

Internal tree cycling and atmospheric archiving of mercury: examination with concentration and stable isotope analyses

Author's Response.

REVIEWER 1 (R1)

R1C1: The manuscript entitled "Internal tree cycling and atmospheric archiving of mercury: examination with concentration and stable isotope analyses." by McLagan and co-authors examine THg concentrations and stable isotopes in two coniferous tree species, Norway spruce and European larch, surrounding a legacy Hg contaminated site in the German Black Forest. The goals of this study are to investigate if historical records of the industrial activities correlate with elevated THg concentrations in tree rings of sampled trees, to examine potential source related variations in MDF and MIF across the tree ring records and physiological processes that may separate pools of Hg in the transport mechanism from atmosphere to foliage to phloem to tree-ring/bole wood, and to investigate if deposition and sorption of Hg to tree bark is the dominant mechanism for bark Hg. For its overall quality and for being of interest to a large audience, this manuscript is worthy of publication to Biogeosciences. I would like to ask a few questions.

R1AR1: We appreciate the reviewer's feedback and considered all the suggestions and comments this reviewer provided and listed them below (in black text), followed by our replies (in blue text). We also label the comments as follows: *reviewer1 comment1 (R1C1)* and *reviewer1 author response1 (R1AR1)*. The comments and response for reviewer 2 follow the same format.

R1C2: As a research setting, I would like to know why THg concentrations and isotope analysis were not performed for needles and soils as well. I think those data, rather than speculating from the literature, would have allowed us to correctly assess the adsorption and transport of mercury in this type of tree.

R1AR2: We agree that total Hg and Hg isotope data of needles and soils could be interesting for present day Hg concentrations and also with regards to foliar uptake mechanisms. The original goal of the study was to assess atmospheric Hg emissions from a contaminated legacy site by observing changes in THg concentrations in the tree rings of tree species that have been demonstrated as effective archiving species for atmospheric Hg⁰. Tree rings provide an archive for such historical gaseous Hg emissions over several decades, needles and soils on the other hand are not suitable for this archiving purpose; as such they were not sampled. The additional objectives of this study were developed inductively from our initial observations of THg analyses of bole wood extracted using the increment borer cores. These of course were understanding the enrichment of mercury in sapwood and bark (uptake mechanism) samples. After these initial observations, we were eager to explore stable isotope

analyses of sapwood, heartwood, and bark samples from trees at this site. Nonetheless, we were aware of the challenges associated with detection limits, which required a substantial amount of material (bole wood from increment borer cores are insufficient). We were fortunate to obtain tree “cookies” from forestry workers that were actively felling trees, but we could neither obtain foliage from these same trees nor soil from where they grew due to their extraction by forest workers and not knowing their exact location within the felled stand. Hg isotopes could be a useful tool to better understand any fractionation that might occur during uptake and downward transport from foliage to bole wood. A closer investigation of the potential fractionation associated with foliage Hg uptake and transformation and subsequent transport to bole wood was not possible in this study, but we agree that this aspect is of great interest for future work.

With regards to soil Hg analysis, we were cognizant to sample only trees that were upslope from the area with contaminated soils and groundwater. Thus, the trees are not situated in contaminated soils; elevated concentrations of THg in tree rings are derived from past atmospheric emissions from the site. Furthermore, root uptake has been demonstrated to have no significant effect on tree tissue Hg concentrations and the dominant uptake route of Hg in several tree species has been shown to be foliar uptake (L62-79). Exploring Hg interactions (potentially with Hg stable isotope analyses) in the rhizosphere is again beyond the scope of this study.

R1C3: Have you confirmed that mercury does not escape from samples that have been freeze dried, and that Hg isotope does not fractionate during freeze dry? Is it possible to correctly evaluate the original mercury concentration if the moisture content differs between the inside and outside of the wood (top and bottom?)?

R1AR3: In dendrochemistry, samples are commonly dried before analysis to allow the results to be reported on a dry-weight basis. Freeze-drying is a standard procedure of pretreatment for measuring Hg in various biological samples including tree tissues. The influence of different drying methods (oven, air, freeze drying) for THg in wood samples has been assessed by Yang et al., 2017 and no loss of Hg was found for freeze drying. Similar assessments were made for THg in soils and reference materials (Hojdova et al., 2015) as well as soils and sediments containing MeHg (Kodamatani et al., 2017). Fractionation of Hg isotopes is only possible if there is significant loss, which has not been reported.

