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Notes

Lichen monitoring as a potential tool in environmental forensics: case study of the Cu smelter and former mining town of Karabash, Russia

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Abstract: The aim of this paper is to showcase the use of lichens in environmental forensics from an assessment of atmospheric deposition in and around the Cu smelter and former mining town of Karabash, Ural Mountains of Russia. *Hypogymnia physodes* was collected on its bark substrate in July 2001 from a 'reference' site (c. 25 km SW of Karabash) and transplanted to 10 stations along an approximately 60 km SSW–NNE transect centred on Karabash. Transplants were collected after 2 and 3 month exposure periods. The elemental compositions of *Hypogymnia* and potential sources of particulates in the study area (smelter blast furnace and converter dusts, wastes, tailings, road dusts, metallurgical slags and top soils) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) and quadrupole ICP mass spectrometry (ICP-MS), and the Pb isotope compositions of the lichens and smelter dusts by multicollector ICP-MS. Particulates on lichen surfaces were analysed by scanning electron microscopy with energy-dispersive X-ray analysis (SEM-EDX).

The method of lichen transplantation, combined with multi-element and surface particle elemental analysis, high-precision Pb isotope ratio determinations and modelling, was shown to be useful for the tracing of the smelter signal, and components from different smelter processes, for more than 25 km from Karabash town. The lichen monitoring methodology is discrete and comparatively low cost, enabling atmospheric deposition from natural and anthropogenic sources to be determined over short (<3 month) periods, and is therefore a valuable qualitative tool for environmental forensics.

Key aspects of the nature and biology of lichens

Lichens are mutualistic associations involving at least one fungus (mycobiont) and an alga or cyanobacterium (photobiont). Overall, this relationship is very successful as lichens are found in almost all terrestrial habitats from the tropics to polar regions. Lichens are classified as fungi and vary from less than 1 mm² to over 2 m long. They are poikilohydric organisms whose water status varies passively with the surrounding environmental conditions. Most species live for decades or hundreds of years and, as perennials, are subject to the cumulative effects of pollutants. Unlike many vascular plants, lichens have no deciduous parts and, hence, cannot shed these to expel potential toxins. Furthermore, the lack of stomata and a protective cuticle in lichens means that atmospheric deposits may be absorbed over the entire thallus surface (Fig. 1). During dry periods, solutions may be concentrated to the point

that levels of toxic substances are sufficient to alter the balance between photobiont and mycobiont. This may lead to a breakdown in the association and death of the lichen. Lichen species distribution patterns may therefore reflect varying levels of air pollutants, as well as a variety of unrelated biotic and abiotic factors. Lichens are efficient accumulators of metals and persistent organic pollutants, and are frequently used to monitor the deposition of such substances (e.g. Farmer 1993; Nimis *et al.* 2002; Nash 2008), and have even been used in minerals prospecting (Chettri *et al.* 1997). Some species are highly tolerant of pollution, including from smelters (e.g. Purvis *et al.* 2000).

Historical background

Pioneering biomonitoring using lichens investigated fallout from nuclear testing in the 1950s (James 1973). Studies in the late 1960s and the early 1970s identified a correlation between atmospheric

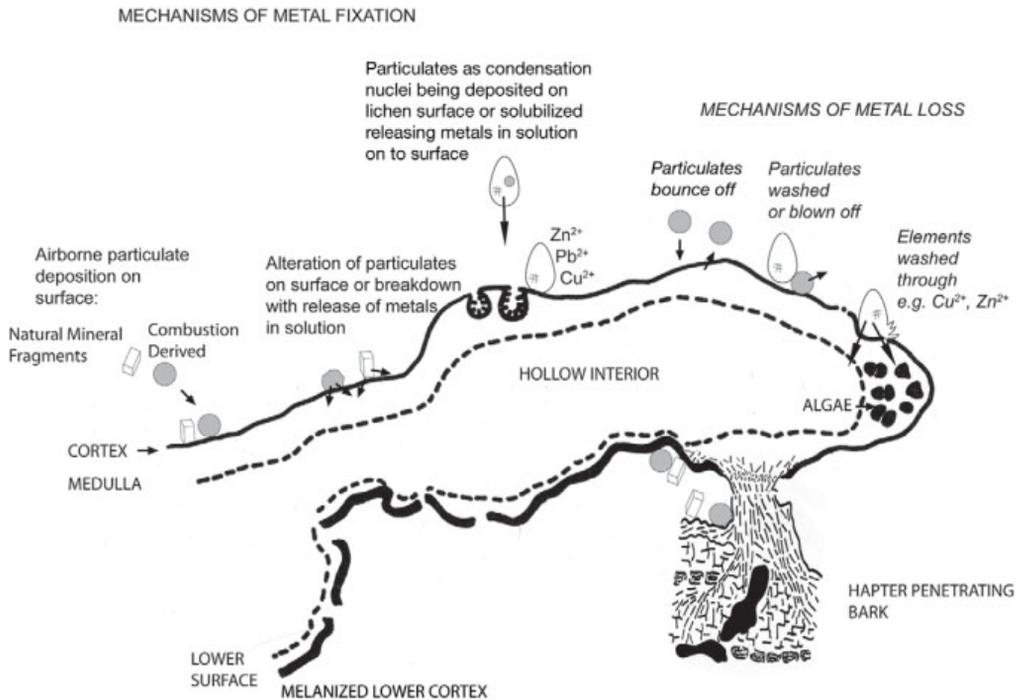


Fig. 1. Diagrammatic representation of section of *Hypogymnia physodes* showing the main mechanisms by which metals may be deposited on or lost from surfaces (adapted from Beltman 1978; Williamson *et al.* 2004a), not to scale.

