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Lichen transplants as a suitable tool to identify mercury pollution from waste incinerators: a case study from NE Italy

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Abstract A lichen transplant study aimed at investigating a strong increase in mercury concentrations in lichens was run in a territory of NE Italy where background values were very low only 8 years before. Thalli of the lichen *Pseudevernia furfuracea* collected in a pristine area were exposed for 1.5, 3 and 6 months at 31 sites selected according to the observed pattern of Hg concentrations, location of the suspected source (a new waste incinerator) and prevailing wind direction. Hg strongly increased at eight sites after 1.5 months, at 12 after 3 months and at 20 after 6 months. The highest values were always located SW and S of the incinerator, in good agreement with the prevailing night wind direction. It was concluded that, although the immediate risk for the population living close to the incinerator is low, long-term hazard due to Hg accumulation in the surrounding environment should be seriously taken into account.

Keywords Active biomonitoring · Elemental composition · Incinerator · *Pseudevernia furfuracea* · *Robinia pseudacacia*

Introduction

Threshold limits are at the core of the current environmental policy of EU countries but are often far from sufficient to safeguard clean production processes and environmentally benign products. One of the reasons the legal requirements are lacking is that damages to the environment are often difficult to quantify because there is a time lag between emission and effects (Hylander and Goodsite 2006). Furthermore, the impact of an emitting source actually depends on the total emission load and the fate of the emitted substances. Thus, plants emitting large

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volumes of exhausts with low pollutant concentrations may affect the environment even more than those emitting smaller quantities at higher concentrations. This is particularly true for molecules scarcely subjected to degradation such as dioxins, furans, polychlorinated biphenyls etc. and for potentially toxic elements, whose loads in the environment are often very low and need expensive analytical methods to be ascertained. Biomonitoring techniques offer several advantages to verify the effects of the release of persistent pollutants at low concentration on the territory. The analysis of natural matrices with high affinity to a specific category of persistent pollutants in fact can give precious information on distribution and destiny over time (Bargagli 1998). Lichens, in particular, are excellent biomonitors because gas exchange occurs through the whole body surface, and it is not reduced by stomatal resistance, as in plant leaves (Loppi 2006). The basic assumption for quantitative biomonitoring with lichens is that, inside thalli, trace element concentrations reach an equilibrium state with the levels in the atmosphere (Sloof 1995), with concentrations reflecting environmental levels of the elements (Herzig et al. 1989).

Mercury is a highly mobile environmental pollutant that is toxic both to humans and wildlife at low levels (WHO 1990, 1991). Because of its high mobility, small environmental releases of Hg can result in significant exposure, hence the importance of monitoring Hg sources in the environment and the fate of the emitted quantities (Carpi 1997). The classic physicochemical approaches used for the latter purpose (e.g. Munthe et al. 2001) are demanding and expensive, whereas the use of lichens as biomonitors of Hg pollution offers several interesting advantages because these organisms are excellent Hg bioaccumulators (Bargagli and Barghigiani 1991), predominantly at photobiont level (Rinino et al. 2005).

In this paper, we discuss the use of lichen transplants to identify a Hg-emitting source in an area of NE Italy where Hg in autochthonous lichens had increased from 0.071 ± 0.046 μg g^{-1} in 1999 (Tretiach and Baruffo 2001; $n = 36$) to 0.173 ± 0.079 μg g^{-1} in 2007 (Tretiach and Pittao 2008; $n = 41$), with maxima ca. two times higher. The purported source might be a new waste incinerator (WI), which is operating, although intermittently since 2000 because a statistically significant linear correlation was observed between the Hg concentration values measured in lichens and the distance of the collection sites from the WI (Tretiach and Pittao 2008). The local authorities for environmental policy and protection, however, considered this hypothesis as insufficiently robust because (a) the data were circumstantial, (b) the current incinerator emissions comply with threshold limits and (c) the contamination might have been caused by a stochastic event, not necessarily connected to the incinerator activity. For these reasons, we decided to continue our investigation with two main aims: (a) to verify whether there is still an ongoing release of Hg and (b) to test the incinerator as the main source of atmospheric Hg pollution in the nearby area.

The active biomonitoring technique of lichen transplants was selected as it offers several advantages (Bargagli 1998): (a) the material can be exposed according to the best rational scheme, (b) it is possible to calculate enrichment rates since pre-exposure values are known and (c) the monitoring can be repeated at request, allowing repeated sampling to demonstrate reduced emissions due to improvements at the source or—in absence of any change—the worsening of the situation over time.

Materials and methods

Study area

The study area is located in the northern Friulian plain (NE Italy), extends over 112 km^2 and lies at 110–130 m a.s.l. Red argillaceous and brown soils cover Quaternary deposit beds of carbonate gravels and sand banks, ca. 400 m deep (Cucchi et al. 2008). The climate is relatively rainy (1,500 mm/year) and temperate, with cold winters and warm summers, mitigated by frequent thunderstorms. Northern winds prevail in the western portion, whereas southwestern winds prevail in the eastern portion.

