Lichen transplants as indicators of gaseous elemental mercury concentrations

Fabrizio Monaci[1*], Stefania Ancora[2], Luca Paoli[3], Stefano Loppi[1], Frank Wania[4]

[1] Department of Life Sciences, University of Siena, Via Mattioli 4, Siena, Italy

[2] Department of Physical Sciences, Earth and Environment, University of Siena, Via Mattioli 4, Siena, Italy

[3] Department of Biology, University of Pisa, Via Luca Ghini, 13, 56126 Pisa, Italy

[4] University of Toronto Scarborough, Department of Physical and Environmental Sciences, 1065 Military Trail, Toronto, ON, Canada, M1C1A4

[*]Corresponding author: Fabrizio Monaci (fabrizio.monaci@unisi.it)

Abstract

Lichens play an important role in the biogeochemical cycling of mercury (Hg) and are commonly used as indicators of Hg enrichment in remote and anthropogenically impacted environments. To assess their capacity for Hg uptake and accumulation, we determined the concentration of gaseous elemental mercury (GEM) in air and the concentration of total Hg (THg) in transplanted thalli of two lichen species. Lichen transplants and passive air samplers (PASs) were concurrently deployed, side by side, at 10 sites within an abandoned mining area, characterized by large gradients in atmospheric Hg contamination. Highly variable time-weighted GEM concentrations determined by the PASs, ranging from 17 to 4,200 ng/m^3, were mirrored by generally high Hg concentrations in transplanted thalli of both *Xanthoria parietina* (174-8,800 ng/g) and *Evernia prunastri* (143-5,500 ng/g). Hg

concentrations in the two species co-varied linearly indicating about 60% greater Hg accumulation in *X. parietina* than in *E. prunastri*. Whereas Hg uptake in the fruticose *E. prunastri* increased linearly with GEM, a power law equation with a fractional exponent better described uptake in the foliose *X. parietina*. Extrapolating the relationships observed here to higher GEM levels yielded concentrations in lichen that agree very well with those measured in an earlier fumigation experiment performed under laboratory-controlled conditions. The uptake model of *X. parietina* was further verified by correctly estimating GEM concentrations from the THg measured in autochthonous thalli collected from the urban area adjacent to the mine site. Passive sampling can effectively provide time-weighted data of suitable spatial resolution to quantitatively describe GEM assimilation by lichens. Therefore, the combined use of passive sampling and lichen transplants can contribute to a more comprehensive understanding of the role of lichens, and potentially also of other cryptogams, in the deposition of atmospheric Hg to terrestrial ecosystems.

Keywords: terrestrial ecosystems; passive sampling; mercury uptake; biomonitoring; mining

1 Introduction

In the natural environment, lichens are exceptionally adaptative symbiotic associations that may become dominant in challenging high altitudes or polar ecosystems, as well as other dry lands (Nash, 2008). Poikilohydry (i.e., the absence of effective epidermal tissues and vascular system) is a prominent trait that makes lichens dependent on the atmosphere for mineral nutrition and water supply (Porada et al., 2014). Placed at the interface between atmospheric and terrestrial compartments, lichens have been recognized as effective receptors of atmospheric deposition (Nash, 2008) and a crucial part of biogeochemical cycles, both at local and global scales (Elbert et al., 2012; Porada et al., 2014). Accordingly, lichens have become important tools for studying changes in the atmospheric load of nutrients (e.g., nitrogen) or anthropogenic inputs of persistent pollutants in

terrestrial ecosystems (e.g., heavy metals; Dresler et al., 2021; Ellis et al., 2022; Paoli et al., 2021; Root et al., 2021).

Of particular ecological significance is research using lichens to monitor atmospheric Hg deposition at urban and industrial sites, as well as in uncontaminated remote areas (e.g., Klapstein et al., 2020; López Berdonces et al., 2017; Paoli et al., 2015; Zvěřina et al., 2018). Mercury is an ecosystem pollutant whose biogeochemical cycle has been dramatically altered by human activities (Chen et al., 2018). Moreover, Hg is especially capable of pervasive environmental impacts, as it is distributed globally through its gaseous elemental form (GEM), which is predominant (approx. 95%) and long-lasting (6-18 months) in the atmosphere (Agnan et al., 2016; Gworek et al., 2020).

