

# Variations in mercury concentration within and across lichen *Xanthoparmelia* spp. individuals: implications for evaluating histories of contaminant loading and sampling design

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**Environmental context.** Lichens have been widely used as biomonitors of atmospheric pollution in the absence of high-density ambient monitoring networks. This study examines the potential for the lichen *Xanthoparmelia* spp. as a recorder of temporal histories of mercury deposition to the landscape.

**Abstract.** Effects of thallus size and internal zonation on the Hg concentration in the foliose lichen *Xanthoparmelia* spp. were investigated. Size and zonation effects, if present, provide the potential for temporal records of atmospheric deposition to be recorded in lichens. Our results ( $n = 49$ ; 0.4–13.8 cm in diameter) indicated no significant relationship between Hg and size, although thalli less than 2 cm in diameter tended towards lower Hg concentrations; and no zonation of Hg within thalli. Distinct zonation of Hg in thalli has been reported in some studies, but not in others, indicating regulatory mechanisms result by which Hg is released or relocated within the thallus under certain conditions. A secondary objective was to evaluate the variability of Hg in lichen individuals to drive future sampling designs. Within a size range of 2–8 cm in diameter, we observed  $\text{Hg} = 154 \pm 30$  ppb (mean  $\pm$  s.d.,  $n = 38$ ). Bootstrap analysis of this dataset indicated that for a sample size of  $n = 3$  thalli, we can expect a 94 % probability that the variability in our sample set will be at least as low as that observed in other studies of Hg in lichen (s.d.  $\approx 50$  ppb Hg).

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## Introduction

Lichens have been widely used as biomonitors of air pollution. Because they derive their water and nutrition from atmospheric sources, lichens are inherently well suited to accumulate airborne particulate and vapour pollutants under both dry and wet deposition environments.<sup>[1]</sup> Lichens are slow-growing and perennial and thus have the potential to integrate contaminant signals over long periods of time. Lichens are suitable for biomonitors of air pollution if the concentrations observed in the lichens are in some way proportional to the delivery of contaminants over time. One aspect of the time dimension is the principle that older thalli may be expected to accumulate higher concentrations of contaminants, assuming there are no release mechanisms, or release is slow. The lichen *Xanthoparmelia* grows outward from a central zone, so within a single individual there are older structures and newer structures. Several investigators have reported higher contaminant concentrations in inner parts of the thallus than outer parts.<sup>[2–5]</sup> Sampling

methodologies usually take this into account. Some methodologies specify collection of only the outer band of the thallus. Bargagli et al., for example, recommended sampling the outer band of the thallus to a width of 2–4 mm.<sup>[1]</sup> Other investigators sample and homogenise entire thalli, but control for the size effect by only sampling within a narrow range of thallus diameters.<sup>[6]</sup>

Selecting only the youngest regions of the thallus, or homogenising entire thalli within a tight size (age) range can normalise for variability in contaminant concentration due to differences in thallus age, however this may result in the loss of otherwise useful information. If older parts of the thallus indeed record pollution over a longer time period than younger parts, it should be possible to quantify the relationship between age and contaminant concentration, either across individuals of different sizes, or within a single individual. This relationship should be similar among individuals in a particular location and may reflect a time-integrated uptake rate of the contaminant and

provide a temporal record of magnitudes of contaminant loading. Spatial differences in recent contaminant loading may be reflected as differences in the relationship between contaminant concentration and either size of the individual or position within individuals.

Lichens have been used as temporal recorders of contamination using several different approaches. Agnan et al.,<sup>[7]</sup> for example, compared archived lichen samples from the early 20th century with recently sampled lichens to infer changes in atmospheric deposition of pollutants. A far more common approach has been to transplant lichens from pristine locations and monitor temporal changes in the uptake of contaminants.<sup>[8–11]</sup> Transplantation studies have yielded information on uptake rates of contaminants and provided insight on mechanisms of capture of pollutants, as well as to inform objectives of evaluating pollutant loading at a particular location within a narrowly constrained time period. To make a broad generalisation, transplantation studies revealed that contaminant concentrations in lichens reach equilibrium with atmospheric contaminant concentrations within several months of transplantation.<sup>[10]</sup> A third strategy for temporal contaminant monitoring using lichens, similar to the first, has been simply to revisit locations at time intervals and compare contaminant concentrations in lichens.<sup>[12,13]</sup> Transplant studies and temporal sampling campaigns enabled investigators to develop models for lichen growth<sup>[14]</sup> and exchange dynamics of contaminants.<sup>[9,12]</sup>

