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Integrated biomonitoring of airborne pollutants over space and time using tree rings, bark, leaves and epiphytic lichens

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ABSTRACT

The integrated use of tree rings and outer tissues, and lichens, was tested for monitoring how pollutant concentrations vary in space and over time nearby an incinerator in industrial area in Central Italy. Trace elements in thalli of lichen *Xanthoria parietina* and in leaves, bark, wood of *Quercus pubescens*, as well as carbon, oxygen and nitrogen isotope ratios in tree rings were analyzed. Some trace elements in the leaves differed significantly between the plots, though this was not the case in lichens and bark. The values of $\delta^{13}C$ and $\delta^{18}O$ showed the same trend in all plots, while the values of $\delta^{15}N$ were higher in the distal plot. The results indicated that trace elements were intercepted and collected by tree bark and leaves, as well as lichens, at low concentrations, and that they hardly entered into tree xylem tissues during the growing season to be stored into the woody tissues. Indeed, the study did not highlight marked changes over time and space, in accumulation of airborne pollutants in the selected biomonitors, most probably due to the low levels of industrial development. Nevertheless, the analysis of tree ring cores in combination with bark and leaves, and lichens might potentially contribute to depict historic impacts of airborne pollutants at pronounced concentrations.

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1. Introduction

Humans and environments are continuously exposed to pollutants via natural and anthropogenic processes. Although the evidence base is as yet weak, there is accumulating evidence that exposure to pollutants is associated with adverse effects on human health, affecting particularly the cardiovascular system (Heal et al., 2012). Plants also exhibit an evident symptomatology when exposed to phytotoxic concentrations of pollutants with altered growth, physiology and reproductive patterns, or productivity and distribution (e.g., Lin and Xing, 2007). However, urban vegetation may efficiently remove airborne pollutants simply as a function of deposition velocity, pollution concentration, and tree structure (biomass) (e.g., Chen et al., 2015). Urban and peri-urban trees are considered, therefore, providers of ecosystem services and monitoring tools; these biomonitors give quantitative or qualitative indications of pollution (Wolterbeek, 2002). For example, leaves have been studied to assess the effects of atmospheric pollution, and sensitive species can be used as biomonitors of trace elements (e.g., Tomăsevíc et al., 2011). The concentration of essential and non-essential elements in leaves provides information on the incidence of each element in the environment (Ugolini et al., 2013), since temporal trends in element accumulation of leaves can be consistent (Aničić et al., 2011). Bark also accumulates atmospheric particulate matter in its outermost surface (e.g., Catinon et al., 2011). However, the mechanisms and physical-chemical characteristics of the deposition of elements of atmospheric origin and their accumulation in bark remain poorly understood (Suzuki, 2006).

Tree rings have been used to monitor stress-induced changes in trees (Savard, 2010), providing signals of past pollution in areas where the monitoring of emissions has been short term or not existent (Ferretti et al., 2002). Tree rings provide information about chemical changes in the concentration of pollutants over time, as well as about how variations in atmospheric deposition affect some key physiological processes and how trees adapt under changing ecological conditions (Novak et al., 2007; Leonelli et al., 2012).

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Long-term tree-ring series represent useful archives, recording past environmental conditions and physiological responses, which are not always directly measurable in urban areas (Battipaglia et al., 2010). The analysis of tree rings for monitoring trace metal pollution is based on the element concentrations in the tree that, therefore, represents the temporal evolution of element availability in the environment in which the tree grows (Baes and McLaughlin, 1984; Padilla and Anderson, 2002; Doucet et al., 2012). Trees may accumulate anthropogenic pollutants either from the atmosphere, by deposition onto foliage and/or bark, and/or following deposition on the soil and subsequent uptake by roots. Pollutant-induced datable signals in tree rings can be useful to reconstruct environmental pollution over time.

Lichens have been widely used as bioaccumulators of trace elements, since their metabolism is very dependent on pollutant atmospheric exchanges and they are metal tolerant (Bargagli and Nimis, 2002). Lichens are able to accumulate trace elements in very high levels, and the concentrations of the trace elements inside the thalli of several lichen species seem to be directly correlated with the environmental levels of these elements (Paoli et al., 2013).

While several studies have focused on both lichens and mosses as bioaccumulators, which take up and retain metals differently (e.g., Szczepaniak and Biziuk, 2003; Giordano et al., 2013), few have used a multi-source approach. Some have compared lichens and bark (e.g., Santitoro et al., 2008) and lichens and leaves (e.g., Aprile et al., 2010), but none have considered combining lichen thalli and tree rings.

In this study, a novel approach to integrated biomonitoring has been developed that uses the chemical traits and ecophysiological processes in two different biomonitors, lichens and trees, in temporal and spatial combination. Bioaccumulation data on trace elements in leaves, bark, lichens and tree rings was used to evaluate the potential of this coordinated approach for monitoring the spatial and temporal impact of anthropogenic activities. Moreover, stable isotopes ratio of C, N and O in tree rings have been used as long-term and sensitive indicators of environmental factors, including pollutants, that might influence the plant functionality (e.g., Guerrieri et al., 2011). Changes in assimilation rate and stomatal conductance are detectable through the variation in stable isotope ratios of C, δ^{13}C, and O, δ^{18}O, respectively, from tree rings (Scheidegger et al., 2000; Saurer and Siegwolf, 2007). Whereas, NOx emissions and N deposition related to anthropogenic activities can affect N cycle, changing δ^{15}N signature in tree rings (Saurer et al., 2004).

The potential of the wood matrix to determine any possible dioxin contamination was also explored. Dioxins are known to be formed during the combustion of industrial and domestic waste, and to escape from incinerators into the environment via exhaust gases (Shibamoto et al., 2007). Tree rings may show environmental signals over time in reaction to different industrial activities, e.g., dioxin accumulation.

To test the validity of this monitoring protocol, hypothesizing that biomonitors would yield similar information, we gathered data on a line transect across an area affected by the emissions of a nearby incinerator plant. The aims of this study were: i) to assess whether pollutants are accumulated differently in lichens, leaves, bark and tree rings according to their specific sensitivities; ii) to determine whether the increasing distance from the incinerator plant affects pollutant accumulation profiles in biomonitors; iii) and to test whether temporal patterns recorded in biomonitors can be used to retrospectively reconstruct pollution disturbance (dendrochemistry for long-term interval, lichens, bark, and leaves as annual indicators).

2. Materials and methods

2.1. Site description

The study area is located in the Venafro Plain (Molise, Central Italy; 41°29′ N, 14°03′ E, 222 m a.s.l.). The climate is temperate sub-continental, with mean annual temperatures in the range of 10–14 °C and precipitation around 700 mm, N–NE and SW winds prevail throughout the year.

The local industry consists of about 30 small companies, which manufacture and process metals, chemicals, plastics, electronics and agri-food products, among other things, there is also a municipal solid waste incinerator, combined with an electric power plant for energy generation from waste burning. Data on the deposition of some airborne pollutants during the study period were available from ARPA Molise (2012): NO_2 deposition varied between 8 and 26 $\mu g\, m^{-3}$, SO_2 0.1–1.4 $\mu g\, m^{-3}$, PM_{10} 20–43 $mg\, m^{-3}$, NH_3 1–10 $\mu g\, m^{-3}$, As, Cd and Ni < 0.5 $ng\, m^{-3}$ and Hg < 0.001 $ng\, m^{-3}$.

The incinerator of Venafro, located NE of the town, has been in operation since 1997 until 2005. It mainly incinerated nut residues, mostly walnut, as well as residues from other fruit including those from olive oil production. The incinerator has diversified its activity in the last 15 years, and currently produces energy from the combustion of Refuse Derived Fuel (RDF).

The airborne emissions from the incinerator are continuously monitored instrumentally (http://www.energonut.it/emissioni_online.php). Punctual measurements of emissions since 2010 have consistently found values lower than the limits for pollutants specified in the national law (Italian Legislative Decree 133/2005).

2.2. Sampling

We aimed at detecting pollutant signals in different biomonitors, taking into account the different length of exposure in the environment, i.e., seasonal in leaves (sampled at the beginning and end of the vegetative season in 2012), a few years in lichen thalli (every 4 years), soil and bark (2012), and long term (several years) in wood. To compare the biomonitors, 2012 was considered a representative year for the completeness of available information, including on the deposition of airborne pollutants (ARPA). Three sampling plots were located at 380 m from each other along a transect from the incinerator to NE (Fig. 1) according to: (i) the localization of the solid waste incinerator, (ii) the dispersion model of PM_{10} (see Buonanno et al., 2010), assuming vegetation as the recorder of air pollution, (iii) the wind frequency distribution (data from: http://energonut.it), (iv) the horography and urbanization of the neighborhood, and (v) the two hilly chains bordering the area from NE to SW. Elevation ranges from 200 m in the lower area up to 900 m in the surrounding hills.

