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Congruence Evaluation of Mercury Pollution Patterns Around a Waste Incinerator over a 16-Year-Long Period Using Different Biomonitors

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Abstract: To date, there has been an ever-increasing interest in complementary air monitoring techniques, which may fill the deficiencies of air quality networks. The present work reports the results concerning five biomonitoring surveys (BSs) performed in the proximity of a waste incinerator (WI) over a 16-year period. Hg emission related to the WI activity was monitored by means of both active and passive BSs based on three photosynthetically-active biomonitors (i.e., two epiphytic lichens: *Pseudevernia furfuracea* and *Xanthoria parietina*; one vascular plant: *Robinia pseudoacacia*) collected or exposed before and/or after the WI installation, and after a four-month period of inactivity. Hg concentration values observed in bimonitor samples varied according to the implemented species and to the status of WI plant (active vs. inactive). Our data demonstrate that, in the same pollution scenario, *P. furfuracea* accumulates three times more Hg than both *X. parietina* and *R. pseudoacacia*. The results are discussed in the context of the actual European Union directives concerning air pollution monitoring and assessment, revealing that both active and passive BSs are efficient tools to provide a reliable estimation of the spatial changes of Hg concentrations in the environment.

Keywords: bimonitor; Hg pollution; *Pseudevernia furfuracea*; *Robinia pseudoacacia*; waste incinerator; *Xanthoria parietina*

1. Introduction

Mercury (Hg) represents a severe threat to human health and the environment as both atmospheric elemental mercury (Hg⁰) and methylmercury accumulate through the aquatic and terrestrial food webs. Owing to its toxicity and long-range transport, Hg pollution has long been recognized as a global issue [1]. Many international efforts have been spent at developing legally-binding treaties (see the work of [2]) in order to reduce Hg emission in the environment [3]. In Europe, the current air quality directives state the need to reduce Hg emissions by regulating the industrial activities through integrated pollution prevention and control programs and, in parallel, by improving air quality monitoring and assessment programs [4–7]. To date, Hg air pollution has mainly been assessed at the continental and global level by carrying out punctual measurements with manual or automated air quality monitoring stations [8], applying highly standardized physical–chemical methodologies, defined at legislative level [4]. However, because of the high costs related to elemental analysis of bulk deposition, the monitoring networks of atmospheric Hg are still characterized by a low spatial and temporal coverage. For instance, in the European Union (EU), only 14% of national air pollution monitoring networks provide air concentration data for this element on an annual basis [9]. Therefore,

there is an ever-increasing interest in complementary air monitoring techniques, which may fill the deficiencies of the extant air quality networks by providing reliable time-integrated estimations of air pollution in a given area [10,11].

An example of a less expensive, but reliable alternative to the direct measurement of air and/or particulate matter (PM) is given by the use of plant biomonitors [12], which can provide a spatial, time-integrated assessment of Hg pollution. Both vascular plants and cryptogams, like lichens and mosses, have been successfully used as Hg bioaccumulators [13]. Although studies have mainly focused on comparing the performances of allochthonous biomonitors in accumulating air pollutants [14–17], there is still a lack of studies specifically aimed at comparing the results of biomonitoring surveys (BSs) carried out in the same pollution scenario, but using different biomonitors.

The present work reports the results of one of the longest biomonitoring studies of Hg air pollution ever carried out at a local scale. The data presented herein concern five BSs performed in the proximity of a waste incinerator (WI) over a 16-year period according to both active (biomonitor transplants) and passive (sampling native biomonitor populations) techniques.

The aim of this work is (a) to provide an overview about the efficiency of different organisms as biomonitors of Hg pollution within the same pollution scenario, and (b) to test the effectiveness of both passive and active biomonitoring techniques in tracking Hg emission from a point source. The results are also discussed in relation to the European Union directives, revealing that BSs, when properly planned and executed, are efficient tools, which provide reliable estimation of the spatial-temporal changes of Hg concentrations in the environment.

2. Material and Methods

2.1. Study Area

The study area is located in the Friulian floodplain (NE Italy), circa 10 km south of the Carnic pre-Alps (Figure 1a). The climate is relatively rainy (1500 mm/year) and temperate, with cold winters and warm summers mitigated by frequent thunderstorms. North-blowing winds prevail in the western portion, whereas southwestern winds prevail, especially during night, in the eastern portion [18]. The land use of the study area is relatively homogeneous and is characterized as follows: 70% is covered by corn, soy bean and vineyard fields, highly specialized grape nurseries, and stable meadows; 16% by natural vegetation, restricted to the two river banks that mark off the study area east- and westward (Tagliamento and Meduna, respectively); and the remaining 14% is covered by industrial and urban settlements, the largest one being Spilimbergo, with circa 10,000 inhabitants. The industrial activities are small manufactures mainly concentrated in two industrial zones, or scattered in the territory, intermingled with the urban fabric (Figure 1b).

The waste incinerator (WI), located SW of the town of Spilimbergo, was built in 1978, closed in 1985, and rebuilt in 1999. In 2002, the authorization was renewed in order to implement the total quantity of disposable waste at 25,000 tons year⁻¹. The WI incinerates wastes at 980–1300 °C, and related pollutant emissions are mitigated by fabric filters; flue gas flow is approximately 10.25 Nm³ s⁻¹ at 192 °C from a 23 m high stack. In Italy, the legislative limit threshold for Hg emission is 0.05 mg Nm⁻³ [D.M. 25 February 2000, n. 124; <http://www.gazzettaufficiale.it/eli/id/2000/05/18/000G0171/sg>] and the actual emission value is certified quarterly by a private company.

