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Lichens as environmental biomonitors of pollution by heavy metals and PAHs

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Riassunto

I licheni, una simbiosi tra un fungo e alghe verdi e/o cianobatteri, sono privi di uno strato cuticolare e possono perciò assorbire nutrienti e contaminanti tramite deposizioni atmosferiche, sia umide che secche. Per questo motivo sono tra gli organismi più spesso utilizzati quali biomonitori di inquinamento atmosferico, in particolare di metalli pesanti. Negli ultimi anni vengono però sempre più spesso utilizzati anche come biomonitori di composti organici persistenti quali diossine, furani, policlorobifenili e idrocarburi policiclici aromatici (IPA), che stanno ricevendo sempre maggiori attenzioni per le loro proprietà mutagene e cancerogene e per la tendenza a persistere nell'ambiente.

Il presente progetto di dottorato è finalizzato a: *i*) testare la validità dei licheni come biomonitori di IPA in ambienti semi-naturali, generalmente considerati incontaminati, mediante campionamenti del lichene autoctono *Pseudevernia furfuracea* (L.) Zopf. var. *furfuracea* a distanza crescente dalla strada (0 m, 120 m e 300 m) in 7 passi montani delle Dolomiti che portano verso aree turistiche più o meno note e quindi con diverse intensità di traffico; *ii*) verificare la presenza di eventuali pattern di distribuzione degli IPA in un territorio del NE Italia con uso del suolo altamente eterogeneo mediante trapianti di *P. furfuracea* in 37 stazioni disposte ai nodi di un grigliato di 700 m di passo e verificare l'esistenza delle differenze stagionali del contenuto degli IPA in due diversi periodi dell'anno (invernale ed estivo); *iii*) testare un metodo innovativo di identificazione delle fonti di inquinamento da metalli mediante misure delle proprietà magnetiche del particolato contenuto nei trapianti lichenici che, abbinato all'analisi del contenuto di metalli in traccia, potrebbe permettere di associare il particolato a determinati processi produttivi.

Il lichene epifita *P. furfuracea*, molto comune su rami di conifere nella fascia montana e subalpina, è stato scelto come specie target in quanto sopporta bene i trapianti, ha delle ottime capacità di accumulo ed è spesso utilizzato come biomonitore di metalli in traccia e IPA.

Si è dimostrato che: *i*) i licheni possono evidenziare efficacemente l'inquinamento da IPA anche in ambienti montani con bassi livelli di contaminazione; in particolare è stato possibile osservare delle differenze significative sia tra i passi con diversa pressione di traffico, sia a diverse distanze dalla strada dello stesso passo; l'inquinamento da IPA può raggiungere livelli non indifferenti lungo i passi con un'elevata intensità di traffico, tendendo però a diminuire allontanandosi dalla strada grazie all'effetto barriera della vegetazione; *ii*) in un territorio altamente eterogeneo è stato possibile ricondurre pattern di distribuzione degli IPA a specifici processi produttivi, confermando che la concentrazione ambientale degli IPA è di un ordine di grandezza maggiore durante il periodo invernale rispetto a quello estivo; ciò è dovuto sia all'assenza di determinate fonti emissive durante il periodo più caldo dell'anno (p.es. riscaldamento domestico)

sia al fatto che gli IPA vanno incontro a degradazione chimica e fotochimica causata da intenso irraggiamento UV, elevate temperature e specie reattive dell'ossigeno quali l'ozono, tutte condizioni che si manifestano tipicamente durante il periodo estivo; *iii*) i dati di mineralogia magnetica dei trapianti lichenici, in sostanziale accordo con quelli del loro contenuto elementare, non hanno permesso di discriminare tra le diverse fonti emittenti presenti sul territorio; è stato però possibile supportare in maniera convincente l'ipotesi che una fonte in particolare non è causa di rilasci importanti nell'ambiente.

I licheni si sono rivelati degli ottimi bioaccumulatori di IPA, sia in ambienti prossimo-naturali, sia in zone industriali e urbane. Gli ambienti di alta montagna, che generalmente vengono percepiti come naturali e incontaminati, possono anch'essi subire un inquinamento da IPA non indifferente dovuto al traffico veicolare. Tenere conto del periodo dell'anno durante il quale eseguire uno studio di biomonitoraggio è fondamentale per evitare il rischio di sottostime dell'inquinamento da IPA. La caratterizzazione magnetica dei campioni, affiancata alle analisi di contenuto elementare, si è rivelata una tecnica molto innovativa e promettente che potrebbe permettere di associare l'origine del particolato contenuto nei licheni ad un determinato processo produttivo. Le misurazioni sono sensibili, veloci e relativamente economiche, e permettono l'identificazione di zone di interesse che necessitano di ulteriori rilevamenti più approfonditi.

Abstract

Lichens, a symbiotic association between fungi and algae and/or cyanobacteria, unlike plants, lack a cuticle layer that limits the inflow of pollutants and thus absorb contaminants and nutrients from both wet and dry atmospheric deposition. They are among the best and most frequently used biomonitors of airborne pollutants, such as heavy metals and, recently, also of various persistent organic pollutants, such as dioxins, furans, polychlorobiphenyls and polycyclic aromatic hydrocarbons (PAHs). The latter are receiving increasing attention due to their mutagenic and cancerogenic properties and the tendency to persist in the environment.

The aim of this project was to: *i*) test the validity of lichens as PAH biomonitors in semi-natural environments, generally considered unpolluted, by collecting autochthonous samples of the lichen *Pseudevernia furfuracea* (L.) Zopf. var. *furfuracea* at increasing distances from the road (0 m, 120 m e 300 m) in 7 mountain passes with different traffic intensities in the Dolomites; *ii*) verify the presence of PAH distribution patterns in a mixed land-use area of NE Italy with the use of transplants of *P. furfuracea* in 37 exposure sites located at the intersections of a 700 m step grid, and 3 further sites located in the nearby urban centers, and verify the existence of seasonal differences of the PAH content in two different periods of the year (winter and summer); *iii*) test an innovative and very promising method of identification of particulate matter (PM) pollution sources through characterization of the magnetic properties of the PM accumulated by lichen transplants that, integrated with the element content analyses, might permit attributing the origin of the PM to one or more known specific emission sources.

The epiphytic lichen *P. furfuracea*, very common on conifer branches in the mountain and subalpine belt, was chosen as the target species because of its resistance to transplantation, excellent accumulation abilities and frequent use as biomonitor of both metals and PAHs.

It was proven that: *i*) lichens can very efficiently detect pollution by PAHs even in mountain areas with low levels of contamination; in particular it was possible to observe significant statistical differences among passes with different loads of traffic pressure, and among increasing distances from the road of the same pass; PAH concentrations may reach rather high levels along passes with elevated traffic pressure, that tend to decrease with increasing distance from the road due to the filtering function of the vegetation; *ii*) in a highly mixed land-use area it was possible to determine PAH distribution patterns and attribute their origin to specific emission sources, confirming that environmental levels of PAHs are an order of magnitude higher during the winter period compared to the summer levels, due to the absence of certain pollution sources during the warmest period of

the year (e.g. domestic heating) and due to that fact that chemical and photochemical degradation processes of PAHs are promoted by high temperatures, intense UV radiation and oxidative conditions (e.g. presence of ozone), all conditions that typically occur during the summer period; *iii*) the data on the magnetic properties of the lichen transplants were in substantial agreement with the element content, it was possible to identify the primary heavy metal pollution source in the area, but it was not possible to discriminate between PM originating from different pollution sources due to very modest emissions from the other potential sources thus confirming their low environmental impact.

Lichens have proven to be excellent PAH bioaccumulators, both in semi-natural environments, and in industrial and urban areas. Mountain environments, that are generally considered as natural and unpolluted, may as well suffer rather high levels of PAH pollution due to vehicular traffic. The seasonal differences should be taken into account when planning a PAH biomonitoring survey in order to avoid the risk of underestimating their environmental load. The magnetic characterization of the lichen samples, integrated with the element content analyses, has proven to be a very innovative and promising method that could permit to attribute the origin of the PM to a specific anthropic activity. The measurements are very sensitive, rapid and relatively cheap and can allow to identify areas of interest for further, more detailed monitoring.

Introduction

Air pollution is contamination of the indoor or outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere (WHO, 2016). More than 2 million premature deaths each year can be attributed to the effects of urban outdoor and indoor air pollution (caused by the burning of solid fuels; WHO, 2005). According to WHO (2005; 2014; 2016) fine particulate matter (PM) has the greatest effect on human health. The evidence on airborne PM and its public health impact is consistent in showing adverse health effects at exposures that are currently experienced by urban populations in both developed and developing countries. It is associated with a broad spectrum of acute and chronic illness, such as lung cancer, chronic obstructive pulmonary disease (COPD) and cardiovascular diseases. All population is affected, but the susceptibility may vary with health or age. The risk has been shown to increase with exposure and there is little evidence to suggest a threshold below which no adverse health effects would be anticipated. Particles with a diameter of 10 μm or less are the most health-damaging since they can penetrate deep inside the lungs. PM_{10} (2.5 – 10 μm) originates primarily from mechanical processes such as construction activities, road dust re-suspension and wind, while $\text{PM}_{2.5}$ (< 2.5 μm) originates from combustion processes (both fossil fuel and biomass). Numerous epidemiological and toxicological studies have shown that the smaller the size of PM the higher the toxicity through mechanisms of oxidative stress and inflammation (Valavanidis et al., 2008).

Airborne PM consists of a complex mixture of solid and liquid particles of organic and inorganic substances suspended in the air, and it is usually referred to as aerosol (Bargagli, 1998; WHO, 2005). This study focusses on its trace element content, with a particular interest in heavy metals, and polycyclic aromatic hydrocarbons (PAHs).

Trace elements in the airborne particulate matter

Natural emissions of atmospheric PM generally involve slow biogeochemical cycles of major constituents of the atmosphere which are relatively unreactive and have fairly uniform concentrations. They contribute to about 85-90% of the total atmospheric aerosols. The main natural sources of atmospheric trace elements are considered to be wind-borne soil particles, volcanic emanations, sea sprays and wild forest fires (Bargagli, 1998).

On the other hand, anthropogenic activities can release large amounts of trace elements in very short time intervals causing them to become highly concentrated. Human activities considered to be their main sources are mining, smelting of metallic ores, industrial production and application

of iron and non-ferrous metals, fossil fuel combustion, waste incineration, cement production and vehicle emissions (Bargagli, 1998; Järup, 2003; WHO, 2016).

Trace elements are removed from the atmosphere through wet and dry depositions. In temperate regions, rain and snow are generally considered the main scavenging processes. At high altitudes above the ground, atmospheric particles can act as nucleating centers for water droplets (in-cloud scavenging), while in atmospheric layers below clouds, PM can be removed by wash-out when precipitating rain and snow absorb the pollutants and carry them to the ground (below-cloud scavenging). Fog and dew play an important role as well. Dry depositions take place by gravitational settling. For example, huge gravitational sedimentation of larger particles occur around cement plants, mines, smelting and metallurgical plants; fine particles are continuously removed from the air accumulating on exposed surfaces (Bargagli, 1998; Bargagli and Mikhailova, 2002).