R1C4: L196-197: Apple Leaves and China Soil were used for quality control. Although the major components of those two RMs and trees are very different, is it appropriate to use these two species as a confirmation of the pretreatment method? The same question applies to the RMs used in the isotope analysis.

R1AR4: The aim of including two RMs was to show good recoveries for the different matrix of these RMs including NIST 1515 apple leaves which is frequently used by studies investigating Hg in wood samples such as tree rings (e.g. Gustin et al., 2022; Scanlon et al., 2020; Yanai et al. 2020). While we acknowledge there are some

differences between the matrices (particularly China soil), we deem the differences between bole wood and leaves (tree tissues; apple leaves RM) to be relatively minor, which is supported by the application of this RM (apple leaves) in the other studies assessing mercury archiving in tree growth rings as mentioned in the previous sentence. We are also unaware of any more appropriate and established RMs for woody material with a reference value for Hg.

To assess the recoveries of Hg by the combustion and trap method, additional RMs were assessed in a previous study by our research group using the exact same instrumentation and method: BCR-482 (lichen): $103 \pm 12 \%$, $n = 13$; CC-141 (loam soil): $95 \pm 4 \%$, $n = 16$; NIST-3133 (Hg standard solution): $102 \pm 4 \%$, $n = 12$ (see McLagan et al., 2022).

The goal in this previous and our current study was to demonstrate the efficient trapping for RMs with different matrices. We believe we have done this effectively across this range of matrices that include the most applicable matrix (apple leaves and lichen) that we could find and had ready access to.

Minor comments

R1C5: L165: "Bole wood (tree ring)..." A description of "Bole wood (tree ring)..." is found in L165, but A first appeared in L57. The explanation in parentheses should be placed in L57.

R1AR5: The explanation in parentheses will be placed in L57 in the revised manuscript

R1C6: L167, L172: Why is the height of tree samplings different between the two?

R1AR6: Sampling with the increment borer was conducted at breast height (standard procedure). The tree cookies were collected on the day of tree felling from the forestry workers. They cut the trees at a lower height and provided the tree cookies from the bottom of the freshly cut bole. Obtaining tree cookies is a much greater challenge than taking increment borer cores as we are not able (nor wanted) to fell trees simply to extract a tree slice/cookie at the appropriate "breast" height.

R1C7: L180: Heating temperature and duration of THg measurement are written in sample preparation part. Information on combustion should be included in the measurement, not in the preparation.

R1AR7: We have updated the manuscript accordingly. Additionally, we clarified at which stage of preparation/analysis process samples were freeze dried for the THg and Hg stable isotope analyses (please see the updated manuscript).

R1C8: The "Spruce" in "Spruce *" and "Spruce ISO*" is better to be capitalized (* is number).

R1AR8: We have capitalized "Spruce" in the revised version of the manuscript.

R1C9: L238: Parenthese is not written. It should be “(no larch trees reached the 1stIP),”

R1AR9: This typo has been corrected in the revised version of the manuscript

R1C10: L241: It may be necessary to add and between sentences as following; “from tree rings in the BGP, and up to 521...”

R1AR10: The sentence will be rewritten in the revised version: “The THg concentrations ranged from $\approx 1\text{--}10\ \mu\text{g}\cdot\text{kg}^{-1}$ in heartwood tree rings from the BGP, and up to $521\ \mu\text{g}\cdot\text{kg}^{-1}$ in a heartwood sample dated from 1951—1953 during the 1st IP in spruce 1, [...]”

R1C11: L302: saplings => samplings

R1AR11: The study by Yamakawa et al. 2021 investigated one-year-old spruce shoots (saplings).

R1C12: L422: “phloem (first layer of bark)...” A description of “phloem (first layer of bark)...” is found in L422, but A first appeared in L91. The explanation in parentheses should be placed in L91.