The natural vegetation (16% of the total surface) is mainly restricted to river banks. Agricultural areas (71%) prevail over urban (9%)

and industrial (2%) areas. Farming consists of vineyards, highly specialised grape nurseries, stable meadows and corn and soy bean fields. Urban areas, except for Spilimbergo (8,000 inhabitants), are small in size and host all together ca. other 8,000 inhabitants. Small manufacturers are concentrated in two industrial zones, or are scattered in the territory, intermingled with the urban fabric. The largest industry is a limekiln active since 1995 (www.unicalce.it), which is using a methane driven kiln of 200 ton day^{-1} and in 2008 produced 43,839 and 3,574 ton year^{-1} of burnt and slave lime, respectively. The WI Mistral FVG (www.mistral-fvg.it) is located SW of Spilimbergo, in an industrial area which also hosts mechanical laboratories, metal structural works and wood carpentries. The WI was built in 1978, closed in 1985 and rebuilt in 2000. It destroys ca. 20,000 tons year^{-1} (2004–2008), at 980–1,300°C, having fabric filters as pollution control equipment; flue gas flow is 10.25 Nm^3 s^{-1} at 192°C from a 23-m-high stack. Limit thresholds (for Hg 0.05 mg Nm^{-3}, see D.M. 25 February 2000, n. 124, www.camera.it/parlam/leggi/deleghe/testi/00124DM.htm), certified three times per year by a private company, have always been respected and imply an hypothetical maximum legal release of more than 16 kg Hg year^{-1} (0.05 mg Hg Nm^{-3} multiplied by the estimated potential emission volume per year, 323.3 × 10^6 Nm^3).

Exposure design

The change in element composition of lichen transplants was assessed at 31 sites, subdivided in agricultural (20), urban (five) and industrial (six). The potential agricultural sites were distributed at 1 km from each other along three transects (A–C in Fig. 1c), traced according to (a) the 2008 pattern of Hg contamination in the in situ epiphytic lichen *Xanthoria parietina* (Fig. 1b), (b) the localization of the waste incinerator and the limekiln and (c) the wind frequency distribution as recorded in three nearby meteorological stations (Arba, Vivaro, Azienda Rinascita). Aerial 2003 ortophotos and field surveys identified the best available deciduous trees for potential exposure. The trees had to be (a) as close as possible to the potential site, (b) at more than 50 m from roads with heavy traffic and (c) isolated or facing a field. When more trees were available, the selection was made by sorting. Sampling sites at urban and industrial areas were selected randomly.

Lichen material

For this study, the epiphytic lichen *Pseudevernia furfuracea* (L.) Zopf var. *furfuracea* was selected on account of its good availability, increasing use in biomonitoring studies (Basile et al. 2008; Bergamaschi et al. 2007; Pirintsos et al. 2006), good resistance to SO_2 (Miszalski and Niewiadomska 1993), NO_x (Marti 1983) and potentially toxic elements (Tretiach et al. 2007) and recent use in some experimental works dealing with heavy metal sequestration (Ates et al. 2007).

On May 11, 2008, ca. 600 short pieces of larch (*Larix decidua* Mill.) twigs bearing *P. furfuracea* thalli were collected at 1,500 m a.s.l. in an area unaffected by geochemical anomalies or local anthropogenic sources of trace metals (Lateis, Carnic Alps, NE Italy). In the laboratory, the twigs were tied up together two by two with plastic bands. A nylon thread was fixed to each pair to suspend it. These multiple thalli were treated as in Tretiach et al. (2007). Twelve of them were randomly selected for pre-exposure element analysis; the others were hung in groups of three on 40-cm-long plastic hoses, which were singularly closed in plastic envelopes to avoid contamination. In the meantime, two stainless steel rods (1 m long) were mounted on two external branches of the selected trees at 4 ± 0.5 m above ground. On May 27, 2008, the plastic tubes were inserted on their respective rod. After 1.5, 3 and 6 months, one multiple thallus from each rod was retrieved. The material from each site was closed in a plastic envelope and transported to the laboratory, where ca. 300 mg of the most external lobe portions were cut with porcelain scissors and powdered in an agate mortar with liquid nitrogen. Each sample was dried out for 24 h over silica and preserved in closed Eppendorf containers before analysis. During the exposure, two multiple thalli were lost. In one case, the whole series of multiple thalli was lost before the first samples were collected. They were not reinstated.