Soil samples (n = 3) in the form of three sub-samples per plot were collected. These were taken from the upper 5 cm of soil, the soil layer most sensitive to changes in atmospheric deposition (Augusto et al., 2010). The samples were sieved through a 2 mm screen, transferred to glass bottles to prevent adsorption by plastic, protected from sunlight and stored at 4 °C. The biological samples (leaves, bark, wood and lichen thalli) were collected from the same replicate trees (n = 3, each constituted by 6 sub-samples; n = 6 for dendrochemistry) in each plot. Healthy leaves of *Quercus pubescens*, the dominant trees in the local forest ecosystem, were collected in May at the beginning and in November at the end of the growing season 2012. Leaves were not washed in order to determine the total deposition, such as small particles incorporated into epidermal leaf structures and large agglomerates trapped onto the surface wax (e.g., Schreck et al., 2012).

Bark samples of *Q. pubescens*, in the form of a thin layer (<1 mm), were collected in three sub-samples at the end of June, after two

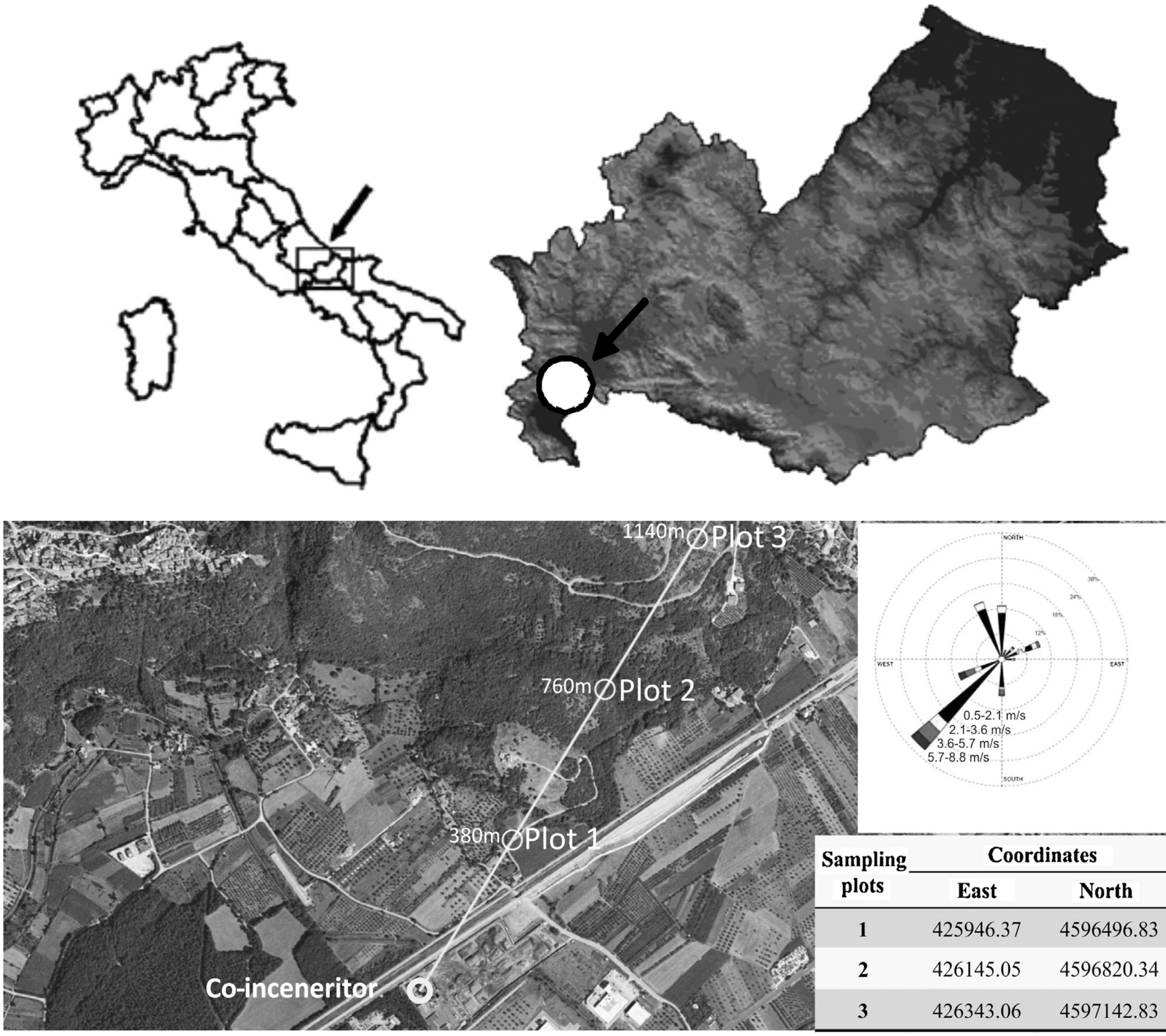

Fig. 1. Map of the study site showing the location of the sampling plots.

sunny weeks, at 130 cm above ground on the side of the stem facing the incinerator.

Two cores from six *Q. pubescens* trees in each plot were collected for dendrochemical analysis to detect the signature of the temporal evolution of pollution stress over long periods. To identify the effects of the incinerator activities on tree growth, we divided the dataset into three periods: prior to building the incinerator (1990–1996) (interval I); the period of biomass combustion (1997–2004) (interval II); the period in which RDF was burned (2005–2012) (interval III).

Thalli of the foliose lichen, *Xantoria parietina*, which is widespread in the surrounding area and which has been widely used in similar studies (e.g., Nimis et al., 2000; Brunialti and Frati, 2007) were collected annually in the period 2010–2013 at the end of June, after two sunny weeks, at 130 cm above ground. Lichens were collected on the side of the stem facing the incinerator (n = 3) in three sub-samples. Samples were not washed in order to measure the elements trapped onto the lichen surface (e.g., Lucadamo et al., 2015).

2.3. Chemical elements

The soil, leaf and bark samples were air-dried to avoid element volatilization, and ground to a fine powder, while lichens were air-dried in the lab and carefully cleaned under a binocular microscope to remove extraneous material deposited on the surface.

To determine any trace elements, samples were powdered and homogenized by grinding in a mill with Teflon balls, and about 150 mg were mineralized with high-quality, ultrapure HNO_3, for 8 h at 120 °C in a pressurized digestion system (Teflon bomb). Total trace element concentrations were determined for: Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, P, Pb, Sn and Zn.

The element concentrations in the mineralized and diluted solution (10 ml) were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, Perkin Elmer, model Plasma 400, Ontario, Canada) for Al, Mg and Mo, and P; by electrothermal atomic absorption spectrometry with Zeeman background correction (ZETAAS, Perkin Elmer, model 4100 ZL, Ontario, Canada) for Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn and Zn; and by AAS (Perkin-Elmer Analyst 100), using the cold vapour technique for As and Hg. The data were expressed on a dry weight basis (mg

