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Insights into seasonal variations in mercury isotope composition of lichens☆

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ABSTRACT

Lichens are commonly used to assess mercury (Hg) concentrations in air because of their cost-effectiveness. However, recent research has revealed temporal variations in the isotopic composition of Hg. Previous work on this topic leaves open questions about the repeatability of data over multiple seasons, different types of sampling (transplantation or in-situ collection), and diverse locations. This study aims to address these issues by conducting a high-frequency sampling campaign of in-situ and transplanted lichens and atmospheric particulate matter (APM). Sampling sites included a range of areas, from pristine to Hg-contaminated sites. Isotopic analysis showed that the isotopic composition of Hg in lichens undergoes mass-dependent fractionation and changes with time. The heaviest isotopic composition was observed in summer and the lightest in winter. These trends were consistent across polluted and unpolluted environments, as well as in both in-situ and transplanted lichens and in APM. The results further indicated towards a correlation between changes in Hg concentrations and isotopic composition in lichens and environmental factors. All of these variables seem to be changing at the same frequency and may have not just correlation but also causation relationship. Environmental factors seem to be influencing the Hg concentrations and isotopic composition. The summer high temperatures might be influencing the heavier isotopic fingerprint observed in lichens during the same season. Similarities with APM-bound Hg suggest a common underlying mechanism. This study highlights the importance of considering temporal and seasonal trends, as well as the method of lichen sampling, when interpreting results. Researchers using lichens as proxies for atmospheric Hg concentrations or isotope ratios should consider these findings when designing their studies.

1. Introduction

Mercury (Hg) is a recognized environmental pollutant of considerable concern due to its toxicity. Addressing the imperative need for comprehending the cycling of Hg in the environment, a global initiative, outlined in guidelines for Hg measurement in the atmosphere, adopts a multi-tiered approach encompassing diverse monitoring methods, including bio-monitoring (UNEP, 2022). Passive bio-monitoring, employing lichens as indicators, holds significant promise owing to their exclusive reliance on atmospheric nutrients, and this approach is extensively utilized for assessing various pollutants (Abas, 2021; Berdonces et al., 2017; Garty, 2002; Szczepaniak & Biziuk, 2003), including Hg (Barre et al., 2015, 2018, 2020; Mlakar et al., 2011). The use of lichens in bio-monitoring offers distinct advantages over alternative methods. Lichens can be retrospectively sampled post-pollution events in their natural habitats, obviating the need for electricity, and are less susceptible to vandalism, maintaining a minimal ecological footprint (Garty, 2002). This makes lichens a compelling alternative to some other passive or active methods, which exhibit up to 12% variations in Hg concentrations across different brands (Naccarato et al., 2021) and differences exceeding a permille in mass-dependent fractionating isotope fingerprints (Szponar et al., 2020).

However, the suitability of lichens as proxies for atmospheric isotopic composition raises certain concerns. Lichens may be differentially impacted by various types of Hg deposition, contingent on whether it is wet or dry (Graydon et al., 2008), as well as the chemical forms of atmospheric Hg species, including Hg bound to particles, oxidized, or elemental Hg (Lyman et al., 2020; Si & Ariya, 2018). Lichens have the potential to fractionate Hg isotopes, potentially deviating from the true atmospheric fingerprint. Initial indications of this phenomenon were observed by Demers et al. (2013), who proposed that foliage may fractionate Hg towards lighter isotopes during the uptake process. Yuan

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et al. (2019) documented the gradual accumulation of Hg in leaves over the growing season, while Fu et al. (2019) noted concurrent shifts towards heavier isotopes in the atmosphere and lighter ones in foliage during the summer months. Moreover, global fluctuations in Hg concentrations attributed to global Hg uptake during summer, akin to CO2, have been observed (Jiskra et al., 2018). These observations have contributed to the evolving understanding of the Hg cycle in forests and foliage (Liu et al., 2022; Wang et al., 2021; Zhou et al., 2021). Changes in atmospheric Hg have been noted not only in foliage but also in atmospheric particulate matter (APM), influenced by a combination of global and local factors (Guo et al., 2021; Li et al., 2020; Xu et al., 2019). This raises questions about the reflection of these trends in lichens and whether their utility as bio-monitors and proxies might be compromised. The key questions revolve around whether the isotopic composition of lichens varies seasonally and, if so, whether it mirrors changes observed in other foliage, atmospheric gaseous Hg, or APM. In essence, do lichens enrich in lighter isotopes during the growing season like the rest of the foliage, or do they exhibit enrichment in heavier isotopes in alignment with the atmospheric trends?