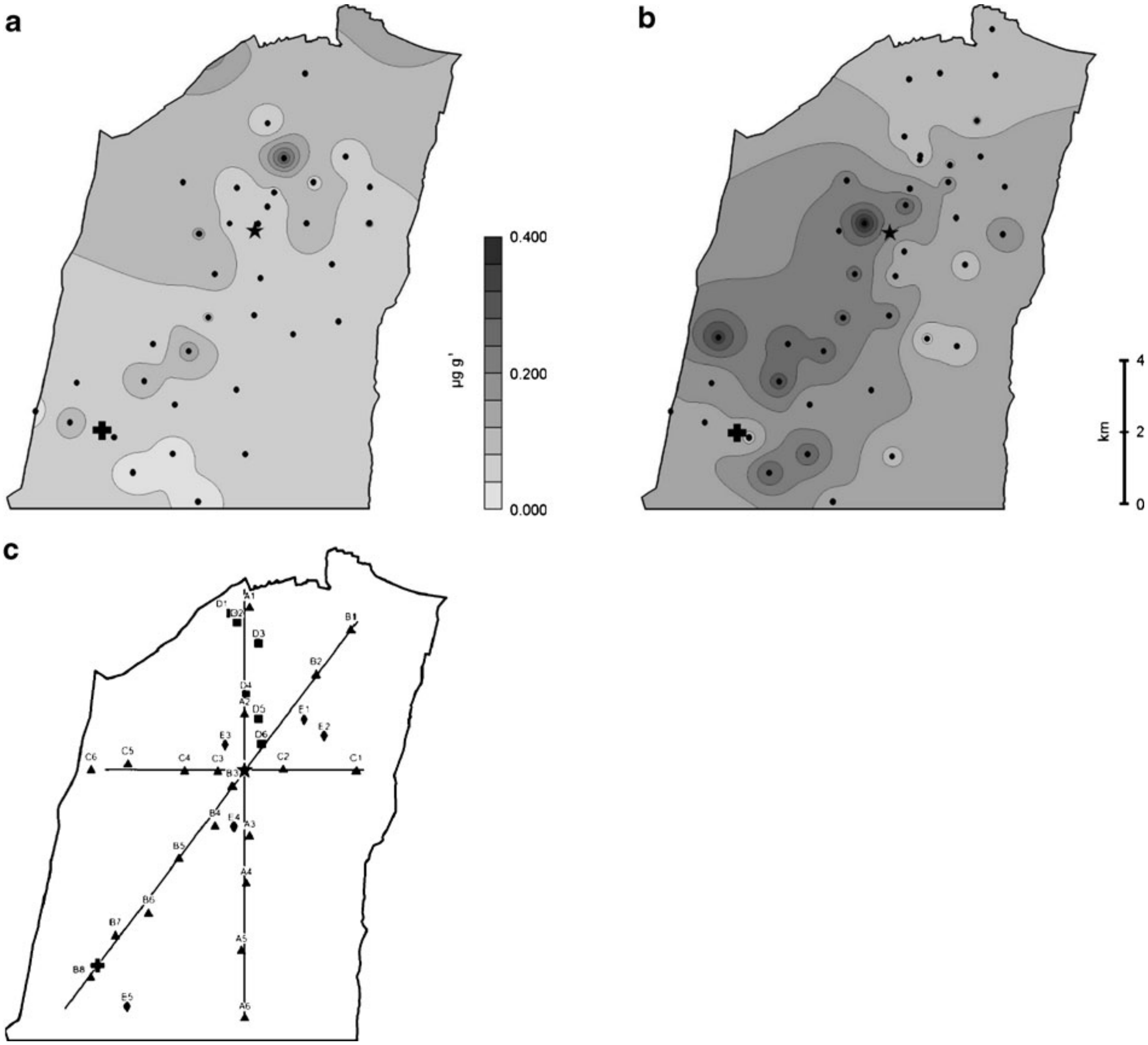

Fig. 1 Pattern distribution of Hg (micrograms per gram dry wt.) in autochthonous thalli of the epiphytic lichen *X. parietina* collected in 1999 (**a**; redrawn from Tretiach and Baruffo 2001; mean value 0.071 ± 0.046 μg g^{-1}, $n = 36$) and 2007 (**b**; redrawn from Tretiach and Pittao 2008; mean value: 0.173 ± 0.079 μg g^{-1}, $n = 41$), with localization of the waste incinerator Mistral FVG (*star*) and the limekiln Unicalce (*cross*); **c** localization of exposure sites of *P. furfuracea* transplants: A–C (*triangle*), agricultural; D (*square*), industrial; E (*diamond*), urban (e); the three segments identify the A–C transects

Plant material

For comparison, 2 weeks before the end of the last exposure, leaves of black locust (*Robinia pseudoacacia* L.) were collected in all the exposure sites of the B transect, from twigs immediately adjacent the lichen transplants. Leaves of ivy (*Hedera helix* L. subsp. *helix*), cherry plum (*Prunus cerasifera* Ehrh. subsp. *cerasifera*) and Scotch elm (*Ulmus glabra* Huds.) were collected in B3, the site which experienced the highest increase in Hg after the first two exposure periods. The leaves were air-dried for 1 week at room temperature in a dark room and for 1 day (ivy only) in a box containing drying silica and then treated and analysed as for lichens.