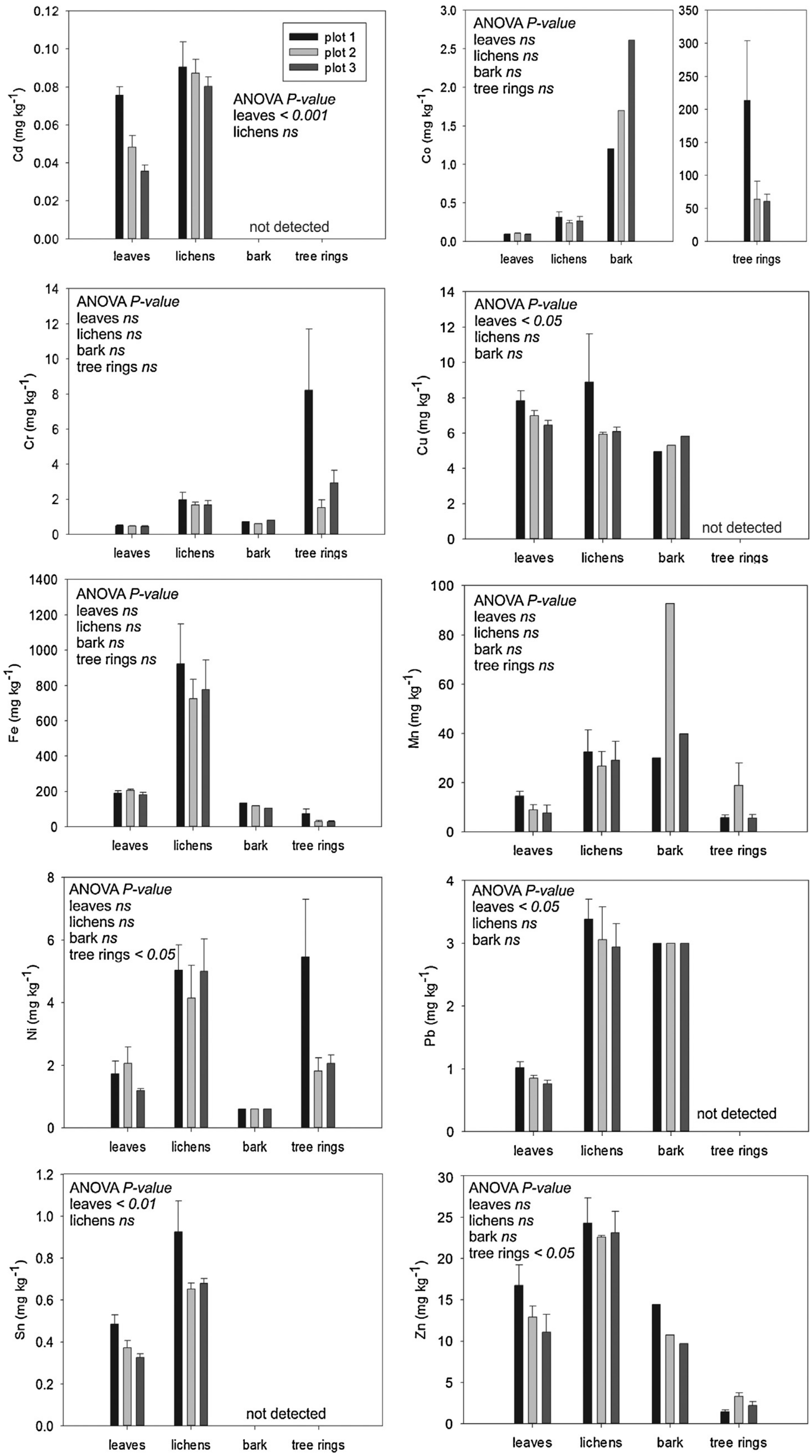

Fig. 2. Contents of trace elements in the leaves, lichens, bark and tree rings in the plots (mean values ± standard errors). One-way ANOVA was applied to measure significant differences between plots (p-level values are given).

Table 1
Pearson's correlation between elements in leaves measured in 2012 (n = 3) (sampling of November) in each plot (correlation coefficients, R higher than 0.54 that are significant at the 95 confidence level, are provided in bold and italic).

Plot 1	Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sn	Zn
Al	–												
As	0.30	–											
Cd	0.40	0.17	–										
Co	***0.74***	***0.56***	0.11	–									
Cr	0.10	0.02	0.04	0.35	–								
Cu	0.12	0.52	***0.87***	***0.91***	0.45	–							
Fe	0.31	0.10	0.02	0.03	0.03	***0.67***	–						
Hg	0.50	0.20	0.02	0.43	0.06	***0.91***	0.02	–					
Mn	***0.74***	0.41	0.04	0.16	0.19	***0.98***	0.08	0.05	–				
Ni	0.13	0.26	***0.96***	***0.96***	0.33	0.02	0.53	***0.92***	***0.78***	–			
Pb	***0.74***	0.08	0.38	***0.58***	0.34	***0.77***	0.31	0.46	0.43	***0.84***	–		
Sn	0.29	***0.94***	***0.76***	***0.63***	***0.87***	0.25	***0.74***	0.31	0.43	0.12	0.53	–	
Zn	0.05	0.35	***0.85***	***0.95***	0.25	0.00	0.47	***0.72***	***0.83***	0.02	***0.90***	0.29	–
Plot 2	Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sn	Zn
Al	–												
As	0.36	–											
Cd	0.16	0.02	–										
Co	***0.68***	0.39	0.43	–									
Cr	0.07	0.02	0.02	0.51	–								
Cu	0.23	***0.94***	***0.87***	***0.56***	***0.57***	–							
Fe	***0.68***	***0.67***	***0.62***	0.00	***0.75***	0.48	–						
Hg	0.04	***0.83***	***0.61***	***0.52***	0.23	0.06	0.42	–					
Mn	0.01	0.39	0.18	0.33	0.16	0.25	0.29	0.07	–				
Ni	0.23	0.09	0.19	0.05	0.10	0.32	0.11	0.27	0.12	–			
Pb	0.13	***0.56***	0.19	0.15	0.36	***0.56***	0.10	0.17	0.04	0.23	–		
Sn	***0.63***	***0.85***	0.47	0.24	***0.56***	0.51	0.19	***0.95***	***0.92***	0.41	***0.98***	–	
Zn	***0.79***	***0.57***	***0.61***	0.17	***0.66***	***0.96***	0.27	***0.94***	***0.86***	0.50	***0.59***	***0.91***	–
Plot 3	Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sn	Zn
Al	–												
As	***0.91***	–											
Cd	0.08	***0.98***	–										
Co	***0.54***	0.17	0.52	–									
Cr	***0.88***	0.08	***0.91***	0.40	–								
Cu	0.15	***0.73***	***0.85***	***0.69***	***0.78***	–							
Fe	0.46	0.05	0.77	0.04	0.16	***0.73***	–						
Hg	not detectable												
Mn	0.14	***0.60***	***0.81***	***0.67***	***0.60***	0.09	***0.56***	–	–				
Ni	0.50	0.07	0.50	0.35	0.01	0.52	0.19	–	0.55	–			
Pb	***0.68***	0.09	***0.75***	0.46	0.33	0.34	0.06	–	***0.63***	0.35	–		
Sn	0.48	0.37	0.13	***0.57***	***0.87***	***0.96***	0.39	–	***0.74***	0.47	0.19	–	
Zn	0.29	***0.60***	***0.86***	0.53	***0.77***	0.07	0.15	–	0.06	***0.81***	0.06	0.30	–

per kg dry weight). The procedure was validated with reference materials of lichens (IAEA 336) and poplar leaves (GBW 07604). The certified values are reported in the Supplementary material. The values obtained with the proposed method are consistent with those certified, within the uncertainty of the measurements.

2.4. Stable isotopes

Tree-ring wood samples were milled in a centrifugal mill (ZM 100, Retsch, Germany), and <0.05 mm (in diameter) of wood powder for each year was put in porous Teflon envelops and the cellulose extracted according to the Loader et al. (1997) method. Finally, 0.6–0.8 mg of cellulose were weighed for isotope analysis and placed in tin and silver capsules.

The samples were analyzed by combustion in an elemental-analyzer connected in continuous-flow mode to an isotope-ratio mass-spectrometer (Delta-S, Finnigan, Bremen, Germany). The isotope concentrations of $\delta^{15}N$, $\delta^{18}O$ and $\delta^{13}C$, were measured according to the procedure described in Saurer et al. (2004). The carbon isotope records were corrected for anthropogenically induced changes in the $\delta^{13}C$ values in the atmosphere due to increasing in CO_2 concentration (the "^{13}C Suess effect") using the annual records of past atmospheric $\delta^{13}C$ obtained from ice cores (Francey et al., 1999).

2.5. Dioxins

The dioxin analyses were carried out in wood samples, where the matrix is exposed longer to local conditions and is suitable for split analysis per year in relation to the period of incinerator activity. Before the dioxin analysis, the wood samples, corresponding to the rings formed between 1997 and 2012, were dried and ground into fine particles and passed through a 20-mesh sieve. PCDDs and PCDFs were analyzed using high-resolution gas chromatography, coupled with high-resolution mass spectrometry (HRGC-HRMS, Thermo Electron GmbH, Bremen, Germany), using an in-house adaptation of the USEPA Method 1613 (1994). The PCDD/Fs were analyzed at the Chelab Lab-Testing and Analytical Services in Resana (Treviso, Italy), which has an accredited process to determine the amount of dioxin species, according to the UNI CEI EN ISO/IEC 17025:2005 standard. The cumulative concentrations of PCDDs and PCDFs were expressed as dioxin toxicity equivalents (TEQ), calculated according to the WHO-TEF 2005 (Van den Berg et al., 2006).

2.6. Naturalness analysis

The level of naturalness is defined as the degree of self-functioning of the natural processes and the intensity of human interventions on the function and structure of ecosystems (MCPFE,

Table 2
Pearson's correlation between elements in lichens measured in 2012 in each plot (n = 3) (correlation coefficients, R higher than 0.54 that are significant at the 95 confidence level, are provided in bold and italic).