In terrestrial ecosystems, atmospheric Hg deposition takes place via wet and dry deposition, sorption of GEM to Earth's surfaces or via vegetation assimilation (Zhang et al., 2009). The latter is globally considered dominant (1,310-1,570 Mg/yr), even exceeding wet terrestrial deposition (730-1,070 Mg/yr), and is reckoned to be the main source of Hg transferred to soils and watersheds (Zhou et al., 2021). Recently, the important role of cryptogams, and among them of lichens, in the assimilation of atmospheric Hg by vegetation has been recognized by a modeling study (Zhou and Obrist, 2021). For example, in forests these organisms may account for 798 ± 202 Mg/yr of Hg uptake, which is approximately 40% of atmospheric Hg assimilated by foliage (Zhou and Obrist, 2021).

Despite the extensive use of lichens for monitoring atmospheric Hg deposition since the 1970s (Siegel et al., 1975; Steinnes and Krog, 1977), little is still known about the ecophysiological processes that govern Hg uptake by lichen thalli, as well as the quantitative relationships between Hg in the air/deposition and in exposed lichens. GEM uptake may involve oxidation of GEM to Hg^{2+} with subsequent immobilization in the lichen thallus for at least 4-5 weeks (Zhou et al., 2021). Laboratory experiments conducted at uncommonly high GEM levels (10-45 $\mu g/m^3$) have shown species-specific trends between GEM and Hg concentrations in the foliose (leaf-like) lichen *X. parietina* and the fruticose (upright and shrubby) *Pseudevernia furfuracea* and *E. prunastri* (Vannini et al., 2014). More recently, a field study exploring relationships between lichens and atmospheric Hg in two

contaminated areas of Spain observed different trends in Hg accumulation in fruticose (genus *Ramalina*) and foliose autochthonous thalli (genus *Xanthoria*) (López Berdonces et al., 2017).

A challenge in quantitively assessing the kinetic of Hg uptake by lichens, is the lack of adequate spatially distributed and temporally resolved GEM measurements, essential to appropriately capture long-term processes of assimilation by lichens of highly fluctuating Hg in the air. Meteorological factors, such as ambient temperature, precipitation as well as wind intensity and direction, are extremely variable over short timescales and affect both GEM concentrations and physiological conditions of lichens that control Hg^0 uptake (Bargagli, 2016; Mlakar et al., 2011). Acquisition of such as variability has been attempted in previous field studies by the means of sporadic atmospheric Hg measurements associated to both in-situ and transplanted lichens (e.g., Bargagli and Barghigiani, 1991; López Berdonces et al., 2017; Mlakar et al., 2011). For example, in their field study with autochthonous lichens, López Berdonces and co-workers regularly determined GEM concentrations with a portable spectrophotometer, although only for a limited period of time (2 min/day, 3 days per week, for 13 weeks) (López Berdonces et al., 2017). However, in contaminated areas, GEM concentrations can be extremely variable, by as much as one to three order of magnitudes on a spatio-temporal scale of few seconds and meters (Vaselli et al., 2013). Under these conditions, active monitoring instruments, like the Tekran® 2537 series, capable of high temporal sampling resolution, are generally used. Such an instrumental approach, however, is not well suited for ecological studies with lichens, as technical constraints (e.g., power and technical gas requirements) and economical limitations (e.g., expensive purchasing and maintenance costs) render the necessary multiple concurrent measurements over long periods unachievable (McLagan et al., 2015).

A common approach for overcoming limitation of automated instrumental approaches and for a reliable quantitative assessment of airborne persistent pollutants is passive sampling (Marć et al., 2015; Wania and Shunthirasingham, 2020). Recently, a precise, low-cost and practical passive sampler for GEM monitoring has been conceived (McLagan et al., 2016). Following a multi-year testing and evaluation process, carried out in the lab, and in the field under different environmental

conditions, this sampler has been proven highly effective and capable of reliable, co-occurrent, time-weighted measurements of GEM. Validity of the system has been verified for various periods of time (i.e. from hours to several months) and for a wide range of atmospheric Hg concentrations, ranging from background (0.5-1.5 ng/m^3) to hotspot levels (10.000 ng/m^3) (McLagan et al., 2016, 2018, 2019; Naccarato et al., 2021; Snow et al., 2021).

In this study, this sampler and the thalli of two lichen species, *X. parietina* and *E. prunastri*, commonly used in biomonitoring studies, were deployed side-by-side for a period of 33 days at a contaminated site characterized by high GEM emissions. The working hypothesis of the study was that GEM concentrations determined with passive samplers on an identical time-weighted and space-based resolution to that of transplanted lichen thalli provided the appropriate data for studying Hg assimilation by lichens. Specific aims of this research were: *i*) characterizing the Hg accumulative capacity of the two transplanted lichen species, which are distinguished by their growth habitus (foliose/fruticose), with respect to different levels of contamination; *ii*) inferring, from experimental data obtained under field conditions, quantitative relationships of Hg uptake and accumulation in the two transplanted lichen species for a wide range of GEM concentrations ; finally, *iii*) predicting, from the identified uptake model of *X. parietina*, GEM concentrations on the basis of Hg concentrations in autochthonous thalli of the same species.

