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Urban/Peri-Urban Aerosol Survey by Determination of the Concentration and Isotopic Composition of Pb Collected by Transplanted Lichen Hypogymnia physodes

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In the northeastern part of France, around the city of Metz, lichens (Hypogymnia physodes) have been transplanted from a single reference site to five different sites: (i) the reference site itself, (ii and iii) two peri-urban sites, (iv) a site in the proximity of a highway, and (v) a final one close to an industrial site. The dynamics of two different system set-ups (one covered and one uncovered) were evaluated. Samples have been collected 14, 34, and 68 days after transplantation. Lead concentrations already accumulated in the thallus, and the corresponding Pb isotopic compositions have been measured by quadrupole-based ICP-mass spectrometry. A systematic difference between the two setups is found for Pb concentrations, with the higher concentrations measured in lichens from the uncovered devices. Lead concentrations in lichens from the covered devices were found to be lower than or equal to the original concentration. Also the Pb isotopic compositions show a systematic difference between the devices, with the Pb isotopic composition present in lichens from the uncovered device being more radiogenic. Substantial changes in the isotopic composition of Pb are recorded for lichens from the uncovered device (from about 1.15 up to 1.22 for the $^{206}$Pb/$^{207}$Pb ratio), in comparison with Pb isotopic composition using ICP-mass spectrometry. A systematic difference between the two setups is found for Pb concentrations, with the higher concentrations measured in lichens from the uncovered devices. Lead concentrations in lichens from the covered devices were found to be lower than or equal to the original concentration. Also the Pb isotopic compositions show a systematic difference between the devices, with the Pb isotopic composition present in lichens from the uncovered device being more radiogenic. Substantial changes in the isotopic composition of Pb are recorded for lichens from the uncovered device (from about 1.15 up to 1.22 for the $^{206}$Pb/$^{207}$Pb ratio), in comparison with Pb isotopic composition using ICP-mass spectrometry.

Introduction
Lichens are known to be sensitive to various pollutants and are considered a good biological indicator of the air quality and thus, they are widely used in environmental studies (1–4). Among a vast array of lichens, epiphytic lichens integrate the atmospheric fallout signal and generally contain much higher concentrations of trace metals than, e.g., rain, which makes lichens more comfortable for concentration and isotope ratio measurements. Moreover, lichens are very widespread and easy to sample, although not found everywhere. To compensate for the absence of lichens in certain areas, in which they were either originally absent or where air pollution has decreased the amount of lichens available to near to zero, transplantation techniques, consisting of the importation of lichens from one area to another one, have been developed and are now commonly used in environmental surveys (5–15).

The sensitivity of lichens to heavy metals is species-dependent and mainly influenced by morphological and structural features (16). Also the exposure time of transplanted lichens, as well as the exposure conditions, may play a role in the accumulation of heavy metals (15, 17, 18). Since a few years, the isotopic composition of Pb present in lichens has been measured in order to identify the different lead sources of aerosols (19–24) and more recently, this approach has also been extended to Zn (25, 26). Even when lichens are brought into an entirely different exposure environment, the lichen dynamics seem fast and the exposure times used are typically lower than 3 months (24). However, both the mechanism and the timing of Pb isotope ratio turnover in lichens are still not well constrained. Moreover, as a recent study has highlighted the importance of the aerosol particle size distribution in the trace element accumulation dynamics (27), one more parameter to control is introduced.

In order to assess the Pb dynamics in different environments, transplanted lichens originating from a reference site were exposed during 68 days on four sites, covering various areas under significant anthropogenic influence. In order to monitor the influence of the rain and/or of the aerosol particle size, two types of devices, one covered and one uncovered, were designed and used on each site. The lichens from two sites, including the reference site, were also sampled 2 years after the experiment to estimate the long-time exposure effect on the transplanted lichens. From all sites and both devices, transplanted lichens were analyzed for their Pb concentration and Pb isotopic composition using ICP-mass spectrometry.

