on line 255.

Reply: We agree with the Referee's comment, and we have split the paragraph as suggested (see line 322).

Line 272: The metaphor of lakes as "conveyor belts" does not quite make sense to me. Streams and rivers seem like conveyor belts, but lakes seem more like storage and processing sites.

Reply: We agree with the Referee's comment. In our revision we have removed the comparison to 'conveyor belts' and instead focused on the potential role of lakes as 'traps' for microplastics (see lines 336-340). We have also incorporated relevant references into the text to provide a more robust foundation for our argument.

Line 277: Again, a very long paragraph, which could be split at either/both lines 283 and 291. The topic sentence is a method. See previous suggestions for stronger topic sentences.

Reply: We agree with the Referee's comment, and we have split the paragraph and changed the text to provide a more insightful topic sentence (see lines 347, 355).

Line 291: Regarding point 2 for the vulnerability of lakes, I am not convinced there are data indicating human exploitation. While most ecologists would agree it's likely true, such a statement needs to be supported with evidence.

Reply: We agree with the Referee's comment, and we have removed 'human exploitation' from the sentence and from the abstract because we have not tested for the relationship between large lakes and human exploitation, as the reviewer correctly pointed out (see lines 357).

Line 303: Similar to previous points about topic sentences – is the news really that all lakes have plastic? I think it is more that lakes may be "sentinels of plastic pollution," and this issue demands additional study.

Reply: We agree with the Referee's comment and, as a result, we placed more emphasis on the concept of lakes as 'sentinels of plastic pollution'. We have also added this concept to the abstract (see lines 361-364).

Finally, the last 3 paragraphs of text could be combined into a shorter and punchier series of points about why microplastics in lakes deserves more attention.

Reply: We agree with the Referee's comment, and we have made significant revisions to the last three paragraphs of the manuscript. We attempted to enhance the relevance of our discussion and make it more engaging for readers. We have reworked the text to ensure that it presents a more compelling and insightful analysis of our research outcomes (see lines 361-393).

Figures

Figure 1: Larger text in all panels, please. In "a" the dot sizes all look the same to me.

Reply: We increased the size of the text, and we modified the caption because the dots are all equally sized as correctly noted.

Figure 2: For (a) I find the combination of text in the colored boxes and yes/no answers to be a confusing combination. To route my brain through high population density and high lake area, I had to answer "no" twice. That's weird. Why not split each of those boxes and eliminate the yes/no? For example, one box as "Population <24.6 inhabitants km⁻²" and a second box as "Population >24.6 inhabitants km⁻²."

Reply: We agree with the Referee's comment, and we changed the figure accordingly.

Methods

Line 524: So what was the value for n?

Reply: The value of "n" varied based on the number of particles detected in each lake. To avoid confusion, we included an additional sentence to explain this (see line 586). "Polymer composition was identified on a subset of 2,295 (~25%) particles." This information can be found in the main text at lines 208-209. Data about the characteristics of individual particles and polymer identification for each lake are available in the open dataset linked (see https://osf.io/du3sc/?view_only=858cbb89d75c4ec2a530c234401cb798).

Referee #3:

Key results: Please summarise what you consider to be the outstanding features of the work.

This is a very interesting paper wherein 38 lakes and reservoirs were sampled at their outflows with a standardized procedure to collect and characterize plastic particles less than 250 µm in diameter. Studies of plastics in oceans have already been conducted, but lakes and reservoirs also play an important role in the plastic pollution cycle. The results indicate that lakes in densely populated and urbanized areas and very large lakes/reservoirs are more likely to have higher concentrations of plastic fragments. While the results are not surprising, this work shines a light on the important problem of plastic accumulation in lakes and the ecosystem impacts that such pollution can cause.

Reply: We thank the Reviewer for the positive and constructive comments to the manuscript.

Validity: Does the manuscript have flaws which should prohibit its publication? If so, please provide details.

See concerns as detailed in the comments that follow. There is no major overarching fundamental flaw in the work that would prevent publication.

Originality and significance: If the conclusions are not original, please provide relevant references. On a more subjective note, do you feel that the results presented are of immediate interest to many people in your own discipline, and/or to people from several disciplines?

I believe that the results would be of interest to a general audience.

Data & methodology: Please comment on the validity of the approach, quality of the data and quality of presentation. Please note that we expect our reviewers to review all data, including any extended data and supplementary information. Is the reporting of data and methodology sufficiently detailed and transparent to enable reproducing the results?

There is one major drawback of the study in terms of its study design, and the authors may be able to address it. The authors do not describe how the 38 lakes/reservoirs were selected, so there could be some selection bias in this sample such that the conclusions reached for these lakes/reservoirs cannot be applied to the population of lakes/reservoirs throughout the world. They note that there is overrepresentation in their sample of lakes/reservoirs in the northern hemisphere and Europe. Furthermore, the wide range of variability in the features of these 38 lakes/reservoirs (see lines 147-149) makes me question whether or not this sample size is large enough to be representative of all lakes and whether or not the analysis of this set of lakes is skewed towards those with extreme features. Are the features of the sampled lakes listed on lines 464 to 468 representative of all lakes?