2 Materials and methods

2.1 Study area and design

The study has been carried out in the mining area of Abbadia San Salvatore (42.87 °N, 11.66 °E; 876 m a.s.l.), a town of about 6,000 inhabitants, in the South of Tuscany Region, central Italy. The mine is part of the district of Mt. Amiata (1,738 m a.s.l.), an extinct volcano inlaid with a belt of Hg mineralization (cinnabar, HgS) which has been mined since antiquity until recent times. For most of the twentieth century, the mine was among the largest global manufacturers of liquid Hg; the production officially ceased in 1984, and since then the area, and especially the metallurgical plant therein, has continued to be a significant source of GEM at the global scale, recent estimates ranging

between 80 and 150 kg GEM/year, in the cold and hot season (McLagan et al., 2019). According to recent assessments performed in the area of Mt. Amiata (Monaci et al. 2022; Nuvolone et al., 2021), Hg represents the one and only atmospheric pollutant affecting the air quality of Abbadia S.S.

Based on the GEM concentration distribution map created by McLagan and co-workers (McLagan et al., 2019), 10 deployment sites were selected within the mining area, with estimated GEM concentrations ranging from 5-17 to 1,680-3,600 ng/m^3 (interpolations from 6/29/2016 to 7/7/2016 monitoring campaign, average temp. 20.3 °C, cumulative precipitation 0 mm; Supplementary Material, Table S1, Fig S1). The deployment of both lichen transplants and PASs lasted 32.9 ± 0.06 days, from 9/9/2016, 11:32 AM to 10/12/2016, 12:50 PM. During this period the average ambient temperature was 8.7 ± 2.85 °C and the total precipitation was 212 mm, as measured at the Laghetto Verde Meteorology Station (42.8823°N, 11.6616°E, 910 m a.s.l), located immediately adjacent to the mining area (Supplementary Material, Table S2).

2.2 Lichen transplants and PASs deployment within the mining area

Lichen transplants were prepared from thalli of the foliose *Xanthoria parietina* (L.) and the fruticose *Evernia prunastri* (L.) Ach. collected on 9/7/2016 in a remote area in Southern Tuscany, central Italy, far from known pollution sources. In this area, twigs of the shrub *Prunus spinosa* (ca. 15 - 30 cm in length) carrying at least two or three thalli of *X. parietina* and/or *E. prunastri* were pruned from a total of 15 trees. These lichen species were chosen for their different growth habitus (foliose or fruticose). Moreover, they are widely available, and autochthonous thalli of *X. parietina* are commonly adopted in biomonitoring surveys of airborne heavy metals (Achotegui-Castells et al., 2013; Paoli et al., 2017; Parviainen et al., 2019); thalli of *E. prunastri* are often chosen for transplanting, as this species is easily collected and prepared for this purpose (Cecconi et al., 2019; Paoli et al., 2015). The two lichen species have also been used in previous studies comparing GEM concentrations and Hg content in lichen thalli (López Berdonces et al., 2017; Vannini et al., 2014).

In the laboratory of the University of Siena, the collected thalli were cleansed of potentially present exogenous material (e.g., larger soil particles and woody debris) then a representative sample, i.e., about 5-6 transplanted thalli of each species, to be used as reference material before transplanting, were put in a paper bag and stored at -20 °C until later Hg analysis. On 9/9/2016, the lichen transplants and the PASs were transported to the study area for deployment. Each sampling site was inspected, the geographical position recorded, and existing structures (i.e., poles, fences, or trees) suitable for the deployment of the PAS and the lichen transplants were identified. Where this was not possible, a custom metallic pole was planted in the ground to reach a height of 1.5 m. Using plastic cable ties, the passive samplers and the lichen transplants were fixed at the supports identified at the 10 sites. The PAS activation was carried out by removing the solid polytetrafluoroethylene (PTFE) screw cap that prevents air circulation in the internal airspace of the sampler (containing the microporous diffusive barrier made from high-density polyethylene and the sulphur-impregnated activated carbon sorbent). The solid PTFE cap was substituted with an open-mesh screen (diamond openings, height of 5 mm, width of 4 mm) that remained in place for the entire length of the deployment. There, transplanted thalli were installed with their substrate at the supports *in situ* by using plastic threads, bonding three twigs per each lichen species. At each site, a passive sampler was installed to the same support using a custom L-bracket and plastic cable ties. The samplers remained on site for the entire period of deployment of the lichens; only at sites BIO_09 and BIO_10, where very high GEM concentrations were expected, two sequential sampler deployments were used; thus, on 9/26/2016 the samplers of the two sampling stations were replaced with new ones, which stayed in place until the completion of the experiment, on 10/12/2016. That day, all the deployed PASs and lichen transplants were retrieved: the open-mesh lids of each PAS were removed and exchanged with a solid PTFE cap to prevent further Hg uptake; then the PAS was wrapped with PTFE tape, and sealed in a ziplock bag.Each lichen transplant was put in a large paper bag. Each bag with retrieved samplers or lichen thalli were identified by the type of sample, the sampling site, and the time of deployment.