Table 1 Concentration values (micrograms per gram dry wt.; mean ± standard deviation) of trace elements in pre- and post-exposure transplants of the lichen *P. furfuracea* in the sites of the agricultural stratum

	Pre-exposure ($n = 12$)			Post-exposure								
				1.5 months ($n = 19$)			3 months ($n = 19$)			6 months ($n = 16$)		
	Mean ± SD	% c.v.	Max	Mean ± SD	% c.v.	Max	Mean ± SD	% c.v.	Max	Mean ± SD	% c.v.	Max
Al	475 ± 109	23	680	560 ± 212	38	1,299	357 ± 68.0	19	519	$640 \pm 117^{aa,cc}$	18	921
As	0.25 ± 0.09	38	0.50	0.27 ± 0.04^{a}	16	0.37	$0.41 \pm 0.47^{a,b}$	116	2.34	$0.38 \pm 0.04^{aa,c}$	11	0.44
Ca	$9{,}927 \pm 3{,}034$	31	14,713	$16{,}863 \pm 6{,}414^{aa}$	38	30,417	$10{,}942 \pm 4{,}009$	37	18,527	$13{,}635 \pm 5{,}252^{c}$	38	26,984
Cd	0.19 ± 0.11	58	0.53	0.28 ± 0.32^{a}	117	1.60	$0.27 \pm 0.08^{aa,b}$	29	0.46	$0.35 \pm 0.04^{aa,c}$	11	0.43
Co	0.24 ± 0.05	22	0.33	0.29 ± 0.06	20	0.4	0.30 ± 0.07^{a}	24	0.53	$0.45 \pm 0.08^{aa,cc}$	18	0.65
Cr	1.29 ± 0.38	30	2.03	1.53 ± 0.44	29	2.63	1.78 ± 0.36^{a}	20	2.38	$2.73 \pm 0.35^{aa,cc}$	12	3.44
Cu	6.03 ± 1.29	21	8.60	13.8 ± 16.2^{aa}	117	79.1	$18.3 \pm 22.2^{aa,b}$	121	88.6	16.6 ± 15.6^{aa}	94	60.8
Fe	220 ± 63	28	350	288 ± 75^{a}	26	450	315 ± 59.6^{a}	19	451	$487 \pm 66.5^{aa,cc}$	13	629
Hg	0.18 ± 0.04	22	0.27	0.47 ± 0.95	202	4.37	$0.58 \pm 1.12^{aa,b}$	193	5.13	0.60 ± 1.18^{aa}	198	5.00
Mn	159 ± 52	33	250	177 ± 47.4	27	276	117 ± 49.6	42	237	143 ± 50.4	35	263
Ni	2.34 ± 3.07	131	12.07	1.68 ± 0.4	24	2.73	1.53 ± 0.21	14	1.9	1.58 ± 0.18	11	2.03
Pb	5.72 ± 2.49	44	10.53	8.46 ± 5.08^{a}	60	24.5	6.69 ± 1.71	26	9.39	$7.69 \pm 1.43^{a,c}$	18	11.5
Sb	0.10 ± 0.03	34	0.17	0.11 ± 0.03	30	0.17	$0.14 \pm 0.02^{aa,b}$	18	0.2	$0.17 \pm 0.03^{aa,c}$	15	0.22
Sn	0.29 ± 0.10	33	0.50	0.32 ± 0.08	24	0.5	$0.41 \pm 0.06^{a,b}$	15	0.57	$0.70 \pm 0.06^{aa,cc}$	9	0.82
V	1.35 ± 0.42	31	2.23	1.19 ± 0.33	27	1.9	1.48 ± 0.27^{b}	18	2.06	$1.82 \pm 0.38^{a,c}$	21	2.55
Zn	44.2 ± 12.9	29	68.0	50.1 ± 11.9	24	80.6	55.7 ± 12.1^{a}	22	86.4	$69.7 \pm 6.40^{aa,cc}$	9	85.9

aa,a: post-exposure vs. pre-exposure; bb,b: second vs. first exposure; cc,c: third vs. second exposure, for $p < 0.001$ and 0.05, respectively

c.v. coefficient of variation, Max maximum value

Chemical analyses

Element concentrations were determined in pre- and post-exposure samples. One hundred fifty milligrams of powdered and homogenized dried sample were digested with 6 ml HNO_3 65% (v/v), 0.2 ml HF 50% (v/v) and 1 ml H_2O_2 30% (v/v) using a closed Teflon vessel at high pressure in a microwave oven with stepwise power application. The mineralised and diluted solution was analysed by flow injection mercury system (Hg only) and inductively coupled plasma-mass spectrometry (all other elements, see Table 1). Element concentrations were determined by comparison with standard solutions and are expressed as micrograms per gram on a dry weight (dry wt.) basis. Procedural blanks were always below the detection limit. The accuracy of digestion and analytical procedures was checked by routine determination of element concentrations in standard reference material (BCR 482, *P. furfuracea*; Institute for Reference Materials and Measurements,