Trace elements are often essential for a proper growth, development, and physiology of the living organisms, but many of them can also cause toxic effects (aluminum, antimony, barium, bismuth, chromium, cobalt, copper, gold, iron, lithium, manganese, platinum, silver, thallium, tin and zinc). The main threats to human health and the environment are associated with exposure to arsenic, cadmium, lead and mercury. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO (2016) that includes them in the list of 10 chemicals of major public concern.

Polycyclic aromatic hydrocarbons (PAHs) in the airborne particulate matter

Polycyclic aromatic hydrocarbons (PAHs) are a complex group of persistent organic pollutants (POPs) composed of two or more fused aromatic rings. The compounds range from volatile (2-, 3-ring), semivolatile (4-ring) to solid phase molecules (5 or more rings), but the partitioning between gas and particulate phases depends on environmental conditions, mainly vapor pressure and temperature (Augusto et al., 2015; Harvey, 1991). They may exist with a great number of structures and, depending on the complexity of the PAHs, in a large number of isomers. They are lipophilic, a property that increases with increasing complexity of the compounds, with tendency to bioaccumulate and persist in the environment, and can be found in high concentrations in soil and biota due to long term atmospheric deposition (Augusto et al., 2013a; Harvey, 1991).

PAHs are ubiquitous and can occur naturally in the environment generated by forest fires and volcanic eruptions, but the largest amounts originate from anthropogenic sources as byproducts of incomplete combustion processes of biomass and fossil fuels (e.g. industrial activities, road traffic, domestic heating, incineration, petroleum cracking) (Harvey, 1991; Mastral et al., 2003).

Low molecular weight PAHs (2-, 3-ring) are usually emitted by petrogenic sources (without combustion, e.g. unburned fuel, lubricating oil), while high molecular weight PAHs (4 or more rings) are usually related to pyrogenic sources (with combustion). Once emitted, they will disperse in the atmosphere and travel for long distances depending on their weight and air temperature (Beyer et al., 2003), undergoing photochemical and chemical degradation when exposed to UV radiation and oxidative molecules such as ozone (Alves, 2008; Boström et al., 2002; Brown and Brown, 2012; Jung et al., 2010; Schauer et al., 2003; Wild et al., 2005). Like trace elements, PAHs are removed from the atmosphere through wet and dry depositions as well (LeNoir et al., 1999; Welsch-Pausch et al., 1995).

PAHs are toxic organic compounds, whose impact on human health is mainly related with carcinogenic, mutagenic and teratogenic effects. Several of them have been classified by the International Agency for Research on Cancer (IARC) as carcinogenic (Group 1), probably carcinogenic (2A) or possibly carcinogenic (2B) to humans (IARC, 1987). The U.S. Environmental Protection Agency (EPA) has edited a list of 16 priority PAHs (EPA-PAHs) to be monitored in the environment.

Lichens as biomonitors of trace elements and PAHs

Biological accumulators are organisms that reflect the chemical content of their environment. Hundreds of studies carried out since the 1970s confirm that lichens are among the most reliable accumulators of airborne particulate matter, their use as biomonitors enables coverage of large and remote areas, it has low costs when compared to establishing and managing automatic monitoring stations, and provides current and retrospective information on the integrated effects of atmospheric pollutants and other environmental factors (Bargagli and Mikhailova, 2002). Lichens are a symbiotic association of fungi and green algae and/or cyanobacteria, and are distributed in a wide range of ecosystems all over the world. They lack a cuticle layer and a root system, so that the acquisition of nutrients occurs through the entire surface of the thallus directly from wet and dry depositions. For this reason, lichens are exposed to any contaminant present in the atmosphere and the more resistant species have a remarkable ability to accumulate elements and PAHs without exhibiting damage (Augusto et al., 2013a; 2013b; Augusto et al., 2015; Bargagli, 1998; Bargagli and Mikhailova, 2002).

Element uptake occurs essentially by three mechanisms: extracellular ion exchange, intracellular accumulation and particulate trapping, the latter being the most important due to entrapment of particulate material in the large intercellular spaces of the medulla (Bargagli, 1998). The mechanisms of PAH accumulation are still largely unknown, but it is expected that similar

processes are responsible for their uptake as well, when they are in the particulate phase (Augusto et al., 2013a; 2013b). A recent study by Augusto et al. (2015) showed that gas phase fluoranthene (Fth; 4-ring) and benzo[a]pyrene (BaP; 5-ring) migrate across the lichen thallus and accumulate within the algal layer. Fth migrated more quickly than BaP, probably due to their different molecular weights, but after 16 days from exposure both compounds remained within the algal layer and were not removed even by washing. Given their lipophilic nature, it is assumed that gas phase PAHs accumulate onto algae surface and/or inside algae cells, in the lipid bodies (pyrenoglobuli) (Augusto et al., 2015).

***Pseudevernia furfuracea* (L.) Zopf as the target species**

Lichen accumulation capacity depends to a large extent on their morphological features such as thallus type (fruticose, foliose or crustose), branching, wrinkling, roughness, size of pores in the epicortex and hyphae density (Bargagli and Mikhailova, 2002). The epiphytic fruticose lichen *Pseudevernia furfuracea* (L.) Zopf. var. *furfuracea* was selected as the target species because it is easy to identify, rather common and widespread on conifer branches in the European mountain and subalpine belts (Nimis, 1993), stress-tolerant and with a good resistance to transplantation (Tretiach et al., 2007). It is morphologically prone to intercept particulate matter both due to its fruticose growth form and due to the development of a myriad finger-like vegetative propagules (isidia) that cover its upper cortex and increase its surface/weight ratio (Tretiach et al. 2005). It is frequently used as biomonitor of trace elements and, more recently, PAHs (e.g. Blasco et al., 2011; Gallo et al., 2014; Giordano et al., 2005; Guidotti et al., 2003; 2009; Protano et al., 2014; Sorbo et al., 2008; Tretiach et al., 2011).

Project aims

PAH biomonitoring studies are conducted mostly in industrial and/or urban areas (e.g. Augusto et al., 2010; Augusto et al., 2013b; Guidotti et al., 2003; 2009), while there is very few data on PAH levels accumulated by lichens in pristine and semi-natural areas (Aragon and Aspe valleys in the Pyrenees, Blasco et al., 2008; 2011; Southern Shetlands, Antarctica, Cabrerizo et al., 2012; western U.S. National Parks, Usenko et al., 2010). The Dolomites are an UNESCO World Heritage Site and they are generally perceived by the public as an uncontaminated environment. However, they can be subjected to high touristic pressure during both summer and winter, particularly along the mountain passes leading to tourist villages due to vehicular traffic. The first aim of this thesis was to test the validity of lichens as PAH biomonitors in this semi-natural environment (Southern Limestone Alps, NE Italy). Autochthonous samples of *P. furfuracea* were

collected in 7 mountain passes with different traffic intensities to test if differences in the PAH concentrations could be detected, and at increasing distances from the road (0 m, 120 m e 300 m) in order to test if there is a PAH concentration gradient in the lichen material.

The use of the transplant technique in biomonitoring studies offers several main advantages compared to the use of autochthonous material (Ayrault et al., 2007; Bargagli, 1998; Bargagli and Mikhailova, 2002): (i) the samples can be repeatedly exposed for known time periods, (ii) a high density sampling design can be applied, (iii) the possibility to cover those areas where autochthonous macrolichens are usually absent (e.g. urban centers), (iv) enrichment rates during exposure can be calculated on the basis of pre-exposure values (Fрати et al., 2005). Most PAHs biomonitoring studies were conducted using autochthonous material. The second aim was to verify if the application of the lichen transplant technique allows to identify PAHs distribution patterns and, thus, possible emission sources in a highly heterogeneous territory where all the primary PAHs sources are present (different industrial activities, road traffic and domestic heating). Samples of *P. furfuracea* were collected in a pristine area of the Carnic Alps and exposed for two months in 37 exposure sites located at the intersections of a 700 m step grid, and 3 further sites located in the nearby urban centers. Seasonal variations of the PAHs content in lichens are also largely unknown, so that the study was repeated in two different periods of the year (winter and summer).

Eventually, even though biomonitoring studies of trace elements and heavy metals have been conducted since the 1970s, when several anthropogenic activities are present in a relatively small area characterized by a mixed land use, it becomes difficult to determine with certainty the source of pollution relying only on the element composition of PM (Capozzi et al., 2016). It is well known that PM may contain magnetic particles which originate from many anthropogenic activities such as metallurgy, mining, abrasion or corrosion of materials and industrial, domestic and vehicle combustion of fossil fuels that contain iron impurities (Rai, 2013). The third aim of the thesis was to test an innovative and very promising method of identification of pollution sources through characterization of the magnetic properties of the PM accumulated by lichen transplants and to test the effectiveness of the methodology in attributing the origin of the PM to one or more known specific emission sources present in a study area characterized by mixed land use.

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Patterns of traffic polycyclic aromatic hydrocarbons pollution in mountain areas can be revealed by lichen biomonitoring: a case study in the Dolomites (Eastern Italian Alps)

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Highlights

- Road traffic is among the main sources of impact in high elevation ecosystems.
- Lichens were used to detect road traffic pollution in the Dolomites.
- Lichens of Dolomite passes have higher pollution than those in remote sites.
- Pollution in the lichens decreases with increasing distance from the road.

Abstract

In mountain areas of touristic interest the evaluation of the impact of human activities is crucial for ensuring long term conservation of ecosystem biodiversity, functions and services. This study aimed at verifying the biological impact of polycyclic aromatic hydrocarbon (PAH) emissions due to traffic along the roads leading to seven passes of the Dolomites (SE Alps), which were recently declared a UNESCO World Heritage Site. Thalli of the epiphytic lichen *Pseudevernia furfuracea*, collected at increasing distances from the roads, were used as biomonitors. Our study revealed a gradient of decreasing PAH pollution within 300 m from the roads. Differences among passes were evident mainly for samples collected nearest to the roads, but PAH concentrations at 300 m were almost always higher than those of undisturbed reference sites, indicating that traffic PAH pollution may impact natural ecosystems and lichen diversity at relatively long distances from the emission source.

Keywords: air pollution, bioaccumulation, epiphytic lichens, PAH pollutants

1. Introduction

The evaluation of the impact of Polycyclic Aromatic Hydrocarbons (PAHs) is receiving increasing attention due to their mutagenic and carcinogenic properties (Boström et al., 2002) that can seriously threaten human health (IARC, 1983). Due to their relatively stable molecular structure, PAHs tend to persist in the environment for long periods and are accumulated by organisms. The US Environmental Protection Agency (EPA) addressed 16 PAHs as priority pollutants to be monitored (USEPA, 1993). The occurrence of PAHs in the environment is mainly related to human activities (Edwards, 1983), including industrial processes, domestic heating, and traffic. Road traffic is one of the main sources of PAHs pollution (Boström et al., 2002) also in semi-natural mountain areas of touristic interest (e.g. Blasco et al., 2006, 2008), where human pressure is concentrated around villages and skiing, or trekking facilities. In these landscapes, the evaluation of the impact of human activities, also in terms of PAHs pollution, is crucial for ensuring long-term conservation of biodiversity, ecosystem functions and services.