SO₂ concentrations and sulphur content in samples of the lichens *Parmelia saxatilis* (Gilbert 1965, 1968, 1969) and *Hypogymnia physodes* (Hawksworth *et al.* 1973; James 1973), and a correlation between the atmospheric deposition of heavy metals and metal content in lichens (Jenkins & Davies 1966). Lichens have since been increasingly used to monitor spatial and temporal patterns of heavy metal dispersion from industrial sources and roads, for example by Seaward (1973), Nieboer *et al.* (1975), Richardson (1995), Garty (2001) and many others; see the list of studies abstracted in the Recent Literature on Air Pollution Lichen series published in *The Lichenologist* over the period 1974 to 2000 (Hawksworth 1974; Henderson 2000). In relation to a smelter, Nieboer *et al.* (1975) were amongst the first to show a curvilinear relationship for Cu, Ni, Zn and Fe content in metal-tolerant lichen species, with distance confirming this as their point source. There is a general consensus that the trapping of relatively large particles in large medullary intercellular spaces accounts for the bulk of accumulated metals in thalli near industrial sites (Seaward 1973; Garty *et al.* 1979).

The widely distributed epiphytic foliose (leaf-like) macrolichen *Hypogymnia physodes* has been extensively used in the source apportionment of

anthropogenic and natural substances deposited in the environment. These substances may originate in the lichens either by dry and/or wet atmospheric deposition or stem flow and/or canopy through-fall (Bargagli 1998). In spatially extensive surveys of remote regions where it is impractical or too costly to employ large numbers of recording/sampling devices, lichen biomonitoring may be one of the only ways to determine patterns of atmospheric deposition (Nimis *et al.* 2002; Nash 2008). Various methods may be applied including monitoring species abundance, diversity and health, which is rapid and relatively low cost. Where lichens are not naturally present (e.g. the environment being too contaminated), transplants may be used. These are sampled from a 'background' or 'reference' site and transplanted to monitoring stations elsewhere for a fixed exposure period. The lichen transplants can then be analysed, and their compositions compared with samples from the reference site to determine and/or map relative levels and sources of metals and other species. It should be noted, however, that *Hypogymnia* and other lichens should not be regarded as 'inert depositors'; that is, they are not 'passive filters' (Johnsen 1981; Glenn *et al.* 1991; Roszbach *et al.* 1999; Nimis *et al.* 2002). Complex, often poorly understood, biogeochemical

processes may contribute to the fixing and localization of metals in lichens (e.g. Garty 2001; Haas & Purvis 2006; Purvis & Pawlik-Skowrońska 2008), and there are a number of ways metals can be lost and/or gained during the transplant exposure period (Fig. 1). The method of lichen transplant monitoring therefore should be regarded as qualitative, although it may be possible to quantify the relative signature of different source inputs (see the subsection on 'The modelling approach' later). The earliest transplant studies using *H. physodes* were by Schönbeck (1969) and others in the early 1970s (Mikhailova 2002; Rusu *et al.* 2006a, b). Subsequent developments in analytical techniques have led to further innovation.

Sampling and analytical methods used to assess metal contents in lichens

The analysis of whole-lichen samples is practical, sensitive and is relatively cost effective. It generally requires sample homogenization and dissolution followed by wet chemical analysis, most commonly by ICP-OES and ICP-MS (Rusu 2002). During the sampling and analysis of lichens, rigorous quality assurance/control procedures should be followed at all times. Lichens must be sampled from equivalent sites, including distance from roads, vegetation/woodland type and similar substrates (Bargagli 1998; Garty 2001; Bargagli & Nimis 2002). Adequate numbers of replicate samples must be collected in order to establish the precision of the methodology. Certified reference materials should be analysed at the same time as the collected samples to establish the accuracy of the analytical procedures being used. Data obtained from whole-lichen analyses can be visualized in a number of different graphical plots (see example figures in this paper) or subject to multivariate statistical analyses to help identify the sources of contained elements (Sloof 1995; Faus-Kessler *et al.* 2001; Yenisooy-Karakas & Tuncel 2004, 2008).

Data from whole-sample wet chemical analysis, however, cannot generally be used to identify the chemical and mineralogical form of contained elements, which may provide important clues as to their source and mode of deposition. The presence and nature of metal-rich particles on thallus surfaces and within their interiors has been directly determined using SEM, often combined with X-ray elemental analysis (Garty *et al.* 1979; Olmez *et al.* 1985; Bargagli & Mikhailova 2002; Williamson *et al.* 2004a).

Increasingly, isotopic analysis including that for sulphur (Krouse 1977; Spiro *et al.* 2002), lead and zinc isotopes (Monna *et al.* 1999; Spiro *et al.* 2004, 2012; Weiss *et al.* 2004; Dolgoplova *et al.*

2006b; Sondergaard *et al.* 2010), and rare earth element signatures have been utilized in source apportionment (Chiarenzelli *et al.* 2001; Zschau *et al.* 2003; Dolgoplova *et al.* 2006a; Purvis *et al.* 2010).

The aim of this paper is to showcase the use of lichens in environmental forensics, which follows on from a recent study on the use of fungi for such studies (Hawksworth & Wiltshire 2011). We present a case study illustrating the assessment of atmospheric deposition from a copper smelter and mining-contaminated areas in the town of Karabash, Ural Mountains of Russia (Fig. 2). Such point sources can provide natural laboratories to investigate the effects of pollutants and geology on vegetation (Bell & Treshow 2002). Methods used in our study included multi-element, isotopic and microscopical analysis of native lichens (*Hypogymnia physodes*), and transplants of this species on birch (*Betula*), with the transplants exposed at the *transplant* stations for 2 and 3 month periods. Previous publications from the Karabash study include general pollution aspects (Udachin *et al.* 2003), characterization of particulates on lichen transplant surfaces (Williamson *et al.* 2004a), biogeochemical signatures and source apportionment (Purvis *et al.* 2004, 2006; Williamson *et al.* 2008), and the application of Pb isotopes to determine the sources of Pb in lichen transplants (Spiro *et al.* 2004, 2012).

Study area

The smelter and former mining town of Karabash lies in the Chelyabinsk region of the South Urals of Russia. It sits within a NE–SW-trending flat-bottomed valley with altitudes ranging from 250 to 650 m. The climate is moderately continental with cold winters and warm summers (mean January and July temperatures are -16 and $+18$ °C, respectively). Precipitation in winter and summer is approximately 130 and 500 mm, respectively, with a snow depth in winter of about 40 cm. Prevailing winds are northwesterly (Stepanov *et al.* 1992). Phytogeographically, the region lies on the border between taiga and forest-steppe zones, consisting of a mosaic of mesic pine woods (*Pinus sylvestris* mixed with *Betula pendula*, *B. pubescens* and *Larix sibirica*) and secondary mesic birch woods that have replaced pine following logging or forest fires.