Temporal studies of contaminants in lichens have always involved some form of re-sampling at time intervals. We hypothesise that a temporal record of Hg loading can be extracted either from individual lichens by sampling older parts of the thallus separately from younger parts, or from groups of lichens of varying sizes. However, this approach is fraught with potential interferences. Elements with limited metabolic significance to the lichen, such as heavy metals, have been observed to accumulate in a time-dependent manner,<sup>[4,8]</sup> a necessary condition for recovery of temporal information on loading from single lichens or groups of lichens collected at a single location and time. However Hg has not always been observed to vary in concentration with age of thallus.<sup>[4]</sup> Metabolic and physico-chemical processes within thalli may result in loss of Hg from the thallus.<sup>[9,10]</sup> Processes occurring as the thallus ages may also compromise the ability of a lichen to record contaminant loading quantitatively over time. Senescence in the older, interior zones of a thallus, for example, may result in the release of contaminants. Fragmentation at the centre of a thallus may result in new growth at the margins of fragments, re-setting the clock of contaminant uptake over time at those sites. As thalli mature fruiting bodies may appear and alter the distribution of contaminants within the thallus.<sup>[14]</sup> In fact, these complexities of lichen growth and maturation have prompted some investigators to only sample the outer, youngest zones of lichen thalli,<sup>[11]</sup> which are the fastest growing<sup>[14]</sup> and represent the most recent atmospheric loading conditions. Nonetheless, observations of zonation in metal accumulation in lichens, with higher concentrations in the older parts of thalli,<sup>[2,4,5]</sup> provide the motivation for our hypothesis that temporal records of contaminant loading can be recovered from lichens.

This study makes an initial attempt to test the hypothesis that thallus age, as indicated by either size of individual or position within an individual, is positively correlated with Hg concentration. A secondary objective of this study was to evaluate the variability in Hg concentrations across a range of thallus sizes

collected at a single location to determine an optimal sampling strategy for future studies.

## Methods

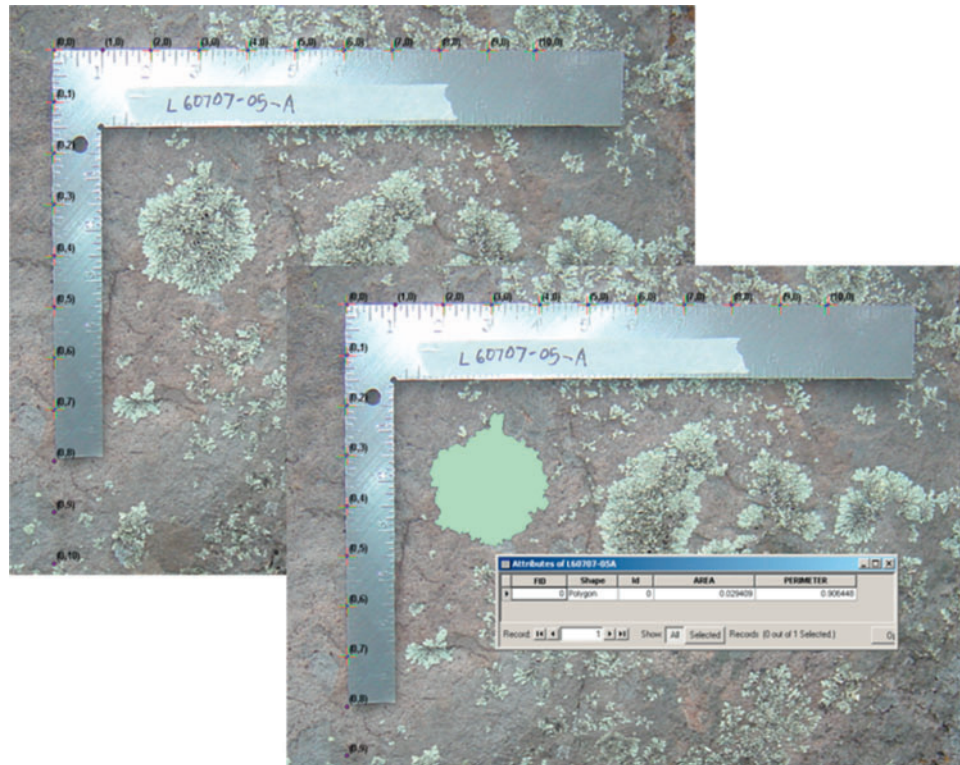
### *Study site and sample collection*

The study site (35°5'30"N, 111°42'19"W, 2050-m elevation) was located ~15 km south of Flagstaff, Arizona (USA), along a south-east-facing slope adjacent to Pumphouse Wash, an intermittent stream. This location was selected to represent conditions without apparent contamination from industrial, urban or motor vehicles. As such it may serve in the future as a reference location. Sampling was conducted in July 2006 along a slope with an open canopy of Ponderosa pine (*Pinus ponderosa*), located in a forested setting, but within 1 km of a residential development. All samples were collected within a radius of ~100 m. We collected all samples from relatively flat regions of basaltic boulders. We selected apparently healthy and isolated *Xanthoparmelia* spp. individuals, located at least 1 m from the ground to avoid effects of soil, and within a size range of <1–20 cm in diameter. Thalli that were greater than ~5 cm in diameter were sub-sampled, starting in the centre, with samples representing ~1 cm of radius per sample. Prior to the collection of each sample, the thallus was photographed, with an X–Y scale placed as close as possible to the thallus (Fig. 1). Thalli were removed from their substrate using a Teflon-coated stainless-steel spatula and stored in labelled plastic sampling bags. Spatulas were cleaned in the field between samples using ultra-pure water and air-dried.