Plot 1	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sn	Zn
As	–											
Cd	***0.74***	–										
Co	0.09	***0.65***	–									
Cr	0.05	***0.79***	0.14	–								
Cu	***0.77***	0.03	***0.68***	***0.82***	–							
Fe	0.02	***0.72***	0.07	0.06	***0.76***	–						
Hg	0.26	***0.99***	0.36	0.22	***0.96***	0.28	–					
Mn	0.26	***1.00***	0.36	0.22	***0.96***	0.28	0.00	–				
Ni	***0.76***	0.50	***0.85***	***0.71***	0.47	***0.78***	0.50	0.50	–			
Pb	0.50	0.24	0.41	***0.55***	0.27	0.48	***0.77***	***0.76***	***0.74***	–		
Sn	***0.96***	0.30	***0.95***	***0.91***	0.27	***0.98***	***0.69***	***0.70***	0.20	***0.54***	–	
Zn	0.09	***0.65***	0.00	0.14	***0.68***	0.08	0.36	0.36	***0.85***	0.41	***0.95***	–
Plot 2	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sn	Zn
As	–											
Cd	0.12	–										
Co	0.39	0.51	–									
Cr	0.47	***0.59***	0.08	–								
Cu	0.18	0.06	***0.56***	***0.64***	–							
Fe	0.41	0.53	0.03	0.05	***0.59***	–						
Hg	***0.74***	***0.62***	***0.87***	***0.79***	***0.57***	***0.84***	–					
Mn	0.02	0.10	0.41	0.49	0.15	0.44	***0.72***	–				
Ni	0.01	0.11	0.40	0.48	0.16	0.43	***0.73***	0.01	–			
Pb	***0.92***	***0.80***	***0.70***	***0.62***	***0.74***	***0.67***	0.17	***0.89***	***0.90***	–		
Sn	0.44	0.32	***0.82***	***0.90***	0.26	***0.85***	0.31	0.41	0.42	0.48	–	
Zn	0.32	0.20	0.71	***0.79***	0.14	***0.73***	0.42	0.30	0.31	***0.60***	0.12	–
Plot 3	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sn	Zn
As	–											
Cd	***0.77***	–										
Co	0.11	***0.88***	–									
Cr	0.36	***0.88***	0.24	–								
Cu	***0.93***	0.17	***0.96***	***0.71***	–							
Fe	0.18	***0.95***	0.07	0.18	***0.89***	–						
Hg	0.02	***0.79***	0.09	0.33	***0.96***	0.16	–					
Mn	0.07	***0.83***	0.04	0.29	***1.00***	0.11	0.05	–				
Ni	0.40	0.36	0.51	***0.76***	0.53	***0.58***	0.43	0.47	–			
Pb	***0.99***	0.24	***0.88***	***0.63***	0.08	***0.81***	***0.97***	***0.92***	***0.61***	–		
Sn	0.49	***0.74***	0.38	0.14	***0.57***	0.31	0.47	0.43	***0.90***	0.50	–	
Zn	0.22	***0.98***	0.10	0.14	***0.85***	0.04	0.19	0.15	***0.62***	***0.77***	0.28	–

2002). Broadly, naturalness can be considered equivalent to environmental quality, and can be measured using specific indicators related to vegetation composition and structure (Petriccione, 2006).

For this study, the interpretative scale of deviation from naturalness proposed by Bargagli and Nimis (2002) was adopted to determine the concentrations of trace elements in lichens, and thus the level of air pollution. For each metal, the degree of deviation (alteration) from background conditions (naturalness) was taken into account. The changes over the sampling period were estimated through a seven-class scale, based on the percentile distribution of several hundred measurements of metal concentrations ($\mu g\, g^{-1}$ dry weight) carried out in foliose lichens throughout Italy (Nimis et al., 2000). Each step of the scale corresponds to a definition of naturality/alteration such that: class 1 refers to "near-natural" (very high naturalness); class 2 to "very natural" (high naturalness); class 3 to "natural" (middle naturalness); class 4 to "not very natural/altered" (low naturalness/alteration); class 5 to "slightly altered" (middle alteration); class 6 to "very altered" (high alteration); class 7 to "altered" (very high alteration). The ratio between the concentrations of each element in the samples collected in 2011 (period I), 2012 (period II) and 2013 (period III) to those collected in 2010 were calculated and then the scale of accumulation/loss proposed by Frati et al. (2005) was used.

2.7. Statistical analysis

The distribution of each population was tested using the Kolmogorov–Smirnov normality test (Razali, 2011). Since it was positive, parametric comparison methods could be used (Razali, 2011). Differences in the trace element concentrations between the plots and at different temporal intervals were analyzed using ANOVA with the statistical package Statistica (StafSoft Inc., Tulsa, OK, USA). The means were compared using the least significant difference (LSD) test. Correlations between the elements in the lichens and the leaves were calculated using the simple Pearson correlation coefficient. To uncover the relationships among trends in different variables and investigate comprehensive pollution patterns, principal component analyses (PCA) were applied to trace elements and stable isotopes, using the package PAST (Harper and Ryan, 2001). PCA is an ordination technique in which the multivariate dataset is projected onto several dimensions, generally two, defined by the axes of maximal variance (Hammer and Harper, 2006). The principal components with eigenvalues greater than 1.0 were retained.

3. Results

3.1. Spatial variation

Trace element concentrations in the soils of the three plots were below the maximum concentrations observed in polluted conditions (Italian Legislative Decree 152/2006). The trace elements in the soil were: As 12.9 ± 0.3 ppb, Cd 0.8 ± 0.1 ppb, Co 13.5 ± 0.2 ppm, Cr 39.5 ± 0.4 ppb, Cu 36.2 ± 0.6 ppb, Hg 0.4 ± 0.0 ppb, Mn 576.4 ± 7.2 ppb, Ni 33.9 ± 0.4 ppb, Pb 44.2 ± 0.6 ppb, Sn 2.8 ± 0.1 ppb and Zn 124.2 ± 1.0 ppb (mean values for the 3 plots).

The trace element contents in the sampled leaves differed significantly between the plots. The contents were higher in plot 1 than in plot 3 for Cd ($P < 0.001$), Cu ($P < 0.05$), Pb ($P < 0.05$) and Sn ($P < 0.01$). In contrast, the trace element contents in lichens and bark did not differ significantly between the plots (Fig. 2). The trace elements in tree rings were higher for Ni in plot 1 ($P < 0.05$) and for Zn in plot 2 ($P < 0.05$) than in the other plots (Fig. 2).

A correlation analysis for the trace elements in leaves (Table 1) and lichens (Table 2) highlighted good correspondences ($n = 3$, each constituted by 6 sub-samples, from 3 plots) ($R > 0.8$) between the elements in each plot.

The ordination diagram (I and II components), resulting from PCA applied to biomonitors (leaves, lichens and tree rings), was grouped in three plots (plot 1, plot 2 and plot 3) for the spatial scale (Fig. 3a), and in sampling periods (seasonal for leaves, annual for lichens and several years for tree rings), for the temporal scale (Fig. 3b). For the spatial scale analysis (Fig. 3a), the first component accounted for over 50% and the second for over 30%. For example, Al and Mn were related to leaves and bark, Cr and Co to tree rings, and Cd, Fe, Ni, Pb and Zn to lichens (Fig. 3a). For the temporal scale analysis (Fig. 3b), the first and second components accounted for 52% and 28% of the total variance, respectively. The temporal scale showed biomonitor-specific trace element patterns (Fig. 3b).

The values of $\delta^{15}N$ were higher in plot 3 (from −1.9, III interval, to 17‰, I interval) than in plot 2 (from 3, II interval, to 6.3‰, III interval) and plot 1 (from −1.6, I interval, to 0.2‰, II interval). The values of $\delta^{13}C$ and $\delta^{18}O$ showed the same trend in all plots: $\delta^{13}C$ ranged from −25.1‰ in plot 1, to −25.2‰ in plot 3 and to −26.3‰ in plot 2; $\delta^{18}O$ values ranged from 28.2‰ in plot 1, to 28.1‰ in plot 3–25.9‰ in plot 2. They remained the same throughout the selected periods of incinerator activity.

3.2. Temporal variation

The temporal variation of the elements was analyzed at three different time scales. On a seasonal basis, Al, Cd and Co contents were significantly higher in leaves collected in November than in May, with Al and Cd concentrations below the detection thresholds in May (changes on a seasonal basis, 2012). The Cu and Zn contents were higher in May than in November. Arsenic, Cr, Fe, Hg, Mn, Ni and Pb did not vary throughout the year (Fig. 4).

Changes detected over the years 2010–2013 (short-term) were found in the content of trace elements in lichen thalli, with significant differences between years for As, Cd, Cr, Cu, Ni, Sn and Zn (Fig. 5).

High percentages of trace element concentrations were included in naturalness class 1 (44%—near-natural) and 2 (27%—very natural). Classes 2 and 3 had similar values (14% vs. 11%) with occasional cases of middle (3%) or high (1%) alteration, in particular for Ni in the last two years (Table 3). The EC ratios (Frati et al., 2005) for the twelve elements are given in Table 4. A natural fluctuation in trace element concentrations (±0.25) was expected in lichens (Loppi et al., 2000), highlighting element accumulation with values ranging between 1.25 and 1.75, severe accumulation with values >1.75, and lack of substantial accumulation with values ranging between 0.25 and 0.75. The Fe, As and Pb contents remained constant during the 4 years; whereas those of Cr, Mn and Co showed accumulation, and Cd and Hg a strong accumulation. The Cu and Sn concentrations remained stable and Zn decreased, as was also observed during the periods II and III.