2.3 Collection of autochthonous lichen thalli from urban green spaces

To compare THg concentrations in lichen transplants with autochthonous lichens, thalli of the two species, *X. parietina* and *E. prunastri*, were collected in the urban area of Abbadia S.S., where possible, in the vicinity of the sampling sites covered by the study of McLagan et al. (2019). The goal was to use GEM concentrations data, available for these sites, as a reference, though not temporally coincident, to support comparison of THg accumulation trends in autochthonous and transplanted lichens and to verify possibility of extrapolation. On 9/21/2016 autochthonous samples of *X. parietina* (n= 17) and *E. prunastri* (n=5) were collected from 18 sites (parks, garden, avenues) in the urban area of Abbadia S.S. (Supplementary material, Fig. S2) and at two sites on Mt. Amiata distant from the mine. At each site, five individual thalli of the two species were randomly collected from the trunks of at least two trees, avoiding areas of the trunk affected by stemflow. Samples were taken at about 2 m from the ground to prevent contamination by resuspended street dust. After sampling, the thalli were stored in paper bags labelled with the site name and the lichen species.

2.4 Sample preparation and analytical determinations

Once in the laboratory, retrieved transplanted and autochthonous lichen thalli were carefully cleaned with nylon forceps under a binocular microscope to remove extraneous material (i.e., adhering bark, other lichen or moss species, senescent parts). After being air-dried to constant weight in a clean room, the peripheral part of the thalli was selected for the analyses, by detaching it with a stainless-steel scalpel. This part consisted of about 2 mm from the lobe tips in the foliose *X. parietina* and 4 cm of the outermost branches of fruticose *E. prunastri*. At least in foliose lichens, such as *X. parietina*, the peripheral part of the thalli is known to be the newest, approximately corresponding to the last year of growth (Gremillion et al., 2013; Loppi and Bonini, 2000). For each lichen species, the composite sample made by the selected thalli fragments were pulverized and homogenized with a ceramic mortar and pestle.

About 400 mg of each homogenized lichen sample were weighted in a polytetrafluoroethylene (PTFE, Teflon®) container; then, 5 ml of concentrated HNO_3 (65% JT Baker®, analytical grade) and 2 ml of H_2O_2 (20%, JT Baker, analytical grade) were added to the PTFE containers which were hermetically sealed and digested in a microwave unit (Milestone® Ethos 1) according to a specific program at controlled temperature (max 210 °C) and pressure (max 160 bar). At the end of the digestion program, the PTFE containers were allowed to cool and then opened inside a suction hood to transfer each solution to a polyethylene (PE) tube, avoiding any contamination or leakage. The solutions were then diluted to a final volume of 10 ml with Milli-Q deionized water (Millipore®). Instrumental calibrations were performed using aqueous reference solutions prepared immediately before use from serial dilution from commercial stock solutions (Merck®) at a concentration of 1 g/L. Concentrations of Hg were determined by the cold vapour technique using a Flow Injection Mercury System (FIMS 400, Perkin-Elmer®). The analytical determinations of the concentrations of each sample were based on three replicate readings. Analytical blanks (digestion solutions) were analysed every 12 samples; the quality of measurements was checked by analysing the Standard Reference Material IAEA-336 "lichen"; precision was 5%, as estimated by the routine analysis of five replicates. Hg concentrations in lichens were blank-corrected and expressed as ng/g on a dry mass basis. The Hg concentrations in the original lichen material of *X. parietina* and *E. prunastri* used for transplants of this study were 61 ± 26 ng/g and 35 ± 11 ng/g, respectively.