### *Laboratory methods*

In the laboratory, samples were placed in 100-mL nalgene containers with ~30 mL of ultra-pure water to remove surface contamination.<sup>[6]</sup> After ~30 min, the thalli were removed from the water and placed on filter-paper discs in a labelled Petri dish and air-dried for at least 24 h at room temperature in a laminar-flow tent ventilated with high-efficiency particulate air (HEPA). After air-drying, samples were crushed using a mortar and pestle and stored in glass vials. Samples were analysed within a month of collection on a LECO AMA 254 cold-vapour atomic absorption analyser (Leco Corporation, St Joseph, MI, USA). The method detection limit for Hg analysis of lichen was estimated using the US Environmental Protection Agency (EPA) method described in 40 CFR.<sup>[15]</sup> Replicate measurements ( $n = 15$ ) of the standard reference material IAEA 336 (lichen) with a sample mass of 23.0 g produced a mean response of 3.95 ng Hg ( $n = 15$ , s.d. = 0.43 ng Hg). These data were used to estimate a minimum detection limit (MDL) of 1.1 ng Hg.

Sample mass for the lichen samples collected averaged 123 mg of dry mass and ranged from 5 to 240 mg, creating a response ranging from ~1 to 46 ng Hg per sample. Four lichen samples produced a response of Hg <1.5 ng and were excluded from further analysis, as they did not contain quantifiable amounts of Hg. Approximately half of the samples were analysed in duplicate or triplicate with replicate precisions averaging 3.3% ( $n = 56$ , relative standard deviation for triplicate samples and relative percentage difference for duplicate samples). Two separate in-house laboratory standards were analysed after every ten unknown samples with relative standard deviations of 3.1% ( $n = 26$ ) and 2.8% ( $n = 25$ ). Certified standard reference materials IAEA 336 (lichen), NIST 2693 (coal) and NIST 2709 (contaminated soil) were also analysed



**Fig. 1.** Photo of lichen in ESRI *ArcMAP* with original photograph in background and digitised polygon of lichen in foreground.

with replicate analyses falling within the certified values of all three reference materials (Table 1).

#### Image processing methods

The surface areas of entire lichen thalli and sub-sampled areas were calculated using *ArcMAP 10.1* (ESRI, Redlands, CA, USA) using a separate file for each sample and a local datum established in inches (the unit of measure of the scales in the photographs). Similar approaches using digital photographic<sup>[16]</sup> and laser (LIDAR)<sup>[17]</sup> data acquisition have been used to quantify lichen size and growth characteristics, but not within a specific context of determining concentration *v.* size or age characteristics. Photographs were added as files to *ArcMap* and georeferenced using scale markings and the *Georeferencing* tool in *ArcMap*. Perimeters were traced as polygons and the area and perimeter of each polygon was calculated using the *Calculate Geometry* tool within the attribute table of the polygon. Units of square inches were converted into square centimetres (Fig. 1). The average diameter of the lichen was calculated as the square root of the area multiplied by  $4/\pi$ .

#### Statistical methods

For this study we collected a relatively large number of samples at a single location. Since the exposure to atmospheric Hg can be assumed to be equal over our closely defined study area, these data can provide an estimate of the variability inherent in the uptake of Hg for *Xanthoparmelia* under the local climatic conditions. Future studies to characterise spatial variability in lichen Hg will require small sample sizes at each location. We used non-parametric statistics to predict the variability we can expect with a small sample size. We treated our collection of samples as though it were a population, and sampled that population with

**Table 1.** Analysis of standard reference materials (SRMs) RSD, relative standard deviation; RPD, relative percentage difference

SRM	<i>n</i>	Mean (ppb)	Standard deviation (ppb)	RSD or RPD (%)	Certified value (ppb)
IAEA 336	33	165.0	14	8.3	200 ± 40
NIST 2693	2	43.6	—	13.4	37.3 ± 7.7
NIST 2709	3	1444	101	7.0	1400 ± 80

a sample size of three observations. We then repeated this sampling for all possible combinations of three samples, with replacement. This is the statistical procedure of bootstrapping.<sup>[18]</sup> We calculated the mean and the standard deviation for each of the three-sample combinations and compiled these data as statistical distributions for the mean and standard deviation. These distributions enabled us to assess variability in Hg associated with a sample size of three in terms of probability.