The Karabash smelter, which lies close to the centre of the town, mainly produces blister copper, around 41 700 tonnes in 2001 (Voskresensky 2002). During the period of the lichen transplant experiment, the smelter was operating blast furnaces and a converter, each with separate stacks. In the

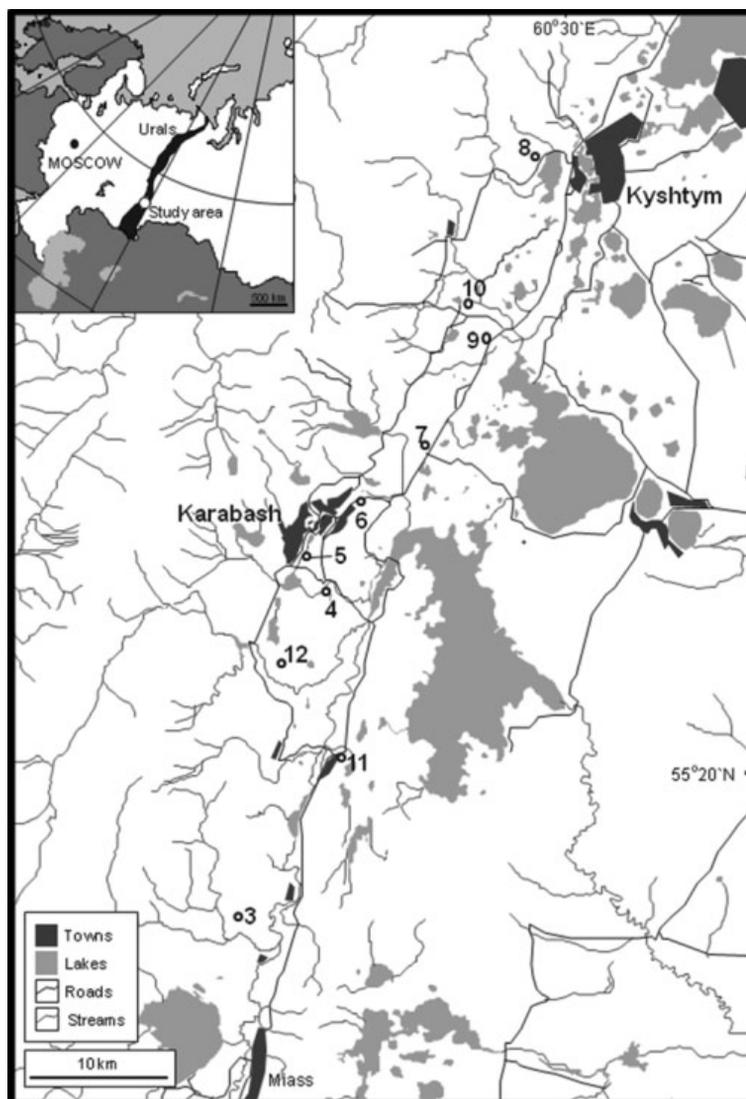


Fig. 2. Locality and drainage map of the Karabash and surrounding areas showing the location of lichen transplant stations.

blast (reverberatory) furnace copper concentrates are melted and silicate slag drawn off to produce a molten sulphide matte. In the converter, however, remaining elements such as iron and sulphur are removed to produce blister copper (*c.* 99% Cu; Williamson *et al.* 2008). From studies of airborne total suspended particulate (TSP) collected in Karabash town in 2001, using air pump apparatus (Williamson *et al.* 2004b), particulates downwind from the smelter was mainly composed of anglesite (PbSO_4), zincite (ZnO), gunningite ($(\text{Zn,Mn})\text{SO}_4 \cdot \text{H}_2\text{O}$), a phase with the composition Zn_2SnO_4 , and

poorly ordered Zn sulphates, with lesser amounts of pyrite, sphalerite, chalcopyrite and galena. This material had a maximum particle size (equivalent spherical diameter) of around $2 \mu\text{m}$ (average of $0.5 \mu\text{m}$, $\text{SD} = 0.2$). From their composition and size, these particles were thought to mostly originate from the smelter converter (Williamson *et al.* 2004b). More Fe–Cu-rich particles on lichen surfaces, with a mean equivalent spherical diameter of $2.2 \mu\text{m}$ ($\text{SD} = 2.4$) and not present in the air filters, are thought to have been mainly derived from the blast furnace (Williamson *et al.* 2004b).

Other possible sources of metal-rich airborne particulates in Karabash are waste dumps and tailings, which lie to the NE, south and SE of the town. Three tailings dumps cover a total area of around 2.1 km². In two of the tailings ponds, around 65% of the material has a grain size <0.074 mm. In the third, the 'Sak-Elga' tailing, around 11% is <0.074 mm. The mineralogy of the tailings is dominated by pyrite, chalcopyrite, sphalerite, tennantite and magnetite, with quartz, feldspar, sericite, chlorite and barite, and a variety of alteration phases. Metallurgical waste dumps cover an area of around 170 000 m² and contain granulated (<0.5 mm) Ca-, Fe-rich silicate glass (slags) and minor amounts of sulphides (Udachin *et al.* 1998). Dumps of pyrite-containing mine waste material cover an area of around 150 000 m². Other major sources of dusts include local roads (commonly gritted using metallurgical slags) and natural wind-blown soil particles from de-vegetated hillsides around the town (Williamson *et al.* 2004b), such as Karabash 'Mountain' (Fig. 3a).