Reply: We thank the Reviewer for the comment and agree that we need to better explain how we selected the study sites, and why we think they are representative of lakes globally. The features of the lakes reported on lines 464-468 (now lines 529-534) are referring to all lakes included in our study.

We are aware of our limited sample size (n=38) compared to the estimates of the number of lakes globally (>1.4 million lakes with surface area > 10 ha, Messenger et al., 2016). However, currently there is no way to obtain a globally representative sample of all the lakes distributed

around the globe. Our approach was to apply a gradient sampling, with gradients being lake features and their catchment characteristics, to retrieve information from a relevant, geographically spread subset that can be placed in a broader global context. To better show the distribution of our dataset in the global context, we added a new figure (see Extended Data Fig. 1), in which we provide the distribution of the most relevant features of the lakes and reservoirs included in our study (i.e., lake surface area, volume, average depth, residence time) in comparison with a published global lake dataset ‘HydroLAKES’ (see Messenger et al., 2016). This global dataset included morphometric information for 1.4 million lakes. As shown by the figure, our dataset covers almost the whole range of these features, and the highest density corresponds to the median value of the global dataset. This addition showed that we had a wide range of variability, but that the features of the lakes and reservoirs are not skewed toward the extremes. We believe that this figure can help put our dataset in a broader context and show the distribution of the features of our study sites. However, we want to underline that we avoided any broader generalisation, as also suggested by the Reviewer, and we do not claim that our results are fully representative for all lakes on the globe. We believe that our study, taking advantage of a truly global and collaborative network existing among limnologists (GLEON, Global Lake Ecological Observatory Network), has generated valuable results that contribute to developing a better understanding of health risks caused by microplastics and humans' impact on lakes and reservoirs at the global scale. Thus, it provides a pathway for future research, as the dataset can be easily supplemented with additional lakes when following our standardised and well-validated protocols.

In terms of reproducibility, the authors state that the data and code are available at https://osf.io/du3sc/?view_only=858cbb89d75c4ec2a530c234401cb798.

I checked this website, and the code requires a dataset called Lakes final dataset REV.csv, but this file is not in the folder given by the authors, so I cannot confirm that the figures generated by the R script match what is in the paper. Furthermore, the authors state that they use the R package RamanMP (line 541) to analyze the polymer spectra, but this code is not provided.

Reply: We agree with the Referee’s comment, and we have made revisions to all the materials that were submitted. Specifically, we have incorporated code for all the figures and included all the pertinent datasets. The 'RamanMP' package has been released on CRAN and is also accessible on GitHub at the following link: <https://github.com/VeronicaNava/RamanMP>. We have included an appropriate reference to the package (see line 614).

Appropriate use of statistics and treatment of uncertainties: All error bars should be defined in the corresponding figure legends; please comment if that’s not the case. Please include in your report a

specific comment on the appropriateness of any statistical tests, and the accuracy of the description of any error bars and probability values.

How was the regression tree fit and selected? In the R code provided, the regression tree was fit to all of the data once, and there was no testing and training set and no leave-one-out-cross-validation. How small was the error of the reported tree in Figure 2? Were there any other possible trees with similar error but different variables chosen and different splits?

Reply: We followed the methods indicated by James et al. (2021) to address this comment by performing model selection and validation. We divided the data into training (80%) and testing (20%) datasets, and we obtained the mean squared error (mse) from the comparison of the performance of the model, created on the training dataset, with the testing dataset. We also compared the model obtained with the pruned tree, and the analysis showed that the original model had a lower mse. More details were added in the ‘methods’ section (see lines 661-670). Moreover, we have updated the R code accordingly.

In the extrapolation of the total number of plastics to the entire lake (see lines 297–301), no uncertainty metric is presented along with the estimate of 817 billion pieces of plastic. The authors do acknowledge that they are ignoring spatial variability and plastics in sediments, but given that the samples that they took were at the outflows of the lakes, and the fact that the concentration of plastics is likely to be greater here, this is likely to be a gross overestimate, and it is misleading.

Reply: We agree with the Referee’s comment that the estimate was highly uncertain. The objective was to offer a tentative idea of the issue of plastic pollution in large lakes scaling up the results. Nevertheless, we decided to omit the estimate, because this sentence could be misleading as the reviewer rightfully pointed out.

One line 522, please be specific in terms of what proportion you are estimating. I assume that it is the proportion of plastics with a particular chemical identity.

Reply: We are referring to the proportion of the different polymers in each sample. We agree with the reviewer’s comment that the sentence was unclear, and we have better specified the meaning in the text (see lines 583-586).

On line 524, what is N set to be equal to? The authors state that it is the global population size.

Reply: We better specify the meaning of N, which represents the total count of plastics in every sample, and this N may differ depending on the specific sample analysed (see line 589).

The description of Variance Inflation Factor on line 572 is incorrect. The VIF is used to remove variables that are highly correlated to the remaining variables in the candidate set, not to “further remove the autocorrelation of independent variables,” which is a contradictory statement in and of itself.