The Dolomites (Southern Limestone Alps, NE Italy), that in 2009 were declared a UNESCO World Heritage Site, are considered among the most attractive mountain landscapes in the world and hence are subjected to high touristic pressure during both summer and winter. The attractiveness and the economic value of the Dolomites closely depend on the long-term maintenance of their landscapes, that includes healthy natural ecosystems with high air quality. Recently, a heated media and political debate has developed around the management of traffic at high elevations, especially along the roads crossing the passes, where a more sustainable management is invoked to ensure the long-term conservation of ecological processes that shape the identity of this territory. However, a monitoring plan for assessing patterns and impacts of traffic pollution in the Dolomites is still lacking.

Lichens, a symbiotic association between fungi and algae and/or cyanobacteria, absorb contaminants and nutrients from both wet and dry atmospheric deposition (Bargagli, 1998; Garty, 2000), accumulating pollutants into their biological matrix. Lichen biomonitoring is widely used for detecting air pollution patterns (e.g. Nimis et al., 2000), especially in remote areas where the use of instrumental recording is hindered by difficult access to sites and difficult management of mechanical and electrical devices. Most lichen biomonitoring studies are focused on accumulation patterns of trace metals, sulfur and nitrogen compounds (Conti and Cecchetti, 2001), providing useful information to detect sites where human health is potentially at risk. However, recent research (Augusto et al., 2009, 2010; Bajpai et al., 2013; Blasco et al., 2006, 2007, 2008, 2011;

Guidotti et al., 2009; Satya et al., 2012; Shukla et al., 2009, 2010, 2013; Studabaker et al., 2012) demonstrated that lichens are also efficient accumulators of semi-volatile organic compounds distributed both in the vapor and solid phases of the air, such as PAHs (Augusto et al., 2013a, b).

This study aimed at testing the use of lichen biomonitoring techniques for detecting patterns of traffic PAHs pollution along the roads leading to some passes of the Dolomites. Based on a replicated experimental design along transects, we tested whether (1) differences could be detected among passes with supposed different traffic loads, and (2) if there is a PAH concentration gradient in the lichen material at increasing distances from the roads. To assess the link between PAH patterns and the risk for biota, the Equivalent Toxicity of the PAHs mixture (Nisbet and LaGoy, 1992) was also included in the analysis.

2. Materials and methods

2.1 Study area

The study was carried out in the Dolomites, a mountain area across Veneto and Trentino-Alto Adige (NE Italy; 46°23'N 11°51'E) covering 142 000 ha and hosting 18 peaks over 3000 m elevation. Mean annual precipitation is 1050 mm, while at an elevation of 2000 m mean minimum temperatures range between -8°C (January) and +7°C (July) and mean maximum temperatures between -2°C (February) and +15° (July-August).

Tourist villages located in different valleys are connected by a network of roads that cross 21 passes located at elevations ranging between 1372 m (Cereda Pass) and 2240 m (Sella Pass). Vehicular traffic is particularly intense in the area around the Sella massif where in summer 2009 the highest traffic intensity was reached (5000 cars during a one-day-spot count). Unfortunately, systematic data on traffic loads for the Dolomites are still lacking, as revealed by a recent study of the European Academy of Bolzano (EURAC, unpublished report).

For our study, the roads leading to seven passes were selected (Table 1; Figure 1), representing a gradient of touristic pressure, based on expert assessment (M. Wagner, personal comment and the authors' personal knowledge of the study area). As an indicator of the popularity of the seven passes we also considered the number of results in Google (www.google.it) for each pass. The two extremes of the gradient are the Sella Pass, notoriously the most visited by tourists, and the little known Staulanza Pass. Fine-tuned differences among the other passes are not easily established, but in decreasing order of touristic pressure we ranked at the same level Pordoi, Falzarego, Rolle and Fedaiia, that share similar loads and popularity, followed by Cimabanche Pass. Fedaiia and Cimabanche have some additional peculiarities: the former is closed during winter (December-April), while the latter, open also in winter, is crossed by a main road with truck traffic

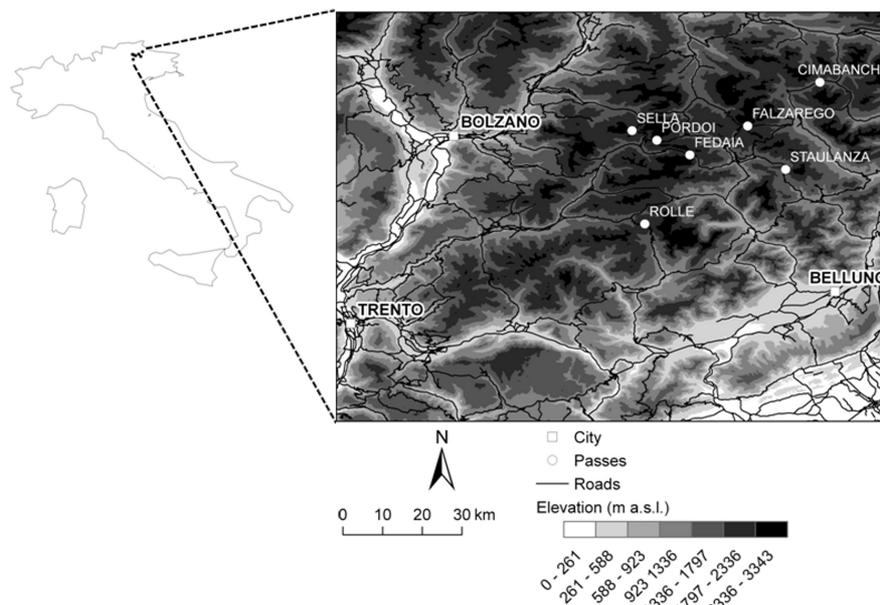


Fig. 1 Location map of the sampling localities in the Dolomites region.

but is not a touristic site where people are concentrated in summer and winter for trekking and skiing. In Table 1, passes are ordered according to this empirical classification. The distance of the seven passes from permanently inhabited villages (74-5931 inhabitants) ranges between 1.7 and 9.5 km.

2.2 Lichen sampling

Along the road leading to each pass, a transect was traced perpendicular to the road, trying to maintain comparable elevations (Table 1) and vegetation conditions, all transects being located within subalpine spruce woods (tree density: c. 450-550 trees ha⁻¹). Along each transect, at 0, 120 and 300 m from the margin of the road, three independent samples consisting of 5-10 thalli of the common epiphytic lichen *Pseudevernia furfuracea* (L.) Zopf were collected, wearing non-talc gloves, on the branches of spruce trees, at 1.5-2.5 m above the ground. Distance from the road of the farthest sampling point was kept relatively small in order to focus on the effects of traffic pollution, minimizing the possible effects of long distance transport of pollutants from villages (Blasco et al., 2008). Three additional sampling sites, located in isolated valleys (Table 1), were selected as reference for background PAH pollution levels.

Sampling was carried out in two consecutive days, in October 2011, under fair weather conditions. In particular, the last rainy event occurred 11 days before sampling with 41±6 mm of rain across the study sites in one day. Average daily temperature of the two sampling days across sites was 5.6±1 and 4.6±0.3°C respectively. Average daily temperature of the previous three weeks was 7.6±4.5°C.

Table 1. Geographic position (UTM, 33T) and elevation (m) of the seven sites along the roads leading to the passes of the Dolomites and the three reference sites selected for the lichen sampling. For the seven passes, the distance to the nearest village (Km) with its number of inhabitants are reported. The passes are ordered according to a decreasing gradient of human pressure.

Site	Longitude	Latitude	Elevation	Distance to the nearest village (km)	Number of inhabitants
Sella	714084	5153044	1810	2.7	1842
Pordoi	719055	5152595	1760	1.7	275
Rolle	712906	5130889	1770	5.5	560
Falzarego	274155	5156367	1820	7	5931
Fedaia	723485	5146450	1575	3.5	224
Cimabanche	283741	5166551	1540	9.5	5931
Staulanza	277822	5145901	1575	2.7	74
Ref1 - Val Ombretta	722146	5145016	1590	-	-
Ref2 - Val Venegia	717034	5132525	1870	-	-
Ref3 - Paneveggio, Val Bona	711530	5130861	1760	-	-

2.3 Samples preparation and chemical analyses

The material was transferred to the laboratory inside paper bags, where it was carefully cleaned from debris and dead or senescent parts, and left to dry out in the dark at 35°C for 3 days. To obtain homogenous samples across sites, each sample was derived from a mixture of moderately isidiate lobes (over-isidiate lobes were excluded since excessive density of isidia may alter the accumulation rate by increasing thallus surface); to ensure samples homogeneity we also avoided the use of lobes with fruiting bodies (apothecia), infected by lichenicolous fungi, or covered by epiphytic algae. Following Tretiach et al. (2007), samples for chemical analyses were prepared using only the 5-6 mm apical parts of the lobes, to ensure as much as possible homogeneous conditions of exposure time. The apical parts used in this experiment roughly represented the annual increment of the thallus. The material was ground in a ceramic mortar with liquid nitrogen and kept at 4°C. The total amount of lichen material available for chemical analyses varied between 0.6 and 0.8 g dry weight.

Sixteen EPA priority PAHs (USEPA, 1993) were identified and quantified in lichen samples by applying Automated Soxhlet extraction followed by SPE clean up and GC/MS analysis. Three instrumental replicates of each lichen sample were analyzed and then averaged.

The analytical standard EPA 8270 PAH Mix 3 containing each of the 16 EPA-PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene, dibenzo[a,h]anthracene) was obtained from Sigma-Aldrich. All the solvents used in the extraction and clean-up steps – acetone,

hexane, dichloromethane - were from Sigma-Aldrich and were for residue analyses. The Supelclean LC-NH₂ SPE tubes (bed wt 500 mg, volume 6 mL), Supelclean LC-Florisil 100–120 mesh and Sodium sulfate anhydrous were from Sigma-Aldrich.

Dried lichen samples were extracted by Automated Soxhlet extraction, according to the EPA Method 3541. The experiments were performed with the Soxtherm/Multistat Rapid Soxhlet Extraction System (Gerhardt). Lichen samples were placed in a cellulose extraction thimble of 33 x 80 mm (Gerhardt) and extracted with 150 mL of 1:1 (v/v) acetone/hexane for 60 min at 140 °C in the boiling extraction solvent. The thimble with sample was then raised into the rinse position and extracted in a reflux for an additional 60 min. Following the extraction steps, the extraction solvent was concentrated to 2 to 5 mL before purification.

The raw extracts containing PAHs and interferences, such as vegetal pigments, were collected in glass vials and purified by SPE clean up, as described by Blasco et al. (2007).

SPE minicolumns were prepared adding approximately 0.05 g of sodium sulfate anhydrous and 0.05 g of florisil to the top of the commercial 500 mg NH₂-SPE tubes. The tubes were conditioned with 6 mL of dichloromethane and 3 mL of hexane. After that, the raw extract was loaded on the top of the tube, the solid bed was rinsed with 0,5 mL of hexane to remove the interfering compounds retained on the column and the analytes were quantitatively eluted using 2 mL of hexane–dichloromethane (65:35). The final extract containing PAHs was concentrated to 0.5 mL under a nitrogen stream before the analysis by GC/MS. All the results are expressed on a dried weight basis.