Materials and Methods
Lichen Sampling. In August 2001, samples of the lichen Hypogymnia physodes were collected next to the city of Metz, in the Moselle River valley, in northeastern France. Although the city comprises about 300 000 inhabitants, the sampling site for this work has been chosen far from any known or potential (e.g., roads, industries) atmospheric pollution sources and referred to in what follows as the reference site (Mont Saint Quentin, Figure 1.). The lichens were collected on a Hawthorne (Crataegus monogyna) tree by cutting small pieces (about 10 cm long and 2 cm wide) of bark located between 1 and 2 m high. To minimize variations due to differences in the substrate, all samples are coming from four shrubs next to each other. Lichen Hypogymnia physodes was chosen because of their important spatial repartition in this area, the rapid development of this species, the possibility of transplantation without altering the thallus, and the fact

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that the thallus is easily removed from the substratum. Lichens have been selected based on their apparent homogeneity. Part of the freshly sampled material has been analyzed to determine the baseline Pb level prior to the transplantation.

**Lichen Transplantation Devices and Exposure Time.** The collected lichens were transplanted onto a suitable setup, installed at five different sites (including the reference site itself) (Figure 1) within the same day of the sampling. The sites have been chosen due to the proximity of an air monitoring station and in such a way that a range of various environments is covered. The reference site (Mont Saint Quentin, StQ, 49°07′11″N, 6°06′42″E, 290 m) has been chosen since it is far from any known or potential atmospheric pollution source and for additional logistic parameters (e.g., accessibility, discretion). The other four sites include one site with dense automotive traffic in the immediate proximity (Thionville-highway, Thi, 49°19′21″N, 6°10′20″E, 155 m), one in proximity of industrial activities (Florange, Flo, 49°19′11″N, 6°08′27″E, 156 m), and two peri-urban areas (Moyeuvre-Grande (Moy), 49°15′53″N, 6°03′06″E, 230 m and Scy-Chazelles (Scy), 49°06′12″N, 6°09′30″E, 169 m). The major wind direction in the area studied along a year is reported elsewhere (20) and is along the north–south axis, with winds blowing from the south to the north occurring more frequently and blowing stronger than vice versa. Winds along the east–west axis are of minor importance.

The transplanted lichens were installed on the roof of an air monitoring station (3 m height), except for the reference site (positioned at 70 cm height, not on an air monitoring station), and two different devices were used on each site, one covered and the other one uncovered (Figure 2). The difference between both devices is the presence of a plexiglas plate as a “roof” for the covered device. This cover plate protects the lichen transplants from rain and the gravitational settling of the majority of particles, but allows contact with fog and mist and the circulation of aerosols (gas + fine particles). Further, the presence of the cover plate does not create additional variations in temperature compared to the uncovered system. Moreover, there is a 100% transmission efficiency for the visible spectrum and more than 90% transmission efficiency for the UV-A radiation (28). Lichens used for this study were found equally active in both devices according to the biological parameters monitored (28).

Lichens were randomly distributed across the two devices of each site and exposed for a 68 days period. After 14, 34, and 68 days of exposure, lichens were randomly sampled from the two devices at all of the sites. Transplanted lichens from the reference site and one peri-urban site have also been exposed much longer and were sampled again after 2 years. Native lichens (on trees) from the reference site have also been collected 2 years later.

Before analysis, the thalli of sampled lichens were washed to remove the superficial deposition and thus keep only the accumulated elements, and were then dried at 105 °C for 1 h (2, 12) and subsequently powdered in an agate mortar while using liquid nitrogen.

