Biomonitoring of lead in Antarctic lichens using laser ablation inductively coupled plasma mass spectrometry

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A method for direct determination of Pb by LA-ICP-MS in lichen samples collected in a heavily anthropogenically impacted area of Maritime Antarctica was developed. The lichen samples were cryogenically ground and digested with nitric acid and hydrogen peroxide. Lead determination by solution-based ICP-MS was validated by the use of 2 certified reference materials. Once the Pb concentrations were measured with adequate accuracy and precision, a method for its direct determination by LA-ICP-MS was developed. The laser ablation parameters were optimized by the use of a Doehlert design matrix. The final optimized conditions were: laser energy (60%), spot size (150 μm) and repetition rate (10 Hz). Lead concentrations of the analyzed lichen samples were plotted against the intensity of the lead isotopes (208Pb, 207Pb and 206Pb) determined by LA-ICP-MS. Satisfactory linear correlation factors were obtained when 13C was used as internal standard. Lead distribution profiles in lichen thalli cross-sections were also obtained. By these results, LA-ICP-MS emerges as a potential analytical tool for Pb concentration estimation in lichen samples. Minimum amount of sample required, bioimaging capability, high analytical throughput, and minimization of waste generation are the major analytical features of this approach. The significant differences between the Pb concentration in the lichen samples from the control site (1.12 ± 0.05 mg kg⁻¹) and from the impacted points (mean = 5.03 ± 0.57 mg kg⁻¹) permitted qualify these organisms as good biomonitor. Elemental bioimaging of lichens demonstrated that the medulla region of lichen thalli is consistently the main atmospheric lead bioaccumulating lichen tissue.

Introduction

Antarctica has been long recognized as the last undisturbed landmass on Earth, notably due to its remoteness, low temperatures, and strong winds hampering logistic operations in this continent.¹ The Antarctic Treaty was signed by 12 countries in 1959 and entered into force in 1961 which is expected to monitor anthropogenic pollution as well as minimization of environmental impacts by the signatory countries.² During the 1990s with the enactment of Madrid Protocol on the Environment Protection in Antarctica all signatory nations are expected to cease any resource exploitation and to maintain and to monitor the Antarctic fragile ecosystems for peaceful and scientific work.³

Fossil fuels are often used for energy generation in Antarctic research stations and for conducting human activities at Antarctica. The massive use of these fuels, their transportation and storage have became significant sources of pollutants to the air,⁴–⁶ soils⁷–⁹ and sediments.⁹–¹² To assess the extent of pollutants coming from atmospheric deposition, chemical analyses of biological tissues such of lichens, mosses, foliage, tree rings and other organisms have been utilized in environmental studies around the world.¹³–¹⁹ Thus, lichen analyses appear as a good option for biomonitoring of organic air pollutants (i.e., polybrominated diphenyl ethers [PBDEs])¹⁰ and trace metals in Antarctica.¹¹ Lichens are formed by a symbiotic association between fungi and algae presenting remarkable characteristics qualifying them as good bioindicators as these organisms are perennial and they present high longevity, immobility and wide geographical distribution.²²,²³ Among them, the absence of protective waxy cuticle and their ability to absorb airborne pollutants along its entire surface are of particular interest.²³

Lead is a toxic metal with no known physiological benefits and it is commonly monitored in urban and industrial areas by lichen
analysis. In spite of the natural occurrence of this element in the Earth’s crust, the production of lead-based materials like ammunitions, batteries, fuels additives and paints have contributed to its enrichment in the environment. These findings are of special relevance due to the well known deleterious effects of lead to the human health such as impairment of the central nervous system, and harmful effects on cardiovascular, hematopoietic, and reproductive systems.

Lead concentration in lichen samples is usually at trace levels. Thus sensitive techniques such as graphite furnace atomic absorption spectrometry (GFAAS) or inductively coupled plasma mass spectrometry (ICP-MS) have been commonly used to determine this element in that lichen matrix. Nevertheless, for GFAAS and ICP-MS determinations, the solid samples must be digested and converted to a representative solution to be introduced into the instrument. To accomplish this step, sample preparation with hazardous reagents such as strong oxidizing acids (HNO₃ and HClO₄) and H₂O₂ are frequently employed. This operation can generate toxic wastes and adversely increase the analysis time and can also contaminate the samples during the tedious preparation process.