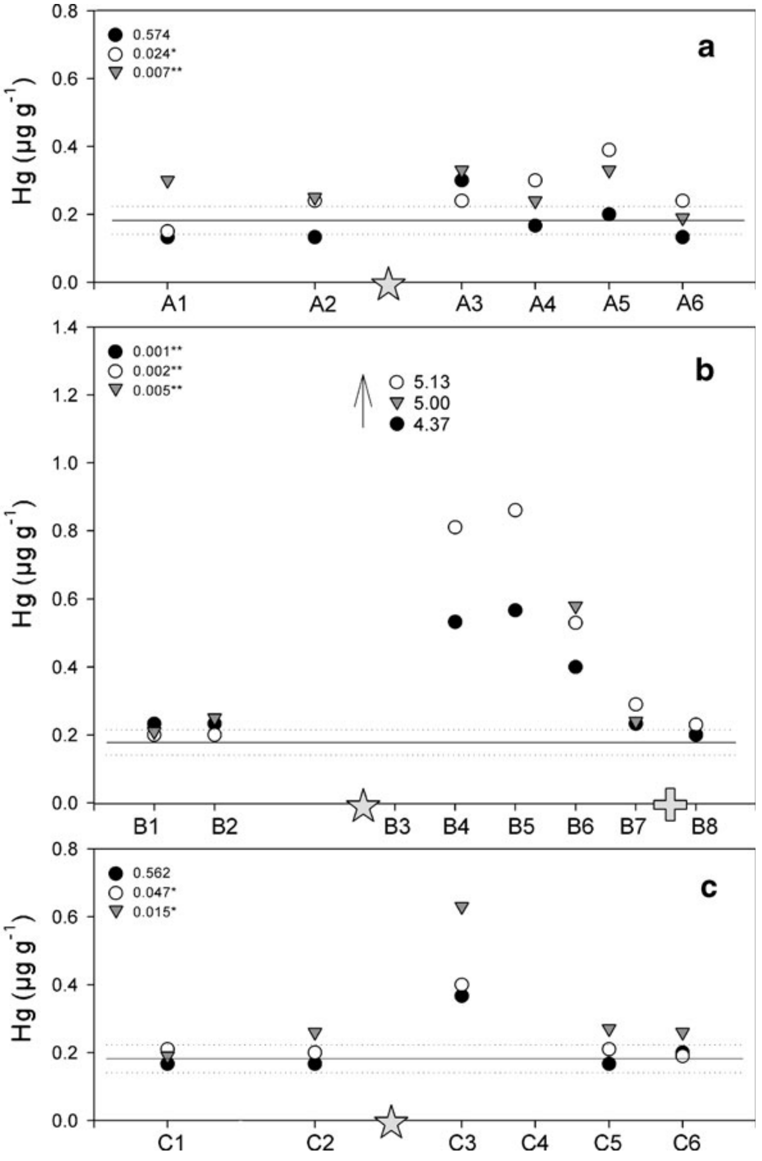

Fig. 2 Mercury concentration (micrograms per gram dry wt.) measured in *P. furfuracea* transplants exposed for 1.5 (*filled circle*), 3 (*empty circle*), and 6 (*filled inverted triangle*) months along the three transects A–C of Fig. 1c. The *star* and the *cross* on the *x*-axis identify the waste incinerator Mistral FVG and the limekiln Unicalce, respectively. *Solid and dotted lines*: mean ± standard deviation of Hg content measured in pre-exposure material ($n = 12$, see Table 1). p values of Mann–Whitney test of post vs. pre-exposure values are reported for each transect ($^{*}p < 5\%$; $^{**}p < 1\%$; $^{***}p < 0.1\%$)

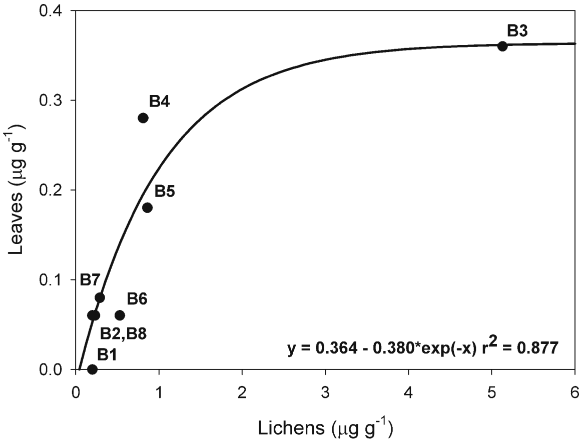

Fig. 3 Relationship between Hg content (micrograms per gram dry wt.) measured in *P. furfuracea* transplants of transect B of Fig. 1c exposed for 3 months (x-axis) and the leaves of black locust, *R. pseudacacia*, collected from the exposure trees in mid-November 2008 (y-axis)

Belgium). Recovery ranged between 93.7 ± 20% (Cd) and 104.3 ± 9.4% (Al); that of Hg was 98.9 ± 2.5%.

Statistics

STATISTICA and EXCEL were used to calculate mean and standard deviation and to carry out the Mann–Whitney *U* test and Wilcoxon signed-rank paired test to verify differences among groups. To identify the predictors which contributed to determine the increase in element content (absolute values, expressed in micrograms per gram), a stepwise logistic general linear model (GLM) analysis was performed for those elements which after 6 months showed severe accumulation, i.e. their post-exposure value was higher than 1.75 times the pre-exposure value (Frati et al. 2005). The predictors are direction of the exposure sites from the waste incinerator (ordinal), exposure (binary: pre—no, post—yes), exposure time (45, 90 or 180 days), distance of the sites along the transepts from the waste incinerator (ordinal) and their multiple combinations.

Results

Pre-exposure element concentrations in thalli of *P. furfuracea* (Table 1) were quite low, with the exception of Al (475 ± 109 µg g^{-1}) and Mn (159 ± 52 µg g^{-1}; for comparison, see Bargagli 1998). Mean Hg concentration was 0.018 ± 0.04 µg g^{-1}, with a median value of 0.017 µg g^{-1}, slightly higher in comparison with two previous samplings carried out in the same site in winter (Adamo et al. 2007; Pittao 2007) but identical to another sampling made in spring (Pittao 2007).