Materials and methods

The lichen transplant methodology

Healthy *Hypogymnia physodes* thalli, about 3–4 cm in diameter, were cut with underlying bark from birch trunks sampled in June 2001 in a birch (mixed *Betula pendula*/*Betula pubescens*) stand from Site 3 ('reference site'), approximately 25 km from Karabash copper smelter (Fig. 2). During sampling, the general guidance in Bargagli & Nimis (2002) was followed, such that: (i) each lichen thallus should have the same probability of being selected from a total population of the same species; and (ii) collected samples suffered no alteration in chemical composition from the original population. Lichens were always removed from the tree bark using a stainless steel knife and plastic gloves to avoid contamination. The transplant stations were established in birch stands along an approximately 60 km transect, with Karabash in the centre (Fig. 2). The predominant wind direction is from the NW; however, due to practical considerations, it was necessary to sample across a NE–SW transect, coincident with the main road in the area. Six birch trees were selected at each transplant site and 10 thalli attached to each tree by carefully gluing (with the all purpose glue 'Moment') their bark substrate (to avoid contaminating the lichen) to within 1 m of the tree base. The transplants were placed in two rows of five thalli facing towards the smelter (see example in Fig. 3b). The transplants were removed after 2 and 3 month exposure periods (September and October 2001). The material was transported to the laboratory in

paper bags, allowed to air-dry, and then sealed in plastic bags before hand-cleaning under a microscope to remove bark flakes and other debris prior to elemental and isotopic analysis (Purvis *et al.* 2004).

Imaging and elemental analysis of particulates on transplant surfaces

Material collected at sites 5 (Karabash) and 3 (transplant/reference site at Severnye Peche) in September 2001 was subsampled for examination. Four thalli collected from different trees were selected from each site and tips of upwards-directed lobes 2–3 mm in length were taken (one lobe from each thallus). Samples were mounted on their lower surfaces onto aluminium 12.5 mm-diameter SEM stubs (Agar, UK), using a thin layer of Araldite, and then each stub was carbon coated. The stubs were loaded into a JEOL 5900LV SEM. The surfaces were imaged in backscattered electron mode in order to distinguish non-organic material from the sometimes complex lichen surface and accumulated organic airborne debris (Fig. 4; Purvis *et al.* 2002). An accelerating voltage of 20 kV and a beam current of approximately 1.4 nA produced a reasonable level of contrast between the thalli (organic) and inorganic surface materials, and a high enough resolution to image particles of <1 µm. Images (×1000 magnification) were obtained for three randomly chosen areas on thalli from each of the four lichen stations from sites 5 (Karabash) and 3 (reference site) (i.e. 12 imaged areas from each site). Elemental analysis was undertaken using an Oxford Instruments INCA energy-dispersive X-ray analysis system. The elemental data obtained are far from quantitative as, amongst other factors, the sample surface is not flat and the particles are irregularly shaped. The data can therefore only be used to infer the mineralogy of individual particles from inter-element relationships (Williamson *et al.* 2004a).

Whole-lichen multi-element analysis

For all transplant stations (except where replicate samples were taken), five thalli from each tree were bulked for chemical analysis for each collection period; that is, 30 thalli per site per sampling period. To determine natural variation at each station, samples of five thalli were bulked (September collection) from each tree from the 'reference' site (Site 3), an 'intermediate' site (Site 12) and the 'impacted' site (Site 5). Native thalli were bulked from each of sites 1, 2, 3 and 8 (July 2001), and both transplants and native thalli from sites 3, 8, 9, 10 and 11 (September and October