An effective method to speed up the sample preparation step permitting direct elemental determination on the solid matrix was the coupling between a laser ablation source to the sensitive ICP-MS elemental detector. LA-ICP-MS approach has gained increased recognition by the scientific community. In this system, a high-energy laser is focused on the solid sample removing particles, atoms, and ions from the surface. The ablation is conducted in an especially designed low pressure ablation chamber. The sample aerosol is directed to the plasma torch of the ICP-MS by a flux of an inert gas. Once the sample aerosol is introduced into the high temperature plasma excitation source, vaporization, atomization and ionization events sequentially take place. The ions related to the analytes of interest are separated by the mass analyzer as a function of its mass-to-charge ratio. Several research fields have employed LA-ICP-MS as a versatile analytical tool such as: proteomics, forensic, environmental, archaeological and cultural heritage, clinical and biological, among others. Many different types of samples are used in these studies including soils, sediments, rocks, tree rings, hair, teeth, bone, plants, and glasses. Up until recently, there is just one paper exploiting the versatility of this technique in lichen analysis. It demonstrated LA-ICP-MS as a potential method for Pb isotope ratios determination in peat and lichen samples for biomonitoring and sample screening purposes but that study did not report total lead concentrations in lichens.

The purpose of this study was to evaluate the feasibility of LA-ICP-MS for direct determination of lead concentration in lichen samples from a heavily anthropogenic impacted area of Maritime Antarctica. For this study, 9 lichen samples were collected around 3 scientific stations located at the Fildes Peninsula. Lichen cross-sections were also prepared to assess the lead distribution profile in the lichen structures. To our knowledge, this is the first study that shows the analysis of lichen samples from Antarctica with LA-ICP-MS presenting the elemental bioimaging of lichen thalli in relation to lead content.

Material and methods

Sampling and preliminary treatments

Fig. 1 shows the Fildes Peninsula highlighting the sampling sites around the Antarctic research stations (n = 8, from P2 to P9) and the control site (P1) located approximately 0.5 km from the nearest scientific station (Escudero Station). In Table 1, detailed information about the sampling sites and the samples are presented.

Lichen samples at selected sites were collected using a stainless steel blade, and kept in paper bags and plastic containers before is sent to the laboratory. These samples were oven-dried at 60 °C for 48 h, pre-ground in a knife mill (A11 basic analytical mill, IKA®, Staufen, Germany) finally being processed in a cryogenic mill (Marconi, Piracicaba, Brazil). The cryogenic grinding procedure was done with 3 stages of freezing (2 min per stage) and 3 stages of grinding (2 min per stage) where liquid nitrogen was used as a cooling agent. One lichen specimen of each sampling site was kept ungrounded to be directly analyzed by LA-ICP-MS. These samples were kept in previously decontaminated polyethylene containers until the chemical analysis were performed.

Reagents and analytical solutions

All reagents were of analytical grade. Deionized water (18 MΩ cm resistivity) obtained in a Millipore system (Bedford, MA, USA) was used to prepare all solutions. All glassware and polypropylene flasks were washed with soap, soaked in 50% v/v HNO₃ overnight, rinsed with deionized water and leave to dry at room temperature in a laminar flow fume hood prior to use. Lead standard solutions (from 0.1 to 100 µg L⁻¹ in 1% v/v HNO₃) were prepared after successive dilutions of the metal standard stock solution (1000 mg L⁻¹, Spex Certiprep, Metuchen, NJ, USA). Ultra-pure nitric acid (ARISTAR® ULTRA, VWR International, Chester, PA, USA) and hydrogen peroxide (30% v/v) p.a. purchased from Fisher Scientific (Fair Lawn, NJ, USA) were also used.

Instrumentation

The LA-ICP-MS experiments were performed using a Nd:YAG deep UV (213 nm) laser ablation system (NWR 213; New Wave Research, Fremont, CA, USA) coupled to an ICP-MS spectrometer PE-Elan 6000a (Perkin Elmer Instruments, Shelton, CT, USA). The detailed experimental conditions are described in the Table 2. The inert gas used to transport the sample aerosol from the ablation chamber to the ICP-MS was a mixture containing argon and helium (50: 50% v/v) delivered at a flow rate of 1.0 L min⁻¹.