The exposures modified the content of most elements (Table 1). Al, which is often used as tracer of soil contamination of samples, was always statistically not differing from the control, ranging between 357 ± 68 µg g^{-1} (second exposure) and 640 ± 117 µg g^{-1} (third exposure). Six elements significantly increased after 1.5 months (As, Ca, Cd, Cu, Fe and Pb), nine after 3 months (As, Cd, Co, Cr, Cu, Fe, Hg, Sb, Sn and Zn) and 12 after 6 months (the previous ones plus Al and V). The increase was severe for seven elements only (Cd, Co, Cr, Cu, Fe, Hg and Sn). A GLM analysis concerning these elements revealed that direction from the suspected source, i.e. the WI plant, was statistically significant only for two of them, Cu and Hg, whereas distance from the WI was statistically significant ($p = 0.004$) for Hg only. Interestingly, Cu and Hg were never significantly correlated with the other elements (data not shown), and their increase was the highest after all the three exposures (Table 1). Concentrations

Table 2 Mercury concentration values (micrograms per gram dry wt.; mean ± standard deviation) in the *P. furfuracea* transplants exposed in the sites of the industrial and urban strata of Fig. 1c

	1.5 months		3 months		6 months	
Industrial stratum	*0.18 ± 0.02*	0.9254	*0.22 ± 0.04*	0.1601	*0.29 ± 0.06*	0.003**
Urban stratum	*0.23 ± 0.11*	0.5621	*0.34 ± 0.19*	0.02*	*0.45 ± 0.38*	0.1023
All strata (agric. included)	*0.37 ± 0.76*	0.1860	*0.47 ± 0.90*	0.0009***	*0.50 ± 0.92*	0.0001***

p values of Mann–Whitney *U* test in italics

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.005$

of Cu doubled after the first exposure and tripled after the second one. The percent increase in Hg was equally important, but not statistically significant for the whole area after the first exposure (Table 1). It was already intense and statistically significant only along the B transect (Fig. 2b), particularly in the segment located SW of the WI plant: In six sites, the Hg content reached 1.6–3.2 times the pre-exposure value, with an outstanding maximum of 4.37 μg g^{-1} (i.e. 24 times) in B3, located at only 300 m from the WI in the SW direction, which accounts for the high variation coefficient (202%). After 3 months, Hg increased in a higher number of sites (16), mainly disposed