GC/MS - Lichen samples were analysed by gaschromatography coupled with mass spectrometry (GC/MS), according to the EPA method 8270D. All analyses were carried out on a Thermo Scientific DSQII single quadrupole GC/MS system equipped with a Restek Rxi-5Sil MS (30 m x 0.25 mm x 0.25 µm ID) capillary column, with helium as carrier gas at 1 mL/min constant flow. The injection in the GC system was performed in splitless mode, splitless time 5 min, and the injector temperature was 250 °C. The GC oven temperature was held at 40 °C for 5 min, increased to 120 °C by a temperature ramp of 10 °C/min, ramped at 3 °C/min to 325 °C and held for 15 min. The transfer line temperature was 290 °C and the ion source temperature 250 °C. Mass spectral analyses and quantification were carried out in SIM (selected ion monitoring) mode using the following characteristic m/z as quantifier ions: naphthalene 128, acenaphthylene 152, acenaphthene 153, fluorene 166, phenanthrene and anthracene 178, fluoranthene and pyrene 202, benzo[a]anthracene and chrysene 228, benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene 252, indeno(1,2,3-cd)pyrene and benzo[g,h,i]perylene 276 and dibenzo[a,h]anthracene 278.

Quantitative results of PAHs were based on the area of their peak compared to the standards. Linear ranges were checked for each of the 16 EPA-PAHs within 100-560 ng g⁻¹.

2.4 Statistical analyses

The effects of locality and distance from the road on PAH pollution, and their interaction were analyzed by analyses of variance (ANOVA). Since an aim was to detect whether roads with different traffic load could have different PAHs pollution, we used locality as a fixed factor and we adopted a multi-factorial ANOVA due to the balanced design. Starting from the full models described above, we simplified the model by manual backward selection ($\alpha = 0.05$). First, we removed the non-significant interaction terms and then the non-significant main effects (unless part of a significant interaction). Residual vs. fitted plots were assessed visually for randomness, and quantile-quantile plots were examined for departures from normality or violation of homogeneity. Response variables were log- or square root- transformed when violation of homogeneity was identified. PAHs profile values were angular transformed prior to analysis (Zar, 1999). Post-hoc comparisons were further used to test the differences within site, distance and their interaction, using the 'glht' (general linear hypothesis test) function in the 'multcomp' package in R (Hothorn et al., 2008). For the interaction term, contrast analysis was performed to test differences among the sites within the same distance class. The comparison between control sites and the seven passes were assessed by one-way ANOVAs followed by a Dunnett's test, at $P < 0.05$ level.

As response variables we used: (1) the total concentration of PAHs (TOT_PAHs; ng g⁻¹); (2) the total concentration of combustion PAHs with 4-6 aromatic rings, characteristic of vehicle exhaust emissions (Blasco et al., 2011; TOT_PAHs_comb) and (3) their relative abundance expressed as percentage on the total PAHs concentration (%_PAHs_comb); (4) the Equivalent Toxicity (TEQ) of the PAH mixture calculated according to the toxicity equivalency factors proposed by Nisbet and LaGoy (1992), and (5) the concentrations of each PAH detected. Only PAHs whose concentrations were quantified in more than 55% of the samples were included in these analyses (Table 2).

3. Results

3.1 General overview

In total, 16 PAHs were detected (Table 2), benzo[a]anthracene/chrysene, and benzo[b]fluoranthene/benzo[k]fluoranthene being treated together due to difficulty in clearly discerning among their peaks. The majority of compounds had concentrations above the detection limit, except acenaphthylene, benzo[a]pyrene, and dibenzo[a,h]anthracene. Acenaphthene and indenopyrene

Table 2. Summary of PAH concentrations (ng g⁻¹) and TEQ in samples of the lichen *Pseudevernia furfuracea* from the seven roads leading to the passes of the Dolomites listed in Table 1. Mean, MIN, MAX = mean, minimum, maximum concentration value of each PAH; SD = standard deviation; %Samples = percentage of samples in which each PAH was detected; N° of rings = number of aromatic rings that make up the molecules of each PAH; Combustion_PAH = PAHs with 4-6 aromatic rings that are characteristic of vehicle exhaust emissions.

PAH	Mean	MIN	MAX	SD	%Samples	N° of rings	Combustion PAH
Naphthalene	9.0	1.4	25.6	5.0	100.0	2	
Fluorene	27.6	2.9	77.3	19.3	80.0	3	
Phenanthrene	249.5	30.6	841.9	141.0	100.0	3	
Anthracene	154.1	7.0	505.5	116	78.0	3	
Acenaphthene	78.0	8.8	175.4	51.0	22	3	
Acenaphthylene	< LOD	-	-	-	-	3	
Fluoranthene	125.7	6.6	471.0	92.4	95.0	4	+
Pyrene	86.1	6.1	412.53	83.8	100.0	4	+
Benzo[<i>a</i>]anthracene+Chrysene	31.6	4.0	146.4	28.6	98.0	4	+
Benzo[<i>b</i>] + Benzo[<i>k</i>]fluoranthene	103.1	10.0	325.5	81.6	57	5	+
Benzo[<i>a</i>]pyrene	< LOD	-	-	-	-	5	+
Dibenzo[<i>a,h</i>]anthracene	< LOD	-	-	-	-	5	+
Indenopyrene	61.3	22.0	188.2	54.3	15	5	
Benzo[<i>g,h,i</i>]perylene	100.5	14.1	249.7	72.0	91.7	6	+
TOT_PAHs	758.0	186.0	2129.5	438.5	-	-	-
TOT_PAHs_comb	325.9	38.6	1270.3	242.0	-	-	-
% PAHs_comb	40.2	16.8	64.0	11.8	-	-	-
Equivalent Toxicity (TEQ)	8.6	0.5	34.1	6.9	-	-	-

were only sporadically over the limit of quantification. Nine PAHs were quantified in more than 55% of the samples, the most abundant being anthracene, benzo[*g,h,i*]perylene, fluoranthene, phenanthrene, and pyrene. Three of them are combustion PAHs. A wide gradient of total PAHs (and combustion PAHs) concentrations was found (Table 2) that was also reflected by the gradient of TEQ (Equivalent Toxicity).

3.2 Comparisons with reference sites

For points located at 0 m from the road, both TOT_PAHs and TOT_PAHs_comb were significantly higher in the roads leading to the passes than in the three reference sites (Dunnnett test $P < 0.05$), except for the pass with the least touristic appeal (see Appendix A in Supplementary data). For sampling points at 120 and 300 m from the road, differences were significant, except for two passes. In general, the roads leading to the passes had higher equivalent toxicity than the three reference sites at the three distances from the road. No significant differences were found for the relative abundances of PAHs_comb between reference sites and the other sampling points for all distances.

Table 3. Results of analyses of variance (ANOVA) testing effects on PAH mixtures, TEQ, PAH profile and individual components of locality, distance from the pass road and their interactions. Non-significant interactions and main effects were removed with a backward elimination procedure ($P > 0.05$).

	Locality		Distance		Locality \times Distance	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
TOT_PAHs	3.406	0.009	8.563	< 0.001	2.165	0.036
TOT_PAHs_comb	5.276	< 0.001	5.546	0.007	-	-
%_PAHs_comb	2.342	0.045	-	-	-	-
Equivalent Toxicity (TEQ)	3.240	0.009	3.622	0.034	-	-
Naphthalene	7.677	< 0.001	1.286	0.288	4.516	< 0.001
Fluorene	-	-	4.716	0.013	-	-
Phenanthrene	3.198	0.012	1.534	0.229	2.510	0.016
Anthracene	-	-	-	-	-	-
Fluoranthene	2.480	0.041	0.681	0.513	2.958	0.006
Pyrene	5.377	< 0.001	15.960	< 0.001	-	-
benzo[a]anthracene+chrysene	2.708	0.024	4.177	0.021	-	-
benzo[g,h,i]perylene	-	-	-	-	-	-
benzo[b]fluoranthene	3.544	0.005	3.643	0.034	-	-

3.3 Patterns of PAHs mixtures and TEQ

A significant interaction between locality and distance from the road was only found for TOT_PAHs (Table 3). Locality and distance from the road had significant effects for both PAHs mixtures and TEQ models. For the model of the relative abundance of PAHs_comb a marginally significant effect of locality was found. The pass with the highest touristic appeal showed higher PAHs pollution and equivalent toxicity as confirmed by post-hoc comparison (Fig. 2a-c). Despite the marginal effect of distance from the road found by ANOVA for the relative abundance of PAHs_comb, the multiple-comparison yielded no significant differences among the seven roads. Sampling sites at 0 m from the road had higher PAHs pollution and equivalent toxicity than those located at 120 and 300 m (Fig. 2e-g). The contrast analysis for the interaction between site and distance from the road revealed a significant difference for TOT_PAHs between the two extremes of the gradient only for the sampling points at 0 m from the road (Fig. 3; and Appendix B in Supplementary data). In general, considering the interaction between locality and distance from the road for TOT_PAHs, higher variability was found among passes for sampling sites at 0 m from the road, while total PAH concentrations were more similar among passes with increasing distance from the road (Fig. 3).

3.4 Patterns of individual compounds

Significant interactions between locality and distance from the road were found for naphthalene, phenanthrene and fluoranthene (Table 3). Locality had a significant effect for

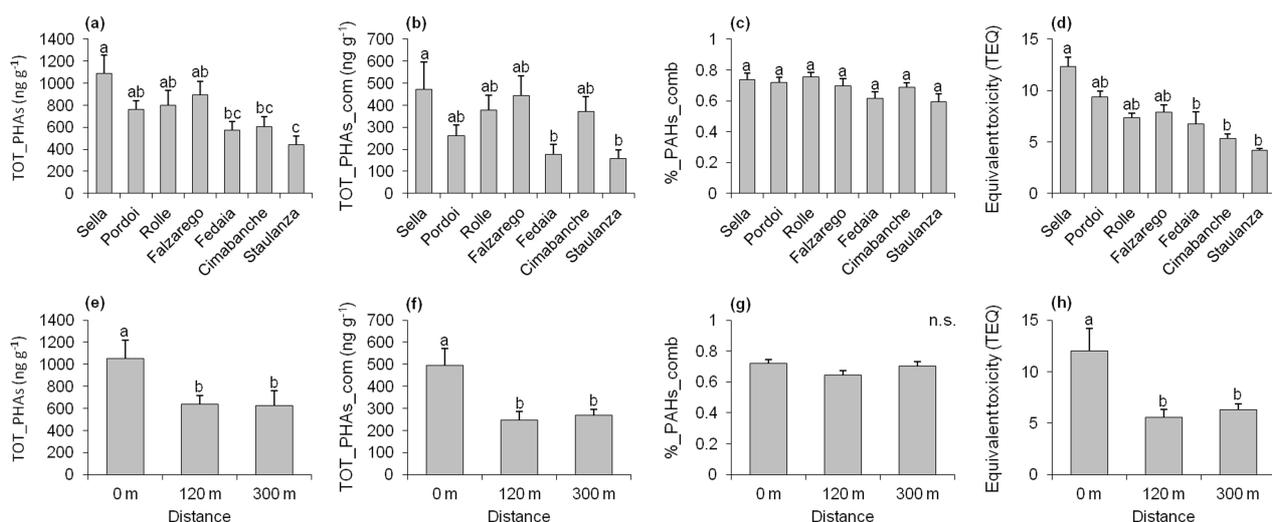


Fig. 2 Mean \pm SE of TOT_PAHs (a, e), TOT_PAHs_comb (b, f), relative abundance of PAHs_comb (c, g), and equivalent Toxicity (TEQ) (d, h) measured in samples of the lichen *Pseudevernia furfuracea* collected along the roads leading to seven passes of the Dolomites (a, b, c, d) at increasing distance from the road margin (e, f, g, h). Different letters indicate significant differences according to the Tukey's multiple comparison test based on the model of Table 3. The passes are ordered as in Table 1.

naphthalene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene+chrysene and benzo[b]+benzo[k]fluoranthene. Distance from the road had significant effects for fluorene, pyrene, benzo[a]anthracene+chrysene, and benzo[b]+benzo[k]fluoranthene. No significant effects were found for benzo[g,h,i]perylene and for anthracene. Post-hoc comparisons showed a higher decrease of high molecular weight PAHs (i.e., pyrene, benzo[a]anthracene+chrysene and benzo[b]+benzo[k]fluoranthene) with increasing distance from the road than those of lower molecular weight PAHs (i.e., naphthalene, phenanthrene, anthracene) (Fig. 4).