Sample digestion procedure

Powdered lichen samples (n = 3) were accurately weighed (100 mg) in perfluorolaxyox (PFA) digestion vessels. Afterwards, 5 mL of ultra-pure HNO₃ conc. were added. A warming step (heating ramp of 3 °C min⁻¹) in a digestor block (Environmental Express, Charleston, SC, USA) was performed at 150 °C during 30 min using PFA reflux digestion vessels. The samples were then cooled
down to room temperature and 2 mL of H₂O₂ were added. The digestion was accomplished at 150 °C under reflux by further 60 min. The digested samples were quantitatively transferred to polyethylene flasks and the final volume was made up to 15 mL. Two Certified Reference Materials (CRMs), the Community Bureau of Reference (BCR, Geel, Belgium) CRM 100 (Beech leaves) and the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) Standard Reference Material (SRM) 1515 (Apple leaves) were digested in same procedure as the samples to check the accuracy of the method.

Lead determination in the digested samples by ICP-MS

For lead determination in the acid digested samples, the ICP-MS instrument and operational conditions are shown in the Table 2. An online addition of Bi (20 μg L⁻¹) was used as an internal standard. The isotopes ²⁰⁹Pb and ²⁰⁹Bi were monitored. Linear calibration functions with correlation coefficients (r²) better than 0.999 were obtained using the Pb concentration range from 0.1 to 100 μg L⁻¹.

Pellets preparation for LA-ICP-MS analysis

To 0.3 g of powdered material (lichens and CRMs) 18 drops of a liquid binder (Chemplex, Palm City, FL, USA) were added. The binder solvent (methylene chloride) was then volatilized leaving the sample in a fume hood at room temperature for 15 min. Using an agate mortar the sample and the binder were homogenized thoroughly. Finally, approximately 0.2 g of sample was pressed into pellets in a pneumatic press using 12 ton pressure for 30 s.

Results and discussion

LA-ICP-MS parameters optimization

A Doehlert matrix design was constructed and applied for LA-ICP-MS parameters optimization. The following variables

<table>
<thead>
<tr>
<th>Sample</th>
<th>Geographic Coordinates</th>
<th>Lichen species</th>
<th>Sampling sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>62° 12’ 15.88” S/58° 57’ 20.23” W</td>
<td>Usnea aurantiaco-ata (Jacq.) Bory</td>
<td>Control site, 0.5 km far from the stations</td>
</tr>
<tr>
<td>P2</td>
<td>62° 12’ 8.87” S/58° 57’ 33.48” W</td>
<td>Usnea aurantiaco-ata (Jacq.) Bory</td>
<td>Intermediate position</td>
</tr>
<tr>
<td>P3</td>
<td>62° 12’ 6.76” S/58° 57’ 40.82” W</td>
<td>Usnea aurantiaco-ata (Jacq.) Bory</td>
<td>Around Escudero Station</td>
</tr>
<tr>
<td>P4</td>
<td>62° 12’ 6.80” S/58° 57’ 42.90” W</td>
<td>Usnea aurantiaco-ata (Jacq.) Bory</td>
<td>Around Escudero Station</td>
</tr>
<tr>
<td>P5</td>
<td>62° 12’ 5.99” S/58° 57’ 45.06” W</td>
<td>Usnea aurantiaco-ata (Jacq.) Bory</td>
<td>Nearest Escudero Station</td>
</tr>
<tr>
<td>P6</td>
<td>62° 12’ 2.49” S/58° 57’ 59.23” W</td>
<td>Usnea aurantiaco-ata (Jacq.) Bory and USnea antarctica Du Rietz</td>
<td>Around Escudero Station</td>
</tr>
<tr>
<td>P7</td>
<td>62° 11’ 56.73” S/58° 57’ 52.70” W</td>
<td>Usnea aurantiaco-ata (Jacq.) Bory</td>
<td>Between Escudero and Bellinghausen Stations</td>
</tr>
<tr>
<td>P8</td>
<td>62° 11’ 47.47” S/58° 57’ 42.27” W</td>
<td>Usnea aurantiaco-ata (Jacq.) Bory</td>
<td>Around Bellinghausen Station</td>
</tr>
<tr>
<td>P9</td>
<td>62° 11’ 47.54” S/58° 57’ 39.37” W</td>
<td>Usnea aurantiaco-ata (Jacq.) Bory</td>
<td>Around Bellinghausen Station</td>
</tr>
</tbody>
</table>
were explored at a number of levels: spot size (from 3 to 250 μm), energy (from 20 to 80%) and repetition rate (from 1 to 10 Hz) as can be observed in the Table 3. The experiments were randomly performed using the previously prepared pellet of the CRM BCR 100 (Beech leaves) which has a Pb concentration of 16 mg kg⁻¹. The laser instrumental conditions as well as the ICP-MS parameters are shown in the Table 2. For each experiment of this optimization step, an ablation line of 1 mm was used and the signals of the Pb isotopes (²⁰⁸Pb, ²⁰⁷Pb, and ²⁰⁶Pb) were monitored. The laser speed (μm s⁻¹) for each experiment was the same numeric value of the spot size in the Doehlert matrix. All experiments were performed in triplicates and the relative standard deviations (RSDs) were calculated. The conditions that led to the best results in terms of sensitivity (accounted by the higher analytical signal) and simultaneously (n = 3) the best repeatability (i.e. lowest RSDs values) were those of experiment 4 (marked with the footnote a in Table 3). These optimized laser conditions with a spot size of 150 μm, energy (60%) and repetition rate (10 Hz) were used for pelletized CRM and lichen samples analysis. In Fig. 2 there are typical analytical signals obtained for 1 mm length ablation line in the P9 sample pellet under optimized conditions.