R1AR12: The explanation in parentheses will be placed in L91 in the revised manuscript.

R1C13: L441: Please describe the variation of a GEM concentration (1.49 ng/m³).

R1AR13: The measurement uncertainty indicated for the EMEP data is indicated to be 0.24 ng·m³ and the standard deviation of the annual means is 0.12 ng·m³.

The variation has been included in the revised version of the manuscript.

R1C14: L466: Delete a space between ISO and 6.

R1AR14: This typo has been corrected in the revised version of the manuscript

R1C15: Table S4.1: “Spruce ISO5 (cont.)” needs to be deleted.

R1AR15: This has been corrected in the revised version of the SI.

REVIEWER 2 (R2)

R2C1: This manuscript aims to understand the Hg variations in tree-ring by isotopes, basically the data are interesting and could provide additional knowledge for us to understand the current challenge by using tree-ring to reconstruct the historical atmospheric Hg trends. However, the methodologies both in sampling and isotopic measurements in this study seem to not as the standard methodology in Hg

dendrochemistry. In addition, the explanations for the variation of Hg isotopic signatures and Hg concentration seem to be not always convinced. I have several important issues that need to the authors to address before this manuscript can be accepted.

R2AR1: We thank the reviewer for their comments, and we have attempted to allay these concerns in the responses below.

Abstract:

R2C2: Basically, the abstract displays the variations of Hg concentration and MDF signatures. This is not enough, for Hg isotopic signatures, the odd-MIF is very important, while the authors did not depict this value any more. In addition, for the findings about the bark Hg sources and enriched in sapwood, the current evidences cannot support.

R2AR2: We will add the following discussion on the MIF to the abstract:

“We also observed a small range of odd isotope-MIF. Differences in $\Delta^{199}\text{Hg}$ between periods of different industrial activities were significant ($\Delta^{199}\text{Hg}$: $1^{st}IP$: $0.00 \pm 0.03 \text{ ‰}$; $2^{nd}IP$: $-0.06 \pm 0.04 \text{ ‰}$, BGP : $-0.13 \pm 0.03 \text{ ‰}$, 1SD), and we suggest MIF signatures are conserved during stomatal assimilation (reflect source MIF signatures).”

The reviewer’s concerns about the bark samples will be addressed below.

Introduction

R2C3: The authors introduce a quite detail information about the vegetation uptake of atmospheric Hg₀, and these introductions can help the researcher to follow the current processes of Hg research communities. Given manuscript aims to focus on the issue of internal tree cycling and atmospheric archiving of mercury, I would like to ask the authors to introduce more processes of Hg dendrochemistry, specifically the current challenge of Hg dendrochemistry to reconstruct historical atmospheric mercury (Hg) trends.

R2AR3: We agree with the reviewer that the challenges of reconstructing historical gaseous atmospheric mercury need to be properly introduced. Lines 80-112 specifically address what the reviewer has asked for in the above statement. These paragraphs describe oxidation in the foliage (and possible re-release), the most likely species/structures/complexes that control Hg associations within the trees, downward transport within the phloem, and translocation from phloem-to-xylem throughout this downward transport (including the specific cells which conduct this translocation). Additionally, lines 100-112 specifically address concerns of lateral translocation between rings citing studies that have observed non-correlated emissions inventories and tree ring THg concentrations and those studies that have observed good correlation between the two. The species specificity of this effect is

also mentioned. Adding more detail beyond this would begin to migrate this from a research based paper to a literature review.

Methods.

R2C4: I have several very important concerns for this section. I carefully read the sampling methodologies of this study, I found that the current description of the methodologies seems not meet our standards in the Hg dendrochemistry. In dendrochemistry, the tree-ring cross-dating is very important to check the absent or false rings in sampled tree ring cores. To guarantee the accuracy of tree-ring cross-dating, we usually sampled tree rings from more 25 tree stands, and dual radii tree-ring cores of south-facing and west- or east- facing sides of each tree were collected at ~1 m height. Then, 180- to 1500-grit sandpapers were utilized to polish one side of the tree ring until the ring boundaries and cells clearly visible. The tree-ring widths were measured. However, the authors without any cross-dating for their samples.