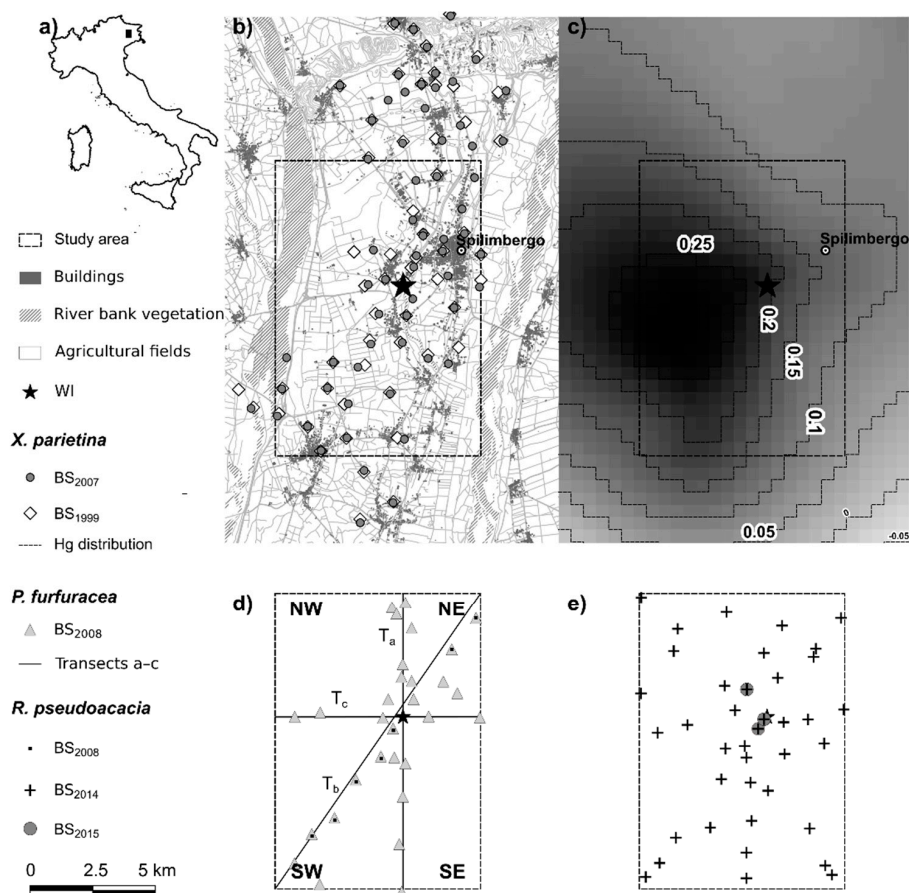


Figure 1. (a) Location of the study area; (b) cartographic details of the study area with the sampling sites of the epiphytic lichen *Xanthoria parietina* collected in 1999 (diamonds) and 2007 (dark grey dots); (c) mercury (Hg) distribution in *X. parietina* samples collected in 2007; (d) line transect design (continuous lines), exposure sites of transplants of the epiphytic lichen *Pseudevernia furfuracea* (light grey triangles), and sampling sites of *Robinia pseudoacacia* leaves (little black squares), carried out in 2008; (e) sampling sites of *R. pseudoacacia* leaves collected in 2014 (crosses) and 2015 (grey circles). BS—biomonitoring survey; WI—Waste incinerator.

2.2. Biomonitoring Surveys

This work summarizes the results of five biomonitoring surveys (BSs) based on two epiphytic lichens, that is, *Pseudevernia furfuracea* (L.) Zopf var. *furfuracea* and *Xanthoria parietina* (L.) Th.Fr., and on the leaves of *Robinia pseudoacacia* L. (black locust tree). The first two surveys were carried out just before (1999) and eight years after (2007) the WI installation, respectively. These two surveys (herein named BS₁₉₉₉ and BS₂₀₀₇) were based on the autochthonous foliose lichen *X. parietina* sampled around the WI according to a random sampling design, in 56 and 64 sites (Figure 1b), respectively, with 73% of sampling sites visited in both surveys.

In 2008, a portion of 97 km² of the original study area was subjected to a lichen transplant survey, aimed at (i) verifying whether the Hg pollution highlighted by BS₂₀₀₇ was still present, and (ii) identifying the source. In this case, the epiphytic fruticose lichen *P. furfuracea* was collected in a remote area (see the work of [18] for details) and exposed for 6, 12, and 24 weeks at 30 sites, 11 of which were selected in urban (n = 6) and industrial (n = 5) areas, whereas the remaining 19 were selected in agricultural areas. The exposure design was based on three transects (T_{a-c}) defined according to the following: (i) the results of BS₂₀₀₇ (Figure 1c); (ii) the wind frequency distribution as recorded in three nearby meteorological stations (Figure 1d; see [18]); and (iii) the location of the putative source, that is, the WI plant.

The sampling of *R. pseudoacacia* leaves was carried out in three out of the five biomonitoring surveys presented in this work: in 2008, during the ongoing of sample exposure of *P. furfuracea* transplants (i.e., BS₂₀₀₈; Figure 1d), and in October 2014 and 2015 (namely BS₂₀₁₄ and BS₂₀₁₅, respectively; Figure 1e). The first leaf sampling was carried out 2 weeks before the end of the 24-week exposure period selected for *P. furfuracea* transplants (see above). In this case, *R. pseudoacacia* leaf samples were collected at eight exposure sites from twigs immediately adjacent to the lichen transplants exposed along T_b, that is, the portion of the study area where *P. furfuracea* samples had the highest Hg concentrations after both 6 and 12 weeks. In 2014, the sampling of *R. pseudoacacia* leaves was iterated at the above mentioned 8 sites and extended to a further 31 sites (total sites n = 39), whose location corresponded as much as possible to the exposure sites selected for the lichen transplant survey (compare Figure 1d with Figure 1e). In July 2015, the WI activity was shut down for an internal incident. After a non-operative period of four months, the sampling of *R. pseudoacacia* leaves was repeated at three sites of BS₂₀₁₄ (Figure 1e) located in the immediate proximity of the WI.