**Table 2 Operational parameters for ICP-MS and LA-ICP-MS determinations**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Laser warm-up time (s)</th>
<th>Laser output (%)</th>
<th>Repetition rate (Hz)</th>
<th>Spot size (μm)</th>
<th>Scan speed (μm s⁻¹)</th>
<th>Energy delivered (mJ)</th>
<th>Fluence (J cm⁻²)</th>
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<tbody>
<tr>
<td>Raster Cross-sections scans</td>
<td>40</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<td>44.5</td>
</tr>
<tr>
<td>Linear scans across the pellet</td>
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<td>60</td>
<td>10</td>
<td>150</td>
<td>150</td>
<td>1.3</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**Table 3 Doehlert design matrix used for LA-ICP-MS parameters optimization with randomly obtained experimental results**

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Spot size (μm)</th>
<th>Energy (%)</th>
<th>Repetition rate (Hz)</th>
<th>²⁰⁶Pb Intensity</th>
<th>²⁰⁶Pb RSD (%)</th>
<th>²⁰⁷Pb Intensity</th>
<th>²⁰⁷Pb RSD (%)</th>
<th>²⁰⁸Pb Intensity</th>
<th>²⁰⁸Pb RSD (%)</th>
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<tr>
<td>1A</td>
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<td>50</td>
<td>5</td>
<td>18269</td>
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<td>7749</td>
<td>17.1</td>
<td>8588</td>
<td>21.5</td>
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<tr>
<td>1B</td>
<td>100</td>
<td>50</td>
<td>5</td>
<td>17932</td>
<td>19.1</td>
<td>7030</td>
<td>6.5</td>
<td>8720</td>
<td>19.2</td>
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<tr>
<td>1C</td>
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<td>50</td>
<td>5</td>
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<td>50</td>
<td>5</td>
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<td>3.8</td>
<td>11695</td>
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<tr>
<td>3</td>
<td>150</td>
<td>80</td>
<td>5</td>
<td>39155</td>
<td>14.3</td>
<td>16789</td>
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<tr>
<td>4*</td>
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<td>60</td>
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<td>50345</td>
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<td>5</td>
<td>646</td>
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<td>317</td>
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<td>40</td>
<td>1</td>
<td>756</td>
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<td>307</td>
<td>44.1</td>
<td>351</td>
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<td>20</td>
<td>5</td>
<td>1751</td>
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<td>35.4</td>
<td>830</td>
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<td>9</td>
<td>150</td>
<td>40</td>
<td>1</td>
<td>2922</td>
<td>9.4</td>
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<td>22150</td>
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<td>10916</td>
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<td>4564</td>
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<td>19.1</td>
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<td>50</td>
<td>60</td>
<td>10</td>
<td>20374</td>
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<td>8733</td>
<td>21.2</td>
<td>9978</td>
<td>23.1</td>
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<td>100</td>
<td>30</td>
<td>10</td>
<td>10534</td>
<td>15.3</td>
<td>4386</td>
<td>18.4</td>
<td>4782</td>
<td>17.0</td>
</tr>
</tbody>
</table>

* Optimized conditions.