**Elemental and Isotopic Analysis.** Pro analysi 14 M HNO₃ and 12 M HCl (Panreac, Spain), used for dissolution of the samples, were further purified by sub-boiling distillation in a quartz still. Ultrapure water, provided by a Milli-Q unit (Millipore, Belgium), was used for the dilutions required. Concentrated HF (analytical reagent grade, 28 M) and suprapur H₂O₂ (10 M) were purchased from Merck (Germany) and used as such.
Variation in the Pb Concentrations. The Pb concentration was measured in the transplanted lichens after 14, 34, and 68 days of exposure for both the covered and uncovered device and for each of the five sites, including the reference site. The initial Pb concentration (i.e., before lichen transplantation) was also determined and found to be 23 ± 3 \(\mu g \cdot g^{-1}\) (sd) \((n = 3\), Figure 3\), a value which is in agreement with the concentration measured for lichen \((\text{Pseudoperidinium physodes, 25} \mu g \cdot g^{-1}, \text{and Ramalina Farinacea, 12} \mu g \cdot g^{-1})\) in the same area \((20)\). The maximum Pb concentrations measured after 68 days of exposure range between 30 and 35 \(\mu g \cdot g^{-1}\) for the uncovered device (Figure 3a, b, and c), whereas it is between 18 and 23 \(\mu g \cdot g^{-1}\) for the covered device (Figure 3a, b, and c). The trend observed for the Pb concentration for the various transplantation sites is the same and is similar to the Pb concentration trend in the lichens transplanted at the reference site itself. However, the trends for the uncovered and covered device are different (Figure 3). For the covered device, there is a systematic, though not statistically significant, diminution after 34 days \((t(\alpha = 0.05, df = 4)) < 2\) and then an increase of the Pb concentration the following 34 days, except for Moy. (Figure 3). On the contrary, for the uncovered device, after a systematic but not statistically significant diminution of the Pb concentration after the first 14 days of exposure, there is a constant increase in the Pb concentration in the transplanted lichens, including those transplanted at the reference site itself (Figure 3). After 68 days, a statistically significant difference in Pb concentration is found compared to the Pb concentration present at the moment of transplantation onto the uncovered device \((t(\alpha = 0.05, df = 4)) > 4.2)\).

Variations in Pb Isotopic Composition. Also the isotopic composition of the Pb present has been determined for the five sites and for both devices, before transplantation and after 14, 34, and 68 days of exposure. The \(^{206}\text{Pb}/^{207}\text{Pb}\) ratio determined before transplantation is 1.147 ± 0.003 (2 s, \(n = 3\), Figure 4), which agrees very well with the ratio determined by means of multicolonlector ICP-MS \(^{206}\text{Pb}/^{207}\text{Pb} = 1.1473\) for lichens from the same area \((20)\). The isotopic composition of Pb present in lichens transplanted onto uncovered devices displays a large variation. For the peri-urban sites Moyeuvre-grande and Scy-chazelles, the \(^{206}\text{Pb}/^{207}\text{Pb}\) ratio reaches values up to 1.206 after 68 days of exposure and for both locations, the trend in variation is very similar, suggesting a characteristic peri-urban signal (Figure 4b). A rapid increase of the \(^{206}\text{Pb}/^{207}\text{Pb}\) ratio is observed already after 34 days of exposure and even after only 14 days for the uncovered lichens from the dense automotive traffic and industrial activities sites (Figure 4). The dense automotive traffic site \(^{206}\text{Pb}/^{207}\text{Pb}\) ratio reaches a value \((1.195)\) close to the peri-urban value, whereas the \(^{206}\text{Pb}/^{207}\text{Pb}\) ratio for the industrial site seems buffered between 1.17 and 1.18 (Figure 4a). The lichens from the covered devices on these sites do not display significant variation of their \(^{206}\text{Pb}/^{207}\text{Pb}\) ratio (Figure 4a). The lichens transplanted onto the reference site maintain the lowest \(^{206}\text{Pb}/^{207}\text{Pb}\) ratio. Although the values are close to the original value, they are outside of the uncertainty interval \((2sd)\) of the original values (Figure 4a, b, and c). Nevertheless, after 2 years of exposure, also the \(^{206}\text{Pb}/^{207}\text{Pb}\) ratio of uncovered lichens for the reference site displays a variation of the Pb isotopic composition and reaches a value close to 1.215 (Figure 4c), whereas lichens freshly sampled from trees...
The transplants from lichens transplanted into covered devices display relatively lower Pb concentration results hence suggest that the accumulation of Pb in the thallus of uncovered lichens is indeed higher than the accumulation in the covered lichens. This implies that the observations made can not only be attributed to differences in terms of direct deposition of particles onto the lichen surfaces, but it has to be concluded that there is a real difference in accumulation depending on the device used. The finest aerosols are more easily fixed and assimilated in the thallus than the coarse particles. In addition, it is reasonable to think that only finest aerosols are found in covered device as it is protected with a plexiglas roof. From these results, it can be concluded that a part of the Pb present in the deposited coarse particles is actually accumulated into the thallus in addition to the Pb present in the finest aerosols, the latter being accumulated by lichens in the uncovered as well as the covered devices, and/or that the direct input of rain leads to a larger dissolved fraction of Pb. Due to the fact that the particle size distribution of anthropogenic aerosols is typically characterized by a larger fraction of small particles, finer aerosols are enriched in Pb compared to coarse particles. Self-evidently, this will also have an impact on the Pb concentrations observed.