2.3. Sample Treatments

Lichen and plant material was pre-treated (cleaning and material selection) and homogenized by manual or automated powdering according to accepted and well-known protocols commonly used in biomonitoring surveys [12,19]. More details about the preparation of analytical samples are reported in Supplementary Material SM1.1–SM1.3.

2.3.1. Characterization of *Robinia pseudoacacia* Leaf Samples

The 2014 sampling of *R. pseudoacacia* leaves was carried out at the end of the vegetative season, when the pigmentation of collected leaves was highly heterogeneous among and within samples. Hence, in order to test whether different leaf pigmentation was related to differences in Hg accumulation, subsamples of yellow (Y) and green (G) leaves collected at eight sites were separately pre-treated for Hg analyses, as explained in Supplementary Material SM 1.3. At the same time, the average specific leaf area (SLA) of Y and G leaves was assessed on 10 randomly selected sub-samples of circa 600 mg each. SLA was measured according to the method reported in the work of [20].

2.4. Mercury Analysis

Overall, nine series of samples relating to three different biomonitors, that is, *X. parietina*, *P. furfuracea*, and *R. pseudoacacia*, were collected. Hereinafter, in order to refer to a specific series of samples the notation reported in Table 1 is used.

Prior to Hg analysis, Xp₁₉₉₉ and Xp₂₀₀₇ (Table 1) samples were digested in Teflon bombs with 2.5 mL of HNO₃ 65% (v/v) at 120 °C (herein, “partial acid digestion”). Hg content of mineralized Xp₁₉₉₉ and Xp₂₀₀₇ samples was measured by means of cold vapor atomic absorption (CVAA) and inductively coupled plasma mass spectrometry (ICP-MS), respectively. Both sets of analyses were carried out at the laboratories of the University of Siena under the supervision of Prof. R. Bargagli. On the other hand, lichen and leaves samples collected in 2008 (i.e., Pf-6-12-24₂₀₀₈ and Rp₂₀₀₈; see Table 1) were digested in Teflon bombs with 6 mL HNO₃ 65% (v/v), 0.2 mL HF 50% (v/v), and 1 mL H₂O₂ 30% (v/v) (herein, “total acid digestion”) in a microwave oven with stepwise power application. In this case, the Hg content in mineralized leaves and lichen samples was measured by flow injection mercury system (FIMS) at the laboratory of the Regional Agency Environmental Protection of Friuli Venezia Giulia (ARPA FVG). Hg concentrations in Rp-G₂₀₁₄, Rp-Y₂₀₁₄, and Rp₂₀₁₅ (Table 1) samples were analyzed by direct mercury analysis (DMA-80, Milestone, Brøndby Kommune, Denmark) at the laboratories of the University of Compostela, under the supervision of Prof. Fernández Escribano. Elemental Hg analyses were always determined by comparison with standard solutions. Hg content in procedural blanks was always below the detection limit. The accuracy of digestion and analytical procedures was checked by routine determination of element concentrations in standard reference material (Table S1 in Supplementary Materials).

Table 1. Notation used to name the nine sample series analyzed in this work. The notation used refers to the following: (i) biomonitor species (Xp: *X. parietina*; Pf: *P. furfuracea*; Rp: *R. pseudoacacia*); (ii) exposure period (i.e., 6, 12, or 24 weeks; See Section 2.2) or, alternatively, to the pigmentation of sampled leaves (i.e., green—G; yellow—Y; see Section 2.3.1); and (iii) year of sampling (i.e., 1999, 2007, 2008, 2014, and 2015). P: passive technique; A: active technique.

Series of Samples	Technique	Year	Biomonitor
Xp ₁₉₉₉	P	1999	<i>X. parietina</i> , native
Xp ₂₀₀₇	P	2007	<i>X. parietina</i> , native
Pf-6 ₂₀₀₈	A	2008	<i>P. furfuracea</i> , transplants
Pf-12 ₂₀₀₈	A	2008	<i>P. furfuracea</i> , transplants
Pf-24 ₂₀₀₈	A	2008	<i>P. furfuracea</i> , transplants
Rp ₂₀₀₈	P	2008	Green leaves of <i>R. pseudoacacia</i> , native
Rp-G ₂₀₁₄	P	2014	Green leaves of <i>R. pseudoacacia</i> , native
Rp-Y ₂₀₁₄	P	2014	Yellow leaves of <i>R. pseudoacacia</i> , native
Rp ₂₀₁₅	P	2015	Green leaves of <i>R. pseudoacacia</i> , native

2.5. Data Analysis

Basic descriptive statistics were calculated for each of the nine series of samples. In order to take into account the effect of the wind regime on the dispersion of WI emissions, both sampling and exposure sites were classified according to their relative position with respect to the WI plant (Figure 1d). Significant increases of Hg concentration values among different series of *X. parietina* or *P. furfuracea* samples were assessed with a generalized linear model (GLM), assuming the year of sampling or exposure period along with the wind regime as categorical predictors, whereas the distance of the sampling/exposure sites from the WI was assumed as a continuous predictor.