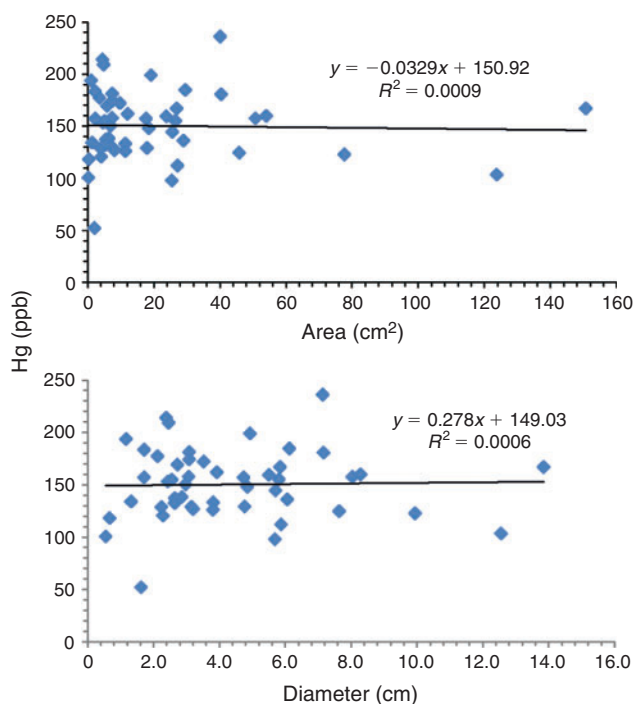
#### Results

A total of 49 *Xanthoparmelia* individuals were analysed, ranging in size from 0.4 to 13.8 cm in average diameter to test for size effects of Hg concentration. Of these, 17 were sampled in concentric circles to test for age effects within individual thalli. The data were normally distributed with a mean of 150 ppb Hg, a median of 153 ppb Hg and a standard deviation of 33 ppb Hg ( $n = 49$ ). Hg concentrations for the 17 sub-sampled thalli are included in all analyses as area-weighted averages. Fig. 2 plots Hg concentration in individual thalli *v.* area (top) and average diameter (bottom) with linear regression equations. The slope of both regression equations was not significantly different from zero ( $P < 0.05$ ).

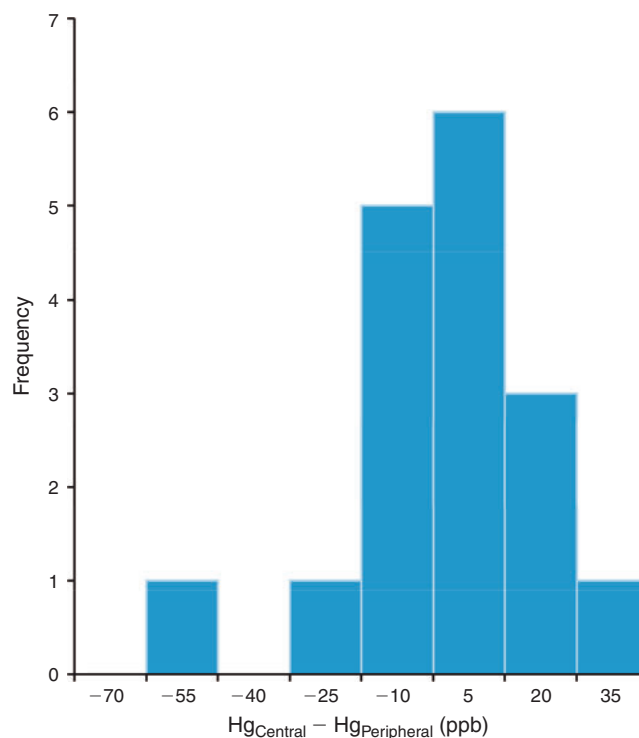
Local data on growth rates of lichens are not available, so the age of the lichen samples was not determined. Armstrong and Bradwell<sup>[14]</sup> reported growth rates for *Xanthoparmelia* in Colorado (USA) ranging from 0.98 to 2.99 mm year<sup>-1</sup>. Depending on elevation and moisture differences, this range may vary considerably from lichens at our study site. However, using these data the smallest individuals were on the order of 4 years old.

To test for variability of Hg within individual thalli, Hg concentration in the various zones of individuals is plotted in Fig. 3, which shows qualitatively both the lack of increase in Hg

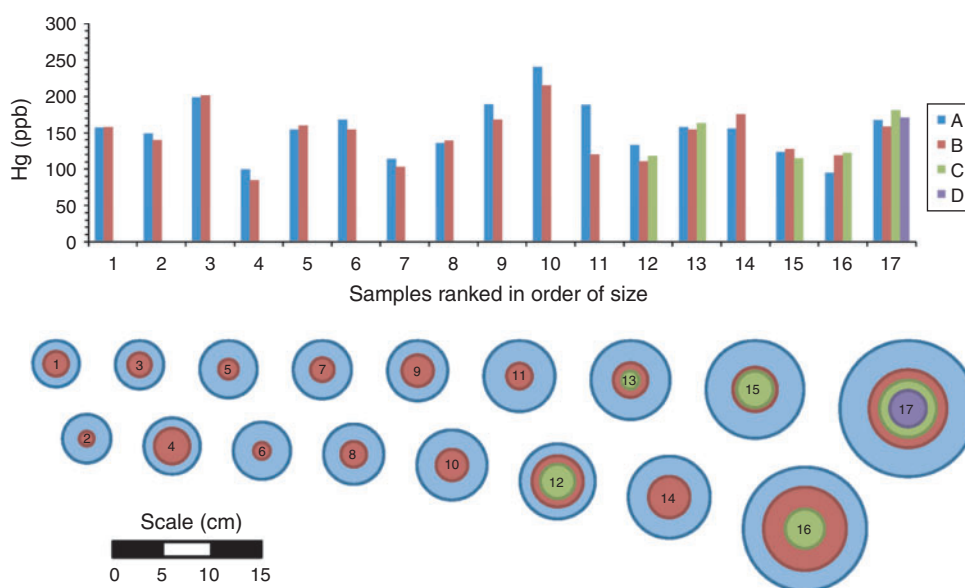
with thallus size observed in Fig. 2 and the lack of a consistent relationship between Hg concentrations in interior and outside zones of thalli. The differences between central and peripheral Hg concentrations (difference = [Hg<sub>Central</sub>] - [Hg<sub>Peripheral</sub>]) were calculated for each of the 17 samples. The results are plotted as a histogram in Fig. 4. The mean difference was -7.1 ppb Hg (s.d. = 21.0, n = 17), indicating that on average, the outer zones contained higher concentrations of Hg than the inner zones. Of the 17 samples, eight had higher Hg in the centre and nine had higher Hg in the peripheral zones. A *t*-test indicated



**Fig. 2.** Hg concentration in individual thalli v. area (top) and average diameter (bottom).



**Fig. 4.** Histogram of the Hg difference between inner and outer zones ([Hg<sub>Central</sub>] - [Hg<sub>Peripheral</sub>]) of lichen thalli (n = 17).