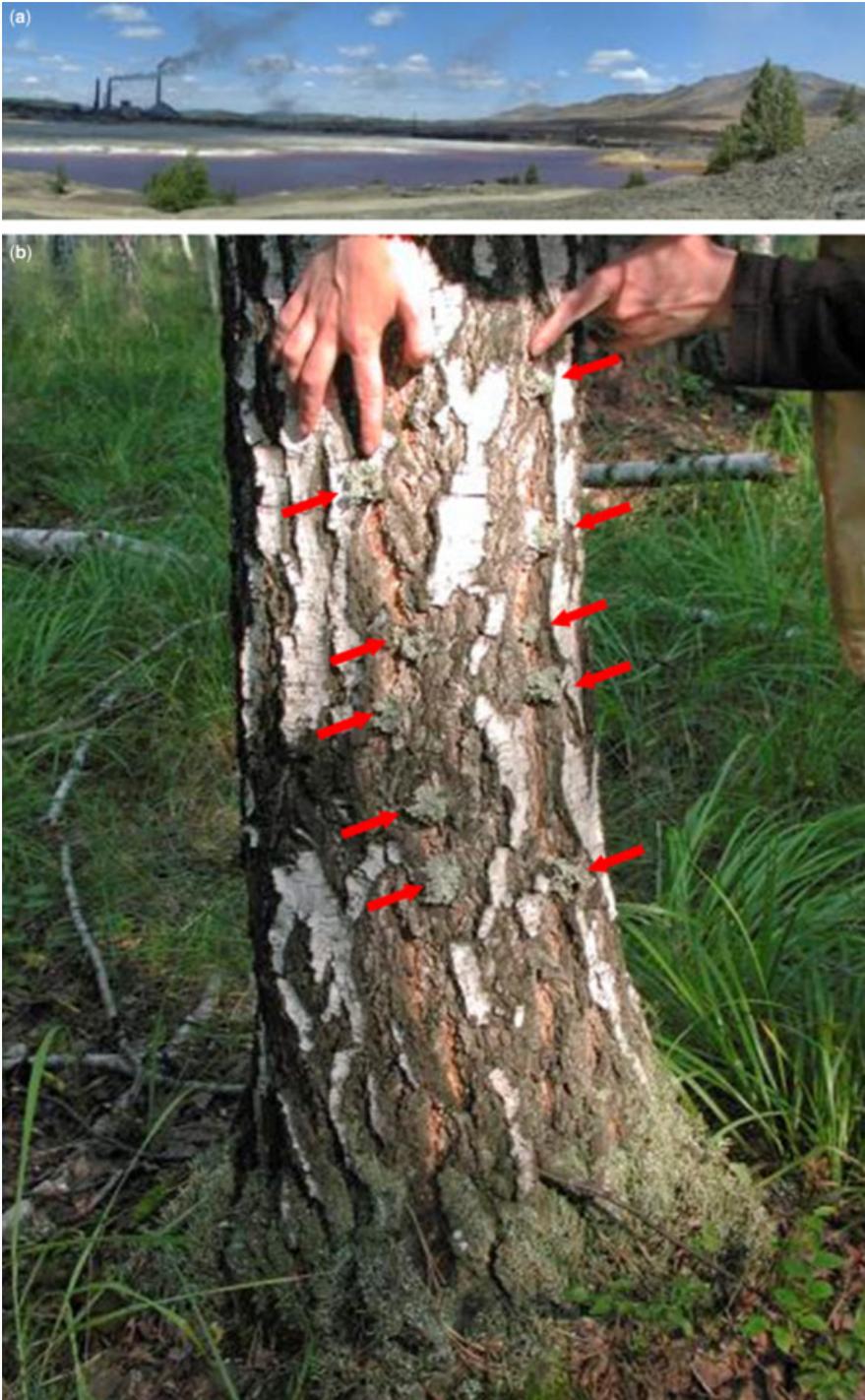


Fig. 3. (a) Photograph looking north towards the Karabash smelter. Note the eroded and de-vegetated slopes of Karabash 'Mountain' to the east; and (b) *Betula* trunk at Site 12 with 10 *Hypogymnia physodes* transplants adhered to the bark with glue. The trunk is colonized by *Cladonia coniocraea* at the base. The understorey was dominated by vascular plants characteristic of acidic habitats (*Vaccinium myrtillus* and *Deschampsia caespitosa*).

LICHENS AND ENVIRONMENTAL FORENSICS

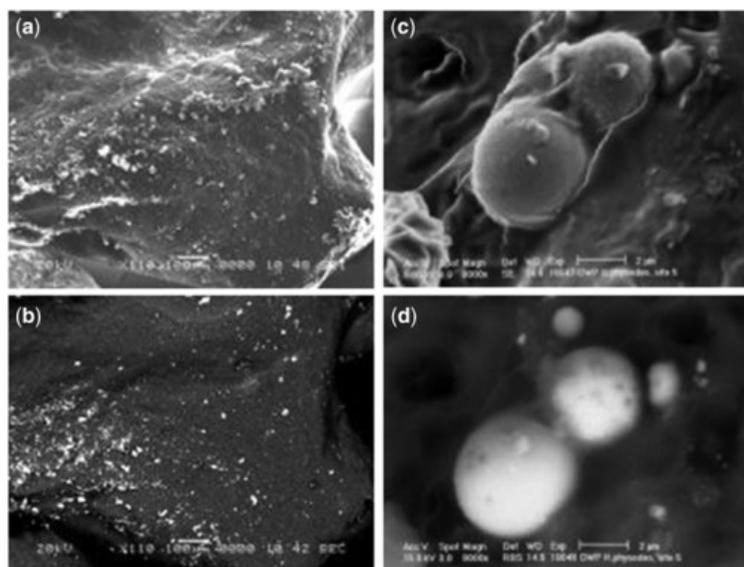


Fig. 4. SEM images of surface of *Hypogymnia physodes* thalli transplanted near the Karabash smelter: (a) and (b) general view from above; and (c) and (d) showing particulates (PM10s), becoming incorporated into the melanized lower surface. (a) and (c) are secondary electron images; and (b) and (d) backscattered (bright areas indicating high atomic number) electron images.

2001). Around 250 mg of *Hypogymnia* thalli were digested in $\text{HNO}_3\text{-H}_2\text{O}_2$ in open vessels under reflux according to method B (Bettinelli *et al.* 1996). Sample solutions were filtered, and the elements Al, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sn, Sr, Ti, V and Zn determined by ICP-OES, and As, Bi, Co, Cs, Ga, Ge, Li, Mo, Rb, Sb, Se, Te, Th, Tl and U by mass spectrometry (ICP-MS). Accuracy was assessed by analysing the lichen reference material CRM482 (Rusu 2002) and was found to be within 10%.

Pb elemental and isotopic determinations

Sample preparation followed the methods of Rusu (2002) and Dolgoplova *et al.* (2004). Samples of lichens were digested using a high-pressure and high-temperature microwave MarsX system with a $\text{H}_2\text{O}_2\text{-HF-HNO}_3$ acid mixture. The Pb was separated from the sample matrix using standard anion-exchange column chemistry with an EiChrom Sr-resin and varying HCl acid strengths. Samples were then measured using a Micromass IsoProbe multi-collector ICP-MS. The mass bias was corrected using thallium (Tl) as external dopant. The long-term 2σ reproducibility of the Pb isotope ratio measurements is below $300 \mu\text{g/g}$ for all measured isotopes ($^{206}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$, $^{208}\text{Pb}/^{204}\text{Pb}$) and the accuracy after daily adjustment of the Tl ratio was more than $100 \mu\text{g/g}$ for

all ratios (Spiro *et al.* 2004). Full details of the procedures for mass spectrometry (e.g. regarding mass bias correction, blank contribution and error propagation) are given elsewhere (Kylander *et al.* 2004; Weiss *et al.* 2004).

Statistical analyses and modelling

Multivariate analysis was carried out using PRIMER 5, and statistical tools within MS Excel and spatial relationships of element concentrations explored. Enrichment factors (EFs) were calculated for elements in relation to average element concentrations in the Earth's upper continental crust (UCC) (Taylor & McLennan 1985) according to the formula: $\text{EF}_{\text{UCC}} = [\text{Element}/\text{Ti}]_{\text{sample}} / [\text{Element}/\text{Ti}]_{\text{UCC}}$. Titanium was selected as the reference element because Sc, frequently used as an indicator of soil contamination and to calculate enrichment factors, was below detection limits of ICP-MS ($<0.4 \text{ mg/kg}$) in all samples (Purvis *et al.* 2006).

To determine which process in the smelter was emitting the highest relative levels of potentially toxic metals (either the blast furnaces or converter), and whether particulates from each showed a different spatial dispersion pattern, a multiple linear least-squares regression mixing model was used (Le Maitre 1979; Williamson *et al.* 2008). The objective of this was to provide data to aid in any future prioritization of measures to reduce

emissions. The assessment involved modelling ('fitting') the elemental compositions (Al, As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sn, Ti and Zn) of lichen transplants from different distances from the smelter by mixing the pattern of the 'reference' transplant lichens with varying proportions of the element compositions of the blast furnace and converter dusts. The model yielded the proportions of: (a) lichen 'reference' composition; (b) smelter blast furnace; and (c) smelter converter compositions to best fit the pattern for the lichen transplants. This type of analysis was not found to be appropriate for determining the relative contributions from minor sources (e.g. waste dumps and tailings). Owing to natural variability between replicate samples from sites 3, 5 and 12, weak source signatures will have been lost amongst the 'noise' (Williamson *et al.* 2008).