Among the BSs based on *R. pseudoacacia*, there was a high discrepancy in terms of the number of collected samples. Therefore, the significant temporal increase or decrease of Hg concentrations in samples leaves was tested with the Mann–Whitney non-parametric test for paired samples. The comparison of the medians was performed between (i) samples collected along T_b in 2008 (Rp₂₀₀₈; n = 8) and 2014 (a subset of Rp-G₂₀₁₄; n = 10), and (ii) samples collected in 2014 (a subset of Rp-G₂₀₁₄; n = 9) and 2015 (Rp₂₀₁₅; n = 9) in the immediate proximity of the WI.

Trends of Hg concentration in each series of biomonitor samples were analyzed according to the distance of sampling/exposure sites from the WI (i.e., trend analysis). Furthermore, the difference between Hg content of Rp-G₂₀₁₄ and Rp-Y₂₀₁₄ samples expressed as a function of SLA was tested by means of a T-test for paired samples.

The results of BSs obtained with different biomonitors were compared depending on the spatial distribution of Hg content measured in six out of the nine series of samples (i.e., Xp₁₉₉₉, Xp₂₀₀₇, Pf-6₂₀₀₈, Pf-12₂₀₀₈, Pf-24₂₀₀₈, and Rp-G₂₀₁₄). Spatial distribution maps were calculated according to ordinary Kriging interpolation algorithms [21]. Both setting parameters and variogram function selected for interpolation analysis are reported in Table S2. The local variability of predicted values (i.e., interpolated values) was expressed as the variance of Hg concentrations [22], whereas their accuracy was expressed as the root mean square error (RMSE) measured between observed and interpolated values [23]. Finally, in order to evaluate the consistency among the six spatial distribution patterns, the degree of correlation among the interpolated values was investigated at 250 randomly selected points selected within the spatial domain of the study area.

3. Results

Table 2 reports basic descriptive statistics of Hg content measured in the nine series of samples collected in the BSs carried out before (i.e., BS₁₉₉₉) and after (i.e., BS₂₀₀₇, BS₂₀₀₈, and BS₂₀₁₄) the WI implanting (2000), and during the non-operational period of the plant (BS₂₀₁₅). Minimum, average, and maximum Hg concentration values observed among BSs differed according to the following: (i) year of sampling, (ii) biomonitor, and (iii) status of the WI plant (active vs. inactive).

Table 2. Basic descriptive statistics of Hg content measured in the nine series of biomonitor samples collected between 1999 and 2015. Exposure period for the epiphytic lichen *Xanthoria parietina* and the leaves of *Robinia pseudoacacia* were estimated according to the works of [24,25]; those of the transplanted lichen *Pseudevernia furfuracea* were taken from the work of [19]. WI: waste incinerator; on: WI active; off: WI inactive; BS: biomonitoring survey; n: number of exposed or collected samples; Min, Max, Mean: minimum, maximum and mean Hg concentration value; St. dev.: standard deviation; CV%: percent variation coefficient.

Parameters	<i>X. parietina</i>		<i>P. furfuracea</i>			<i>R. pseudoacacia</i>			
	off	on	on		on	on		off	
BS	BS ₁₉₉₉	BS ₂₀₀₇	BS ₂₀₀₈		BS ₂₀₀₈	BS ₂₀₁₄		BS ₂₀₁₅	
Sample Series	Xp ₁₉₉₉	Xp ₂₀₀₇	Pf-6 ₂₀₀₈	Pf-12 ₂₀₀₈	Pf-24 ₂₀₀₈	Rp ₂₀₀₈	Rp-G ₂₀₁₄	Rp-Y ₂₀₁₄	Rp ₂₀₁₅
Exposure Period (Weeks)	~52	~52	6	12	24	~40	~40	~40	~40
n	56	64	30	30	30	8	39	8	3
Min	0.011	0.039	0.130	0.150	0.130	0.000	0.017	0.024	0.023
Max	0.270	0.384	4.370	5.130	5.000	0.360	0.326	0.425	0.061
Mean	0.096	0.141	0.371	0.467	0.500	0.135	0.052	0.106	0.040
St. dev.	0.064	0.078	0.764	0.899	0.919	0.126	0.052	0.135	0.019
CV%	66.6	55.5	206.0	192.5	183.9	93.6	101.0	126.7	47.4

Both GLM analyses revealed that the increase of Hg content in autochthonous and transplanted lichens were significantly related to the distance of sampling/exposure sites from the WI (Table 3; Figure 2a). The relationship between Hg concentrations in both lichen and leaf samples and the distance of the sampling/exposure sites from the WI were described according to a power or an exponential function (Figure 2a). The GLM-based comparison between Hg bioaccumulation data of the first two BSs (BS₁₉₉₉ and BS₂₀₀₇) revealed a significant increase of Hg concentration in *X. parietina* native thalli, especially in sites located SW of the WI (Figure 2b). According to this, the interaction term between the year of sampling and the wind regime is highly significant (Table 3).