Table 3
Naturalness classes show the levels of naturalness/alteration, measured as the deviation from the 98° percentile among the distributions with respect to concentrations found in Italy (Nimis et al., 2000). Class 1: very high naturalness; class 2: high naturalness; class 3: middle naturalness; class 4: low naturalness/alteration; class 5: middle alteration; class 6: high alteration. No information is available for Sn.

Year	Plot	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
2010	1	2	1	1	2	1	3	1	2	3	1	3
	2	2	1	1	2	1	4	1	2	3	2	3
	3	2	1	1	2	1	4	1	2	3	1	3
2011	1	2	1	1	2	1	4	1	2	5	2	2
	2	2	1	1	2	1	4	1	2	4	1	3
	3	2	1	1	2	1	4	1	2	4	1	2
2012	1	2	1	1	2	2	4	1	3	5	1	1
	2	2	1	1	2	1	3	1	3	4	1	1
	3	2	1	1	2	1	3	1	3	5	1	1
2013	1	2	1	1	3	1	5	2	2	6	2	1
	2	2	2	1	3	1	4	1	4	4	1	1
	3	2	2	1	3	1	4	4	3	4	2	2

The changes in trace element contents during the whole period of activity of the incineration plant (1990–2012) were recorded in the tree rings, but did not differ significantly in the three different periods of activity (Fig. 6).

Applying PCA to the tree ring data from the three plots and periods of investigation (1990–1996, 1997–2004, 2005–2012) resulted in the ordination diagram (I and II components) shown in Fig. 7. The biplot was used, so both objects (grouped plot and years) and variables (vectors: trace elements, Fig. 7a; isotopes, Fig. 7b) were represented in the same best plane, defined by the first two components, explaining the maximum variance accounted for in the data. The first and second components accounted for 77% and 16% of the total variance, respectively (Fig. 7a). The analysis showed correlations between Mn and Zn in plot 2 from 1997 to 2012; between Al, Co, Cr, Fe, and Ni in plot 1 from 1990 to 2004; and P in plot 2 from 1990 to 1996 and in plot 3 from 1990 to 2012 (Fig. 7a). The first and second components accounted for 63% and 32% of the total variance (Fig. 7b). The first and second components of the isotope ratios were mainly determined by $\delta^{13}C$ and $\delta^{18}O$, correlation with plot 1 in the three periods considered (Fig. 7b). The third component ($\delta^{15}N$) had a less explicit gradient, and was related to plot 3 from 1990 to 2012.

The quantities of dioxins and dioxin-like compounds were very low in the wood matrix (1997–2012). Octachlorodibenzo-*p*-dioxin (octaCDD) values were always below 0.005 $\mu g\,kg^{-1}$, and octachlorodibenzofuran (OctaCDF) below 0.004 $\mu g\,kg^{-1}$. The values of equivalent toxicity (TEQ) were zero in all plots. The analysis was not carried out in the other matrices because they were not exposed for more than a season to the incinerator emissions.

4. Discussion

The air pollution in the study site was low and the levels of naturalness remained unaltered throughout the period 1997–2012, as Paoli et al. (2015) also found for the same area. They recorded the main pollutants of atmospheric origin in transplanted lichens, and directly measured airborne pollutants. The present study was intended to detect common environmental signals in different biomonitors. It is assumed that airborne pollutants in the tree bark, leaves, and lichens, and inside trunk wood would have come from the particulate matter produced at the nearby incinerator (and other pollution sources). Tree bark and leaves, and lichens, exposed directly to airborne pollutants could incorporate both dry and wet deposition of particulate. Airborne particles could, in principle, pass through outer tissues into tree rings, where they might be retained, concentrating into xylem tissues. Encapsulation following deposi-

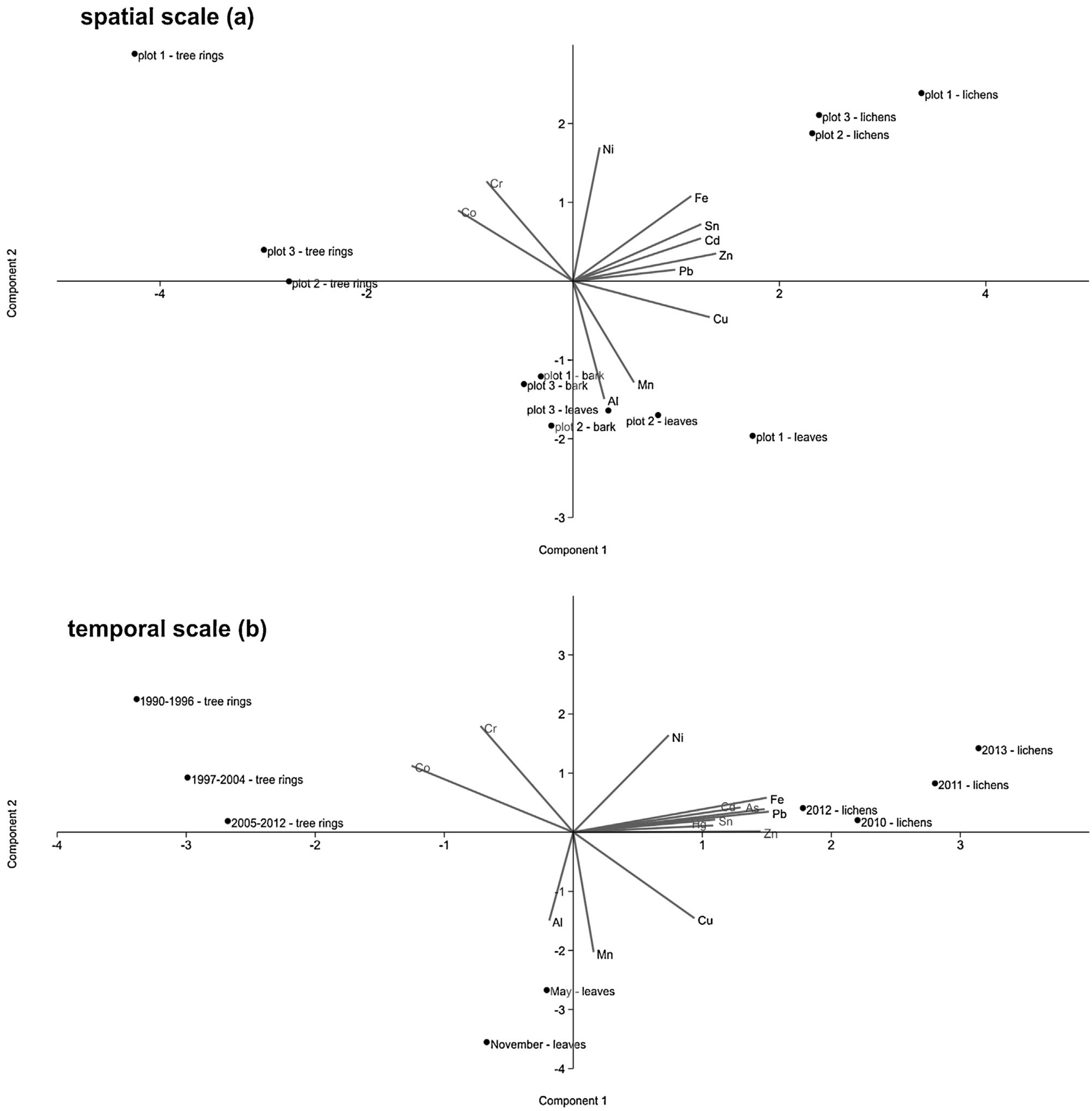

Fig. 3. Biplot diagrams from the Principal Component Analysis on a spatial scale (matrices in each plot) (a) and a temporal scale (time defined by matrix signal) (b). The biplot in the first and second component planes is shown with the elements (dots) and parameters (vectors) measured. Each dot indicates the average for the 3 samples.

tion of air pollutants onto tree outer tissues (foliage and/or bark), and uptake of soil contaminants by tree roots, or a combination of both might act as intake pathways (Anderson et al., 2000; Zhang et al., 2008). Overall, the signals of disturbance detected in lichens, leaves, bark and tree rings were spatially and temporally small, and the soil and naturalness analyses confirmed that the study area was poorly contaminated with trace elements.