Results and discussion

Whole-lichen multi-element analysis

The distribution of 25 elements along the transect showed a characteristic curvilinear decrease with distance from the Karabash smelter suggesting influence from the point source (Purvis *et al.* 2004). In the case of Pb (Fig. 5), over the 3 month exposure period transplants showed more than a five times increase in concentration near the smelter (Site 5, 1200 $\mu\text{g/g}$ Pb) compared with those from the 'reference' site 3 (average of 213 $\mu\text{g/g}$ Pb, $n = 6$) approximately 25 km to the

SW. The average concentration of 15 elements with the highest enrichment factors (see the subsection on 'Statistical analyses and modelling') in replicate transplants from sites 5 and 3 were compared. The most enriched elements (in order of decreasing enrichment) were Te, Cd, Cu, Pb, Se, Zn and Sb. All elements showed curvilinear decreases from Karabash, even those at concentrations below 1 $\mu\text{g/g}$.

Interestingly, 14 of the top 15 elements (Te, Cd, Cu, Pb, Se, Zn, Sb, Sn, As, Bi, S, P, Mo and Ca) with the highest enrichment factors in replicate transplants established at Karabash site 5 were also the most enriched in *Hypogymnia* transplants from along a transect centred on the Cu smelter in Zlatna town, Romania (Rusu *et al.* 2006a). This confirms the association of these particular elements with Cu ore smelting. In contrast, little or no enrichment was observed for Na, Th, Ti, Al, Li, U, Cs and V (Purvis *et al.* 2006). By analogy with moss biomonitoring studies carried out elsewhere (Faus-Kessler *et al.* 2001), the data strongly suggested that the elements U, Th, Cs, Na, Ti and Al were at least partly derived from fossil fuel combustion (Purvis *et al.* 2006). Negative correlations between concentrations of K with S and potentially toxic metals (e.g. Cu, Pb, As) was interpreted to reflect element leakage from lichen tissues as a result of stress (Purvis *et al.* 2004; Williamson *et al.* 2004a).

Element variability amongst *Hypogymnia* transplants from the same site was highlighted through analysing replicate samples from sites 3, 12 and

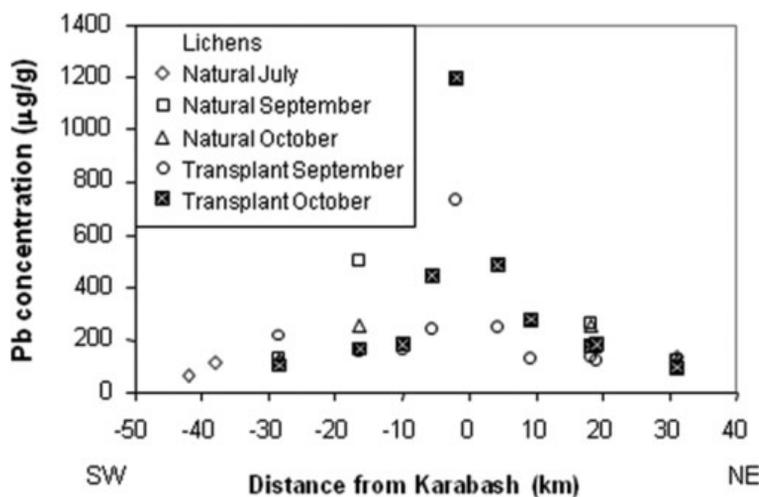


Fig. 5. Concentration of Pb ($\mu\text{g/g}$) in *Hypogymnia* collected from the SW to NE 60 km-long transect centred at Karabash. Native (natural) lichen were collected in July, September and October in 2001. Transplants were deployed for 2 months (September) and for 3 months (October). The transplants deployed for 3 months and the native samples from reference site 3 collected in July were used for Pb isotope analysis.

5. At the 'reference' site 3, Pb content ranged from 68 to 760 $\mu\text{g/g}$; that is, a more than 11 times variation in Pb content amongst the five samples analysed. Nevertheless, the similar trends in enrichment factors at sites 3 and 5 indicate the robustness of the averaging approach.

Imaging and analysis of particles on lichen transplant surfaces

Particulates in the Karabash samples were mainly in the form of 5–200 μm sized, commonly spherical sulphide and silicate metallurgical slag particles. Approximately 20–30% of particulates consisted of aggregates of materials of variable composition, occasionally up to 40 μm in diameter, which appeared to represent surface deposits formed *in situ* on the lichen surface. This may be either through the breakdown of primary particulate material or precipitation from solution. The Karabash samples contained seven times the number of chalcopyrite particles, over nine times the proportion of the Zn–Sn-rich phase, and around half and two-thirds the number of quartz and albite particulates, respectively, compared with the 'reference site'. The discovery of particles of the Zn–Sn-rich phase (thought to be exclusively of smelter origin) in the 'reference' sample (25 km south of Karabash), together with chalcopyrite, indicates wide spatial dispersion towards the SW of particles from the Karabash smelter.

Particulates on the surfaces of the transplants from Karabash were found to be relatively coarse grained and to have compositions similar to dusts from the blast furnace. This observation is at odds with the size ($<2 \mu\text{m}$) and composition (Pb- and Zn-rich) of particles collected on air filters in the town, which are thought to have been derived from the converter process (Williamson *et al.* 2004b). The most likely explanation for this is that the relatively small particles from the converter have a longer atmospheric residence time compared with the generally larger Cu–Fe-rich particles from the blast furnace. Spatial fractionation of airborne particulates according to particle size is considered a major factor affecting elemental inventories in lichens (Zschau *et al.* 2003). This hypothesis was tested by carrying out source apportionment modelling for metals in lichen transplants from different distances from the smelter (see the subsection on 'The modelling approach').