To our knowledge, the first significant study to observe substantial shifts in isotopic composition in lichens during the season was conducted by Božič et al. (2022). In that work, the primary focus was on correlating atmospheric Hg concentrations using active and passive methods on transplanted lichens, transplanted to various locations to elucidate micro-local variations. Since the observation of isotopic composition changes was serendipitous and not the primary focus, certain limitations in the study design required addressing in the present study: (I) Increased sampling frequency from once per season to monthly or more frequent intervals to better delineate differences between periods with heavier and lighter isotopic compositions. (II) An investigation of the natural variability of lichens was undertaken to enhance the overall robustness of observations. (III) A parallel examination of in-situ and transplanted lichens was performed. (IV) Environmental factors such as temperature and precipitation were thoroughly examined. (V) The Hg isotopic composition of APM was compared to lichen Hg levels. To ensure the continuity of measurements, some of the same sites utilized in the 2020–2021 sampling campaign (Božič et al., 2022) were revisited in the subsequent 2021–2022 sampling campaign.

The objective of this study was to expand our comprehension of lichens as atmospheric proxies under diverse conditions, employing both transplantation and in-situ sampling methodologies. The resulting data provides insights into the limitations of lichen-based bio-monitoring. The findings further our understanding of trends in Hg uptake by lichens and the potential seasonal variations in atmospheric Hg concentrations and isotope ratios.

2. Sampling sites

Pokljuka is a plateau in the Julian Alps in Slovenia (Supplementary material A, Fig. S1). It is a forested area that stretches in both north-south and east-west directions for about 10 km. The plateau lies at an altitude between 1000 and 1400 m above sea level and is part of the Triglav National Park, which ensures that it remains as untouched as possible. In addition, the plateau is not surrounded by major industrial facilities that emit Hg, so the local environmental impact of human activities in this area is negligible and largely caused by long-distance Hg transport. During the winter, Pokljuka is the only one among the sampling sites that is constantly covered with snow for many months (Supplementary Material B).

Idrija is a small town in the Dinaric hills of western Slovenia, nestled in a deep valley (Supplementary material A, Fig. S1). The area is characterized by lush forests. The town has a few thousand residents and is today a regional industrial hub. Idrija gained recognition since the end of 15th century due to the discovery of one of the major Hg ore deposits. This has caused significant environmental problems due to the release of Hg into the surrounding area. This is especially concerning as Idrija's location at the bottom of a deep valley creates conditions favourable for the capturing emissions through temperature inversion. Several studies have been conducted to understand the extent of the impact on local ecosystems (Biester et al., 1999; Gnamuš et al., 2000; Gosar & Teršič, 2012; Hines et al., 2000; Horvat et al., 2002, 2003; Kocman, Kanduč, et al., 2011; Kocman, Vreča, et al., 2011; Kocman & Horvat, 2011; Kotnik et al., 2005; Miklavčič et al., 2013; Tomiyasu et al., 2012, 2017; Žagar et al., 2006; Žižek et al., 2007).

The Reactor Centre Podgorica (RCP) is located in the Ljubljana basin, a lowland area in central Slovenia (Supplementary material A, Fig. S1). In winter, this region often experiences temperature inversion start lead to the formation of smog and dense fog. The RCP is located on the so-called Savsko polje, which surrounds the Sava River, about 800 m south of the site. The surrounding area is mainly used for agriculture, with a small forest located about 1 km to the west. There are no forests in the immediate vicinity, the nearest forested area is over 4 km away. The city of Ljubljana is located 3 km to the southwest, and a major highway is located 500 m northwest of the RCP. In the northern vicinity, is a municipal water treatment plant (WWTP) that is also responsible for the treatment of water from the Novartis pharmaceutical factory. The factory used to manufacture Hg-containing pesticides. Limited evidence suggests that Hg concentrations in the air can reach levels as high as 20 ng/m^3 at certain times, compared to the average Hg concnetration of 1–2 ng/m^3. Since these levels are associated with winds blowing from the direction of the WWTP, this increase in Hg concentration appears to be related to the WWTP.