Case-studies associated with *X. parietina* in situ sampling also found similar trace element concentrations in other areas (Nimis et al., 2000). The concentrations of Ni and Zn were comparable to those reported for industrial areas in Adriatic Italy, while those of Cd, Cr and Cu were lower (Brunialti and Frati, 2007). The concentrations of Cd, Cr, Cu, Pb and Zn were below those found with similar biomonitoring in Tuscany (central Italy) around a municipal solid

Table 4
EC Ratio of trace element concentrations in thalli of *X. parietina* collected in 2011 (I period), 2012 (II period) and 2013 (III period) to those collected in 2010. Accumulation (1.25–1.75) is highlighted in italic, severe accumulation in italic and bold (>1.75), a loss (0.25–0.75) in bold. Data refer to Fig. 4.

Year	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sn	Zn
I	1.03	0.93	1.16	1.24	*1.28*	1.21	1.00	1.08	*1.62*	0.83	***4.27***	0.93
II	0.83	0.99	1.08	0.98	*1.45*	0.96	***4.19***	*1.39*	*1.70*	0.82	1.11	**0.51**
III	0.78	***2.63***	*1.26*	*1.68*	0.85	1.23	***13.11***	*1.38*	*1.77*	0.96	1.16	**0.68**

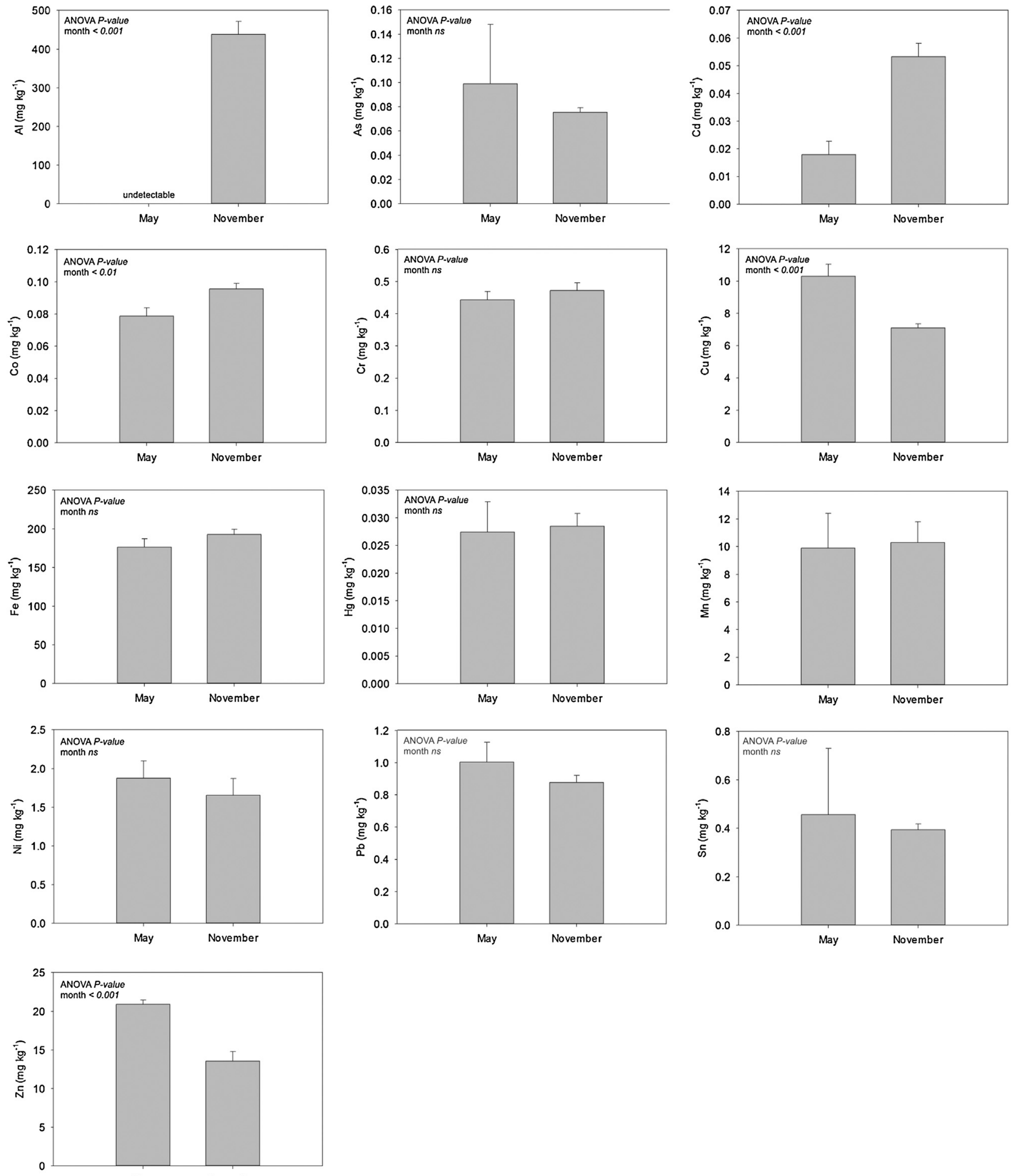

Fig. 4. Contents of trace elements in leaves measured in May and November 2012 (mean values ± standard errors). One-way ANOVA was applied to measure significant differences between months (p-level values are given).

waste incinerator. There, the biomonitor used was the foliose lichen *Flavoparmelia caperata* (L.) Hale (Loppi et al., 2000). The mercury content was within the range of 0.2–0.38 $\mu g\, g^{-1}$ and lower than that found in areas with hot springs and fumaroles (e.g., Loppi and Bonini, 2000), even though sulfur spring water has been found near the study area (Del Prete et al., 2008).

The concentrations of the trace elements Co and Mn in the leaves were comparable to those found in conditions of heavy traffic and industrial activities (Lorenzini et al., 2006). However, the Cr, Cu, Fe, Pb and Zn concentrations were lower than those in polluted urban areas (Aničić et al., 2011). In the bark, the Cr, Co, Fe, Ni and Zn contents were lower than those reported by Guéguen et al. (2011) for sites exposed to various anthropogenic emissions. The

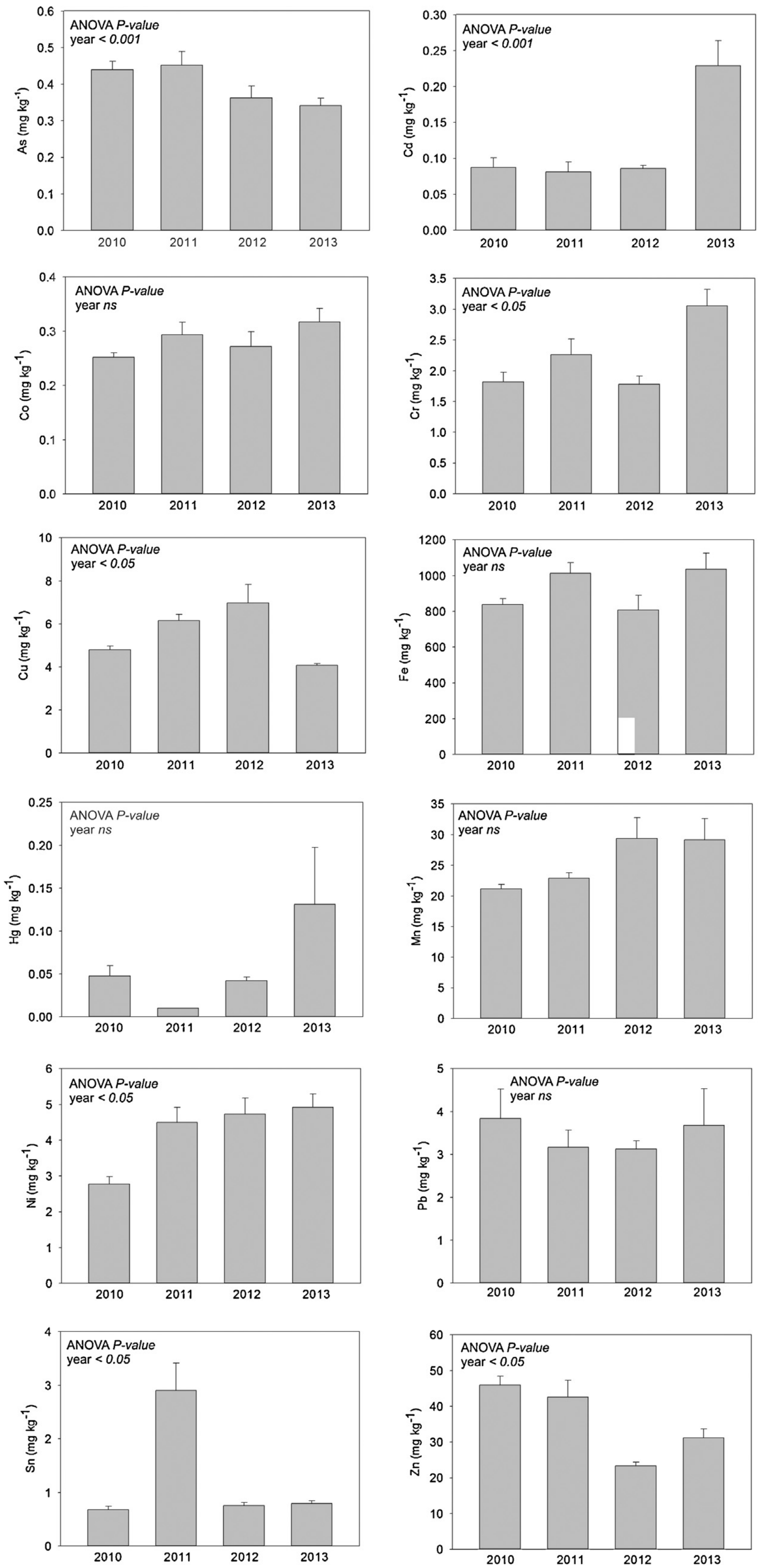

Fig. 5. Contents of trace elements in lichens measured in 2010, 2011, 2012 and 2013 (mean values ± standard errors). One-way ANOVA was applied to measure significant differences between years (p-level values are given).