Interestingly, thallus surfaces from the Karabash and 'reference' sites showed similar particle numbers per unit area (Fig. 6). This was interpreted as indicating that over the 2 month exposure period, the 'input' of particles to the Karabash site samples was apparently balanced by removal of 'background' particles, possibly being washed or blown off, or some types of materials being solubilized (Williamson *et al.* 2004a). A sketch diagram outlining the main mechanisms by which metals may be deposited on the surfaces of *Hypogymnia physodes* is presented in Figure 1.

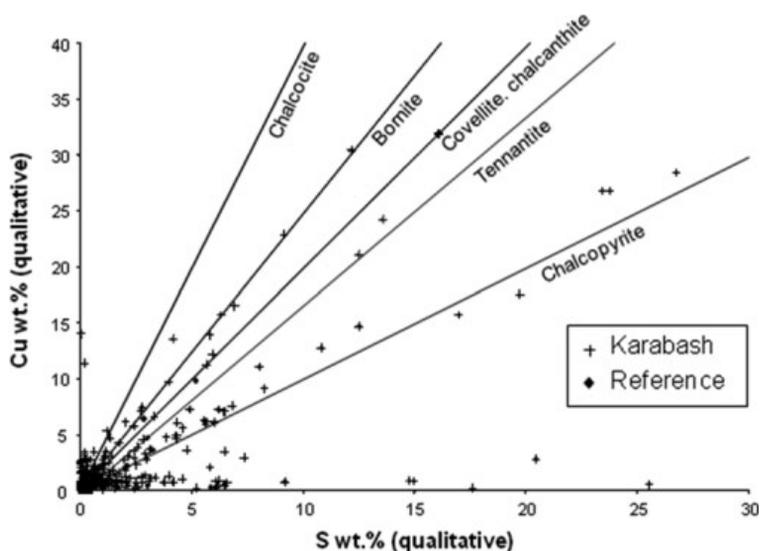


Fig. 6. Diagram of S v. Cu wt% (qualitative) for particulates on transplant thallus surfaces from the Karabash and the 'reference' site. Lines are shown for the stoichiometric ratios of different common Cu-bearing sulphides (modified after Williamson *et al.* 2004a).

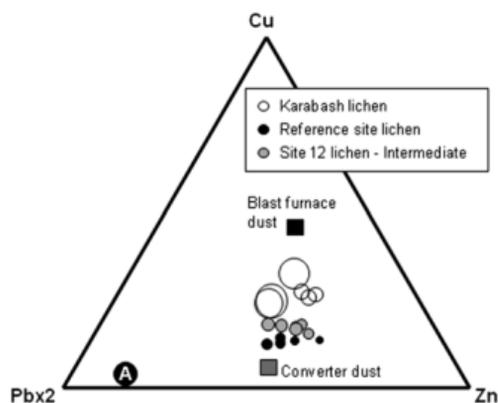


Fig. 7. Ternary diagram for Cu v. Pb \times 2 v. Zn for lichen transplants, TSP filter collections and samples from the blast furnace and converter stacks. The sizes of symbols for lichen transplants and TSP filter collections are proportional to Pb content (relative to other samples from each media type). Point A is an anomalous value for lichen transplants from the 'reference' site (adapted from Williamson *et al.* 2008).

The modelling approach

On the triangular diagram showing relative concentrations of Cu, Zn and Pb in the lichen samples from wet chemical analysis (Fig. 7), the replicate transplants from the Karabash site lie further towards the Cu apex compared with the 'reference' site samples, with the intermediate site transplants in between. Samples from the Karabash site therefore

have a relatively high ratio of Cu to Zn and Pb (Williamson *et al.* 2008). There is a trend in the compositions of the lichen transplants from the 'reference' site to the Karabash samples (Site 5) towards the composition of the blast furnace dust. This was interpreted as indicating that the dominant metal from the smelter is Cu, and that the dominant source of this metal is the smelter blast furnace. Other potential sources (tailings, metallurgical slags, road dusts and soils) were also plotted on this diagram (Williamson *et al.* 2008) but owing to a lack of detailed trends within the replicate datasets, and because some of the compositions for minor sources overlap with those of the smelter dusts, no non-smelter source signatures could be identified with any confidence.

The results of the least-squares modelling are graphically presented in Figure 8, which shows the modelled percentage of blast furnace and converter signature for lichen transplants collected at different distances from the smelter. From this figure, it is clear that close to the smelter the signature of the blast furnace is dominant over that of the converter in the transplants. Further from the smelter, the converter signature dominates (Williamson *et al.* 2008). The same pattern was observed in the September and October sample datasets.

Sources from Pb isotopes data

The blast furnace and converter dusts (smelter dust in Fig. 9) from the Karabash smelter show a $^{208}\text{Pb}/^{204}\text{Pb}$ isotope ratio characteristic of Ural-type

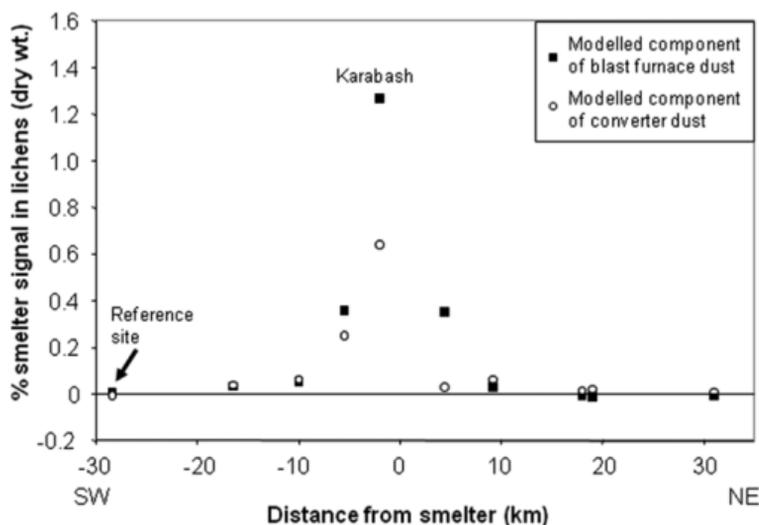


Fig. 8. Results of multi-element (Al, As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sn, Ti and Zn) least-squares modelling for October collections (adapted from Williamson *et al.* 2008).