2.*5 Passive sampler analysis*

The 12 retrieved passive samplers were delivered in an airtight container to the laboratory of the Department of Physical & Environmental Sciences at the University of Toronto Scarborough, where they were cleared from their original protective packaging, the sorbent carefully extracted from the diffusive body, weighted, and analysed for total Hg (THg) concentrations using an AMA254 Total Mercury Analyzer (Leco Instruments). The analytical procedure followed USEPA method 7473 (USEPA, 2007) and is described in detail in previous papers (McLagan et al., 2016, 2017, 2019). The

GEM concentration (ng/m^3) was calculated by dividing the blank-corrected mass of sorbed Hg (ng) by the product of the deployment time (days, hours, minutes) and the sampling rate (0.135 m^3/day) of the sampler (McLagan et al., 2018). The GEM concentrations at BIO_09 and BIO_10 were calculated as time-weighted average of the Hg concentrations resulting from each of the two consecutive deployments (9/9-9/26/2016 and 9/26-10/12/2016). Precision of measurements, expressed as the difference between duplicated samples (n =9), was 2%; accuracy of determinations was assessed by the routine analysis of NIST SRM 2685c Bituminous Coal.

2.6 Data analysis

Exploratory analysis was carried out to check distribution and outliers of the data sets. Linear and nonlinear regression analysis was used to describe the relationships between GEM concentrations (ng/m^3) determined using the PASs and THg concentrations in transplanted lichen thalli of *X. parietina* and *E. prunastri* (ng/g), and to derive Hg uptake rates (ng/g/h) from those relationships. For each species, the best predictive relationship was identified based on the goodness of fit, using Microsoft Excel Solver® to minimize the root-mean square error (RMSE). The best model for each lichen species was used to predict Hg uptake at GEM concentrations of 10, 15, 30, 45 µg/m^3 of an earlier fumigation study (Vannini et al., 2014) and the predicted data were compared with the experimental results. Using the relationship between GEM concentrations and uptake in *X. parietina*, we further estimated GEM concentrations from the THg concentrations measured in the outermost 2 mm of the autochthonous thalli sampled in Abbadia S.S., representing last's year biomass growth. These estimated concentrations were compared with: *i*) GEM concentrations measured in the same urban area using high sampling resolution active monitoring instruments (Lumex RA 915) (Cabassi et al., 2022; Vaselli et al., 2013); *ii*) time-weighted GEM concentrations determined with PAS (McLagan et al., 2019) deployed at the sites (or in close proximity) where *X. parietina* thalli were taken (Supplementary material, Fig. S4).

3 Results and discussion

3.1 GEM concentrations and Hg concentrations in transplanted lichen thalli

GEM concentrations across the 10 deployment sites ranged from 17 to 4,200 ng/m^3 with a median of 382 ng/m^3 (Table 1). Such an extended range (2.5 orders of magnitude) reflects the steep spatial gradient of GEM concentration that characterize the investigated area, as shown by previous studies (McLagan et al., 2019; Vaselli et al., 2013, 2017). To get an understanding of the levels of GEM measured in this study, it is useful to compare them with conventional reference values used for protection of human health. GEM concentrations measured at three sites (BIO_08-10; Figure 1A) were remarkably higher than the non-occupational minimal risk level (MRL) for chronic inhalation (365 days) of GEM, established in Europe (1,000 ng/m^3; WHO Regional Office for Europe, 2000). Two more sites (BIO_06-07) remained far above the stricter MRL of 200 ng/m^3 applied in the USA by the US Agency for Toxic Substances and Disease Registry (USATSDR, 2021). The other five sites (BIO_01-05) were characterized by GEM concentrations (16.9-54.4 ng/m^3) that are consistent with those previously reported in the urban area of Abbadia S.S., adjacent to the mining site (Cabassi et al., 2022; McLagan et al., 2019; Vaselli et al., 2013) and are similar to GEM concentrations commonly found in other post-Hg mining towns (Esbrí et al., 2016; Kocman et al., 2011).

In relative terms, variability of Hg data in lichens was slightly higher, but comparable (coefficient of variation = 1.21-1.41) to that of GEM (1.13). The foliose *X. parietina* reached the highest Hg concentration (8,790 ng/g), while the fruticose *E. prunastri* had maximum concentrations of 5,540 ng/g. The transplanting for 33 days in the study area resulted in a remarkable enrichment of Hg in both lichen species, reaching factors of 4 up to 159 in *E. prunastri* and of 3 up to 144 for *X. parietina*, as calculated from the concentrations originally present in unexposed lichen material (see Material and Methods). The high Hg accumulation trends observed for the two lichen species of this study confirm similar observations obtained by other authors with both transplanted (e.g., Tretiach et al., 2011) and autochthonous lichens (e.g., Bernardo et al., 2021). Ecophysiological processes underlying such a high atmospheric Hg uptake and retention can be ascribed to both fungal and photosynthetic