3. Experimental

3.1. Sample collection

Lichen specimens were gathered under two distinct conditions. Firstly, there were the in-situ lichens, which were collected from the various trees surrounding the research location. Secondly, there were the transplanted lichens, which were essentially the in-situ lichens transferred to a new environment at the start of the sampling period. The transplantation (collection and placement) and the collection of the fists in-situ lichens all occurred in a one-week time window (14th to 21st of November 2021). The transplanted lichens were collected at Pokljuka, where approximately 5 kg of material was mixed by shaking the collection box. To facilitate transplantation, small plastic mesh bags measuring 10 by 30 cm were created. Each bag was divided into three compartments, each measuring 10 by 10 cm. These bags had a depth of less than 0.5 cm when fully loaded, just enough to accommodate a piece of bark with the attached lichen. The bags were interwoven with wire to provide structure and ensure that the lichens faced outward toward the elements while the bark, their substrate, faced inward towards the surfaces they were placed on. Sampling of both in-situ and transplanted lichens occurred approximately once a month (see Supplementary A, Table 1), resulting in around 10 samplings per site over the course of a year-long campaign. Sampling commenced in the beginning of fall 2021 and concluded in the beginning of fall 2022. The sampling sites for Pokljuka and Idrija were consistent with those in (Božič et al., 2022), with the addition of one site at RCP. One of the bags was intentionally designed to be larger to accommodate a greater number of lichens. This larger bag was transplanted to Pokljuka to evaluate the variability in measured concentrations and isotope ratios within the bag. The variability between bags was assessed by preparing four additional bags that were measured at the end of the sampling period. For a detailed description of the sampling procedure, please refer to Supplementary Material A, Fig. S2, and Fig. S3.

APM-collecting filters were conditioned in a room maintained at a constant temperature of 20 °C and a humidity level of 45% before sampling. An APM sampling device (LVS6-RV by Sven Leckel, Berlin) equipped with a filter holder according to CEN EN 12341 standards was utilized. Fourteen sampling events were conducted at RCP, and two

were conducted at Idrija. A 2 μm PTFE filter with a 46.2 mm diameter (Whatman, UK) was employed for sampling. Air was filtered until the filters became clogged and the pump could no longer maintain the specified air flow rate, which was set at 3 m3/h.

Weather data were obtained from the Slovenian Environmental Agency (Slovenian Environmental Agency, 2022) and are presented in Supplementary Material A, Fig. 2, and Supplementary Material B. Temperature data were recorded simultaneously from the PM-2.5 sampling unit.

3.2. Sample preparation

Lichen sample preparation began with removal of the lichens from the bark using polytetrafluoroethylene forceps. These were then placed in plastic containers and immersed in liquid nitrogen to de-vitalize and store them. This step might have removed some of the particulates, but lichens were not thoroughly washed. Prior to digestion, the lichens were lyophilised at −40 °C with a vacuum of 0.27 mbar for 24 h in a Martin Christ Liophilizer (Martin Christ Gefriertrocknungsanlagen, Germany). A microwave digestion system (UltraWave, Milestone, Italy) was used for digestion. A total of 0.3 g of each lichen sample was weighed into pre-cleaned polytetrafluoroethylene tubes, and 3 ml of 65% HNO_3 and 0.5 ml of 30% HCl (both supra-pure grade) were added. The samples were then subjected to closed-vessel microwave digestion at a maximum power of 1500 W and a maximum pressure of 100 bar. The resulting solution was filtered through a 0.45 μm filter, quantitatively transferred into 10 ml polyethylene graduated tubes, and diluted with Milli-Q water.

APM filters were cut in half with pre-cleaned scissors to better fit the digestion chamber. A combination of 1.5 ml HNO3, 0.5 ml HCl, and 0.1 ml HF (per analysis purity) was used for digestion. Samples were digested using the same procedure as for the lichens described above. These samples were then analysed for their Hg concentrations. Since some of the Hg concentrations in the solution were too low for isotopic measurements (a minimum of at least 1 ng/ml of Hg is required for isotopic measurements), some of the samples had to be pooled together into one vial and preconcentrated to be used for isotope determination (Supplementary material A). All APM samples were subsequently filtered and transferred into glass impingers in which HF was neutralised with 10 ml of 5% (w/w) H_3BO_3. The impingers were used for Hg preconcentration using the procedure described by Ali et al. (2023). Briefly, N_2 gas was used to purge the impingers filled with $SnCl_2$ solution, reducing and converting the Hg to gaseous form. This Hg was then trapped into an oxidising solution of concentrated HNO_3, which was diluted to the appropriate concentration for further measurements.

3.3. Analyses

Hg concentrations in the solution were quantified using a Model Hg-201 semiautomated Hg analyser employing cold vapor atomic absorption spectroscopy (CV-AAS, Sanso Seisakusho Co., Japan). Between 0.5 and 1 ml of the sample was transferred into the reduction chamber. A reducing agent consisting of SnCl2 in a 10% w/w mixture of 10% v/v HCl (supra-pure) to Mili-Q water was utilized.