It should be noted that after 68 days of exposure, the amount of Pb accumulated by lichens on the transplantation sites is not significantly different from that on the reference site (t test never significant). This supports the hypothesis that the differences observed for covered and uncovered devices indeed have to be explained in terms of differences in the ingestion of Pb originating (i) only from fine particles, and the dissolved fraction of Pb only for the covered device, while (ii) for the uncovered device, also the coarse particles are a source of ingested Pb. This observation supports the assumption that biological excretion is also partly responsible for the loss of elements observed in lichen transplants after more than 2 months of exposure, such that rain leaching is not the only reason, as was previously suggested (27). The rain events and PM<sub>10</sub> concentration (see Supporting Information Figure S1) obtained from one meteo station (Figure 1) and considered as representative for the entire area studied are not directly correlated with the variation in Pb concentration (i.e., diminution in cumulative precipitation from 37 mm during the first 14 days of exposure to 27 mm in the period between 14 and 34 days of exposure, versus an increase in Pb concentration during the same period). It is reasonable to think that the higher accumulation in lichens on the uncovered devices is to be attributed to the rain that aids Pb from all particles of all sizes to be incorporated into lichens. Moreover, lichens are known to be more efficient in their accumulation of elements from the dissolved fraction than from solid particles, resulting in a higher concentration in the thallus from the uncovered device than from the covered one. Next to that, the amount of Pb deposited onto the lichen surface is, self-evidently, more important in the uncovered device.

Since also the uncovered transplanted lichen on the reference site accumulated Pb, a variation in the air sources may be involved to explain the Pb concentration variation. Indeed, punctual events may be recorded within short time periods of exposure (27). Such variation in the air sources could also be evolved for the transplantation sites. Indeed, the two peri-urban sites Scy-Chazelles and Moyeuvre-Grande show a similar accumulation of Pb in lichens from the uncovered device (Figure 3b). Also, the Pb accumulation observed for the industrial (Florange) and dense automotive traffic (Thionville autoroute) sites is similar, but higher than for lichens from the peri-urban sites. Such a higher Pb accumulation in the industrial and high automotive traffic sites was expected, as these sites are more subject to direct pollution. Again, the difference in Pb accumulation between lichens stemming from the covered and the uncovered devices at those particular sites reflects the real accumulation

**FIGURE 4.** Details of the 206Pb/207Pb ratio measured in the lichens before and after transplantation on all sites and for both devices as a function of the exposure period. Results for (a) the industrial site (Flo) and dense automotive traffic site (Thi), (b) the two peri-urban sites, and (c) the reference site. Filled symbols represent the lichens from the uncovered devices and open symbols the lichens from the covered devices. Error bars are included in the symbol size.