**Lead determination of the digested CRMs**

In order to check the accuracy of the whole digestion procedure for lead determination by ICP-MS, the CRMs (Beech leaves, BCR100 and Apple leaves, NIST 1515) were used. Quantitative recoveries were obtained for both CRMs (see Table 4).

![Fig. 2](https://example.com/figure2.png)

**Fig. 2** Typical LA-ICP-MS analytical signals obtained in a laser scan of a pelletized lichen sample (from the P9 site) under optimized conditions (Energy: 60%; Repetition rate: 10 Hz and Spot size: 150 μm).
Calibration strategies using LA-ICP-MS

In order to evaluate the applicability of LA-ICP-MS as an analytical technique for direct Pb determination, several statistical parameters were calculated. Linear correlation factors, Predict Error Sum of Squares (PRESS) and Root Mean Square Error of Prediction (RMSEP) were calculated after using two calibration strategies. The calibrations were performed using $^{13}$C as an internal standard or by plotting the raw lead isotope intensity against the Pb concentrations of the mineralized and analyzed lichen samples. Unsatisfactory linear correlation coefficients ($r^2$) were obtained using just the raw Pb intensity data against the concentrations (see Table 5).

This finding can be explained by the normal long-term fluctuations of the analytical signal during the analysis as well as by random transport disturbances. On the other hand, the investigated statistical parameters (see Table 5) pointed out for the use of $^{13}$C as a suitable internal standard for Pb determination in lichen samples. As such, the internal standardization is a good analytical strategy in order to correct drifts occurred during the signals acquisition and sampling variations as well as transport effects of the sample aerosol from the ablation chamber to the ICP-MS torch. The $^{13}$C satisfied the core requirements of a good candidate of internal standard which include: must be in the same concentration in all samples (it is naturally present since the carbon content in the lichen samples are almost the same and the C-based binder is added at an uniform quantity to all pellets); must correct fluctuations during the signal acquisition, then it must have a similar behavior with the analyte (this is observed by the very comparable analytical signals shapes for both the $^{13}$C and Pb isotopes as can be seen in Fig. 2); and finally its use must result in better signal accuracy as it is observed by the low PRESS and RMSEP values and better repeatability (see Table 6).

The analytical figures of merit are presented in Table 6. The limits of detection (LODs) for lead determination in the lichen samples were in very low levels (3 μg kg$^{-1}$ using $^{208}$Pb isotope). There were no observed significant differences in the LODs values between both calibration strategies. These LODs values are in the typical range for metal determination in solid matrices and RMSEP values between both calibration strategies. These LODs values are in the typical range for metal determination in solid matrices and RMSEP values between both calibration strategies.

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Once the accuracy of the digestion procedure was validated by the CRMs analysis, the lead content in the lichen samples were determined. The Fig. 3a presents the results obtained for all collected samples. As expected, Pb concentration in the lichen sample from the control site (P1, n = 3) was lower than those points affected by the scientific stations (from P2 to P9). The greatest difference in relation to Pb concentration was just between the control site (1.12 ± 0.05 mg kg$^{-1}$) and the nearest Escudero research station site (see Fig. 1; P5 site – [Pb] = 8.71 ± 0.08 mg kg$^{-1}$). Possible Pb contamination sources in the area could be coming from fossil fuels and waste burning as well as contaminated airborne soil particles in suspension on the air. These results are consistent with data presented by Osyczka et al.$^{58}$ In this study, the authors determined Pb concentration, among other elements, in Usnea antarctica and Usnea aurantiacoatra lichen samples collected around several scientific stations in the King George Island. Lead content of the detectable samples varied from 1 mg kg$^{-1}$ (samples from the Polish Henryk Arctowski station) to 6 mg kg$^{-1}$ (samples from the Bellinghausen station). About a decade before this publication, Olech et al.$^{59}$ determined the Pb content by proton induced X-ray emission spectrometry (PIXE) in lichen samples collected at the Antarctic Peninsula. In this study, lichen samples collected in “pristine” regions of Antarctica were transplanted to intensively impacted sites near the electricity power plant of the Polish Arctowski station. After 6 months of exposure, lead concentrations reached up to 160 mg kg$^{-1}$ in samples with a background Pb concentration of 3.2 mg kg$^{-1}$. As a result, the authors pointed out the