Table 3. Generalized linear model (GLM) summary statistics performed on Hg concentration values observed in samples of the lichens *Xanthoria parietina* (autochthonous) and *Pseudevernia furfuracea* (transplanted) collected or exposed in the study area during the respective biomonitoring surveys (see text). SS: sum of squares; MS: mean squares; F: F-statistic; p: p-value. Significant effect of continuous or categorical predictors are reported in bold

	SS	MS	F	p
<i>X. parietina</i>				
Intercept	0.50	0.50	136.64	<0.001
Distance (D)	0.02	0.02	5.93	0.016
Year (Y)	0.05	0.05	13.01	<0.001
Wind (W)	0.02	0.01	2.15	0.098
Y × W	0.16	0.05	14.23	<0.001
Error	0.41	0.00		
Total	0.67			
Model	0.26	0.03	8.72	<0.001
<i>P. furfuracea</i>				
Intercept	18.21	18.21	30.50	<0.001
D	10.40	10.40	17.41	<0.001
Exposure (E)	0.16	0.08	0.13	0.877
W	13.37	4.46	7.46	<0.001
E × W	0.23	0.04	0.06	0.998
Error	44.19	0.60		
Total	62.60			
Model	18.41	1.53	2.57	<0.001

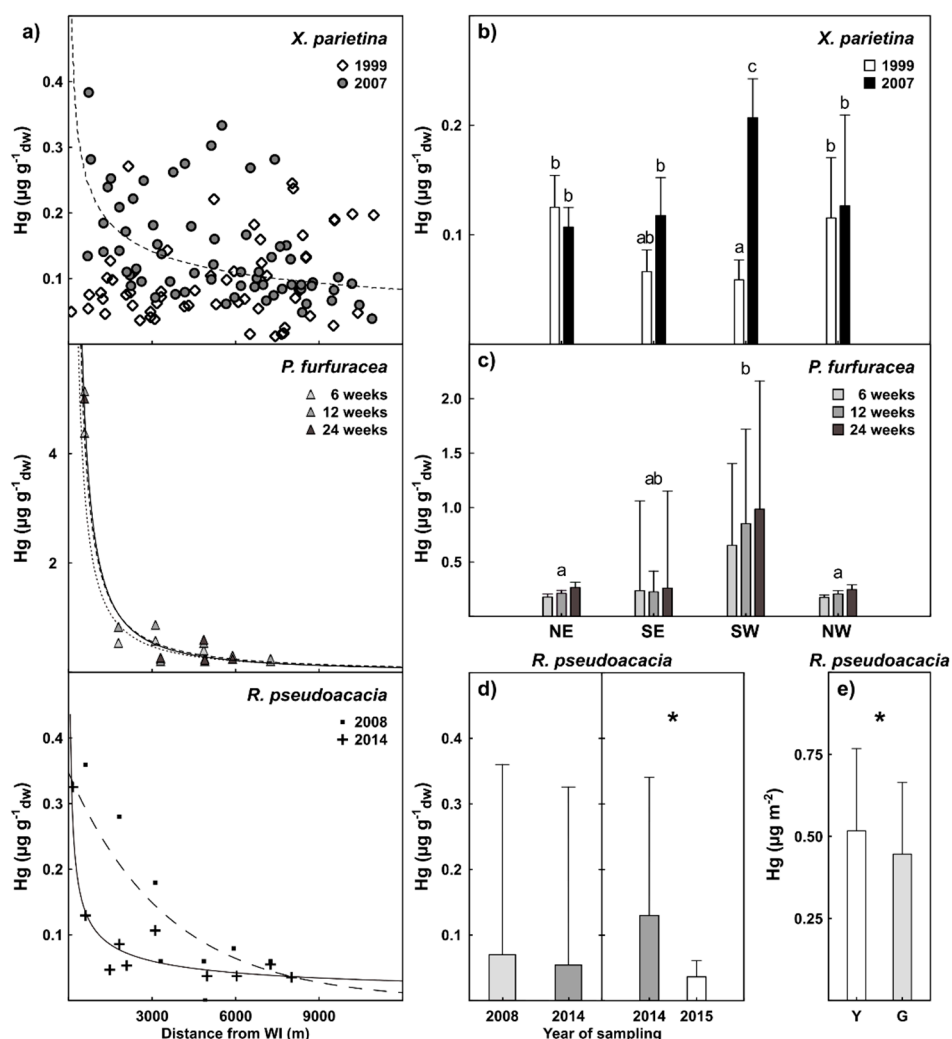


Figure 2. (a) Trends of Hg concentration vs. distance of exposure or sampling sites from the WI; (b,c) results of generalized linear model (GLM)-based comparison performed on samples of the lichens *Xanthoria parietina* and *Pseudevernia furfuracea* (see Section 2.5; data elaborated from the work of [18]); (d) median comparison between leaves of *Robinia pseudoacacia* sampled in 2008 and 2014 in the whole study area and in 2014 and 2015 in three sites close to the WI (see text for explanation); (e) comparison of Hg content in yellow (Y) and green (G) *R. pseudoacacia* leaves collected in the same ten sites in 2014. Different letters in (b,c), as well as asterisks in (d,e), indicate significant difference for $p < 0.05$.

The results of the following BSs strengthened the findings of the 2007 survey, indicating that the Hg pollution was not the result of a stochastic episode. The GLM analysis of the BS₂₀₀₈ data showed a significant high Hg enrichment in *P. furfuracea* samples exposed SW of the WI (Figure 2c), with values that are still the highest ever observed in biomonitoring surveys with *P. furfuracea* transplants (see the works of [26–28]). Nevertheless, there were no significant differences among the three selected exposure periods (Table 3; Figure 2c), although the Hg enrichment pattern was the same in all three series of transplanted samples (i.e., those exposed for 6, 12, and 24 weeks), clearly pointing out a close relationship between wind regime of the study area and WI location.