4. Discussion

Results indicated that natural ecosystems of mountain areas may be impacted by traffic PAH pollution whose patterns can be efficiently detected by lichen biomonitoring techniques. Two main patterns were found: (a) among roads with different touristic pressure, and (b) within roads in relation to the distance from the road margin.

The pattern of PAH pollution detected in lichen material is consistent with that of TEQ, and both reflect the gradient of touristic pressure. The most polluted site was that of Sella Pass, confirming that critic loads of touristic pressure may be reached in this very popular locality. The least polluted site was Staulanza Pass, where PAHs values were similar to those of unpolluted reference sites, reflecting the lower touristic loads. Between these two extremes we found a group of passes with similar PAH pollution, in accordance with their similar loads of touristic pressure.

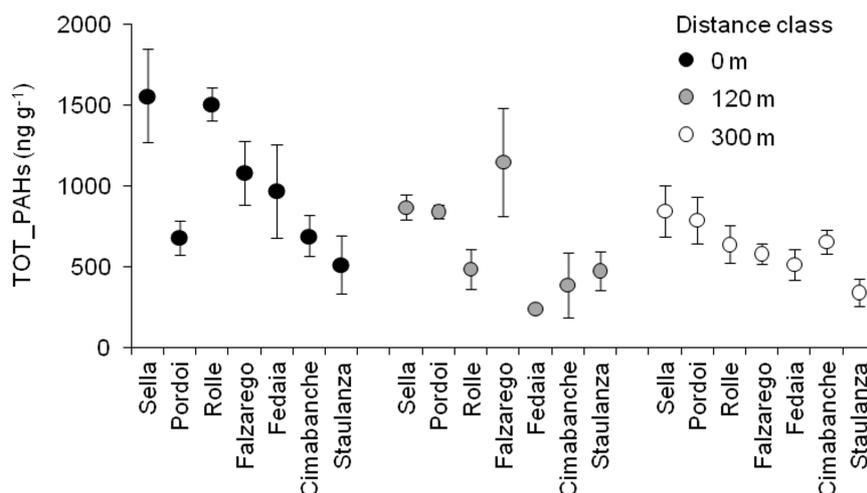


Fig. 3 Mean \pm SE of TOT_PAHs for the seven passes listed in Table 1 measured in samples of the lichen *Pseudevernia furfuracea* collected at increasing distance from the pass road; the passes are ordered as in Table 1.

The incidence of PAHs most likely related to vehicular traffic (combustion PAHs) is similar among our study sites (including reference sites), suggesting that a similar PAHs mixture with different concentrations is potentially impacting the study area.

Within each road there is a pattern of decreasing PAHs pollution with increasing distance from the road within a range of 300 m. PAHs concentrations at 300 m are similar to those of undisturbed reference sites in one pass only, indicating that traffic PAHs pollution may impact natural ecosystems at relatively long distances from the source. It is noteworthy that this may have also direct consequences on lichen biodiversity that is a relevant component of forest biota of mountain areas, contributing to forest functioning (Knops et al., 1996; Pike, 1978) and including several species of conservation concern (Nascimbene et al., 2010; Nascimbene et al., 2012; Nascimbene et al., 2013). In particular, Phenanthrene concentrations similar to those found in our specimens were demonstrated to inhibit photosynthetic processes of the photobiont that are likely related with changes of membrane structure induced by this pollutant (Kummerová et al., 2006).

The pattern of PAHs concentrations at increasing distance from the road is consistent with that of TEQ, confirming a higher risk for the biota within the first meters from the road. Moreover, the pattern of total PAHs concentration and TEQ is partially reflected by those of total PAHs of combustion origin and of individual compounds. This pattern is evident for most of the compounds with higher molecular weight that in our case are mainly of combustion origin. These results support the hypothesis that the observed PAHs pollution along the transects is mainly related to road traffic. However, the relative amount of PAHs of combustion origin is constant along the transects, indicating that this component of the PAHs mixture is homogeneously distributed, due to its long-distance origin.

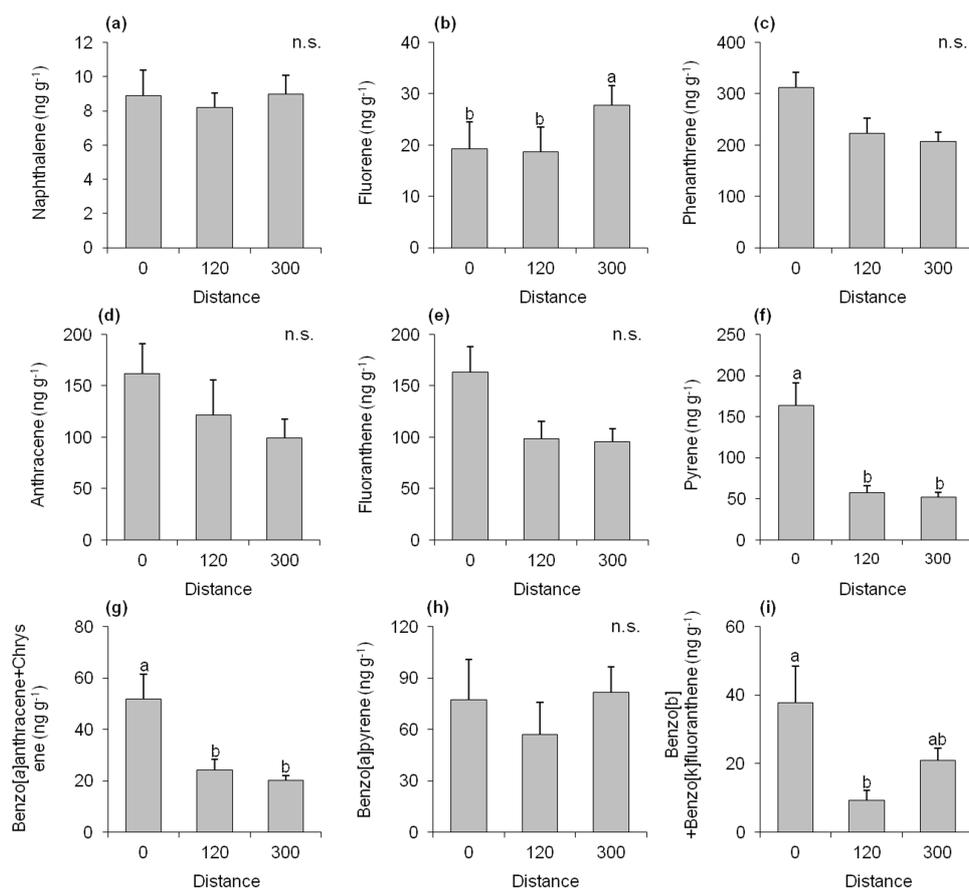


Fig. 4 Mean \pm SE of naphthalene (a), fluorene (b), phenanthrene (c), anthracene (d) fluoranthene (e), pyrene (f), benzo[a]anthracene+chrysene (g), benzo[a]pyrene (h) and benzo[b]+benzo[k]fluoranthene (i) at increasing distance from the pass road. Different letters indicate significant differences according to the Tukey's multiple comparison test based on the model of Table 3. The individual compounds are ordered according to an increasing molecular weight.

The interaction between site and distance from the road for the total concentrations of PAHs indicates that the pattern of PAHs pollution is site specific, which is particularly evident in the most polluted sites. Moreover, differences among sites were evident mainly for samples collected nearest to the road, supporting the hypothesis of a filtering function by forest vegetation (McLachlan et al., 1998), resulting in a progressive reduction of PAHs loads that, at 300 m from the road, are lower and more similar among the seven survey areas. Only in the least polluted one, PAHs concentrations at 300 m was not significantly higher than those of undisturbed reference sites. This situation is consistent with the findings by Blasco et al. (2008) that found relevant concentrations of PAHs within a distance of 500 m from the road in a mountain touristic area of the Pyrenees.

Direct quantitative comparisons in terms of total PAHs concentrations and TEQ with other studies are hindered by the fact that it is not always clear whether autochthonous lichen samples were normalized for exposure time by using only the apical portion of the lobes for chemical analyses (Adamo et al., 2007, 2008), as in our experiment. Furthermore, comparisons in terms of PAHs profile could be partially biased if young parts of lichens would prove to be most active than

old parts capturing low molecular PAHs which mainly exist in the gas-phase. This could lead to a different PAHs profile at the thallus level. However, comparisons in terms of PAHs profile indicate that our results are consistent with those obtained in previous lichen biomonitoring studies that showed the prevalence of PAHs with 3-4 rings in the mixture of pollutants (e.g. Augusto et al., 2010; Blasco et al., 2008, 2011; Guidotti et al., 2009). In particular, as in Blasco et al. (2011) and Guidotti et al. (2009), the most abundant compound was phenanthrene, indicative of traffic pollution (Blasco et al., 2006). The accordance of our PAHs profile with other lichen biomonitoring studies addressing traffic pollution is further supported by the high contribution of some combustion PAHs, such as fluoranthene, pyrene, and benzo[g,h,i]perylene (Blasco et al., 2006, 2008).

5. Conclusions

Our study supports the effectiveness of lichen biomonitoring to reveal both large scale and local patterns of traffic PAHs pollution in mountain areas where chemical-physical monitoring is often hindered by logistic constraints. Local patterns are more evident in the most impacted sites where PAHs pollution has a steeper gradient with distance from the road. These results indicate that lichen biomonitoring can be used to prioritize sites for management (i.e. traffic control and limitation) and to evaluate the local impact of traffic on the biota that may alter ecosystem functions and services.