283 component of the lichen association (Bargagli, 2016). Observations from field studies (Adamo et al.,
284 2008; Cecconi et al., 2021) and laboratory experiments (Balarama Krishna et al., 2004; Vannini et
285 al., 2014) indicated that GEM assimilation have a non-reversible component which may involve an
286 active, enzyme mediated conversion of Hg^0 to Hg^{2+} with subsequent complexation to sulfhydryl
287 groups of proteins (Bargagli, 2016).
288 In general, concentrations of Hg in lichen thalli > 1,000 ng/g, such as those recorded in about half of
289 the transplants of this study, are rare in the scientific literature; in *X. parietina*, data exceeding this
290 threshold have only been found in previous biomonitoring studies conducted in Abbadia S.S. or near
291 the chlor-alkali plant of Flix (SE Spain) (Bargagli and Barghigiani, 1991; Esbrí et al., 2015; Loppi et
292 al., 2006; Rimondi et al., 2020). This threshold of 1,000 ng/g seems also to reflect the central tendency
293 of Hg concentrations in autochthonous *X. parietina* (median = 1,110 ng/g) and *E. prunastri* (1,130
294 ng/g) from public gardens and other urban sites of Abbadia S.S. (Table 2; Supplementary material,
295 Fig. S2). Hg concentrations in lichen thalli transplanted to sites BIO_01-05 (ranging in *X. parietina*
296 between 170 to 400 ng/g and in *E. prunastri* between 140 and 210 ng/g; Fig. 1A) correspond to Hg
297 contamination conditions commonly found in environments impacted by industrial activities other
298 than mining (Table 2).
299 The scatterplot of Hg concentration datasets of transplanted *X. parietina* and *E. prunastri* revealed
300 the anomaly of site BIO_10 (Fig. 1B). Hg data from this site deviate remarkably from the trend of the
301 relative Hg accumulation in the two species. Site BIO_10 is characterized by the most extreme
302 conditions of the study area, as it was positioned close to, if not almost within, the Hg hotspot of the
303 mining complex (McLagan et al., 2019). Here, differently from the other deployment sites that were
304 all in the open air, transplanted lichens were adjacent to heavily contaminated built structures, such
305 as the metallurgical facilities building which has been exposed for a long time, and to a lower extent
306 continues to be exposed, to GEM-rich fumes and vapours. Concrete, paint, mineral wool and Moplen
307 polymer (condensers) from that building of site BIO_10 were found to be highly enriched with Hg
308 (up to 10,800 mg/kg) (Vaselli et al., 2017). Therefore, it can be assumed that airborne fragments

from these materials, if trapped by the transplanted thalli, cause heavy enrichment with Hg. Site BIO_10 was also located upwind of the main source of GEM (the metallurgical plant) during the deployment period as the prevailing winds were recorded to come mostly from W to E (Supplementary material, Fig. S1 and Fig. S3). Those winds largely blew from the metallurgical plants towards BIO_09, where in fact the maximum GEM concentration of the study was recorded (Fig. 1A).

After the data for site BIO_10 was excluded from the linear regression, the relationship between Hg concentrations in transplanted thalli of the two lichen species explained a considerable proportion of the variance (R^2 =0.981), in a range of concentration up to 3,660 ng/g in *E. prunastri* and 8,790 ng/g in *X. parietina.* From this, it can be deduced that, on a mass basis, the foliose lichen *X. parietina* accumulates, on average, 60% more THg than the foliose *E. prunastri* at any GEM concentrations of this study. The difference in Hg accumulation potential between the two species appears even larger when the specific surface area to dry weight ratios for *X. parietina* (50 g/m^2; Loppi et al., 2006) and for *E. prunastri* (189 g/m^2; Gries et al., 1997) are taken into account. This result apparently conflicts with previous observations under which a higher surface/volume ratio, typical of fruticose lichen or moss, results in more efficient accumulation of atmospheric Hg (Adamo et al., 2007; Bargagli, 2016; Tretiach et al., 2005). In our field-study, species-specific ecological features of the two lichens may have played a more predominant role in Hg assimilation than their morphological structure, indicated by the surface/volume ratio. For instance, *X. parietina* is known as meso-xerophytic and capable to tolerate strong solar radiation as well as eutrophication, while *Evernia prunastri* is a hygro-mesophyte which prefers indirect sunlight and poorly tolerates even moderate levels of eutrophication. Differences in THg accumulation from GEM concentrations have been observed experimentally between *X. parietina* and *E. prunastri* in relation to the ambient temperature (Vannini et al., 2014). Therefore, it can be assumed that temperature or other meteorological conditions encountered during the transplanting period, were more favourable for Hg assimilation or for physiological acclimation to *X. parietina* rather than to *E. prunastri*.