The result of the Mann–Whitney-based comparisons between the Rp₂₀₀₈ and Rp-G₂₀₁₄ samples was not significant, revealing that the load of Hg pollution in the study area did not decrease between 2008 and 2014 (Figure 2d). Along the transect Tb of Figure 1d, the Hg content of *R. pseudoacacia* leaves in 2014 was still strictly related to the distance from the WI. However, the Hg concentration trend in *R. pseudoacacia* leaves collected in 2014 was slightly different to that observed in leaves collected in 2008 (Figure 2d), possibly for the different analytical techniques (in 2008: FIMS; percent recovery:

100%; in 2014: DMA; percent recovery from 80% to 115%). Interestingly, a significant decrease of Hg content was observed in *R. pseudoacacia* leaves collected in October 2015, four months after the WI had been shut down as a consequence of an accident (Figure 2d). Therefore, by sampling *R. pseudoacacia* leaves during this non-operative period in 2015, it was possible to incontrovertibly demonstrate that the WI activity was significantly related to the fluctuation of Hg content observed in *R. pseudoacacia* leaves (Figure 2d).

The spatial distribution maps of Hg content in the biomonitor samples are reported in Figure 3. Overall, the interpolation values calculated for six out of the nine series of biomonitor samples (i.e., Xp₁₉₉₉, Xp₂₀₀₇, Pf-6₂₀₀₈, Pf-12₂₀₀₈, Pf-24₂₀₀₈, and Rp-G₂₀₁₄) were predicted with a more than satisfactory accuracy (RMSE between the 0.009 µg g⁻¹ of Rp₂₀₁₄ samples to the 0.3375 µg g⁻¹ of Pf-6₂₀₀₈ samples) and with a low local variability (Table 4). Most importantly, the correlation analysis among the six series of interpolated values (see Section 2.5) revealed a good spatial consistency, with highly significant values of Pearson’s *r* correlation coefficient among the bioaccumulation data collected between 2007 and 2014 (Table 4).

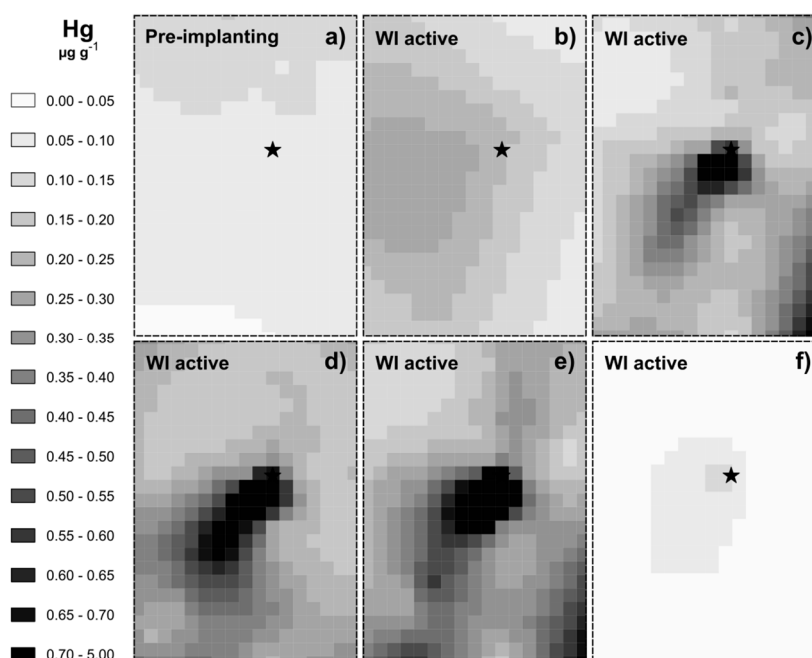


Figure 3. Result of kriging interpolation analyses based on (a) Xp₁₉₉₉, (b) Xp₂₀₀₇, (c) Pf-6₂₀₀₈, (d) Pf-12₂₀₀₈, (e) Pf-24₂₀₀₈, and (f) Rp-G₂₀₁₄ samples. The notation of samples is reported in Section 2.4.

Table 4. Summary of interpolation analyses carried out on Xp₁₉₉₉, Xp₂₀₀₇, Pf-6₂₀₀₈, Pf-12₂₀₀₈, Pf-24₂₀₀₈, and Rp-G₂₀₁₄ datasets. RMSE and VAR are expressed in µg g⁻¹ and correspond to the root mean square error and the local variability, respectively. Significant correlation values among interpolated values are reported in bold.

Set of Samples	RMSE	Variance Range	Xp ₁₉₉₉	Xp ₂₀₀₇	Pf-6 ₂₀₀₈	Pf-12 ₂₀₀₈	Pf-24 ₂₀₀₈	Rp-G ₂₀₁₄
Xp ₁₉₉₉	0.0315	≈0	1					
Xp ₂₀₀₇	0.0345	[0.002, 0.003]	−0.054	1				
Pf-6 ₂₀₀₈	0.3375	[0.029, 0.247]	−0.220	0.023	1			
Pf-12 ₂₀₀₈	0.0892	[−0.124, 0.122]	−0.218	0.327	0.90	1		
Pf-24 ₂₀₀₈	0.334	[−0.176, 0.663]	−0.271	0.121	0.970	0.934	1	
Rp-G ₂₀₁₄	0.0093	[0.017, 0.152]	−0.341	0.546	0.546	0.662	0.616	1

4. Discussion

4.1. The Pattern of Hg Pollution

Waste incinerators have frequently been associated with Hg emission by means of both biomonitor surveys [29] and direct instrumental records [30]. In the Mediterranean basin, WI plants are estimated to emit in the atmosphere approximately 16.6 t yr^{-1} of Hg^{2+} , 5.5 t yr^{-1} of particulate Hg (Hg_p), and 16.6 t yr^{-1} of Hg^0 [31]. By-products of waste incineration, for example, slug (mostly used for road construction, noise barriers, concrete production, or landfill material), could contain relatively high quantities of Hg (from 0.02 to 7.75 mg kg^{-1}), which can be partially released into the atmosphere [32,33]. The authors of [34] identified the Hg source of the Spilimbergo WI mostly in the incineration of hospital wastes, which accounted for 57.6% of the total material treated by the plant.