Recent research on PAHs pollution monitoring by lichens suggests the possibility of relating PAHs values in lichens with their atmospheric concentrations which would give the chance to use this information for regulatory purposes; however, the relationships between PAHs in lichens and in air have to be tested for their regional patterns and may also vary depending on the lichen species (Augusto et al., 2013). Further studies are needed for a more accurate estimate of the air pollution risk in mountain areas of touristic interest.

Conflict of interest

We declare that we have no conflict of interest related with this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2013.12.090>

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Appendix A. Comparison of TOT_PAHs, TOT_PAHs_comb, equivalent Toxicity (TEQ) and %PAHs_com (PAH profile) between control sites and the seven passes for each distance from the road separately. Means of each response variable are provided for control site and the seven passes. Asterisks indicate that the value is significantly different from control site (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; Dunnett's test).

	Distance 0 m		Distance 120 m		Distance 300 m	
	Mean	<i>P</i> -value	Mean	<i>P</i> -value	Mean	<i>P</i> -value
TOT_PAHs		0.001		0.008		0.003
<i>Control</i>	194.4		194.4		194.4	
Sella	1559.1	***	868.9	**	845.3	**
Pordoi	682.3	*	842.6	**	791.1	**
Rolle	1506.7	**	486.8	*	639.4	*
Falzarego	1083.5	*	1148.6	**	581.6	*
Fedaia	969.4	**	239.9		515.9	*
Cimabanche	693.2	*	476.0	*	656.4	**
Staulanza	514.1		389.3		342.4	
TOT_PAHs_comb		< 0.001		0.005		< 0.001
<i>Control</i>	59.6		59.6		59.6	
Sella	835.0	***	389.4	**	327.5	**
Pordoi	310.2	*	335.3	*	358.1	**
Rolle	659.7	**	211.1	*	310.6	**
Falzarego	558.2	**	411.2	**	276.5	**
Fedaia	467.6	*	59.3		174.6	
Cimabanche	228.3	*	168.1	*	311.0	**
Staulanza	187.3		154.1		133.1	
%_PAHs_comb		0.349		0.175		0.340
<i>Control</i>	0.342		0.342		0.342	
Sella	0.495		0.450		0.409	
Pordoi	0.440		0.392		0.461	
Rolle	0.438		0.465		0.498	
Falzarego	0.515		0.316		0.477	
Fedaia	0.449		0.247		0.315	
Cimabanche	0.332		0.418		0.467	
Staulanza	0.334		0.301		0.321	
Equivalent Toxicity (TEQ)		0.002		0.025		< 0.001
<i>Control</i>	1.68		1.68		1.68	
Sella	20.88	***	8.03	**	8.14	***
Pordoi	6.30		7.10	*	9.80	***
Rolle	12.11	*	6.00		5.60	**
Falzarego	13.36	*	8.48	**	5.47	**
Fedaia	14.81	*	1.56		3.87	
Cimabanche	5.56		4.05		5.97	**
Staulanza	3.59		3.59		5.31	**

Appendix B. Contrast analysis testing differences on TOT_PHAs among the sites within the same distance from the road based on the model of Table 3.

	Estimate	SE	<i>t</i> -value	<i>P</i> -value
Distance from the road 0 m				
Cimabanche vs. Falzarego	0.485	0.454	1.067	0.996
Cimabanche vs. Fedaia	0.295	0.321	0.917	0.999
Cimabanche vs. Pordoi	-0.003	0.321	-0.008	1.000
Cimabanche vs. Rolle	0.812	0.359	2.260	0.505
Cimabanche vs. Sella	0.810	0.321	2.520	0.345
Cimabanche vs. Staulanza	-0.371	0.321	-1.153	0.992
Falzarego vs. Fedaia	-0.190	0.454	-0.418	1.000
Falzarego vs. Pordoi	-0.487	0.454	-1.073	0.996
Falzarego vs. Rolle	0.327	0.482	0.679	1.000
Falzarego vs. Sella	0.325	0.454	0.715	1.000
Falzarego vs. Staulanza	-0.856	0.454	-1.882	0.754
Fedaia vs. Pordoi	-0.297	0.321	-0.925	0.999
Fedaia vs. Rolle	0.517	0.359	1.439	0.949
Fedaia vs. Sella	0.515	0.321	1.603	0.896
Fedaia vs. Staulanza	-0.665	0.321	-2.071	0.633
Pordoi vs. Rolle	0.815	0.359	2.267	0.501
Pordoi vs. Sella	0.812	0.321	2.528	0.341
Pordoi vs. Staulanza	-0.368	0.321	-1.145	0.992
Rolle vs. Sella	-0.002	0.359	-0.006	1.000
Rolle vs. Staulanza	-1.183	0.359	-3.291	0.074
Sella vs. Staulanza	-1.181	0.321	-3.673	0.029
Distance from the road 120 m				
Cimabanche vs. Falzarego	0.638	0.579	1.101	0.995
Cimabanche vs. Fedaia	-0.622	0.482	-1.290	0.978
Cimabanche vs. Pordoi	0.930	0.508	1.831	0.784
Cimabanche vs. Rolle	-0.510	0.508	-1.004	0.998
Cimabanche vs. Sella	0.141	0.482	0.293	1.000
Cimabanche vs. Staulanza	0.646	0.482	1.340	0.970
Falzarego vs. Fedaia	-1.260	0.557	-2.263	0.503
Falzarego vs. Pordoi	0.293	0.579	0.505	1.000
Falzarego vs. Rolle	-1.148	0.579	-1.982	0.691
Falzarego vs. Sella	-0.496	0.557	-0.892	0.999
Falzarego vs. Staulanza	0.008	0.557	0.015	1.000
Fedaia vs. Pordoi	1.552	0.482	3.220	0.087
Fedaia vs. Rolle	0.112	0.482	0.231	1.000
Fedaia vs. Sella	0.763	0.454	1.680	0.863
Fedaia vs. Staulanza	1.268	0.454	2.790	0.212
Pordoi vs. Rolle	-1.441	0.508	-2.835	0.195
Pordoi vs. Sella	-0.789	0.482	-1.637	0.883
Pordoi vs. Staulanza	-0.285	0.482	-0.590	1.000

Rolle vs. Sella	0.652	0.482	1.352	0.968
Rolle vs. Staulanza	1.156	0.482	2.399	0.415
Sella vs. Staulanza	0.504	0.454	1.110	0.994
Distance from the road 300 m				
Cimabanche vs. Falzarego	-0.605	0.557	-1.087	0.995
Cimabanche vs. Fedaia	-0.558	0.454	-1.227	0.986
Cimabanche vs. Pordoi	0.166	0.454	0.366	1.000
Cimabanche vs. Rolle	-0.860	0.482	-1.784	0.810
Cimabanche vs. Sella	-0.579	0.454	-1.274	0.980
Cimabanche vs. Staulanza	-0.329	0.454	-0.724	1.000
Falzarego vs. Fedaia	0.048	0.557	0.085	1.000
Falzarego vs. Pordoi	0.772	0.557	1.386	0.961
Falzarego vs. Rolle	-0.255	0.579	-0.440	1.000
Falzarego vs. Sella	0.026	0.557	0.047	1.000
Falzarego vs. Staulanza	0.276	0.557	0.496	1.000
Fedaia vs. Pordoi	0.724	0.454	1.593	0.900
Fedaia vs. Rolle	-0.302	0.482	-0.627	1.000
Fedaia vs. Sella	-0.021	0.454	-0.047	1.000
Fedaia vs. Staulanza	0.229	0.454	0.503	1.000
Pordoi vs. Rolle	-1.027	0.482	-2.130	0.593
Pordoi vs. Sella	-0.746	0.454	-1.640	0.881
Pordoi vs. Staulanza	-0.496	0.454	-1.090	0.995
Rolle vs. Sella	0.281	0.482	0.583	1.000
Rolle vs. Staulanza	0.531	0.482	1.102	0.994
Sella vs. Staulanza	0.250	0.454	0.550	1.000

Seasonal variations of PAHs content and distribution patterns in a mixed land use area: a case study in NE Italy with the transplanted lichen *Pseudevernia furfuracea*

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Highlights

- Seasonal differences of the PAHs accumulation in lichen transplants were investigated.
- Important differences in concentration and distribution were observed.
- In summer PAH degradation processes are more pronounced and some sources are inactive.
- Environmental monitoring of PAHs should be carried out in winter.

Abstract

The seasonal differences of the polycyclic aromatic hydrocarbons (PAHs) content in lichen transplants were investigated in an area of ca. 40 square kilometers in NE Italy characterized by different land use. Two sets of samples of the epiphytic lichen *Pseudevernia furfuracea* were collected in a pristine area of the Carnic Alps and transplanted to 40 exposure sites for a period of two months, respectively in late winter and in summer. Results revealed a pronounced difference between the two seasons in terms of PAH content and distribution patterns. After the summer exposure the PAH concentrations in the transplants were more than one order of magnitude lower than after the winter exposure (ranging from 48.22 to 272.73 ng g⁻¹ dw and from 289.73 to 1,575.85 ng g⁻¹ dw in the summer and winter samples respectively). Also the main emission sources changed, mostly due to the drastic reduction in the emissions by wood burning for domestic heating and to the different meteorological conditions. In summer PAHs degradation was enhanced by intense UV radiation, high temperatures, and presence of ozone. The implications of these findings for the biomonitoring of PAHs pollution are addressed.

Keywords: biomonitoring, domestic heating, lichen transplants, PAHs degradation, seasonality.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants that originate mainly from anthropogenic sources such as industrial activities, road traffic, and domestic heating through processes of incomplete combustion of biomass and fossil fuels (Harvey, 1991). They have received much attention in the last decades due to their mutagenic and carcinogenic properties (Augusto et al. 2012; Boström et al., 2002) with tendency to bioaccumulate and persist in the environment (Augusto et al., 2013a). Once emitted, they will disperse in the atmosphere and travel for long distances depending on their weight and on air temperature (Beyer et al., 2003), undergoing photochemical and chemical degradation (Alves, 2008; Boström et al., 2002; Brown and Brown, 2012; Jung et al., 2010 and Schauer et al., 2003).

Lichens, a symbiotic association between fungi and algae and/or cyanobacteria, unlike plants, lack a cuticle layer that limits the inflow of pollutants and thus absorb contaminants and nutrients from both wet and dry atmospheric deposition (Bargagli, 1998; Garty, 2000). They are among the best and most frequently used biomonitors of air-borne pollutants, so that a norm for their use has just been edited by the European Committee for Standardization (CEN, EN 54 16413:2014). Lichens are valid biomonitors of PAHs because (i) they bioaccumulate both vapor- and solid-phase PAHs (Augusto et al., 2013a; Guidotti et al., 2003, 2009; Nascimbene et al., 2014; Protano et al., 2014; Shukla and Upreti, 2009; Van der Wat & Forbes, 2015); (ii) they show a “memory” effect with a retroactive period of 45 to 60 days, and correlations can be made between the concentrations found in lichens and in the air (Augusto et al., 2013b); (iii) the low costs of material recovery allow to apply a high sampling density that improves the experimental design of field investigations; (iv) by using transplants, it is also possible to cover those areas where autochthonous macrolichens are frequently absent (e.g. urban centers); (v) based on the PAH profile it is possible to identify the source(s) of emission (forest areas are dominated by 3-ring PAHs, urban by 4-ring PAHs, industrial pyrogenic by 5- and 6-ring PAHs and industrial petrogenic by 2-ring PAHs, see Augusto et al., 2009; Shukla et al., 2012).