335 *3.2 Quantitative relationships between GEM and Hg in lichens*

336 The trend of Hg assimilation in *X. parietina* and *E. prunastri* transplanted thalli, reported as THg
337 concentration (ng/g; left y-axis) and uptake (ng/g/h; right y-axis), across the range of GEM
338 concentrations of this study is shown in Fig. 2. The two species, representative of different biological
339 forms (foliose/fruticose), were distinguished, under identical environmental and atmospheric
340 contamination conditions, by specific assimilation kinetics of Hg. Mercury uptake in *X. parietina* was
341 described by a power law equation having an exponent of fractional order (n = 0.765). Assimilation
342 kinetics of Hg by *E. prunastri* was instead best described by a linear equation with a slope
343 approaching unity (0.839) and an intercept value roughly indicating the baseline THg concentration
344 (129 ng/g) for this species. The best goodness-of-fit was found for the non-linear model of *X.*
345 *parietina* (R^2 = 0.984), while the linear model of *E. prunastri* showed a less explicative fitting (R^2 =
346 0.770), which improved remarkably when the datum of BIO_10 was excluded from computations (R^2
347 = 0.994; Fig. 2). Interestingly, López Berdonces and co-authors (López Berdonces et al., 2017),
348 described the uptake of Hg by eight samples of autochthonous thalli of the foliose *X. parietina*
349 collected around the chlor-alkali plant of Flix (NE Spain) by a power equation with exponent n = 1,
350 thereby essentially indicating a linear uptake of Hg within the range of the measured GEM
351 concentrations (from ~ 80 to ~ 600 ng/m^3). In our study, the relationship between THg concentration
352 in transplanted *X. parietina* and GEM concentrations could also be acceptably described by a linear
353 equation kinetics (y = 2.237x + 367; R^2 =0.969), although less accurately than the nonlinear model
354 reported in Fig. 2.

355 The validity of the two species-specific uptake models identified above was examined by comparing
356 the uptake rate curves (ng/g/h; Figure 3) with data of the same type for both *X. parietina* and *E.*
357 *prunastri* from an experiment by Vannini and co-authors (Vannini et al., 2014). Although the latter
358 data originate from a shorter (12 days) controlled fumigation study at very high GEM concentrations
359 (10-45 µg/m^3), it agreed remarkably well with the respective uptake curves of our field study based

on time weighted GEM concentrations determined using PAS (R^2 = 0.998 and 0.959 for *X. parietina* and *E. prunastri*, respectively; Fig. 3). Especially the power law model for *X. parietina*, is capable of predicting, with minimal error, the Hg assimilation by the thalli exposed to GEM occurring in an extraordinary range of concentrations of four orders of magnitude (17 ng/m^3 – 45 µg/m^3), if the concentration intervals of our study and that of the fumigation experiment (Vannini et al., 2014) are combined. The *E. prunastri* model appears to be slightly less accurate in quantifying the Hg uptake at the GEM concentrations of the fumigation study (Fig. 3).

Since the outermost part of the thalli of *X. parietina*, which corresponds to one year of exposure (Bargagli et al., 1987; Nimis et al., 2001), was analyzed in this study, it is possible to apply the Hg uptake model for this species to estimate GEM concentrations in air from the THg concentrations of autochthonous thalli (Table 2; Supplementary material, Fig. S2). Estimated GEM concentration, shown in Fig. 4A as a box-and-whisker plot, resulted in a median concentration of 25 ng/m^3 (min-max = 3.8-66 ng/m^3) for lichen thalli collection sites located 100 – 1,300 m (mean = 454 m) away from the mining area. When compared with results from GEM monitoring campaigns conducted in the same urban area (Cabassi et al., 2022; Vaselli et al., 2013), autochthonous *X. parietina*-based estimations fell in the same concentration range obtained with a dedicated GEM analyzer (Lumex RA-915; Fig. 4A). Moreover, estimated GEM concentrations at the 17 sites of collection of *X. parietina* thalli realistically reflect the physical processes of GEM dispersion as represented in Fig. 4B by the exponential decay trend of Hg concentrations with respect to the distance (m) from the mining site. In general, all of estimated GEM concentrations remain below the GEM limit of 100 ng/m^3 which was identified by Vaselli and co-workers in an instrumental monitoring survey (Vaselli et al., 2013), as the limit distinguishing the range of GEM concentrations commonly found within the urban perimeter of Abbadia S.S. from that (exceeding 100 ng/m^3) occurring inside the nearby mining area. Data from *X. parietina* thalli collected at more than 6 km from the mining area of Abbadia S.S. resulted in estimated GEM concentrations of 1.24-2.55 ng/m^3 (Fig. 4B) that are representative of

typical background values reported for Mt. Amiata (estimated ranging between 3-5 ng/m^3; Cabassi et al., 2022; Ferrara et al., 1988).