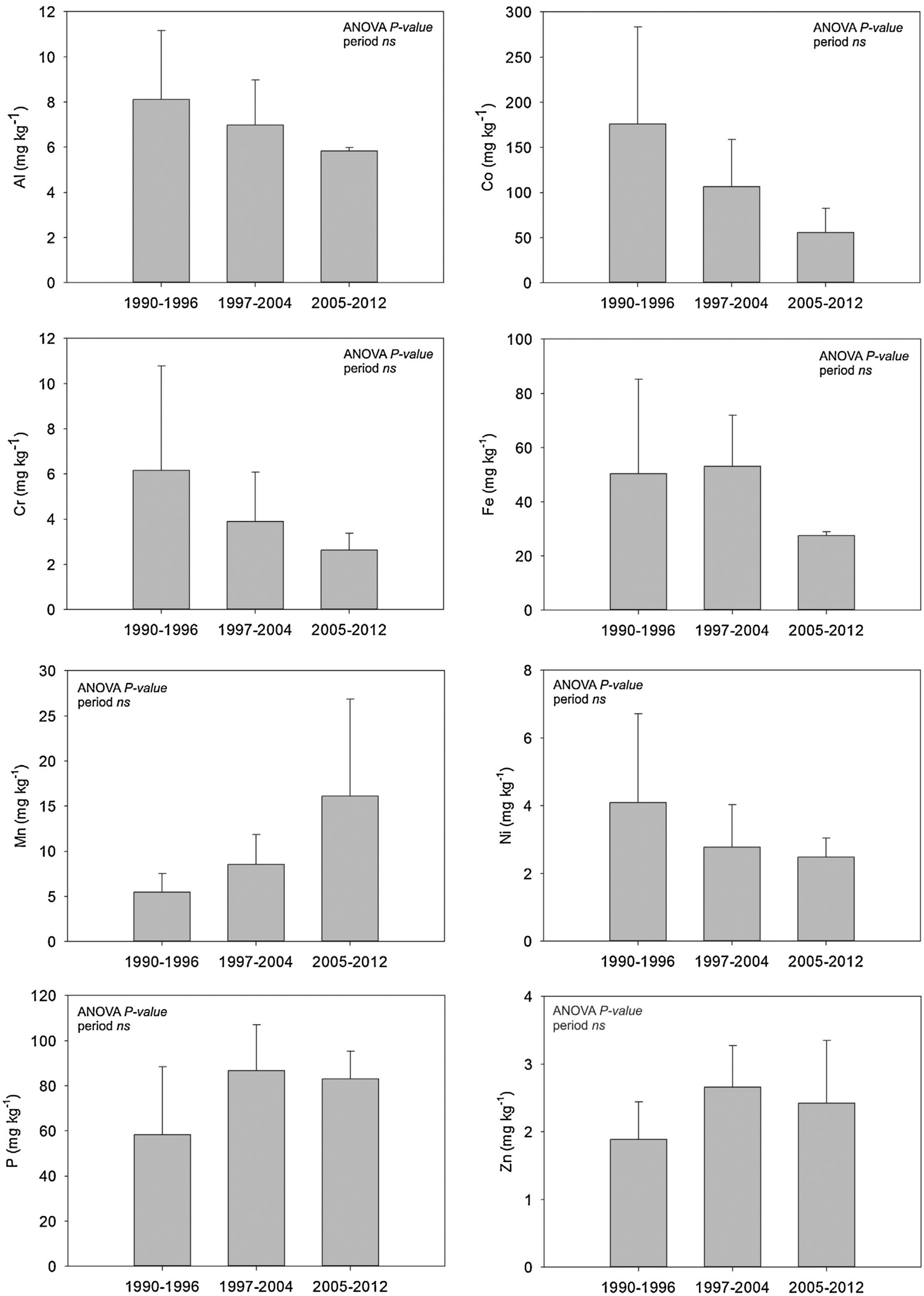

Fig. 6. Contents of trace elements in tree rings formed in periods 1990–1996, 1997–2004, and 2005–2012 (mean values ± standard errors). One-way ANOVA was applied to measure significant differences between periods (p-level values are given).

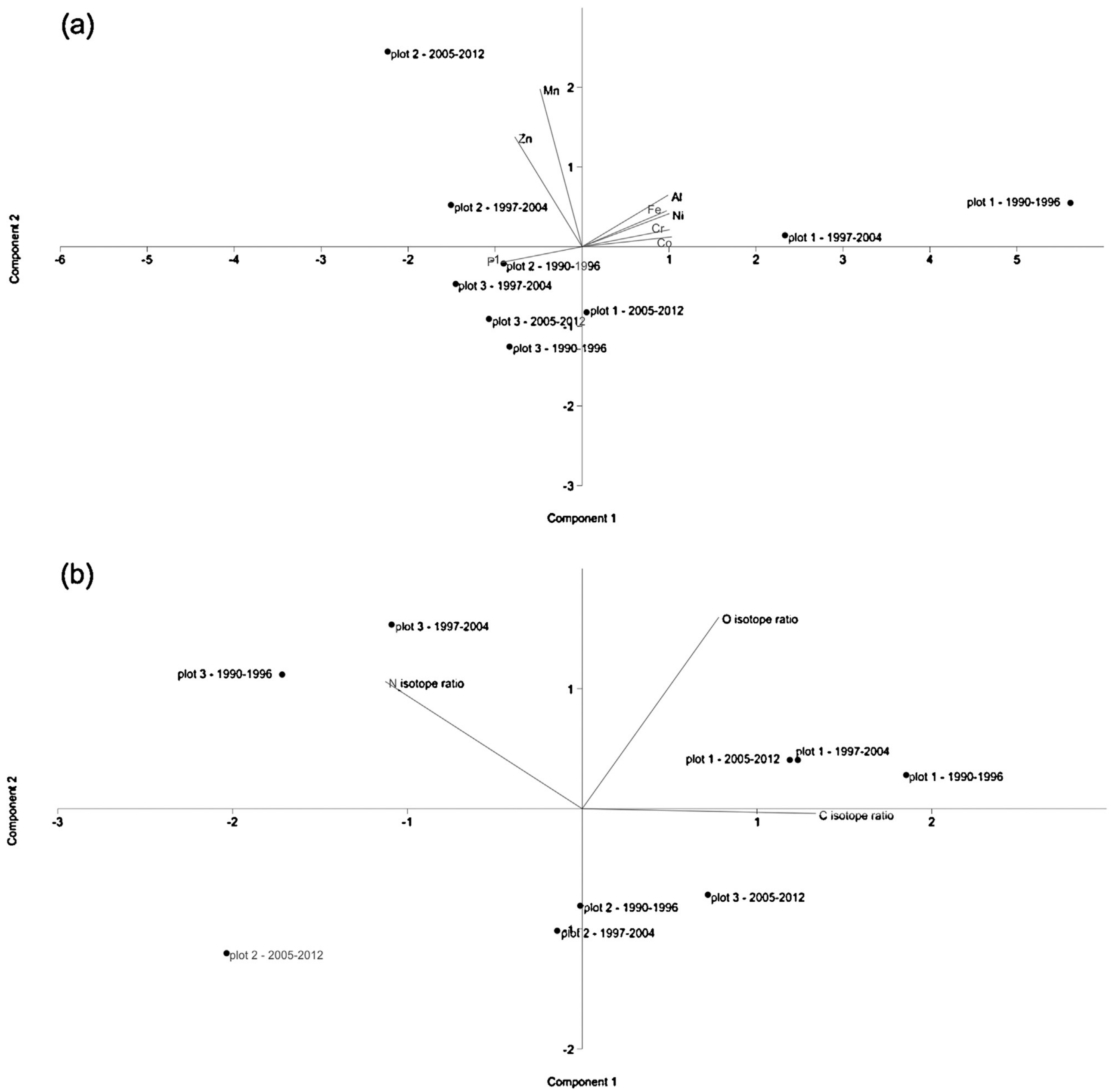

Fig. 7. Biplot diagrams from the Principal Component Analysis of the trace elements (a) and isotopes ratios (b) per plot and in the different activity periods (1990–1996, 1997–2004, 2005–2012). The PCA was applied to the three periods for each plot, and the resulting biplots in the first and second component planes show the periods (dots) and parameters (vectors) measured. Each dot indicates the average for the 3 samples.

concentrations of other elements were below the thresholds found in analogous studies (Catinon et al., 2011). Higher contents of Cd, Fe, Pb, Sn and Zn were in lichens than in leaves, bark and tree rings (Fig. 2), confirming the attitude of lichens to accumulate higher levels of pollutants (Aprile et al., 2010).