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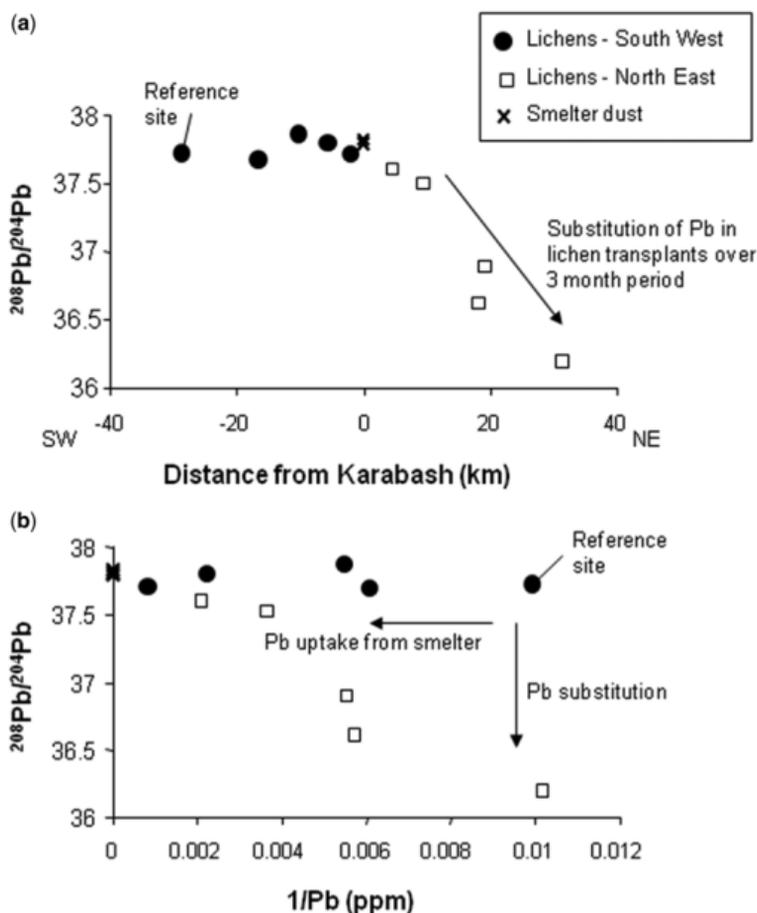


Fig. 9. (a) Pb isotope composition of *Hypogymnia* transplants exposed for 3 months (2001). Each lichen sample is bulked from five individual thalli from each of six trees; $^{208}\text{Pb}/^{204}\text{Pb}$ isotope ratios are relatively constant in the SW and change NE of the smelter (Spiro *et al.* 2004). (b) Plot of $^{208}\text{Pb}/^{204}\text{Pb}$ v. $1/\text{Pb}$ in lichen transplants and smelter dust showing that the isotopic composition in lichen transplants are independent of the Pb concentration in samples collected from the SW part of the transect (black circles) but not in the NE part (open quadrangles). This suggests the presence of an additional dispersed source to the NE, most probably soil.

volcanogenic massive sulphide deposits (Tessalina *et al.* 2001). To the SW of the transect, isotope ratios in transplanted and native lichens show this same signature, indicating that the smelter was the main source of Pb in these samples. However, to the NE, the Pb isotope ratios of the lichens changes markedly indicating a contribution of Pb from alternative sources. This is most clearly discernible on the plot of $^{208}\text{Pb}/^{204}\text{Pb}$ v. $1/\text{Pb}$, which presents two arrays (Fig. 9). The array of transplants to the NE shows a decreasing isotope ratio with decreasing $1/\text{Pb}$, whereby at low Pb concentrations the $^{208}\text{Pb}/^{204}\text{Pb}$ ratio decreases to the value of 36.199, and at high Pb concentrations it reaches that of the smelter dust samples (37.821). This

suggests a mixing of two end members; one end member being the smelter dust, while the other has a low concentration of Pb that may be associated with soil. This indicates that the Pb content from the background site was largely replaced, and that the transplants therefore provide a transient record reflecting a continuous fixation and loss of environmental Pb (Spiro *et al.* 2004). By using tri isotope plots $^{208}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{206}\text{Pb}/^{204}\text{Pb}$, distinct sources of lead in the native and transplanted lichen were identified (for details see Spiro *et al.* 2004). A study of the Orlovka–Spokoinoe mining site, Transbaikalia, Russia identified atmospheric transfer of lead from distant sources to the lichen, in addition to

local components mobilized by mining and ore-processing activities (Dolgoplova *et al.* 2006a, b).

Conclusions

This study of transplants and native thalli of the lichen *Hypogymnia physodes* as a potential forensic tool focused on the area in and around the Cu smelter and former mining town of Karabash, South Urals of Russia. The main source of heavy metals was known (the smelter), and therefore the study focused on the indicative, largely spatial, characteristics of atmospheric dispersion, the relative contribution of metals in the lichens (with distance) from each of the main processes in the smelter (blast furnace or converter) and what other sources contributed to the environmental load of heavy metals in the area. A smelter signal, and relative contributions from the blast furnace and converter, could be identified for up to 25 km from Karabash town. The most prevalent additional source signature was from the burning of coal (Spiro *et al.* 2004).

In conclusion, *Hypogymnia* transplants are dynamic systems that trap and lose particles deposited on their surfaces. They are therefore useful as monitors of deposition for limited time periods when transplanted to environments where they would not naturally grow. The present study suggests that a 2–3 month period is useful for the heavily polluted area in the vicinity of the Karabash smelter. However, other periods and other species may be useful for the biomonitoring and sampling of atmospheric deposition in other settings. This case study demonstrates the efficacy of the approach in general, and that it could be used for different compounds and with different lichens. Provided there is sufficient background research, and rigorous sampling and analytical protocols are followed, we suggest that the information derived from lichen studies could be used in a forensic situation and challenged in court.

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