**Discussion**

**Origin of Lead Concentration Variation.** For each of the transplantation sites, including the reference site, a systematic difference in Pb concentration is observed for lichens stemming from the uncovered device on the one hand, and from the covered device on the other hand (Figure 3). The lichens stemming from the uncovered device display a systematically higher Pb concentration. This strongly suggests a direct influence of the type of device used (covered versus uncovered) on the amount of Pb accumulated in lichens as already observed in a previous work (27). The Pb accumulation is higher in lichens from the uncovered device, which suggests that rain leaching is not very prominent during the first 68 days of accumulation after transplantation. Moreover, in this study, the lichens were washed before analysis to remove deposited particles onto the surface so that only the Pb accumulated in the thallus was monitored. The Pb concentration results hence suggest that the accumulation
of a larger part of Pb from the finest aerosols and/or a part of the Pb in the coarser aerosols in the uncovered thallus. These experiments seem to confirm the efficiency of lichen transplantation in air monitoring studies. The higher Pb accumulation observed in uncovered devices is explained by both (i) a more efficient accumulation of Pb present in the finer aerosols—which contain the highest concentration of Pb—owing to the impact of rain on their dissolution, and (ii) the fact that in the uncovered devices, also Pb from the coarse particles is taken up. A way to reinforce the conclusions drawn on the basis of Pb concentrations is to investigate the isotopic composition of Pb in the same samples.

Difference in Particle Size, Air Sources Variation or Biological Effect? The Pb isotopic composition displays large variations in Pb isotopic composition of lichens from the covered and the uncovered devices is observed (Figure 4), with lichens from the uncovered devices displaying higher $^{206}$Pb/$^{207}$Pb ratios than lichens from the covered devices. This observation suggests a pronounced difference in Pb isotopic composition of the coarse particles on the one hand and the finest aerosols and dissolved part on the other hand. The systematic variation in Pb isotopic composition on the one hand and that in Pb concentration on the other—with the highest degree of variation for uncovered devices—actually points in the same direction, which seems to confirm the hypothesis of variations in Pb sources and of the impact of the particle size distribution on the Pb isotopic composition, as is well illustrated in Figure 4. The two peri-urban sites display the same variations in terms of Pb concentration and show a similar $^{206}$Pb/$^{207}$Pb ratio of about 1.205 (Figure 4b) for lichens in the uncovered devices, whereas the $^{206}$Pb/$^{207}$Pb ratio stays lower than 1.160 in the covered device. However, such a radiogenic value for the uncovered device at the peri-urban site was not expected, mainly because Scy-Chazelles is close to the reference site and the latter did not record significant variations of the Pb isotopic composition, neither in transplanted lichens for both the covered and the uncovered set-ups, nor in “native” lichens 2 years later. Nevertheless, a variation in Pb isotopic composition is recorded in the transplanted lichens on the reference site after 2 years of transplantation (Figure 4c), giving rise to a $^{206}$Pb/$^{207}$Pb ratio around 1.215 for the uncovered lichens, which is the same ratio as that for uncovered lichens from the Scy-Chazelles peri-urban site. In Scy-Chazelles, both devices display similar $^{206}$Pb/$^{207}$Pb ratios and similar Pb concentrations of around 53 µg·g$^{-1}$ (not shown), suggesting an equilibrium of the air in both devices with the ambient air, so compensating for the differences between particles of different size. Nevertheless, a difference still exists between the covered and uncovered device on the reference site after 2 years (Figure 4c). This can be due to the difference in the height of the location where the lichens were transplanted to (70 cm instead of 3 m for the other sites) or by a lower variation in anthropogenic activities at the reference site. The results in lichens after 2 years of transplantation actually tend to confirm that the difference in terms of Pb concentration and Pb isotope ratios is mainly due to the dissolved fraction incorporated. Indeed, the covered lichens have access only to mist and gases, such that it takes them longer to become equilibrated with the dissolved part. The reason why the native lichen on the reference site does not record such a pronounced variation in Pb isotopic composition ($^{206}$Pb/$^{207}$Pb ratio of 1.154) remains unclear. It is probably due to a lower rate of Pb accumulation or Pb renewal in the native lichen because the native lichen does not loose Pb following the transplantation, so that the Pb turn-over rate is lower in the native than in the transplanted lichen. This could have an implication on the use of lichen as reliable bioindicators, mainly when the isotopic composition is used. An alternative explanation is a possible biological effect in the transplanted lichens. Indeed, one can imagine that the biological equilibrium of the lichens is destabilized after the transplantation and that this induces an effect on the Pb isotopic composition of the lichen.