Reply: We agree with the Referee's comment, and we changed the sentence accordingly (see line 660).

Conclusions: Do you find that the conclusions and data interpretation are robust, valid and reliable?

Lines 234-237: The authors suggest that red plastic may have been less frequently observed in their sample because they are more visible and were likely ingested. Another reason that red plastic may have been less frequently observed is simply that it occurs less frequently in the waste stream cycle and was already present in lower proportions than other colors.

Reply: We agree with the Referee's comment that the variation in colour observed in the waste stream cycle may contribute to the difference in detected colours of plastics. As such, we have modified the sentence to reflect this potential explanation (see lines 300-302).

Lines 255-256: The statement "The effect of human population density on the total concentration of plastic is self-evident." is too strongly worded. One must remember that this sample is likely to be strongly biased.

Reply: We agree with the Referee's comment, and we removed the sentence.

Line 303-304: The word "affect" should be changed to "are linked to". The authors have evidence from their sample indicating a relationship, but to say that one thing affects another ignores the uncertainty in the sample and the data.

Reply: The text has been changed and the sentence was removed.

Suggested improvements: Please list additional experiments or data that could help strengthening the work in a revision.

None to suggest.

References: Does this manuscript reference previous literature appropriately? If not, what references should be included or excluded?

As far as I can tell, yes.

Clarity and context: Is the abstract clear, accessible? Are abstract, introduction and conclusions appropriate?

Inflammatory material: Does the manuscript contain any language that is inappropriate or potentially libelous? No.

Minor Comments:

1. Abstract, Page 3, line 119: ... likely due to of their elevated ... → ... likely due to their elevated...

Reply: We changed it accordingly.

2. Line 284: I believe that the reference of Fig. 2c should be Fig. 2b.

Reply: Yes, we changed it accordingly.

3. Figure 1a: The dots are all the same size, but the captions says that they are sized differently.

Reply: We changed the caption.

4. Figure 1b: The word “boxplot” is spelled as both “boxplot” and “box plot” in the caption.

Reply: We changed to “boxplot”, and we checked throughout the whole text.

5. Figure 1c: When printed, the text is too small to read, and when I zoom in on the pdf, the resolution of the figure is so poor that some of the words are not legible.

Reply: We increased the size of the text, and we provided high-resolution images. The figures have been also added to the repository with the code and the dataset of the publication.

6. The sentence on line 492 is repeated verbatim on line 495.

Reply: We removed the repetition.

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

I think the authors have answered queries well and strengthened the paper. I don't have anything more to add. I think it's acceptable for publication.

Referee #2 (Remarks to the Author):

The authors have adequately addressed all of my comments.

Referee #3 (Remarks to the Author):

A. Summary of key results: Please see previous review, which captured this well.

B. Originality and significance: The impact of the work comes through as a consequence of the revisions. The authors did an excellent job responding to criticisms regarding some of the writing. The messages about the scope of the plastics problem and how it relates to human/landscape features are much clearer. Overall, this is a relatively simple, and yet very important, set of findings that open the door to much future research.

C. Data and methods: fine

D. Appropriate use of statistics: Yes. Again, relatively simple data set, and authors extracted and interpreted the right amount of information from it.

E. Conclusions: As in previous review, these are robust, valid, and reliable.

F. Suggested improvement: Just a couple of minor typos, and a request for data availability as follows:

Line 388: two commas in succession

Figure 1. Would be good to match the colors between panels C and D. Perhaps D is just mislabeled?

Data availability: It is not clear to me whether the referenced URL provides a persistent identifier (PID), such as a DOI. If not, it should. For discoverability purposes, once a persistent identifier (e.g., DOI) has been obtained, it should be cited in the text and included in the references, which will make the data much more discoverable. In addition, future use of the dataset can more easily be traced back to this original publication.

G. References: Fine, except to include data citation in main references if at all possible, which will

improve discoverability.

H. Clarity and context: Huge improvement over previous draft!

Author Rebuttals to First Revision:

Manuscript number: 2022-12-19147A

Manuscript title: Plastic debris in lakes and reservoirs worldwide

Authors' response to reviewers' comments

We would like to thank the Editor who handles the manuscript and the reviewers for the time that have dedicated to the manuscript.

Referee #1:

I think the authors have answered queries well and strengthened the paper. I don't have anything more to add. I think it's acceptable for publication.

We thank the reviewer for the positive comment and the time dedicated to the revision of the paper.

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G. References: Fine, except to include data citation in main references if at all possible, which will improve discoverability.

H. Clarity and context: Huge improvement over previous draft!

We thank the reviewer for the positive comment and the time dedicated to the revision of the paper. We have corrected the typos identified by the reviewer. In particular, we removed the comma at line 388 and we matched the colours between figure 1c and 1d.

We provided a DOI with all the data and script used for the publication reserved on Zenodo. We uploaded all the data that have been made available for review purposes. As soon as the paper is accepted, the repository will be made public. Following the suggestion of the reviewer, we have added the reference to our dataset in the text.