The study area has no geochemical anomalies [35] and, in parallel, the Hg content of lichen samples was never significantly correlated with Al (data not shown). Consequently, the significant increase of Hg in native *X. parietina* observed between 1999 (when the average value in the study area corresponded to the Italian background level) and 2007 (when some sites resulted heavily polluted, with max. values of circa $0.384 \text{ } \mu\text{g g}^{-1}$), not certainly the result of soil contamination, was—Almost unavoidably—referred to the new perturbation factor present in the study area, that is, the WI, which started to operate a few months after BS₁₉₉₉ [36]. Overall, the magnitude of concentrations corroborated the occurrence of an important environmental pollution. In particular, by comparing the maximum Hg values with the threshold values of the “naturalness”/alteration scales proposed by the authors of [37], the entity of Hg pollution was classified as “middle alteration from natural conditions”, with single sites falling in the class of “maximum alteration” [36]. It is noteworthy that single concentration values were comparable to those observed in the same biomonitor from highly polluted urban and industrial areas of central Italy [38,39], northern France [40], and north-eastern Turkey [41].

In general, the statistical and geostatistical elaboration of BS data can provide reliable, accurate, and robust information, useful to evaluate the temporal changes in the distribution pattern of pollutant emissions from a punctual source only if high-density probabilistic samplings are adopted and the potential bias related to the biology of the biomonitor are taken into account. In our case study, the robustness of our geostatistical analyses (with a $500 \times 500 \text{ m}$ spatial resolution; Table S2 Figure 3) is fully corroborated by the tight relationship between Hg content measured in biomonitor samples and the distance from the putative Hg source (Figure 2a). Interestingly, similar relationships were observed in lichen samples collected at increasing distance from abandoned cinnabar mining site or geothermal source placed in central Italy [42,43]. This is noteworthy because, although the WI plant adopted the best available technologies accordingly to IPPC legislation, Hg emissions persisted for 16 years with a contamination radius of $\sim 2 \text{ km}$. On the other hand, the robustness of our BSs' results is supported by the high consistency of interpolation values predicted with different series of samples (i.e., Xp₁₉₉₉, Xp₂₀₀₇, Pf-6₂₀₀₈, Pf-12₂₀₀₈, Pf-24₂₀₀₈, and Rp-G₂₀₁₄). This is an important result because, for the first time, it has been possible to demonstrate that although different biomonitors were used to detect Hg pollution, the spatial relationship among Hg content measured in biological samples collected or exposed at different sites is well-preserved also over a prolonged period of time. Nevertheless, the interpolation analyses performed on *P. furfuracea* samples were characterized by high values of RMSE and were partially inconsistent with the interpolation elaborated for Xp₂₀₀₇ and Rp-G₂₀₁₄ samples (Table 4; Figure 3). This disagreement (low values of correlation in Table 4) might depend on both the different spatial structure of the dataset (i.e., the location of the sampling and exposure sites; compare Figure 1d vs. Figure 1e) and the respective sampling density [44]. Certainly, the presence of astonishing extreme values in the *P. furfuracea* sample series, besides to impose a log-transformation of our data prior to interpolation analysis, made it difficult to find a proper variogram function without causing an over- or under-estimation of interpolation values. The reliability of interpolation methods for biomonitoring purposes have been widely discussed by the authors of [45], pointing out the

sensitivity of these methods to the presence of outliers [46], as well as to the distribution function of the data.

4.2. Comparison Among BSs' Results

In both cryptogams and vascular plants, Hg accumulation has widely been investigated for Hg^0 and only to a lesser extent for Hg^{2+} and Hg_p . Hg^0 fluxes in leaves are bi-directional, allowing a continuous exchange between air and leaves [47,48]. There are data suggesting that Hg^0 accumulation in lichens is ruled by similar mechanisms [49]. Therefore, although Hg^0 is not stably absorbed and accumulated onto leaves or lichen matrices over a prolonged period, high amounts of Hg in biological matrices inevitably reflect a continuous availability of air Hg, as has been described for a volcanic environment, in which different taxa of both lichen and plants accumulate Hg in similar amounts [50]. In this study, the geostatistical comparison among the results of the five BSs clearly highlighted that the study area was affected by a well-defined and wind dependent spatial pattern of Hg over a period of 16 years (Figure 3). Interestingly, despite that *P. furfuracea* samples were exposed for short periods (i.e., 6, 12, and 24 weeks, respectively), some of them accumulated 10 times more Hg than *R. pseudoacacia* and *X. parietina* samples, which were exposed to the environmental conditions for longer periods of time (circa 40 and 52 weeks, respectively, see Fortuna and Tretiach 2018) (Table 2). On the other hand, our data did not allow us to provide a reliable comparison of Hg accumulation capabilities between the two lichen biomonitors, which were collected and exposed, respectively, in the same study area, but not at the same time—the pollution scenario between 2007 (when *X. parietina* was collected) and 2008 (when *P. furfuracea* was transplanted) might have been significantly different in terms of both rate of Hg emission and Hg content in the moieties of burned waste. However, previous laboratory experiments demonstrate that *P. furfuracea* significantly accumulates more Hg^0 than *X. parietina* [51]. Also, in this case, the high Hg accumulation performance observed for *P. furfuracea* has been brought back to the isidia, that is, minute fingerlike outgrowths, with a high chlorophyll content, which considerably increase the external exchange surface of the lichen [52,53], and thus CO_2 gas exchange between thallus and atmosphere. All these factors increase gross photosynthesis, which, if compared with non-isidiate species such as *X. parietina*, is almost two times higher [52]. In addition to improving the exchange of gaseous Hg, isidia might also increase the entrapment of air particulate matter, with all Hg species eventually adsorbed to it (e.g., Hg^{2+} and Hg_p). Therefore, the high Hg content observed in *P. furfuracea* suggests that the main Hg species emitted from the WI might be Hg^{2+} (e.g., HgCl_2) and Hg_p .