**Fig. 3.** Column chart of Hg concentration in each zone of thalli that were sampled in concentric circles (top) and a graphical representation of the relative sizes of concentric samples and thallus sizes (bottom).

that at the 95 % confidence level the differences were not significantly different than zero.

### Discussion

The objectives of this study were to test for Hg variability within and across *Xanthoparmelia* individuals collected at a single location to determine the nature of variability with size and to evaluate this variability within the context of sampling design for future studies.

#### Variability due to size and zonation

Tests for variations in Hg concentration as a function of size of thallus (and presumably age) and of position within individual thalli both indicated insignificant response. It is clear from Fig. 2 that a systematic positive relationship between Hg concentration and thallus diameter does not exist. Nearly all of the individuals collected (47 out of 49) were 9.0 cm in diameter or smaller, so responses relevant to older and larger individuals may not have been observed. However, the size range captured reflects the available population. The substrate of basaltic boulders provided limited area for lichen growth and larger thalli were more likely to intersect with other individuals in the competition for substrate.<sup>[19]</sup>

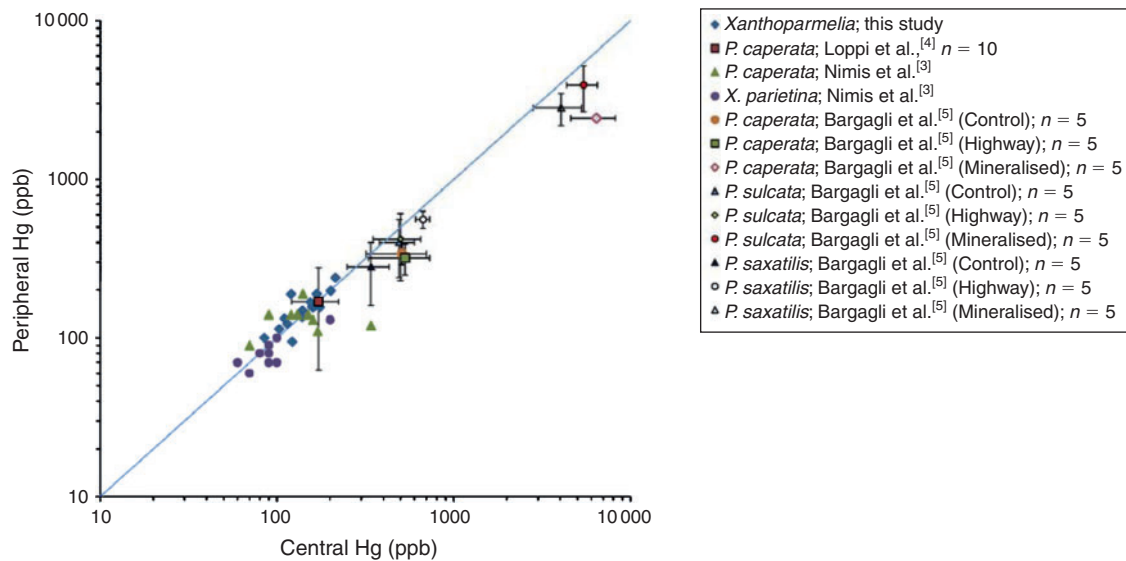
Relationships between atmospheric contaminants and size of lichen thallus have been conducted in only a limited number of studies.<sup>[10,20,21]</sup> Most studies instead examined contaminant distributions within individual thalli.<sup>[4,5,10]</sup> Of those considering size effects, Senhou et al.<sup>[20]</sup> observed strong size effects in the foliose lichen *Evernia prunastri* in Morocco, although they did not monitor for Hg. In contrast, Armstrong<sup>[21]</sup> found no relationship between thallus size and accumulation of elements. Neither study analysed Hg.

The absence of an increase in Hg with thallus size (Fig. 2) was also reflected in an apparent absence of increase in Hg concentration with age within thalli (Fig. 3). Comparisons between the innermost and outermost locations with the thallus indicated no difference in Hg concentration (Fig. 4) regardless of the size of lichen sampled. Zonation of contaminants has been widely reported,<sup>[2,4,10]</sup> but the degree to which this occurs varied

considerably.<sup>[10]</sup> In addition, relatively few studies included Hg as an analyte. The results of several of the few studies that did include Hg are shown in Fig. 5, which plots Hg in peripheral (younger) v. Hg in central (older) locations within thalli. Observations falling below the 1 : 1 line show higher Hg concentrations in central regions. Of these, Bargagli et al.<sup>[5]</sup> observed the greatest differential of Hg between inner and outer parts of the thallus in a comparison of unpolluted and highly polluted locations. Even in the control (uncontaminated) location, the innermost tissues were enriched in Hg by a factor of 1.50 (150 % higher in inner zones). Enrichment factors varied for other investigators, and were almost equally distributed above and below the 1 : 1 line. Exceptions are Nimis et al.,<sup>[3]</sup> who reported a high enrichment value for common greenshield (*P. caperata*) and Bargagli et al.,<sup>[5]</sup> who observed high enrichment at all sites.