The GEM concentrations predicted from the autochthonous thalli of *X. parietina* can also be compared with GEM concentrations obtained from the extensive PAS measurements conducted in the Mt. Amiata area, and specifically in Abbadia S.S, by McLagan and co-workers (McLagan et al., 2019). Data of this study make site-to-site comparisons, though not concurrent, possible at nine locations within the urban area where PAS deployment sites (McLagan et al., 2019) and *X. parietina* collection sites coincided (Supplementary material; Fig S4). This comparison is illustrated in Fig. 5 which combines seasonally averaged GEM concentrations determined using the PAS and, for the same locations, GEM estimations from autochthonous thalli. The figure highlights seasonal variability of GEM data, especially at sites near the mine (e.g., "a0") and at sites that were only monitored with 1-week-long deployments (e.g., "a40"). It is therefore important to acknowledge that inherent differences of the two datasets, originating in different methodological approaches (passive sampling/*in situ* biomonitoring), limit their comparability. However, GEM concentrations predicted from autochthonous thalli of *X. parietina* (Fig. 5) are in a good agreement with GEM concentrations measured with PAS during the "cold seasons". The linear relationship between them has a slope close to 1 and a small intercept close to zero (Fig. 5). These result reflects the poikilohydric nature of lichens, which have no control over uptake or loss of water; their metabolism, and with it the gas exchanges with the atmosphere, switch on and off in relation to their hydrated and desiccated state (Nash, 2008). *Xanthoria parietina* is considered a meso-xerofitic species that can tolerate direct and intense solar radiation. The trends shown in Fig. 5 suggest that autochthonous thalli of this species, under the climatic conditions of Mt. Amiata, can provide a good quantification of exposure to GEM for those sites that are characterized by generally low or moderate year-long levels contamination (i.e., < 20-30 ng/m^3). Poorer predictions are instead expected at the most contaminated sites (i.e., site "a0"), characterized by very high GEM concentrations during hot periods, which practically are not "recorded" by lichens in dry state.

Quantitative relationships shown in the present study demonstrated the benefits of the recently developed Hg PAS (McLagan et al., 2016) to overcome challenges in studying kinetic relationship in GEM assimilation in lichens. In the first place, the analytical performance of the sampler (e.g., high sensitivity, wide operational range) was found to fit well the remarkable accumulative capacity of transplanted lichen thalli. However, it should be noted that the reported quantitative relationships can be considered valid only for the specific environmental conditions in which our study has been conducted and further, more detailed studies are needed to disentangle the effects of seasonal and meteorological effects on GEM assimilation by lichens. On this ground, the combined use of lichens and PAS under a specific experimental design has allowed us to: *i*) quantify accumulation of Hg in two lichen species to different GEM concentrations; ii) characterize species-specific kinetic relationships between GEM and THg in lichens and finally, *iii*) verify the suitability of uptake models identified for *X. parietina* to predict GEM concentration on the basis of a Hg concentrations dataset of autochthonous thalli of the same species.

4 Conclusions

In this study, passive air samplers have been concurrently deployed with lichen transplants, at very different atmospheric GEM contamination. In this way, we could, for the first time, compare real-world atmospheric and bioaccumulation Hg data produced with identical temporal and spatial resolution. This is essential for a reliable characterization of Hg enrichment at the atmosphere/lichen interface and for a precise determination of the mass of Hg available for biological assimilation. The adopted experimental design allowed us to define species-specific, quantitative relationships between GEM concentrations and the amount of Hg accumulated in the lichen thallus, which can be used to quantify the impact of atmospheric Hg primarily at a local scale, near sources of contamination. To allow for a more comprehensive assessment at a regional scale, these relationships should be tested in the future, by using similar side-by-side deployments of passive samplers and transplants, with special regard to: *i*) lower GEM concentrations than those of the present study (i.e., from regional background levels, 1-2 ng/m^3, to industrial, non-mining values < 50 ng/m^3), *ii*) longer periods of

exposure including different seasonal conditions (i.e. 3, 6 or even 9 months) and finally, *iii*) accumulative responses of autochthonous lichens.

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