Quality assurance and quality control (QA/QC) procedures were implemented by analyzing every tenth sample in duplicate. For calibration, a 1 ng/ml NIST 3133 standard Hg solution was prepared, and the volume transferred was adjusted until the signals matched within 50% of the signal from 1 ng/ml of the NIST 3133 solution. Prior to usage, pipettes were calibrated, and a reagent blank value of 4% was subtracted from all measurements. Procedural blank values were subtracted separately for each batch of lichen and APM samples. For lichen samples, a matrix-matched reference material, BCR 482, was utilized, yielding a recovery of 92.6% (RSD 8.8%, N = 8). As no direct reference material was available for APM, NIST 1648a (urban particulate matter) was used, resulting in a recovery of 98.5% (RSD 13.4%, N = 6). The concentration values' uncertainty was assessed using the ISO GUM approach and amounted to 4.1% (k = 2).

Isotopic ratios of Hg were determined using a multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) provided by Nu Plasma II (Nu Instruments, UK). All measurements followed the sample–standard–sample bracketing technique (Peel et al., 2008). The measurements are denoted with δ and Δ symbols, as detailed in Equations (1) and (2) (Blum et al., 2014; Blum & Johnson, 2017). The correction factor "f" was applied, with values of 0.2520 for Δ^{199}Hg, 0.5024 for Δ^{200}Hg, 0.7520 for Δ^{201}Hg, and 1.4930 for Δ^{204}Hg. The atomic mass of an isotope was represented by "xxx."

$$\delta^{xxx}Hg = \left(\frac{\frac{^{xxx}Hg}{^{198}Hg_{sample}}}{\frac{^{xxx}Hg}{^{198}Hg_{NIST3133}}} - 1 \right) \times 1000 \quad (1)$$

$$\Delta^{xxx}Hg = \delta^{202}Hg - \delta^{xxx}Hg \times f \quad (2)$$

Operating conditions of the instrument can be found in Supplementary Material A, Table S1. The long-term uncertainty of the measurements was determined using NIST 8610 (UM-Almadén, (National Institute of Standards & Technology, 2017), with accuracy reported as the standard deviation of repeated NIST 8610 measurements. All ranges for isotopic values are presented with an expanded factor (k = 2). Detailed results of the isotopic analysis of NIST 8610 and BCR 482 (lichen) matrix-matched reference material are provided in Supplementary Material A, Table S2. For instance, for the commonly used isotope pair for Mass-Dependent Fractionation (MDF), δ^{202}Hg, the difference between our repeated (N = 32) measurements of NIST 8610 and the NIST-reported value was less than 0.00‰, with an expended standard deviation between measurements (k = 2) at 0.14‰. Similarly, for the most commonly used isotope pair for Mass-Independent Fractionation (MIF), Δ^{199}Hg, the difference was 0.03‰, with an expended standard deviation between measurements (k = 2) at 0.03‰. Unfortunately, we have found no published NIST 1648a findings, but our data for its isotopic composition is provided for potential further comparison by peers in Supplementary Material A, Table S2.

APM Hg concentrations were calculated by determining the volume of air drawn through the sampling device based on time and pump flow rate, and then dividing the Hg concentration by these values. The concentration of APM in the air was determined by dividing the weight of APM on the filters by the volume of air pumped. Weights of the APM were calculated by subtracting the weight of clean filters from the weight of dirty filters. All weighing procedures were conducted in environmentally controlled clean room conditions. Normalized Hg values were computed by dividing the Hg concentrations in APM by the concentrations of APM in the air.

4. Results and discussion

4.1. Comparison of sampling variabilities

Multiple sources of variability must be taken into account for both in-situ and transplantation sampling techniques. In the case of in-situ lichens, natural variability at the collection site was assessed in the study conducted by Božič et al. (2022). This assessment revealed a relative standard deviation of 21.8% for Hg concentrations and standard deviations (k = 2) of 0.58‰ for δ^{202}Hg and 0.18 for Δ^{199}Hg (N = 5). In the present study, we examined two sources of variability: (I) among different bags within 1 m of each other, and (II) within a single bag. The between-bag variability was calculated at 8.9% for Hg concentration, and 0.34‰ and 0.06‰ for δ^{202}Hg and Δ^{199}Hg, respectively (N = 4). Within-bag variability for Hg concentrations was slightly lower at 8.7%, while isotopic ratio variability exhibited a lower figure for δ^{202}Hg at 0.14‰ and a slightly higher value for Δ^{199}Hg at 0.09‰ (N = 4).

A comparison of the within-bag and between-bag variabilities with instrumental variabilities indicates that their standard deviations are roughly similar (as discussed in Section 3.3 and Supplementary Material A, Table S2). However, it's noteworthy that in-situ variabilities tend to be higher, suggesting that transplanted lichens offer greater precision compared to in-situ lichen samples. These findings imply that the utilization of transplanted lichens could potentially lead to more dependable and consistent measurements. Furthermore, these variability values offer qualitative evidence regarding the significance of observed trends and offer insight into the reliability and robustness of the presented data.