The absence of accumulation of Hg in older parts of the thallus suggests some combination of the following factors: the supply of Hg is too low at the study site for bio-accumulation to be recorded, even in the oldest structures; the supply of Hg has been inconsistent during the life of the lichens; there are mechanisms for transport and relocation of Hg within the thallus; or there are processes of release that altered Hg concentrations over time. With regard to the possibility that the supply of atmospheric Hg was too low to record differential accumulation, the concentrations of Hg observed in this study for lichens in the 2–8-cm range (mean Hg = 150 ppb) fall well within the range of lichen Hg concentrations at relatively unpolluted or remote sites<sup>[10]</sup> (3–500 ppb) and are in fact higher than lichen Hg concentrations observed at some urban and industrial sites (60–29 000 ppb). Even so, our average of Hg of 150 ppb is well below concentrations reported by Bargagli et al.,<sup>[5]</sup> who observed the highest differential of Hg between old and young regions of the thallus (Fig. 5). It is possible that only under high burdens of Hg loading do lichens retain concentrations proportional to their age.

With regard to variations in supply, long-term monitoring of atmospheric Hg has not been conducted near our study location, however from a mass-balance perspective, the inner regions of the thallus always have the opportunity to accumulate a greater



**Fig. 5.** Peripheral v. central Hg concentrations in lichen thalli for study and literature data. The diagonal line indicates  $[Hg_{\text{Peripheral}}] = [Hg_{\text{Central}}]$ . Error bars  $\pm 1$  s.d. are included for studies reporting the mean of more than one value. Literature data cited are Loppi et al.,<sup>[4]</sup> Nimis et al.<sup>[3]</sup> and Bargagli et al.<sup>[5]</sup>

burden of contaminants than younger regions. Unless there is a release or relocation mechanism for contaminants, an equal concentration of Hg in all parts of the thallus would require a recent and dramatic increase in atmospheric loading (in fact similar to conditions observed in transplant studies), which has not occurred near the study location.

Also relevant to considerations of changes in pollutant supply is the concept of *memory length*,<sup>[21]</sup> or *rememberance time*,<sup>[22]</sup> in lichens. A common study design has been to transplant lichens from a relatively pristine location to one known to have higher atmospheric pollutant loads. Results have varied, but some level of equilibrium in the concentration of contaminants has been observed after several months of exposure. The phenomenon of equilibrium, rather than constant increase, suggests that more complex mechanisms than passive accumulation of contaminants occur in lichens.

Although many studies have considered the biochemical<sup>[10,22]</sup> and physicochemical<sup>[23]</sup> capture and transport in lichens, Loppi et al.<sup>[4]</sup> provide a succinct explanation of the relevant processes. In a multi-element study of the foliose lichen *Parmelia caperata*, they concluded that elements of limited metabolic significance (they measured Al, Cd and Pb) had higher concentrations in interior parts of the thallus, whereas elements essential for lichen metabolism (Co, Cu, Mo and Zn) had higher concentrations in peripheral parts of the thallus.<sup>[4]</sup> The same study reported, however, that other trace elements (As, Cr, Fe, Hg, Mn, Ni and Sb), also presumably of limited metabolic significance and including Hg, showed no significant pattern in zonation. In their studies of *Flavoparmelia caperata* lichens in Portugal, Godinho et al.<sup>[2,8]</sup> also observed that some elements tended towards zonation more than others. They concurred with Loppi et al.<sup>[4]</sup> that lichen metabolism affects transport and sometimes release of certain contaminants. They concluded that time-dependent accumulation may indicate 'progressive passive uptake', whereas the absence of accumulation over time indicates 'regulation mechanisms'.<sup>[2]</sup> Their study did not include Hg, but Hg would be included in a class of metals subject to translocation and internal regulation. For some sort of equilibrium to occur between Hg deposition and concentrations of Hg in the atmosphere, loss mechanisms are necessary within the lichen thallus. Goyal and Seaward<sup>[23]</sup> describe some of these regulatory processes, but not specifically for Hg.