The variation in the isotopic composition of Pb, reported on a three isotope diagram (Figure 5), could be attributed to an influx of Pb displaying the Pb isotopic composition of the natural crust (continental crust and Peri-industrial aerosol, Figure 5) (22, 31, 32), or to Pb of a particular source, similar to the steel plant reported on previously (33) (Figure 5), both sources having a similar $^{206}$Pb/$^{207}$Pb ratio but a different $^{208}$Pb/$^{206}$Pb ratio. The trend displayed by the transplanted lichens is fitting the trend defined by native lichens over two years in the same area (20, 26) (Figure 5), suggesting that the same source of Pb described before is at the origin of the variation. This trend is actually defined with a mixing between (i) radiogenic Pb from natural origin or particular industries such as steel plants and (ii) unradiogenic Pb coming from leaded gasoline, which is either old Pb present in the thallus or recycled Pb that was recently accumulated there. As a result, it is impossible to reveal whether the Pb accumulated in the thallus is of natural or of anthropogenic origin on the basis of the Pb isotope ratios measured along this study. However, considering that enrichment factors, calculated with Cerium, are slightly increasing from 4 up to 8 (Table S1, Supporting Information), it is more likely that the anthropogenic Pb contributing to the Pb isotope composition acts as an end-member than that the Pb is of natural origin.

At the two other sites (industrial and high automotive traffic), the $^{206}$Pb/$^{207}$Pb ratio is lower than for the two peri-urban sites. In the uncovered device at the industrial site (Florange), where the Pb accumulation is the highest, the $^{206}$Pb/$^{207}$Pb ratio is around 1.17 (Figure 4a), most probably reflecting the typical Pb isotopic composition of the local industries. Nevertheless, the covered device does not reflect this local Pb isotopic composition, suggesting that this composition is mainly represented in the dissolved part. A radiogenic Pb isotopic composition is also found in the lichen transplanted onto an uncovered device at the site characterized by dense automotive traffic (Thionville-highway), with a $^{206}$Pb/$^{207}$Pb ratio of 1.195. According to the increase in the Pb concentration in the same sample, an unradiogenic Pb isotopic composition was expected, corresponding to the Pb previously added to the French leaded gasoline ($^{206}$Pb/$^{207}$Pb ratio around 1.09–1.10 (22, 33–36), mainly due to the recycling of old Pb. The Pb coming from unleaded gasoline

![FIGURE 5. Three-isotope plot: $^{206}$Pb/$^{207}$Pb ratio versus $^{208}$Pb/$^{207}$Pb ratio. Each potential Pb source to atmospheric aerosol is indicated with a gray area. Diamond data for Pb originating from steel plant, small open circles are Pb isotope ratios measured in peat bogs and small filled triangles are Pb isotope ratios measured in lichens from the same areas in 2001 and 2003. Big filled circles represent the data obtained for lichens before and after transplantation (this work).](image)
is not an issue to explain such a variation since the Pb concentration emitted is really low and the Pb isotopic composition is variable, with 208Pb/206Pb ratios varying from around 1.09 to 1.17 (20, 36) and thus, not in agreement with a radiogenic value as recorded in the transplanted lichens. A small contribution of the Pb coming from gasoline (leaded and/or unleaded) can be involved, considering that the background is represented by the peri-urban site, where the Pb sources are more diffuse. In addition, it should also be noted that analysis of PM10 (26) and air bus filters (20) previously realized in the same area never revealed any influence of such radiogenic Pb, leading to the question of the biological effect of lichens.

More experiments on the Pb isotopic composition of explanted lichens will be needed to find out the actual biological effects in lichens during bioaccumulation and equilibration with the environment.

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Supporting Information Available

An additional figure and table. This material is available free of charge via the Internet at http://pubs.acs.org.

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