4.2. Lichen Hg concentrations

The lichens transplanted to Idrija showed a similar response to the lichens in the 2020–2021 sampling campaign. In both cases, it took approximately one year for Hg concentrations to reach those of the in-situ lichens (Fig. 1 A). The highest concentrations in the in-situ lichens were observed in winter. The reason for the highest Hg concentrations in Idrija lichens could be the biomass burning during the coldest months of the year. Since the local biomass is contaminated with Hg (Kotnik et al., 2015), Hg concentrations in the air are potentially elevated in winter. This effect was exacerbated by the local temperature inversion in the Idrija valley, which is typical for winter.

An opposite trend in Hg concentrations was observed at RCP and Pokljuka in both transplanted and in-situ lichens. At these sites, the lowest Hg concentrations were observed in winter. The difference between winter and summer Hg concentrations was also smaller than in Idrija, ranging from 0.1 to 0.2 μg/g. Such large variations in Hg concentrations were not detected in previous sampling campaign at any of the locations (Božič et al., 2022), possibly due to the lower temporal resolution. The reason for this decrease in concentration during the winter months is not known. Lichen activity is dependent on moisture, temperature, and solar radiation, with lichens being most active at temperatures above freezing (Honegger, 2007). Therefore, they may take up less Hg during the winter months. Some of the potentially relevant environmental factors, such as precipitation and temperature, are shown in Supplementary Material A Fig. S4 and Supplementary Material B.

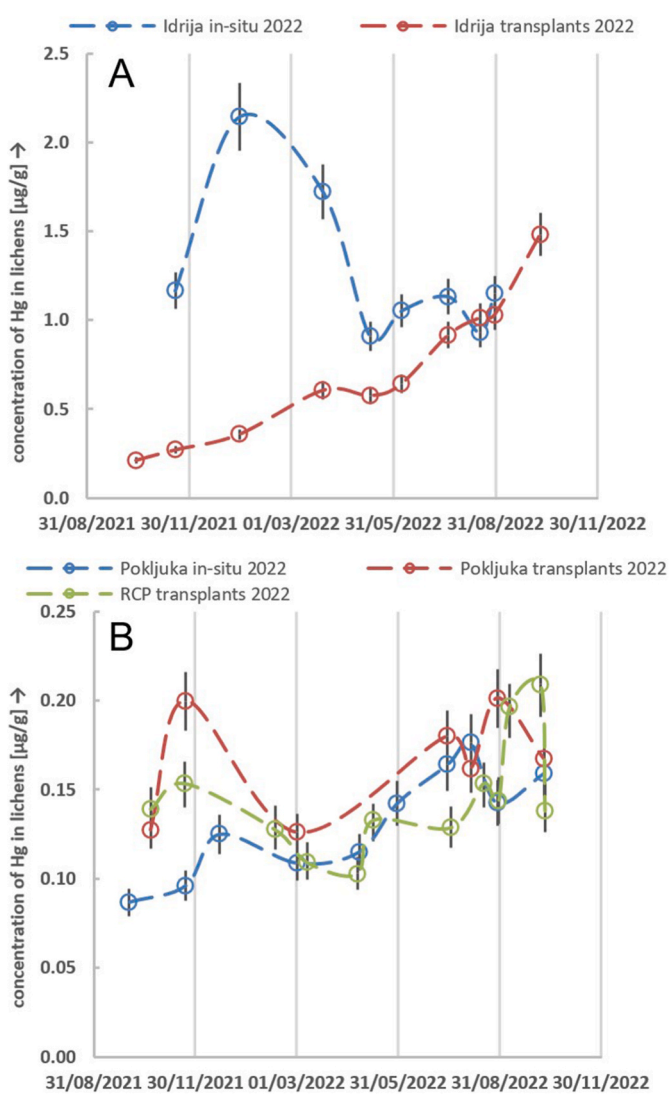

Fig. 1. The concentrations of Hg in the lichens.