However, the extent of the seasonal variation in the PAHs content of lichens is largely unknown. Several physical-chemical and biological factors certainly interplay to determine a multi-faceted figure. Lichen growth forms (Blasco et al. 2011), age (Bargagli et al. 1987), lipid content (Zhu et al. 2015), development of reproductive structures (Adamo et al. 2007) are all factors that may influence the bioaccumulation capability. This is further complicated by the variable amounts

of PAHs released into the environment, and by the different fate in the atmosphere of e.g. 2- and 6-ring PAHs.

The objective of this study was to verify the seasonal differences of the PAH content in lichen transplants in a mixed land use area of NE Italy selected in order to have:

- all the primary PAH emission sources (different industrial activities, road traffic and domestic heating);
- to evaluate the effects of the different meteorological conditions on the ability of lichens to accumulate PAHs;
- to observe if and to what extent the PAH distribution changes with location between the seasons.

2. Materials and methods

2.1 Study area

The study area is located at the foot of the Carnic pre-Alps (NE Italy; 46°10'N 12°44'E; Fig. 1), at an altitude of 200 - 380 m a.s.l. It covers almost 40 km² and hosts a resident population of ca. 16,240 inhabitants. It was selected in order to include different land uses: natural, agricultural, urban, and industrial, intermingled to form the typical mixed land use patchwork that extends over large areas of N Italy. The main potential pollution sources of the study area are: (i) an isolated, medium-sized cement plant (clinker production: 556,000 ton year⁻¹ in 2012; M. Vicenzetto, personal communication); (ii) a large industrial zone that hosts two steel works, several knife manufacturing factories and a chemical plant producing pesticides; (iii) vehicular traffic, concentrated in the main urban centers and along a national road ("464 - Spilimbergo") that crosses the northern part of the study area; (iv) domestic heating emissions.

In the study area 37 exposure sites were located at the intersections of a 700 m step grid, and 3 further sites were located in the nearby urban centers (Fig. 1). Following the Corine Land Cover (2006) classification, 21 sites belonged to the agricultural stratum, 10 to the urban stratum, 5 to the industrial stratum and 4 to the forest stratum. Detailed information about the exposure sites is listed in Tab. SD-1 in the Supplementary Data.

The data on the meteorological conditions during the exposure periods was retrieved from the closest weather station, that is located outside Arba (Fig. 1), and is run by the Civil Protection Service.

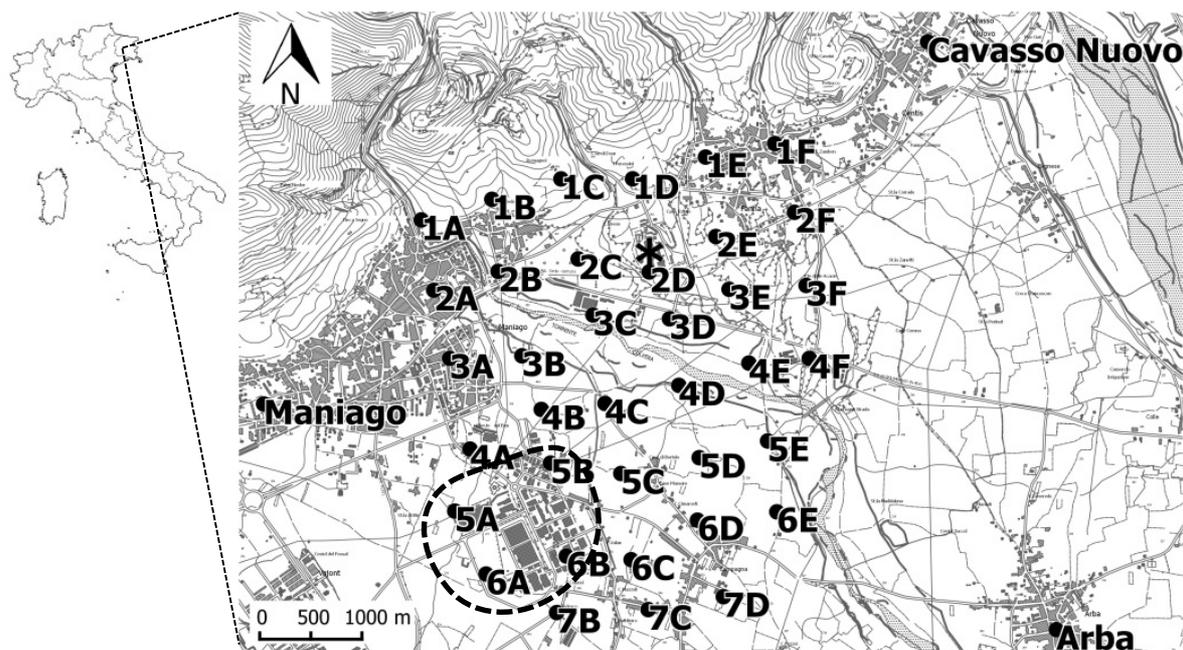


Fig. 1. Study area with location of the 40 exposure sites. The cement plant is indicated by an asterisk, the industrial area by a closed dashed line.

2.2 Lichen sampling, exposure and recovery

The epiphytic lichen *Pseudevernia furfuracea* (L.) Zopf. var. *furfuracea* was selected because of its commonness, good resistance to transplantation (Tretiach et al., 2007), and frequent use as biomonitor of trace elements and PAHs (Tab. SD-2 in the Supplementary Data). Samples were collected in the Carnic Alps (Casera Razzo, Belluno; 46°25'N 12°44'E) at 1,700 m a.s.l., far from local anthropogenic sources (Adamo et al., 2008; Tretiach et al., 2011), from branches at 2-4 m above ground of solitary larch (*Larix decidua* Mill.) trees, in two distinct periods: 9th January 2012 for the late winter exposure (see below), and 8th June 2012 for the summer exposure (see below). The material was collected along with ca. 15-20 cm long piece of the supporting twig using garden shears. The material was transported to the laboratory in paper bags and left to dry out in dim light at room temperature. Moderately isidiate samples were selected avoiding those over-isidiate or with apothecia (because these structures may alter the surface/weight ratio), infected by lichenicolous fungi or covered by epiphytic algae (Tretiach et al., 2007, 2011).

One twig carrying 1-3 thalli was attached to a 120 cm long bamboo stick using plastic bonds (both first rinsed with distilled water) and sealed in plastic bags until exposure to avoid contamination.

Two exposure periods were selected, one in late winter (15th February-17th April 2012), and the other in summer (19th June-21st August 2012), using two different sets of samples. At each site two bamboo sticks were attached to the external branches of deciduous trees at approximately 4 m above ground using plastic bonds. In both cases, the exposure was done within 12 hours of field

work. After the two-month exposure the samples were retrieved with a piece of the bamboo stick, sealed in plastic bags, and transported in a coolbag within 6 hours to the laboratory, where the bags were opened, and the samples were left to dry out at room temperature for 12 hours. 600 mg of ca. 2.5 cm long lobe portions were selected for each sample, sealed in glass cans and kept in the dark at -20°C until analysis minimizing potential losses of PAHs from samples (Augusto et al. 2013b). Samples of the site 4B were lost during the winter exposure and samples of the sites 1E, 2C and 5B were lost during the summer exposure.

2.3 PAHs analyses

The analysis procedure is based on the protocol of Domeño et al. (2006), slightly modified as follows.

Before extraction the samples were allowed to defrost and then finely chopped with scissors. The extraction was performed using an Accelerated Solvent Extractor (ASE X-100, Dionex): ca. 600 mg of dried sample was placed in the extractor cell together with 600 mg of dried Na₂SO₄ and 600 mg of Florisil. A solution of surrogate standards of deuterated PAHs (100 µL, 1.2 µg mL⁻¹ Wellington L429-IS) was added for the evaluation of recovery rates. The extraction was performed in a mixture of dichloromethane/acetone 1:1 for trace analysis (Pestanal, Sigma-Aldrich) at 140 °C and 100 atm (3 extraction cycles of 10 min). The extract (ca. 120 mL) was rotary evaporated under reduced pressure to ca. 1 mL. The reduced extract was purified by column chromatography on dried alumina activated at 400 °C for 6 h, eluting with dichloromethane. The eluate was evaporated to dryness by a gentle nitrogen stream and the residue dissolved in approximately 2 mL of cyclohexane for trace analysis (Pestanal, Sigma-Aldrich). An exact amount of Pyrene-D (100 µL of a 20 µg mL⁻¹ solution, Aldrich 490695) was added as internal standard to every extracted solution in order to avoid bringing the final volume exactly up to 2 mL.

The analysis was carried out by a GC-MS system (Agilent 6890/5973 Inert, Agilent DB 5MS UI capillary column 30 m × 0.25 mm i.d. × 0.25 µm film thickness) with helium as carrier gas. The system was equipped with an autosampler (Gerstel MPS2).

The GC oven temperature program started at 55 °C then was ramped to 200 °C at 25 °C/min, to 320 °C at 10 °C/min and to 325 at 25 °C/min, with a final isothermal stage held for 10 min. The mass spectrometer operated in selective ion monitoring (SIM) mode. The following mass ions were used for the quantification of individual PAHs: fluoranthene (Fth, m/z 202), pyrene (Pyr, m/z 202), benzo[a]anthracene (BaA, m/z 228), chrysene (Chr, m/z 228), benzo[b]fluoranthene (BbF, m/z 252), benzo[k]fluoranthene (BkF, m/z 252), benzo[a]pyrene (BaP, m/z 252), indeno[1,2,3-cd]pyrene (Ind, m/z 276), dibenzo[a,h]anthracene (DahA, m/z 278), and

benzo[g,h,i]perylene (BghiP, m/z 276). The PAH recoveries were calculated respectively by fluoranthene-d10 (m/z 212, for Fth and Pyr), benzo[a]anthracene-d12 (m/z 240, for BaA and Chr), benzo[b]fluoranthene-d12 (m/z 264, for BbF and BkF), benzo[a]pyrene-d12 (m/z 264, for BaP), indeno[1,2,3-cd]pyrene-d12 (m/z 288, for Ind and DahA), benzo[g,h,i]perylene-d12 (m/z 288, for BghiP). Six calibration solutions with concentrations ranging from 5 ng mL^{-1} to 100 ng mL^{-1} for each PAH were prepared in triplicate by appropriate dilution of the certified PAHs standard mixture (PAH-EPA 610 by Sigma-Aldrich) in cyclohexane for trace analysis (Pestanal, Sigma-Aldrich) in order to quantify the PAH concentrations. A mid-range calibration standard solution was re-measured every six samples in order to ensure that the instrument was still calibrated.