<table>
<thead>
<tr>
<th>Calibration strategy</th>
<th>Linear correlation factor ($r^2$)</th>
<th>PRESS</th>
<th>RMSEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Pb] versus $^{206}$Pb intensity</td>
<td>0.7815</td>
<td>16.73</td>
<td>1.36</td>
</tr>
<tr>
<td>[Pb] versus $^{207}$Pb intensity</td>
<td>0.7856</td>
<td>16.33</td>
<td>1.35</td>
</tr>
<tr>
<td>[Pb] versus $^{208}$Pb intensity</td>
<td>0.7771</td>
<td>17.17</td>
<td>1.38</td>
</tr>
<tr>
<td>[Pb] versus $^{206}$Pb/$^{13}$C</td>
<td>0.9132</td>
<td>5.66</td>
<td>0.79</td>
</tr>
<tr>
<td>[Pb] versus $^{207}$Pb/$^{13}$C</td>
<td>0.9141</td>
<td>5.59</td>
<td>0.79</td>
</tr>
<tr>
<td>[Pb] versus $^{208}$Pb/$^{13}$C</td>
<td>0.9102</td>
<td>5.88</td>
<td>0.81</td>
</tr>
</tbody>
</table>

$^a$ PRESS – Prediction Errors Sum of Squares; RMSEP – Root Mean Square Error of Prediction.

Table 4  Recoveries of Pb in the Certified Reference Materials

<table>
<thead>
<tr>
<th>Certified Reference Materials</th>
<th>[Pb] certified (mg kg$^{-1}$)</th>
<th>[Pb] found (mg kg$^{-1}$) n = 3</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST SRM 1515 (Apple leaves)</td>
<td>0.470 ± 0.024</td>
<td>0.461 ± 0.017</td>
<td>98 ± 4</td>
</tr>
<tr>
<td>BCR 100 (Beech leaves) 16$^a$</td>
<td>16$^a$</td>
<td>15.4 ± 0.8</td>
<td>96 ± 5</td>
</tr>
</tbody>
</table>

$^a$ information value.
necessity of continuous air pollution biomonitoring in the Antarctic region by lichen analysis.

In Fig. 3b it can be seen that the ratio between the Pb isotopes signals in relation to $^{13}$C intensity obtained with LA-ICP-MS and follows the Pb isotopic abundance distribution. The same pattern of intensity ratio and Pb concentrations were observed for the sampling sites under study (see Fig. 3a and 3b). Lichens samples from the P1 site (control site, see Fig. 1 and 3a) have the lowest Pb concentration as well as the lowest Pb signals (see Fig. 3b). The most contaminated points (P3 and P5, see Fig. 3a) are those with highest Pb signals (see Fig. 3b). As demonstrated clearly here, it can be assumed that LA-ICP-MS is a feasible analytical approach for Pb concentration estimation in lichen samples.

Lead distribution profile in lichen thalli cross-sections

Two lichen thalli with similar diameters from each of the two most distinct sites (P1 and P5, see Fig. 3a) were randomly selected and carefully sliced with the help of a stainless steel blade. In order to assess the Pb distribution profile along the lichen thalli, one sample of each site was selected. A rectangular grid was made in the lichen thallus cross-section aiming to cover all anatomical structures of the organism: outer cortex, medulla and central cord (see Fig. 4a and 4c). These raster grids had the following dimensions: 310 x 360 μm (for the P1 lichen thallus, see Fig. 4a) and 250 x 320 μm (for the P5 lichen thallus, see Fig. 4c). Before laser sampling the grid scan to assess the Pb distribution, a pre-ablation scan in the same grid area was made with “mild” ablation conditions in order to eliminate potential contaminants added to the material surface during its handling. For this purpose, the laser operational conditions are the following: energy (40%), spot size (10 μm), repetition rate (10 Hz), speed (10 μm s⁻¹) and depth (5 μm). Afterwards, optimized analytical laser conditions (80% of energy, 10 Hz of repetition rate and 10 μm of spot size) were applied to record the signal intensities of $^{208}$Pb and $^{13}$C by the ICP-MS in the same grid area. These laser ablation conditions were employed to achieve a good spatial resolution and satisfactory analytical signals in order to build the elemental bio-image of Pb distribution. Three line scans on the beech leaves CRM pellet were also performed with the same laser conditions and the $^{208}$Pb and $^{13}$C signals were also recorded by the ICP-MS equipment software. The stored elemental intensity (cps) and time (s) data were transferred to a Microsoft Excel® (Richland, WA, USA) spreadsheet. The sample analytical signals were converted to concentration by the use of a single point calibration with the beech leaves CRM signals. With this approach semi-quantitative data were obtained. Finally, spatial Pb distribution bio-images were constructed using the Origin Pro8 software (Northampton, MA, USA).