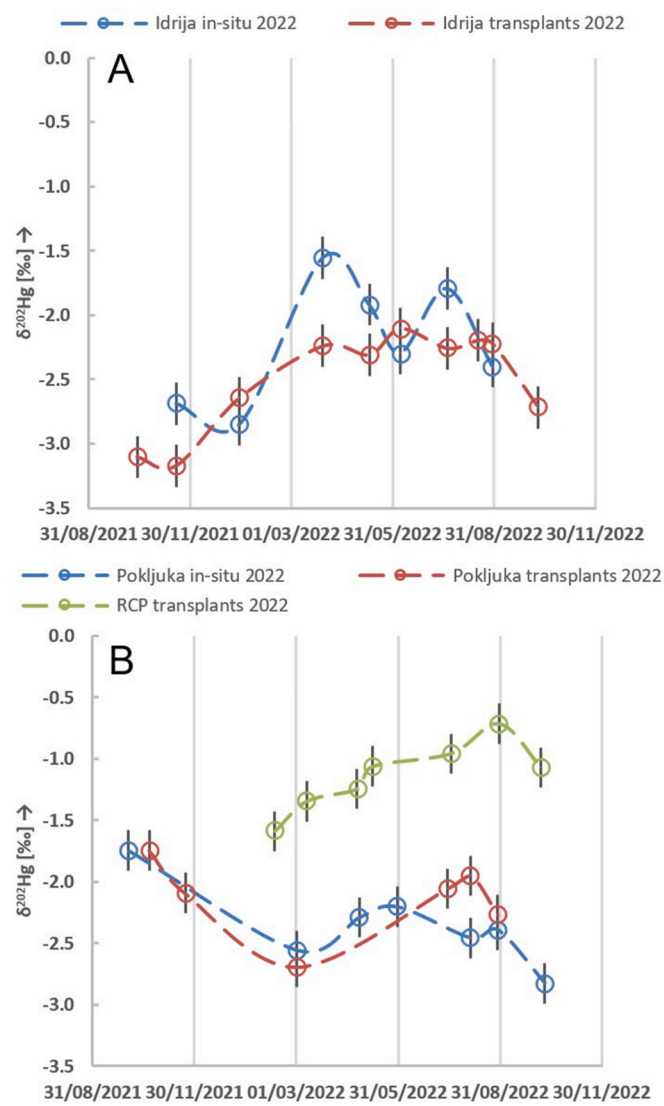

Fig. 2. Mass dependant mercury stable isotope ratios, as δ^{202}Hg values, in lichens. Top: Idrija in-situ and transplants. Bottom: Pokljuka transplants and in-situ and RCP transplants.

4.3. Hg isotopes in lichen

During the sampling campaigns conducted between 2020 and 2021, a conspicuous trend in isotopic composition emerged, characterized by a skewing of values towards heavier isotopes during the summer months, resulting in a range of approximately 3‰ for δ^{202}Hg (Božič et al., 2022).

In the subsequent campaign spanning 2021–2022, the maximum range between the heaviest and lightest isotopic compositions narrowed to about 1.5‰ for δ^{202}Hg, as observed at the Idrija site (in-situ) and illustrated in Fig. 2A. Nevertheless, all sampling sites exhibited a consistent seasonal pattern, with lighter isotopic compositions prevailing during the winter months, akin to the findings reported by Božič et al. (2022), where a more comprehensive exploration of potential mechanisms underlying these changes can be found. Notably, the data presented herein unveil an additional observation, namely, that certain sites exhibit significantly distinct Hg isotope fingerprints despite similar Hg concentrations. For instance, the disparity between the RCP and Idrija sites compared to the Pokljuka site is approximately 1.5‰.

No discernible seasonal trends were detected in Δ^{199}Hg, mirroring the observations made in lichen samples from the 2020–2021 sampling campaign (Božič et al., 2022). However, Idrija exhibited significantly elevated Δ^{199}Hg values in comparison to other sites, averaging −0.12 ± 0.07‰ and −0.08 ± 0.11‰ for transplanted and in-situ lichens, respectively. Conversely, Pokljuka displayed values of −0.30 ± 0.11‰ and −0.26 ± 0.15‰ for transplanted and in-situ lichens, respectively. Moreover, Δ^{199}Hg values in RCP lichens closely approximated those at Pokljuka, with Δ^{199}Hg measuring −0.24 ± 0.11‰ (Supplementary Material A, Table S6). Similar outcomes had been previously observed but had not yet been subjected to interpretation in the study conducted by Božič et al. (2022). The elevated Δ^{199}Hg values in Idrija are likely attributed to the presence of Hg originating from geogenic sources, with the Δ^{199}Hg in ores, as reported by Foucher et al. (2009), approaching zero.

In instances where both transplanted and in-situ lichens were subjected to analysis (Idrija, Pokljuka), the distinctions between isotopes that fractionate mass-dependently and independently within the two groups were minimal (Fig. 2). The average difference for δ^{202}Hg in Idrija was 0.06 ± 1.07‰, and for Δ^{199}Hg, it was 0.04 ± 0.11‰. At Pokljuka, the difference for δ^{202}Hg was 0.28 ± 0.54‰, and for Δ^{199}Hg, it was 0.04 ± 0.21‰. This implies that the observed isotopic composition remains consistent whether lichens are transplanted or naturally situated, even within a relatively short timeframe compared to changes in Hg concentrations. This observation aligns with the proposition by Božič et al. (2022) that lichens adapt their isotopic composition to their environment at a rapid pace.