A final remark concerns the differences in terms of Hg content observed between green and yellow leaves of *R. pseudoacacia*. In accordance with what has been observed by other authors (e.g., [54]), our data pointed out that light, but significant differences in Hg concentration in leaves of the same plant species are mainly driven by differences in the specific leaf area (SLA). During senescence, leaves undergo progressive deterioration, connected to the dismantling of the photosynthetic apparatus with progressive reduction of photosynthesis. This implies a reduction of starch deposits, and a progressive lightening of the leaf itself [55]. Actually, SLA of yellow *R. pseudoacacia* leaves was higher ($2.06 \pm 0.46 \text{ m}^2 \text{ g}^{-1}$) than that of green leaves ($1.83 \pm 0.30 \text{ m}^2 \text{ g}^{-1}$). However, in proximity to the pollution source, the difference in Hg content of green and yellow leaves also remained high (25%) when the data were expressed on area (compare the maximum values of Rp-G₂₀₁₄ and Rp-Y₂₀₁₄ samples in Table 1). This suggests that at high Hg air concentrations, small differences in leaf time span can become a further source of variation. However, further investigation is required before considering leaf pigmentation as a potential bias in Hg pollution biomonitoring.

4.3. Effectiveness of Biomonitoring Techniques in Pollution and Monitoring Assessment Programs

At present, in Europe, the national air monitoring networks measure the air concentrations of different Hg species according to highly standardized methodologies such as EN 15852:2010 for total gaseous Hg determination and, limited to a low number of monitoring stations, EN 15853:2010 for determining Hg^{2+} and Hg_p concentrations in bulk deposition [4]. In order to provide a reliable

time-integrated estimation of Hg distribution, the facilities have to be continuously maintained and, at the same time, the related consumables substituted [11]. The high costs associated with these ground-based measurements explain, at least in part, why across Europe, the number of air monitoring stations is low ($n = 24$; [9]) and their temporal data coverage scarce (only 17% of monitoring stations collect more than 75% of data on an annual basis). For this reason, the European directive EC 2004 [4] suggests the implementation of less expensive, reliable alternatives in order to monitor air pollutants, Hg included. Indeed, it specifically encourages the use of “*bioindicators where regional patterns of the impact on ecosystems are to be assessed*”. By exploiting the native population of cosmopolitan bioindicators, biomonitoring techniques are particularly apt to carry out preliminary, rapid investigations aimed at pinpointing potential sources of air pollutants in a given area. Our study clearly indicates that both active and passive BSs can be carried out according to high-density based sampling designs (~ 1 sampling site km^{-2}). This density of spatial coverage is undoubtedly out of reach for the today’s air quality monitoring programs based on ground-based measurements.

Although BSs’ results can be used to easily describe the spatial and temporal pattern of Hg emissions in a given area, a proper way to use these data in order to estimate the exact Hg air concentrations seems to remain a problem without a solution [56,57]. Nevertheless, in order to contextualize the magnitude of the observed pollution scenario, the results of active and passive BSs based on lichens can be expressed as a function of the species-specific background elemental content (BEC; [58–60]). This allows researchers to evaluate pollution phenomena as a relative difference between the pollutant concentrations measured in lichen samples exposed in a polluted area and those collected in unpolluted, background areas (EC ratio, see the work of [61]; now EU ratio, see the work of [60]). If these concepts would be applied to our data, we might classify the observed pollution scenario as an event causing an increase of Hg concentration circa 6.4, 24, and 4 times higher than the usual Hg content observable in the native population of *X. parietina*, *P. furfuracea* (pre-exposure values; see the work of [18]), and *R. pseudoacacia*, respectively. This should be the most effective way to interpret and communicate biomonitoring results to both decision makers and, most importantly, to the public. Furthermore, this way to express the results might fill the shortcomings for the implementation of active and passive biomonitoring techniques even at a legislative level.

5. Conclusions

The different Hg content in collected or exposed lichen or plant samples seems to be mainly dependent on the species-specific morpho-physiological features of biomonitors, but possibly also to their different capabilities in accumulating specific Hg species emitted from the pollution source.

Overall, the high accuracy of spatial distribution pattern of Hg should encourages the use of lichen and plant as bioaccumulators of persistent airborne pollutants in BSs and assessment programs with a threefold aim: (i) fill the shortage of data collection at local and regional scale, (ii) validate the so-called atmospheric dispersion models (ADMs) of air pollutants, and (iii) identify the most appropriate location of air quality monitoring stations (i.e., those sites subjected to the highest deposition of air pollutants).

Finally, our results clearly show that both active and passive biomonitoring surveys carried out according to highly standardized protocols are suitable tools to define whether the measured environmental changes are statistically significant. In this context, legislative organs should take into account the methodological and economic advantages inherent to the application of biomonitoring techniques.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4433/10/4/183/s1>, SM1.1–SM1.3: description of sampling methodology applied in lichen and leaves sample collection, Table S1: list of SRM and CRM used in chemical analyses, Table S2: summary of setting parameters used in kriging interpolation analyses.

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