The elemental bio-images (see Fig. 4b and 4d) clearly demonstrate considerable Pb concentration (from ≈0.003 (LOD) to 3 mg kg⁻¹) present in the medulla region of the lichen thalli cross-section from the control site. Moreover, it is evident that the higher Pb concentrations (from 0.06 to 21 mg kg⁻¹) present in the medulla region of the lichen thalli from the contaminated

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$^{208}$Pb</th>
<th>$^{208}$Pb/$^{13}$C</th>
<th>$^{207}$Pb</th>
<th>$^{207}$Pb/$^{13}$C</th>
<th>$^{206}$Pb</th>
<th>$^{206}$Pb/$^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of detection (μg kg⁻¹)</td>
<td>3.00</td>
<td>3.32</td>
<td>18.96</td>
<td>16.74</td>
<td>12.06</td>
<td>12.39</td>
</tr>
<tr>
<td>Repeatability</td>
<td>12.4</td>
<td>7.9</td>
<td>12.4</td>
<td>7.7</td>
<td>12.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Maximum theoretical analytical throughput (determinations per hour)</td>
<td>60</td>
<td>50</td>
<td>120</td>
<td>45</td>
<td>60</td>
<td>45</td>
</tr>
</tbody>
</table>

LOD – limit of detection following IUPAC (3σblank/slope, n = 10). R.S.D.(%) – average relative standard deviation of the 9 analyzed lichen samples (n = 45 measurements, 5 per sample).
It is apparent that Pb is bioaccumulating in the medulla region which comprises the region between the outer cortex and the rigid central cord. In this region, the algal layer and the fungi lichen coexist. It is well documented that lichens exhibit high tolerance against elevated concentrations of toxic metals. Some metabolites produced by these organisms such as phytochelates, oxalates, organic phosphates, lichen-derived acids (e.g. usnic, pulvinic and rhizocorpic acids) and melanin pigments are able to form stable complexes with metals. These metal–ligand interactions likely take place where the lichen symbiotic organisms are located, i.e., in the medullar structure.

The elemental imaging capability of LA-ICP-MS have been recently exploited in several reports dealing with plants, biological tissues and other diverse types of samples. However, this study is the first attempt of element distribution bioimaging in lichen tissue structures using LA-ICP-MS.

An alternative method for rapid discrimination between Pb contaminated samples from non-contaminated sites and Pb distribution across the thallus was also evaluated. In this case, one lichen thallus from each of the most distinct sampling locations (P1 and P5) was selected and three linear ablation lines through whole cross-sections (see Fig. 5a and 5b) were done with the same laser conditions of the grid approach discussed above. The semi-quantitative data obtained with this scheme is shown in the Fig. 5c. Again, it is a compelling demonstration of the highest levels of Pb present in the lichen medulla region. This observation is in agreement with the raster distributions described previously. At the same time is easy to distinguish between the control sample and the contaminated one by their Pb signals. It appears that this triplicate-lines laser ablation approach is the most suitable technique for rapid Pb screening in lichens with virtually no sample preparation and minimum use of lichen sample amount.
environmental assessment studies using lichens as biomonitor

Best structure to be selected and analyzed by LA-ICP-MS in

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thalli

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concentration estimation in lichen samples. The minimum

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16 E. Simon, M. Braun, A. Vidic, D. Bogyó, I. Fábián and


I. Suchara, J. Sucharova, M. Hola, C. Reimann, R. Boyd,


D. L. Hawksworth, T. Iturriaga and A. Crespo, Rev. Iberoam. Micol.,

2005, 22, 71.

S. Giordano, P. Adamo, S. Sorbo and S. Vingiani, Environ. Pollut.,


2009, 166, 1344.

D. Cuny, L. Davranche, P. Thomas, M. Kempa and C. Van Haluwyn,


International Programme on Chemical Safety, World Health

Organization, Geneva.