The δ^{202}Hg and Δ^{199}Hg values discerned in lichens may signify a distinct isotopic fingerprint in different geographic regions, the influence of divergent mechanisms shaping the isotopic fingerprint, or a combination thereof. It is probable that variations in the atmospheric Hg isotopic fingerprint between sites contribute significantly to the observed discrepancies, given that all lichens were exposed to similar atmospheric conditions. Evidently, lichens exhibit alterations in their isotopic composition over time, irrespective of the sampling site, and display varying δ^{202}Hg and Δ^{199}Hg values across different locations.

4.4. Atmospheric particulate matter

At RCP, concentrations of Hg bound to atmospheric particulate matter (APM) exhibited a pronounced seasonal disparity, with Hg levels in winter (58.6 pg/m3) surpassing those in summer (ranging from 2.57 to 5.22 pg/m3) (Fig. 3A). Considering that APM concentrations in this investigation were also elevated during winter (Fig. 3B), it becomes imperative to factor in the proportional presence of Hg within these APM samples. Normalization of the Hg values revealed that the relative concentrations were notably higher in winter, reaching up to 15 μg/g in contrast to 2 μg/g observed in summer (Supplementary Material A, Fig. S4, Table S3). Comparable fluctuations in APM Hg concentrations were observed near a coal-fired power plant in China, spanning a three-year period, where concentrations ranged from approximately ~200 pg/m3 during summer to ~20 pg/m3 during winter (Sun et al., 2021). In urban settings like Beijing, Hg concentrations within APM can reach several hundred pg/m3 during winter (Huang et al., 2020). The