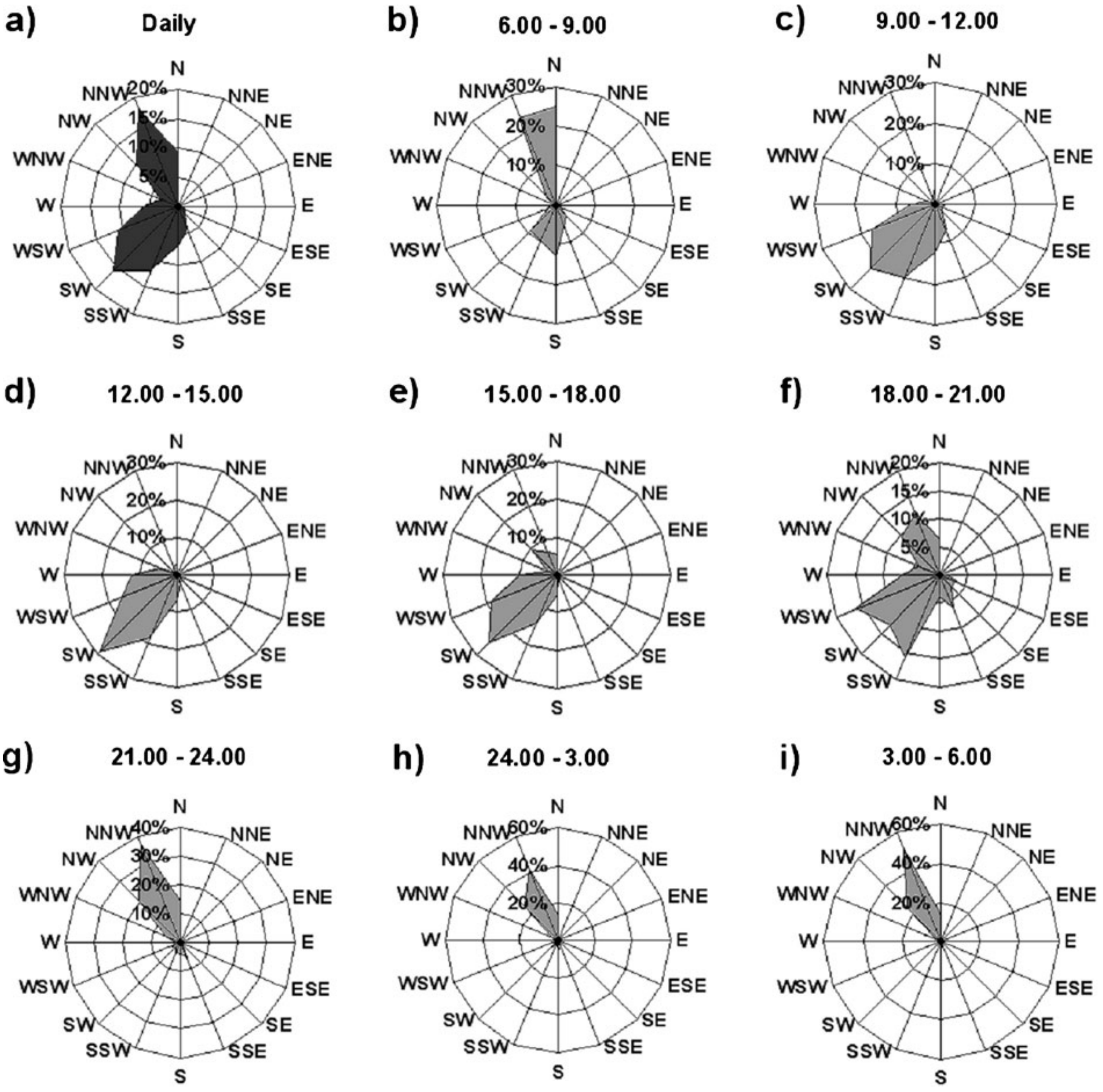

Fig. 4 Relative frequencies of wind direction during the first period of transplant exposure (May 27–July 11, 2008), calculated on a daily basis (**a**, *dark grey*) and for eight belts of 3 h each (**b**–i, *light grey*). Data recorded by an anemometer positioned at ca. 15 m above the ground near the stalk of the waste incinerator Mistral FVG; by courtesy of Mistral FVG

close to the WI plant in the SW direction. In addition, the contamination also extended southward along the A transect and to the three sites nearest to the WI plant in the N, E and NE direction, with a mean value of 0.58 ± 1.12. Not surprisingly, this time the increase in Hg was statistically significant for the whole area (Table 1) and for all the three transects (Fig. 2). The maximum content was again recorded in B3 (at this stage, 28 times the pre-exposure value), with an increase of 0.76 $\mu g\ g^{-1}$ with respect to the 1.5-month value. After 6 months, there was a further small increase in Hg, statistically not significant with respect to the mean value of the previous exposure. However, also in this case, seven sites, most of them belonging to the C transect, witnessed an important increase, up to 100% of the 3-month-exposure values (e.g. A1).

Leaves of black locust also showed an evident increase in Hg approaching the WI plant from SW (transect B), although they accumulated two to three times less Hg than lichens (Fig. 3). The lower efficiency of plant leaves in Hg accumulation is confirmed by the values measured in the other vascular plants sampled in B3 (cherry plum 0.24 $\mu g\ g^{-1}$, ivy 0.48 $\mu g\ g^{-1}$, Scotch elm 1.06 $\mu g\ g^{-1}$).

The industrial and urban strata also experienced statistically significant positive changes in Hg over time (Table 2). The increase was generally less pronounced in the industrial sites, which are spatially restricted to the northern portion of the A transect. Conversely, it was particularly dramatic in the urban sites E4–5, located in the SSW direction from the WI (Fig. 1c), showing final values of 1.06 and 0.60 $\mu g\ g^{-1}$, respectively. The urban sites E1–2, which are located NE from the WI within the town of Spilimbergo (Fig. 1c), were not affected by Hg enrichment.

The hypothesis that the source of Hg release is the WI, fully supported by the GLM analysis, is apparently contradicted by the wind data recorded immediately near the WI stack, at ca. 18 m above ground (Fig. 4), which were retrieved some months after the transplant exposure. On a daily basis, winds were mainly southwesterly and northerly (Fig. 4a). However, a more painstaking analysis of the data set showed that winds were constantly northerly during the night and southwesterly during the day (Fig. 4b–i), due to the presence of the nearby, steep slopes of the Carnic pre-Alps. Differential emissions during the day might well explain the observed Hg distribution pattern. No significant differences were observed when the data were analysed according to wind speed classes.

Discussion

The use of lichens for biomonitoring purposes is not free from shortcomings when the aim is air quality control. The most obvious one is the absence of specific laws accepting the biomonitoring approach per se, or at least indicating reference values. For Hg, the legislation of most countries defines the maximum allowable content in water, air and selected aquatic organisms (mostly those edible, for evident reasons of human health care). However, agricultural products and—more generally—plant matrices are ignored, probably because the human risk of exposure is considered as negligible (EPA 2008). Only few countries have Hg threshold limits for plant matrices (e.g. Finland 0.8 ppm in mushrooms; New Zealand 0.03 $\mu g\ g^{-1}$ in vegetables and fruits), and none of them is for wild plants or auto- or allochthonous lichens (Lorenzini and Nali 2005). To bypass this problem, one approach is the evaluation of deviation from background values, as specifically foreseen by some national legislations. In Italy, e.g. air pollution had already been defined as "any modification of the normal composition of the air [...] which alters the normal environmental conditions" (D.P.R. 24 May, 1998, n. 203, www.apat.gov.it/_Files/NormativaAria/DPR/DPR203_88_aggiornato_D.Lgs.152_2006.pdf). In order to apply this definition, an interpretative scale of deviation from naturalness was proposed for the most frequently analysed elements measured in autochthonous lichens (Bargagli and Nimis 2002). This scale has been used in a number of environmental studies (Loppi 2006) and even in some legal cases (Perotti 2004). From this point of view, the modification of the Hg distribution pattern observed in our study area between 1999 and 2007 (Fig. 1a, b) unequivocally identifies a pollution event (Tretiach and Pittao 2008). The present data, congruent over three cycles of exposures, (a) circumstantiate the phenomenon, (b) confirm that the pollution (defined as an increase in Hg concentration in two different biological matrices, lichen transplants and autochthonous plant leaves) is still ongoing and (c) pinpoint the source (Fig. 2).