J. F. Rosen, Toxicology, 1995, 97, 11.

References

1 P. R. Dingwall (Ed.), in: Antarctica in the environmental era;


scar.org, accessed on May 2011.


4 W. B. Lyons, C. A. Neza, K. A. Welch, S. T. Kottmeier and


5 M. A. Leal, M. Joppert, M. V. Licinio, H. Evangelista, J. Maldonado,


6 M. B. Guerra, C. E. G. R. Schaefer, P. F. Rosa, F. N. B. Simas,

T. T. C. Pereira and E. R. Pereira-Filho, Water, Air, Soil Pollut.,

2011, DOI: 10.1007/s11270-011-0811-z, in press.

7 M. A. E. Chaparro, H. Núñez, J. M. Lirio, C. S. G. Gogorza and


8 M. B. Guerra, C. E. G. R. Schaefer, R. F. M. Michel, P. F. Rosa


9 A. Curto, E. Pelletier, C. L. Vodopivez and W. P. M. Cormack, Sci.


10 M. C. Bicgo, E. Zanardi-Lamardo, S. Taniuchi, C. C. Martins,

D. A. M. da Silva, S. T. Sasaki, A. C. R. Albergaria-Barbosa,


11 C. C. Martins, M. C. Bicgo, N. L. Rose, S. Taniuchi,

R. A. Lourenço, R. C. L. Figueire, M. M. Mahiques and

R. C. Montone, Environ. Pollut., 2010, 158, 192.

12 A. P. Ribeiro, R. C. L. Figueira, C. C. Martins, C. R. A. Silva,

E. J. França, M. C. Bicgo, M. M. Mahiques and R. C. Montone,


2004, 6, 636.

14 L. González-Miqueo, D. Elustondo, E. Lasheras, R. Bermejo and

J. M. Santamaría, Water, Air, Soil Pollut., 2010, 210, 335.

15 M. Milejović, V. Ettler, O. Šebek, O. Šracek, B. Krítek, T. Kyncl,

V. Majer and F. Veselovský, Water, Air, Soil Pollut., 2011, 216,

657.


17 B. Smolić, M. L. Pignata, M. Saiki, E. Cortés, N. Bangfa, B. Markert,

B. Nyarko, J. Arunachalam, J. Garty, M. Vuchtkov,

H. Th. Wolterbeek, E. Steinnes, M. C. Freitas, A. Lucaciu and


18 E. Simon, M. Braun, A. Vidic, D. Bogyó, I. Fábián and


19 I. Suchara, J. Sucharova, M. Hola, C. Reimann, R. Boyd,

P. Filzmoser and P. Englmaier, Sci. Total Environ., 2011, 409,

2281.


2005, 22, 71.

24 S. Giordano, P. Adamo, S. Sorbo and S. Vingiani, Environ. Pollut.,


2009, 166, 1344.

26 D. Cuny, L. Davranche, P. Thomas, M. Kempa and C. Van Haluwyn,


International Programme on Chemical Safety, World Health

Organization, Geneva.

28 J. F. Rosen, Toxicology, 1995, 97, 11.

Conclusions and outlook

The significant differences observed between the Pb concentra-

tions in the lichen samples from the control site (1.12 ± 0.05 mg

kg⁻¹) and from the anthropogenically impacted sampling sites

(5.03 ± 0.57 mg kg⁻¹ in average) permitted qualify these organ-

isms as good biomonitors.

LA-ICP-MS emerges as a potential analytical technique for Pb

concentration estimation in lichen samples. The minimum

amount of sample required (few μg in a laser shot), high

analytical throughput (about 80 samples daily) and minimization

of waste generation are significant analytical advantages of this

approach.

The medulla region of lichen thalli is consistently the main sink

of Pb coming from the atmosphere. We can conclude that it is the

best structure to be selected and analyzed by LA-ICP-MS in

environmental assessment studies using lichens as biomonitor

organisms. This proposed approach is a novel analytical strategy
toward biomonitoring studies using lichens.

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pellet press.

Fig. 5 Lead distribution profile (semi-quantitative data obtained by

a single point calibration using the Beech leaves CRM signals) along

whole lichen thalli cross-sections of 2 selected samples by LA-ICP-MS:

(a) Lichen thalli from the control site (P1). (b) Lichen thalli from the

most contaminated site (P5). (c) Lead (mg kg⁻¹) distribution across both

thalli.