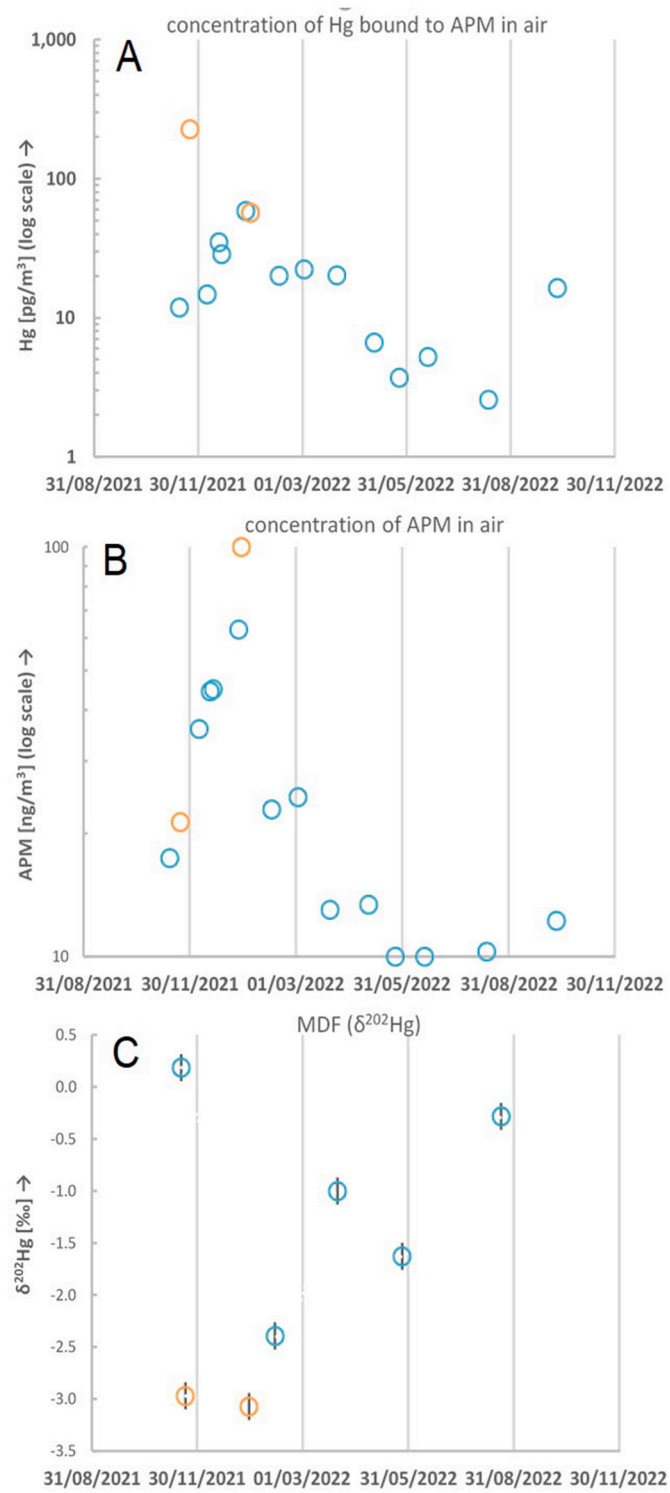

Fig. 3. Mercury and particle mass concentrations, and mercury stable isotope ratios in Idrija (orange) and RCP (blue) APM filters. Top: the concentration of Hg bound to APM in air; middle: the concentration of APM in air; and bottom: the mass-dependent fractionation (δ^{202}Hg) in APM. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

underlying causes for these Hg concentration variations in APM are likely akin to the factors contributing to higher Hg concentrations in lichens in Idrija, chiefly attributed to biomass burning and fossil fuel combustion. Additionally, the operation of wastewater treatment plants (WWTP) could be a contributing factor, although data regarding Hg

releases into the water treatment system is currently unavailable. In Idrija, Hg concentrations in the APM reached up to 0.01% of the APM mass (Supplementary Material A, Fig. S4), possibly stemming from the nugget effect (Kocman, Vreča, et al., 2011), where larger HgS particles or 'nuggets' are present in atmospheric particles.

However, the Hg isotopic composition of APM at RCP exhibited a parallel seasonal trend to that of lichens, featuring a lighter isotopic composition during winter and a heavier one during summer (Fig. 3C). Moreover, similarities in the isotopic signatures indicated that the isotopic composition between Idrija and RCP deviated by approximately ~0.5–1‰ for δ^{202}Hg during the corresponding time frames in both cases. These similarities suggest that both entities may be influenced by a common underlying mechanism. Notably, at RCP, a significant surge in Δ^{199}Hg was detected during the spring and summer months, specifically from May to July, with a Δ^{199}Hg value of 0.60 ± 0.03‰, exceeding the annual average of 0.10‰ observed at other sampling points. Furthermore, a slight decline in δ^{202}Hg was noted in the same sample collected in May (Fig. 3C). These findings align with the observations made by Huang et al. (2021), who reported an increase in Δ^{199}Hg and a decrease in δ^{202}Hg for aerosols exposed to light, attributed to photoreduction. This time period also coincided with the lowest precipitation levels (Supplementary Material A, Fig. S2A) and relatively low cloud cover, coupled with maximum sunshine duration (Supplementary Material B).

5. Conclusion

In summary, this investigation into Hg isotope composition in lichens, encompassing both contaminated and pristine environments and employing transplanting and in-situ collection techniques, has yielded valuable insights into seasonal dynamics and influencing factors on Hg isotope composition and Hg concentrations. These findings, consistently corroborated by prior research (Božič et al., 2022), highlight the similarity of the observed seasonal alterations in Hg isotopic composition through multiple years across diverse environmental conditions and sampling methods. They also suggest that while diverse mechanisms may govern Hg concentrations at various sites, a common set of mechanisms drives changes in isotopic composition. Moreover, the resemblance between APM isotopic data and lichen isotopic fingerprints implies an intriguing interconnectedness between lichens and APM in terms of their Hg isotopic signatures, expanding our understanding of the broader environmental implications and deepening our appreciation of the complexity of Hg bio-monitoring using lichens.

These findings significantly enhance our comprehension of Hg behaviour in lichens and its interaction with environmental factors, fostering a deeper understanding of the intricacies of Hg bio-monitoring using lichens across diverse sites and climates. As this study, conducted under specific geographic conditions, may not be universally applicable to all environments and locations, caution should be exercised when using lichens as proxies for assessing Hg concentrations or isotope ratios in the atmosphere until further investigations are carried out. Future research can build upon these findings to delve into the specific mechanisms underpinning the observed isotopic composition changes and their implications for Hg pollution monitoring. It is advisable to conduct comprehensive studies that consider factors such as natural and seasonal variations in lichen samples and potential local pollution sources. Additionally, extended sampling periods for lichen collections can yield a broader range of variations in Hg concentrations and isotope ratios at a given site. When utilizing transplanted lichens for a particular location, allowing sufficient time for lichens to acclimate to their new surroundings likely important at least in case of the transplantation to a polluted site.

Credit author roles

Dominik Božič;: Conceptualisation, Methodology, Investigation, Data curation, Writing – original draft, Visualisation. Milena Horvat: writing – review, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.122740.

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