The form of deposition of contaminants is also relevant to mechanisms of capture, translocation and release. In some respects Hg behaves more like contaminants delivered in vapour form, such as SO<sub>x</sub><sup>[24]</sup> and NO<sub>x</sub><sup>[25]</sup> as it can be delivered, unlike other metals, in a gaseous (Hg<sup>0</sup>) form,<sup>[10]</sup> although the mechanisms of retention in lichens may be different for Hg than other gaseous compounds. Indeed, the forms and delivery mechanisms for Hg are particularly complex in the arid US Southwest, where a significant amount of annual Hg deposition can be in forms adsorbed to dust particulates as dry deposition,<sup>[26,27]</sup> as well as gaseous oxidised Hg and the Hg<sup>0</sup> form in either wet or dry deposition. These forms of deposition affect retention of Hg in the lichens. Particulate forms tend to be retained effectively through physical entrapment. Dissolved or gaseous forms may be incorporated into cell walls or deeper into lichen structures and consequently not susceptible to loss and likely to accumulate with lichen age. Dry-deposited forms may be more likely to be lost by washing from precipitation events.<sup>[9,10,22]</sup> Yet, Hg may be unique among metals in its retention characteristics in lichens. In a comparison of direct measurements of atmospheric particulates with concentrations of elements in four species

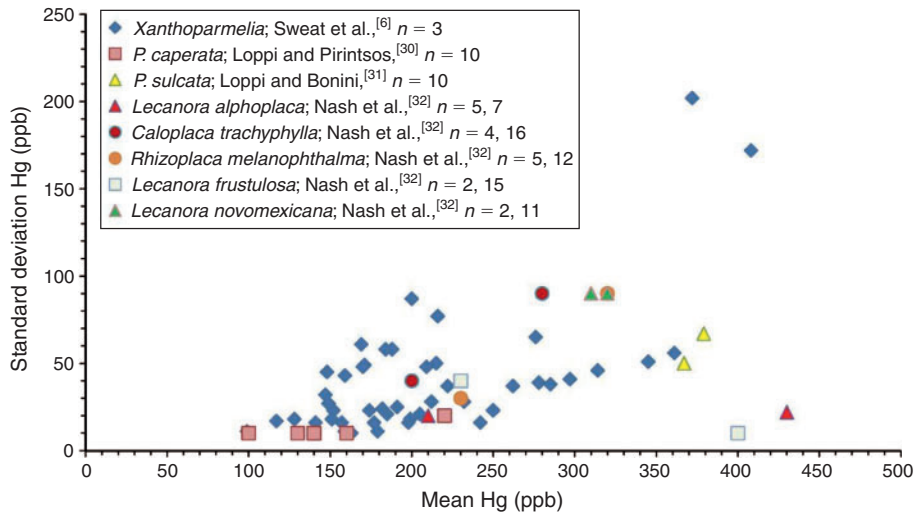
of lichens of the genus *Parmelia*, Saeki et al.<sup>[28]</sup> attributed a lower level of accumulation of Hg, relative to other elements, to the volatility of Hg compounds.

To summarise, processes of entrapment and retention for metals including Hg are complex and can lead to passive accumulation of the element within the thallus resulting in an increase in concentration with age. Alternatively, processes of translocation and loss of metals can result in something resembling equilibrium, where accumulation is offset at least to some extent by removal processes. Our data show that Hg retention appears to be in equilibrium with deposition, as no increase with size across thalli or position within individual thalli was observed. We can conclude that size or zonation effects do not occur for Hg for *Xanthoparmelia* at our study location at the time of the field study. However it would be premature to conclude that this equilibrium can be expected to be maintained in the future at this location or that it is valid for other locations in the US Southwest. In future studies at this and other locations, either sampling for a narrow size range in individuals, or sampling for size and zonation effects can monitor for the occurrence of either passive accumulation or equilibrium effects. Transplantation of lichens from less polluted areas to areas suspected of higher pollution can provide another control on Hg accumulation within lichen.