R2AR4: We thank the reviewer for their input on dendrochronological dating. As mentioned in R1AR2, the original goal of this study was to determine if there were significant emissions of Hg from a non-combustion, HgCl₂ contaminated site. By our review of the literature, we have found no studies that have made any assessment of emissions of Hg to the atmosphere from any such site. We therefore had no knowledge or basis to estimate what the historical atmospheric Hg₀ concentrations at the site might be. As such, we selected two species that have been demonstrated as effective archiving species (*P. abies* and *L. decidua*) and attempted to sample trees as close to the facility as possible to ensure we captured trees with the maximum possible Hg content from these species in multiple directions around the site. The area immediately around the contaminated site is not forested (urban and rural) and multiple trees (from the identified species) could not always be samples. Additionally, the temporal resolution of the information we could obtain on mercury usage at the site (mercury inventory data) was poor (limited reporting) and if atmospheric emissions occurred (which our data confirm) they would be “relatively” constant and non-episodic. Thus, we did not deem it necessary to measure THg concentrations in individual growth rings and reduced the temporal resolution of samples to between 2 and 5 growth rings (years). We compared these data to the three distinct industrial periods that we outlined from review of the literature on the sites. THg concentrations in growth rings agreed well with these changes in industrial activity as we have demonstrated in the study. Since we analyse neither annual THg concentration changes nor annual differences in Hg usage at the site, any uncertainties associated with “absent” or “false” rings that would resolve on an annual temporal resolution would have a minimal if not negligible effect on our results. Indeed, concerns related to lateral movement of Hg across the bole wood (inter-ring migration) and the associated impacts on historical atmospheric Hg₀ archiving would favour including multiple growth rings (2-5 rings) in individual samples as we have done. The following sentence was updated in Section 2.2 of the revised manuscript: “... and not sampled

on an annual temporal resolution (there were multiple tree rings were in each sample)...”.

An additional concern with dendrochronology is the impact coring trees has on the health of the trees themselves as outlined by Tsen et al. (2015). These concerns also include the impact on the timber resource of the trees, and stands surrounding this site are plantation timber, many of which are only young trees that are not old enough to have been living during the industrial periods at this site and of little interest based on the objectives of the study. This was an additional reason we did not sample more trees and more cores within all the sampled trees (although we note, selected trees were sampled with multiple trees at different radial angles; see Section 3.3.4). We believe tree health and resource damage are valid concerns that should be considered when planning dendrochronological studies on the order of that suggested by the reviewer (multiple cores, from multiple trees, from 25+ tree stands). We also note that we contemplated sanding of the tree core and cookie samples, but deemed this unnecessary as the rings of the two species (and even distinction between heartwood and sapwood) were easily discernible; sanding presents the possibility of contaminating the samples as we cannot know if each piece of sandpaper used is completely Hg-free.

R2C5: For the Hg analysis, how is blank for DMA80 boat, and how is the precision of your methodology.

R2AR5: These two sentences have been added to the SI of the manuscript:

“The mean DMA-80 boat blank was 0.019 ± 0.017 ng of Hg. This equated to a method detection limit (MDL) of 0.050 ng of Hg and method quantification limits (MDL) of 0.165 ng of Hg for the total Hg analyses using the DMA-80. All concentrations were above the MQL.”

R2C6: For the Hg isotopic analysis, the current description for the methodology is also not enough because of absent of QA/QC. Have you measured the CRM to assess if the non-unity recoveries resulting from the offline combustion-trapping technique induced discernible isotopic bias?

R2AR6: Please refer to the response to reviewer 1 (**R1AR5**). SRM/CRMs (103 ± 12 % for BCR-482 (n =13); 95 ± 4 % for CC-141 (n = 16) and 102 ± 4 % for NIST-3133 (n=12)) were run throughout the pre-concentration procedure to confirm complete recovery of Hg in the traps. This includes a biota CRM/SRM (lichen; BCR-482) and nominal CRM/SRM used across all isotopic analyses (NIST-3133).