The retrospective analysis showed that none of the signals of dioxins and dioxin-like compounds in the wood of *Q. pubescens* were permanent for the whole activity period of the incinerator. The traces obtained were probably the result of noise around the detection limits of the analytical method and there are no reasons for concern. Nevertheless, the incineration of RDF could be a potential source of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in the environment (e.g., Ren and Zheng, 2009), and long-term monitoring is a wise precaution. The dioxin contents in plants may be closely related to the environments in which the plants grow (Jou et al., 2007; Gworek et al., 2013). Potentially, they could accumulate in tree rings (Padilla and Anderson, 2002), but biomonitoring programs have rarely attempted to detect this pollutant in tree rings.

4.1. Spatial variation

Although the level of trace elements found in the biomonitors in the study area were below the official thresholds for pollution, the correlation between the elements indicated that their sources were the same. The unclear spatial distribution (Fig. 3a) was probably due to the relatively low pollution levels and various pollution sources. The differences in the single environmental signals induced by each pollutant were related to distribution processes, and the origin and history of the emission. Heavy elements can have various anthropogenic sources, including the chemical industry, steel works and waste incineration. The area is crossed by heavy traffic and surrounded by intensive agriculture, which might potentially bias or confound the studied effects of pollutants on trees and lichens.

Correlations between elements were interpreted as signals induced by common sources. For instance, the correlations found in leaves between Cd and Ni, Hg and Ni, and Ni and Pb in plot 1 could be due to incinerator activities, but Hg might also be a signal of natural sulfur emission. Moreover, the correlations in leaves between Al and Cr in plot 3 could be linked to industrial activities, while the correlations between Co and Cu, and Co and Zn in plot 1 may have been artificially increased by process wastes and solid-phase wastes. Several of the correlations, e.g., Cr and Sn, Ni and Pb, Pb and Zn in plot 1; Cu and Zn, Pb and Sn, Sn and Zn in plot 2; as well as Cu and Sn in plot 3 (Table 1) could arise from vehicular traffic emissions, whereas that between As and Cu in plot 2 may be due to agricultural processes (Guéguen et al., 2011; Ali et al., 2013). Weak signals from waste processes were found in the leaves in plot 1 for Co-Zn and plot 2 for Hg-Zn, plot 2 for Hg-Zn, and plots 1 and 2 for Cd-Zn. Traffic could influence the correlation signals for Cr-Sn and Ni-Pb in plot 1, Pb-Sn in plot 2, and Cr-Sn in plot 3, whereas agricultural activities may explain the correlation signals for Cu-Pb in plot 1.

In lichens, the positive correlations between Pb and Hg in plot 1, Cd and Pb in plot 2, and Cd and Hg in plots 1 and 3 (Table 2) could be due to waste incinerator fallout, while the high correlations between Cr-Ni, Fe-Cd and Pb-Ni could be due to the re-suspension of street dust caused by traffic. Heterogeneous sources of pollutants do not always result in a positive correlation between trace elements, since intermediary compounds may be involved in the pathways for the biosynthesis of different secondary plant metabolites (e.g., Rossini Oliva et al., 2009). The unclear distribution of pollutants in relation to the industrial origin, and the sparse industrial activities with several potential pollutant sources, though at low accumulation potential, was reflected in the lack of high concentration of trace elements in the study area, as detected by these biomonitors.

4.2. Temporal variation

The temporal patterns of environmental disturbance were reconstructed by analyzing the time of exposure of the biomonitors studied. Indications of trace element deposition during the vegetative season were determined by sampling the leaves at different times of year. Leaves sampled in May were newly formed and tended to have high accumulations of mainly Cu and Zn; whereas leaves sampled in November had been exposed to the local environment for six months, and tended to have relatively high contents of Al, Cd and Co. Some trace elements may be actively translocated from leaves to other plant compartments if they are microelements useful for the plant's metabolism but a larger portion of non-essential or toxic metals are sequestered into the vacuole of leaf cells (Peng and Gong, 2014).

The trace elements in lichen thalli, which were used to track differences in 2010–2013, generally altered little. In a few cases they remained rather constant (e.g., Ni), but Sn accumulated markedly in 2010, and Cd and Hg in 2013. This pattern requires further investigation. The two highly correlated elements involved could be affected by the incinerator activity. Mercury showed a progressive accumulation and seems to be the best tracer for air pollution caused by the incineration plant.

The PCA analysis for the tree rings revealed a clear grouping of the trace element accumulation in each plot and in accordance with the activity period of the incinerator (Fig. 7). The first group contained Al, Co, Cr, Fe and Ni, the second Mn and Zn, and the third P. This suggests that sources and periods of pollution were different. The first group of elements showed a preferential accumulation in tree rings formed in the interval from 1990 to 2004, i.e. before RDF combustion started, in trees grown in plot 1. The correlation between these elements might be due to the concurrent effect of traffic and industrial activities in the plot located near the incinerator. The Al and Co accumulation may be caused by chemical factories and incinerators, and that of Fe, Cr and Ni by traffic (Ali et al., 2013). The second group showed a preferential accumulation of Mn and Zn (stemming from chemical industries) in the tree rings from plot 2 in the tree growth period from 1997 to 2012. Moreover, in plot 2 (from 1990 to 1996) and in plot 3, some P was found near the industrial area, which was independent of the activity period of the incinerator, which could be associated with livestock.

The spatial and temporal (long-term) patterns found for P and $\delta^{15}N$ in tree rings suggest the most probable origin of the signals is the grazing livestock rather than industrial activities. The tree-ring $\delta^{15}N$ showed an imprint of N deposition in plot 3, especially in the periods 1990–1996 and 1997–2004. No effect on the tree-ring $\delta^{15}N$ signatures that could be attributed to industrial activities was observed in plot 1. Enriched $\delta^{15}N$ in tree rings is a signal of N accumulation, reflecting changes in soil and canopy processes (Guerrieri et al., 2011). $\delta^{13}C$ and $\delta^{18}O$ highlighted an effect on plant physiology in plot 1 for the whole period considered. The $\delta^{13}C$ and $\delta^{18}O$ values in the tree rings is probably indicative of airborne particle deposition, highlighting diverse responses to growth conditions that modify the tree physiological functions, in particular their stomatal function, carboxylation efficiency, photosynthetic rate, respiration rate, and the water uptake by the root system (Savard, 2010). No correlation between $\delta^{13}C$ and $\delta^{18}O$ was detected throughout the whole study period. Thus the $\delta^{13}C$ signal in plot 1 may be due to the proximity of residential areas rather than to incinerator-induced environmental pollution.

5. Conclusion

The study did not show straightforward changes over time and space of pollutant accumulation in biomonitors, which showed specific pollutant accumulation patterns. Pollutants were prevalently intercepted and collected by biomonitors (tree bark and leaves, and lichens) at low concentrations. At these concentrations, we did not find striking evidence of trace elements mobility from external tissues and tree rings, in relation to the specific activity of the incinerator or any other pollution source. Therefore, the retrospective reconstruction of pollution disturbance with a combination of different biomonitors was feasible. However, the entrance of airborne pollutants into tree xylem tissues by translocation during the growing seasons and subsequent accumulation into lignified tree ring tissues cannot be ruled out. This suggests caution in interpreting tree ring data, when other biomonitors are not available.

This integrated biomonitoring approach could have been not sensitive enough to monitor the low pollutant thresholds and to assess the overall spatial variability of air quality in a complex peri-urban area, where industrial activities are dispersed in an agricultural and forestry context. However, the use of this integrated biomonitoring approach could likely contribute to assess historical patterns of air quality and, thus, to the implementation of environmental policies on atmospheric pollution control. Appropriately integrated biomonitoring plans could help to determine temporal and spatial patterns of pollution across the area under investigation, highlighting the affinity between signals and biomonitors, provided that consistent data of the specific sensitivities of each biomonitors are made available.

Conflict of interest

Authors declare that there is no conflict of interest in relation to this study and its findings.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ufug.2016.04.008.

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