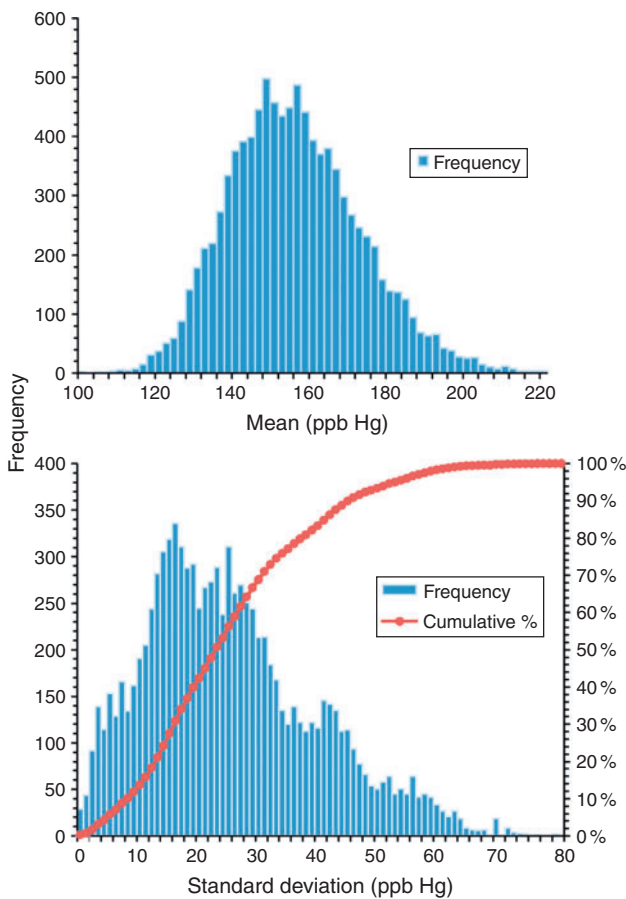
#### Variability of lumped data

The absence of an effect for size or location for Hg in *Xanthoparmelia* thalli indicates that individuals within a broad size range can be assumed to represent Hg in the population of lichens at a particular location. It is therefore relevant to identify the appropriate size range for future sampling studies, and to evaluate the observed variability in that size range for the samples collected. The plot of Hg v. average thallus diameter (Fig. 2) shows that individuals less than ~2 cm in diameter may have lower than average Hg concentrations. Fig. 2 also shows that few individuals greater than 8 cm in diameter were collected, reflecting site conditions favouring smaller lichens. Thalli in the size range of 2–8 cm had a mean of 155 ppb Hg and a standard deviation of 30 ppb ( $n = 38$ ). From a sampling design perspective, it is relevant to evaluate whether this amount of variability is acceptable and to determine an appropriate number of lichens to sample in each location. Analysis of variability across lichens also provides an indication of the inherent local variability<sup>[29]</sup> occurring in our study location. In other studies of Hg in lichens, samples sizes from three<sup>[6]</sup> to ten<sup>[3]</sup> individuals per location were collected and analysed separately. For each of these studies standard deviation was plotted v. sample mean to provide an indication of ranges in observed variability in the literature (Fig. 6). Of these, only one<sup>[6]</sup> analysed *Xanthoparmelia* and two<sup>[6,28]</sup> reported Hg concentrations in lichens collected in the US Southwest.

It would be inappropriate to make a direct comparison of the standard deviation for the complete set of samples collected in this study with the literature data shown in Fig. 6 for two reasons. First, our data likely have a lower amount of variability due to the relatively large sample size of 38. Second, future sampling at multiple locations will require a minimal number of thalli to be collected at each location. To address both of these concerns, we made the assumption that our 38 thalli (size range 2–8 cm) represented the population of lichen thalli at that location. We then set a sample size of three and used the bootstrap techniques<sup>[18]</sup> to create a dataset of all possible combinations (with replacement) of three samples from that population of 38.



**Fig. 6.** Hg standard deviation v. Hg mean for this study and literature data from Sweat et al.,<sup>[6]</sup> Loppi and Pirintso,<sup>[30]</sup> Loppi and Bonini,<sup>[31]</sup> and Nash et al.<sup>[32]</sup>



**Fig. 7.** Histogram for the mean (top) and standard deviation (bottom) results of bootstrap analysis of Hg concentration in thalli of the size range 2–8 cm. All possible combinations of  $n = 3$  samples from a population of 38 lichen thalli are included.

The results of the bootstrap analysis are shown in Fig. 7, with the distribution of sample means (top) and standard deviations (bottom) for the 9880 three-sample combinations. These histograms show that if we had only collected three individuals at our study site location, we would have the highest probability of obtaining a mean Hg concentration of 153 ppb and a standard

deviation of 23 ppb (median values). Fig. 6 shows that for sample means less than ~150 ppb Hg, reported variability expressed as standard deviation ranged from nearly 0 to ~50 ppb for sample sizes ranging from  $n = 3$  to 10. Our re-sampled data (Fig. 7) indicate that 94% of all possible three-sample combinations from our 38-sample population resulted in standard deviations less than 50 ppb. Restated, if we collect three individuals per location, we have a 94% chance of obtaining variability at least as low as other studies reported in the literature. We conclude both that our data compare well with other studies in terms of sample variability and that a sample size of  $n = 3$  individuals in the size range of 2–8 cm in diameter is adequate to characterise Hg concentration at a single location.

### Conclusions

The primary objective of this study was to evaluate whether Hg concentration varied with thallus size or position within individual thalli for the foliose lichen *Xanthoparmelia* collected at a single location in the US Southwest. A secondary objective was to assess Hg variability in general among *Xanthoparmelia* thalli to guide future sampling designs. We observed no relationship in Hg concentration with size, either across individuals or within individuals in a size range of 2–8 cm. It is possible that the supply of Hg was too low at our study site to produce zonation. The observation that older regions of the thallus did not support a higher concentration of Hg than younger regions indicates that accumulation processes were offset by release mechanisms. Processes involved in both accumulation and release are complex, so our observation of an absence of net accumulation with size may not be generalised either spatially or temporally. As a result future sampling will either be constrained to a narrow range of thallus sizes, or a wide range of sizes will be sampled and interpreted within the context of size. In the absence of a size-effect on Hg concentration among thalli, we concluded that thalli in the size range of 2–8 cm reflect the concentration of Hg in the population of lichens at that location. By sub-sampling our set of 38 thalli in that size range, we concluded that a sample size of  $n = 3$  lichens in each location provides adequately low variability to represent Hg at that location. Transplantation of lichens from more pristine areas to locations of known or suspected pollution sources may also improve our understanding of controls on Hg deposition on the landscape.

This study also included two approaches in the collection and analysis of data that show promise in the study of lichens as biomonitors: the use of spatially registered photographs of lichens for accurate measurement of thallus size, and the use of non-parametric statistical analysis to guide in sampling design. Digital photography and image-processing software enables researchers to build photographic, scale-referenced archives of lichen samples, which can be exploited for studies including lichen growth characteristics<sup>[16,17]</sup> and relationships between size and contaminant content or zonation of contaminants. Non-parametric bootstrap analysis enabled us to evaluate the consequences of varying our sampling strategy in a probabilistic manner.

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