Isotopic fractionation is only possible with insufficient trap recoveries. This is detailed in Sections S4 and S5. Additionally, if there were recovery related issues using the purge and trap method we would expect lower precision and more randomised uncertainty across different samples, sessions, and the different stable isotope ratios (i.e., $\delta^{202}\text{Hg}$, $\Delta^{199}\text{Hg}$, $\Delta^{200}\text{Hg}$, etc.). However, this was not the case. 2SD values for the

analyses were consistent throughout and within the typical acceptable range for each of the respective isotope ratio. All traps were diluted to match concentrations within each session and the matrix was adjusted to <10% acid strength. Samples were run using standard sample bracketing with NIST 3133 and instrumental mass bias was corrected with TI doping using NIST 997. During the analyses accuracy and precision (2SD) was assessed by repeated measurement of in house standard "ETH Fluka". Please see Table S4.1, Table S4.2 (in the SI) and L218-223 for further QA/QC during the measurement.

Results and discussion

The section of 3.2.

R2C7: For Hg MDF in tree rings, currently few evidences can support the nearly no MDF occurring during the Hg translocation from leaf to stem. If the MDF occurring during Hg translation in vegetation, this leads to very hard to explain the variations of $\delta^{202}\text{Hg}$. This is because when the growth of tree, the canopy height increases quickly for the young tree periods. This means the Hg transport distance from the canopy to the 1-1,5 stem height sampled the tree rings increase with the tree growth year. For this study, authors subtracted -2.6‰ range of MDF caused by foliar uptake to reconstruct the $\delta^{202}\text{Hg}$ signatures of air Hg₀. Due to unknown the MDF occurring during Hg translocation in vegetation, this methodology seems not to be reasonable and convinced.

R2AR7: Potential MDF occurring during the transport of Hg from the foliage to the stem is a valid concern. However, the reviewer has stated "currently few evidences can support the nearly no MDF occurring during the Hg translocation from leaf to stem", but provided no evidence (literature or otherwise) to demonstrate differences in MDF values between foliage and bole wood. In contrast, Figure 4 shows that background foliage MDF values (ranging from $\delta^{202}\text{Hg}$: ~ -3.5 to -1.0 ‰) compare favourably to all bole wood (growth ring) MDF values (ranging from $\delta^{202}\text{Hg}$: ~ -3.0 to -1.5 ‰) in background trees away from sources (we stress these are background data). Data from Liu et al. (2021) support this further in a single study (See Figure 3; Liu et al., 2021) – foliage $\delta^{202}\text{Hg}$: ~ -3.5 to -2.0 ‰; bole wood $\delta^{202}\text{Hg}$: ~ -3.0 to -2.4 ‰.

Bole wood samples from Wang et al. (2021) are highly negative in $\delta^{202}\text{Hg}$ and similar to those measured in our study during industrial period. Again, similar to our study, samples from Wang et al. (2021) with more negative $\delta^{202}\text{Hg}$ values were associated with higher THg concentrations, which supports our hypothesis that MDF values reflect the more negative MDF signatures of industrial sources when offset for the ~ -2.6‰ $\delta^{202}\text{Hg}$ fractionation associated with foliar uptake of Hg. We note that the study by Wang et al., did also not include foliage samples and used the same foliage offset method to estimate MDF of historical GEM in air from the MDF values in the growth rings.

To our knowledge there is no literature showing MDF during the translocation of Hg from foliage to stem. Nonetheless, at no point within our study do we suggest that this fractionation cannot occur after assimilation of Hg⁰ into foliage. Indeed, this is a research question we encourage exploration of in the future. Nonetheless, based on this summation of data, we argue that all evidence from our study and the literature supports little MDF during downward transport of mercury from foliage to bole wood. Perhaps a better way to state this would be that the very large fractionation induced by Hg⁰ assimilation into stomata and subsequent oxidation and sorption in the foliage far outweighs any fractionation between foliage and bole wood; if this downward transport process is responsible for fractionation, it is of much lesser extent. As stated, addressing the extent of this fractionation is of interest to us, but one we cannot answer within the scope of this study (See also **R1AR2**).