Hg is released in the environment by several human activities, from geothermal exploitation to several chemical processes, to the use of fossil fuels (mainly coal) and to the incineration of Hg-containing waste (EPA 2008). Only the latter activity was identified in the study area. As the WI plant thermodestroys exhausted paints and inks, hospital waste medical materials, the Hg source had to be identified among them. From the 1980s, paints and inks marketed in EU countries contain only extremely low concentrations of heavy metals. Therefore, Hg is most likely to originate from the hospital waste, which accounted for 57.6% of the total amount of material treated in 2008 by the WI plant (Canciani 2008). Although we could not get direct information on the chemical composition of these materials (only CER codes and respective amounts were available), dental amalgam, mercurial disinfectants and pharmaceuticals, thermometers and other medical apparatus containing free Hg might be present in the waste and thus responsible for the emission to the environment. Interestingly, the incinerator of a civic hospital has already been identified as responsible for an important Hg contamination episode (Gasparo et al. 1989), whereas other municipal solid waste incinerators did not cause significant increase in Hg (Loppi et al. 2000).

The Hg enrichment observed in this study seems to be exceptional, at least for Italy. In previous transplant studies with *P. furfuracea* (Adamo et al. 2007; Bergamaschi et al. 2007; Giordano et al. 2009; Pittao 2007), the maximum increase, observed in a mixed urban/industrial area, was an order of magnitude lower than at site B3 (117% against 2,850%). Values comparable to those measured at site B3 have only been reported for autochthonous lichens from areas with exceptionally high Hg occurrence, such as the cinnabar mining and smelting sites of Mt. Amiata or the nearby geothermal fields exploited for electric energy production (Bargagli and Barghigiani 1991). The high contamination of thalli exposed in B3 is probably caused by a predominance of coarse, Hg-rich particulate matter, whereas in all the other sites, contamination might derive from particulate matter of smaller size classes and gaseous Hg forms (Hg° and/or $HgCl_2$, see Carpi 1997). Although only less than 5% of Hg emitted by waste incinerators occurs as particulate matter (Pacyna and Munch 1991), our hypothesis is supported by the variation in Hg content observed among the four vascular plants sampled in B3: The highest value was that of the scabrous, hairy leaves of Scotch elm, which can entrap particulate matter on the external surface, the lowest that of the shiny, smooth leaves of cherry plum. The adhesion on the outer surface is evidently even more pronounced in our lichen, which develops a dense cover of minute, finger-like outgrowths which increase one to two times the total surface of the lobes (Tretiach et al. 2005).

One final point concerns the observed increase in other elements. Rather surprisingly, the WI was not the main source, and with the exception of Hg, only the enrichment in Cu was really astonishing. Interestingly, in this case, data spread was high (variation coefficient higher than 100% in all three exposure data sets of Table 1), suggesting randomized enrichment over the study area. Sites with high post-exposure Cu values were always located near vineyards and/or grape nurseries (e.g. in C1 and B6). This Cu enrichment, observed also in autochthonous lichens (Tretiach and Baruffo 2001; Tretiach and Pittao 2008), might well derive from the practice of spraying these cultures, particularly frequent in the study area, with Cu-containing fungicides.

Conclusions

Once again, the biomonitoring technique of lichen transplants proved an extremely useful tool for verifying the spatial and temporal effects of a pollution point source. The application of this technique confirmed our hypotheses: The enrichment in Hg observed in autochthonous lichens in 2008 (Tretiach and Pittao 2008) was not due to a stochastic event, but it is caused by the WI emissions, most probably attributable to the high content of Hg in the hospital waste treated by the plant. The ongoing release of Hg from the incinerator shows that its pollution abatement system, even though representing the most up-to-date technology in flue gas cleaning, it is non-sufficient to contain pollution at a negligible level.

It seems improbable that the amount released by the incinerator poses an immediate health risk to the population living nearby the plant (ca. 1,000 inhabitants). However, long-term hazards due to Hg accumulation in the surrounding environment should be seriously taken into account. For this reason, a more exhaustive survey of biological and soil matrices is necessary. More efficient abatement strategies or, alternatively, a more painstaking selection of waste treated by the plant should be adopted to avoid further environmental contamination and risks for human and ecosystem health. A repetition of this study could be used to assess environmental effects following technical improvements in the plant abatement system.

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