Section

3.3.1

R2C8: In this section, the authors highlighted that negative $\delta^{202}\text{Hg}$ values which comparable to the tree-rings, and suggested that stomatal uptake, internal transport, and translocation from phloem to inner bark was likely the dominant uptake pathway for Hg stored in bark. Given substantial factors influencing the MDF occurring, the authors provided data cannot support their hypothesis. Given the loose porous structure of bark, the atmospheric Hg⁰ and Hg²⁺ absorption by bark also possibly leads to distinct MDF, which similar to the processes the Hg passing through the stomata and absorbed by the leaf tissues.

R2AR8: Here we argue that the process of foliar assimilation of Hg⁰ and the mechanism suggested by the reviewers of Hg⁰ diffusion into bark and then sorption to bark are NOT similar. We state three distinct reasons as to why the process of direct bark sorption could not cause such a large MDF (on the order of -2.6 ‰).

(1) Hg⁰ assimilation into foliage is NOT a passive process, it occurs during the active process of foliar respiration, whereas uptake of Hg to bark would be a passive process (Zhou et al, 2021). Additionally, foliar assimilation of Hg⁰ is controlled by stomatal conductance, which can be controlled by trees depending on environmental conditions (temperature, relative humidity, soil moisture, etc.; Wohlgemuth et al., 2022); again, direct bark deposition (including diffusion into bark) of Hg would not be dependent upon such factors.

For Hg to continually diffuse into bark there must be a Hg concentration gradient; the inner bark air spaces must be depleted in gas phase Hg (i.e., through sorption) compared to air and the outer bark air spaces (in foliage the Hg⁰ concentration gradient is maintain by oxidation to Hg²⁺ within the foliage cavity). This would suggest preferential sorption of Hg onto the inner bark compared to the outer bark. We see no reason as to why we would expect that to be the case. Also, any Hg sorbing to the outer bark would be undergoing less diffusion; the diffusive path length is less from air into outer bark than air into inner bark. The greater the diffusive path length the

greater the MDF associated with diffusion. In this case, we would expect outer bark samples to be more positive in MDF than the inner bark (less negative diffusive MDF). We analysed inner and outer bark of the same trees for Spruce ISO4 and Spruce ISO5. Spruce ISO4 had an inner and outer bark values of -3.88 ‰ and -3.70 ‰, respectively. This small difference in MDF (-0.18 ‰) that the reviewer is relating to the increased diffusive path length, is less than 10% of the mean fractionation associated with the active foliar uptake process (-2.6 ‰). Spruce ISO5 had an inner and outer bark values of -3.62 ‰ and -4.21 ‰, respectively; the outer bark (with a smaller diffusive path length) has a more negative MDF value, which could not be the case if diffusion into the bark was causing the negative MDF values observed in the bark samples.

(2) Liu et al. (2021) measured THg and Hg stable isotopes in bark of background trees away from Hg emission sources. THg concentrations in bark were an order of magnitude lower (11 ± 4 ng/g) than bark samples from our study, which again suggests Hg in bark in our study is influenced by local industrial Hg emission sources (as we have suggested). Additionally, MDF values in the bark samples from Liu et al. (2021) are negative in MDF (~ -2.5 ‰). If we add the foliar uptake offset to these data, we would get a gaseous Hg⁰ value for air at this site of $\sim +0.1$ ‰, which is within the range of gaseous Hg⁰ MDF values in background air (~ -0.2 to $+1.5$ ‰; see Figure 4 of our manuscript). This led Liu et al. (2021) to conclude (like us) that Hg found in bark is “mainly derived from foliage transport”.

(3) Figure 4 in our manuscript includes MDF and MIF values for precipitation because Hg²⁺ dominates the fraction of Hg in precipitation; thus, Hg²⁺ dominates the wet deposition of Hg (Yin et al., 2016; Jiskra et al., 2021). The data from Figure 4 show positive MIF values for precipitation samples (~ 0.0 to $+1.3$ ‰). The MIF values of our bark samples are negative in MIF (~ -0.2 ‰) and outside of the range for precipitation. This is not reflective of oxidized Hg wet-depositing to bark.

Hence, we disagree that we “provided data [that] cannot support [our] hypothesis”; contrastingly, we maintain that our hypothesis is the most plausible explanation for the observations. Again, we encourage further research on this topic.

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