In the absence of a certified lichen standard material, instrumental signal to noise ratio was calculated on a 10 ng mL^{-1} standard solution in order to determine the limit of detection (LOD) and the limit of quantification (LOQ). LOD is determined as the amount of analyte that generates a signal that is three times the noise in regions of the chromatogram close to the elution time of the peak of interest, that are approximately twenty times the analyte peak width. LOQ is determined as the amount of analyte that generates a signal that is ten times the noise. LODs (expressed in ng g^{-1} of PAHs in a theoretical 600 mg lichen dw) were respectively: 0.10 (Fth), 0.14 (Pyr), 0.19 (BaA), 0.22 (Chr), 0.48 (B(b+k)F), 0.36 (BaP), 0.18 (Ind), 0.27 (DahA), 0.30 (BghiP).

2.4 Statistics

Descriptive statistics were performed using Microsoft Excel (Microsoft Office Professional Plus 2010). Box-plots, principal component analysis (PCA) and Mann-Whitney U Test were performed to verify differences between groups using STATISTICA 8.0 StatSoft Inc. (2007). For samples in which a compound was under the limit of detection (LOD) of the instrument its concentration was assumed to be the LOD value. The maps representing the distribution patterns of the total PAHs were calculated using Surfer (Win32) 6.04 (Golden Software Inc.) with Inverse Distance to a Power as the gridding method and a medium Smooth Contour for the creation of the maps, the intervals between the minimum and maximum concentration values have been divided into 10 classes of equal extent. The equivalent toxicity quotient (TEQ) of the PAHs was calculated according to the toxic equivalency factors proposed by Nisbet and LaGoy (1992). The localization of the exposure sites in the study area was graphically represented using Quantum GIS 1.8.0.-Lisboa (2012).

3. Results

3.1 Meteorological conditions

The main climatic differences observed during the two exposure periods are summarized in Fig. 2. The total rainfall was similar, 202.2 mm in winter and 213.8 mm in summer. The rain events of the winter exposure were concentrated at the end of the period, while those of the summer exposure were more evenly distributed but much briefer and significantly more intense ($p=0.001$; Fig. 2a). The atmospheric pressure was significantly higher during the winter period ($p=0.009$; Fig. 2b). The winds were mainly north-northwesterly all year round (Fig. 2c). The mean temperature was 10.0 °C during the winter exposure, ranging from 5.6 to 15.1 °C (mean daily minimum and maximum, respectively) and 24.6 °C during the summer exposure, ranging from 19.3 to 30.2 °C (Fig. 2d).

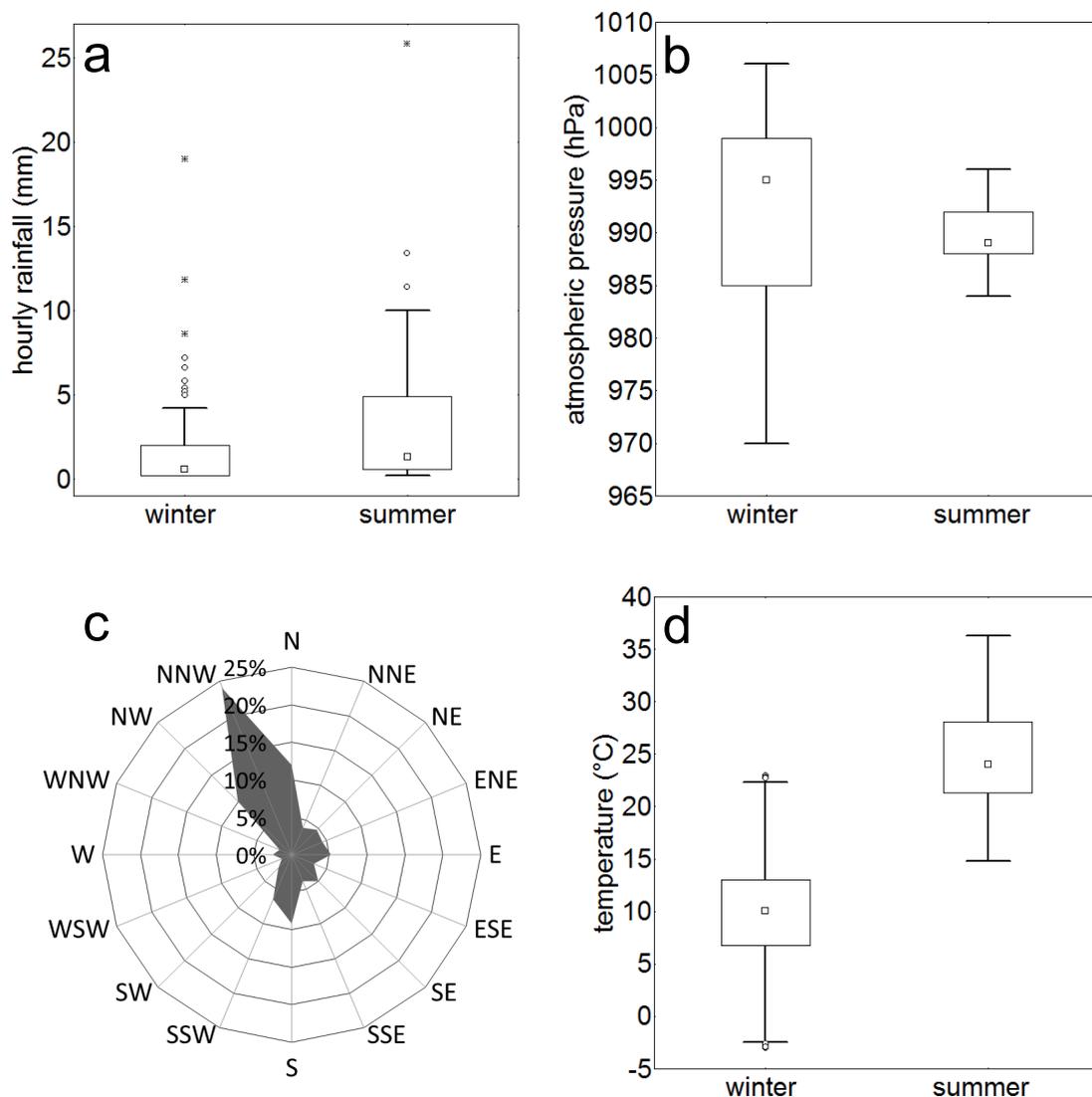


Fig. 2. Hourly rainfall (a), atmospheric pressure (b), overall wind direction (c), and air temperature (d) in the study area of Fig. 1 during the two exposure periods, winter and summer (\square : median; box: 25-75%; whiskers: non outlier ranges; \circ : outliers; $*$: extremes).

3.2 PAH content

In total, 10 PAHs were detected: fluoranthene (Fth), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene and benzo[k]fluoranthene being treated together as benzo[b+k]fluoranthene (B(b+k)F) due to the difficulty in clearly discerning among their peaks, benzo[a]pyrene (BaP), indeno[1,2,3-c,d]pyrene (Ind), benzo[g,h,i]perylene (BghiP) and dibenzo[a,h]anthracene (DahA). Lighter (two and three ring) PAHs – with higher volatilities, smaller octanol-air partitioning and more prone to volatilization from lichens (Simonich, 1994; Obadasi, 2006; Schrlau, 2011) – were not considered for analytical quantification due to both unfavorable temperature dependent lichen-air partitioning in the environment and non-relevant human toxicity in comparison with heavier congeners (Böstrom, 2002; IARC WG, 2010). Moreover, our purification procedure, while guaranteeing adequate recoveries for heavier PAHs, does not allow the removal of certain lichen substances eluting at retention times similar to those of two and three ring PAHs and thus generating undesired noise.

The sum of the concentrations of the 10 PAHs in the pre-exposure winter samples ranged from 65.83 to 211.92 ng g⁻¹ dw (Tab. 1, Tab. SD-3), and were significantly lower than in all the post-exposure winter samples. The concentrations in the latter ranged in fact from 289.73 to 1,575.85 ng g⁻¹ dw (Tab. 1, Tab. SD-3), with BaP under the LOD of the instrument in only one sample, Ind in two samples, BghiP in 53.8% and DahA in 69.2% of the samples. In these samples the concentrations of 5-, 6-ring PAHs, as well as of BaP, were rather low and equally distributed in the study area with no significant differences between land-use strata, whereas 95% of the total PAH content was given by 4-ring PAHs (Fig. 3). The significantly lowest concentrations of 4-ring PAHs were found in the forest stratum (Tab. 2, Fig. 4a), followed by the industrial and agricultural strata (with similar values), while significantly higher concentrations were found in the urban stratum, although the TEQ values denote a low carcinogenicity risk (Fig. 5, Tab. SD-5).

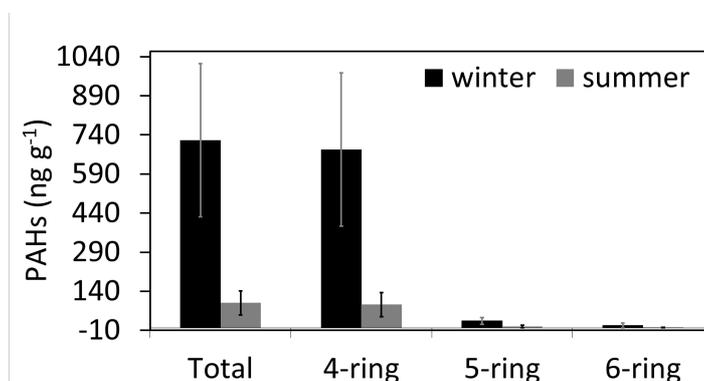


Fig. 3. Average and standard deviation of total, 4-, 5- and 6-ring PAHs content (ng g⁻¹ dw) in the winter and summer samples of the lichen *Pseudevernia furfuracea* exposed in the 40 sites of Fig. 1.

Tab. 1. PAHs content (ng g⁻¹ dw) measured in pre- and post-exposure samples of the lichen *Pseudevernia furfuracea* transplanted in the sites of Fig. 1 subdivided according to land use.

WINTER		pre-exposure	post-exposure			
land use strata			agricultural	forest	industrial	urban
n. of sites			20	4	5	10
4-ring PAHs	mean	156.87	586.59	314.58	702.89	1015.36
	SD	44.18	190.16	84.59	146.47	268.17
	min	55.54	403.62	236.77	509.36	691.06
	max	199.17	1138.41	430.59	905.61	1546.89
5-ring PAHs	mean	14.86	23.84	32.48	21.66	31.77
	SD	6.8	7.05	9.29	7.65	21.58
	min	3.55	13.68	20.38	13.33	19.8
	max	22.12	44.16	41.68	34.01	92.49
6-ring PAHs	mean	1.77	7.49	23.56	4.79	8.03
	SD	1.32	5.05	23.99	1.87	4.54
	min	0.71	0.71	3.26	2.25	3.54
	max	4.6	19.75	58.32	6.68	19.7
Total PAHs	mean	173.5	617.91	370.62	729.34	1055.16
	SD	45.18	190.83	85.03	154.14	270.4
	min	65.83	445.51	289.73	526.25	724.8
	max	211.92	1175.59	464.35	944.75	1575.85
SUMMER		pre-exposure	post-exposure			
land use strata			agricultural	forest	industrial	urban
n. of sites			20	4	4	9
4-ring PAHs	mean	87.12	81.12	57.89	138.97	99.34
	SD	16.12	47.34	8.2	69.32	21.02
	min	55.54	46.88	45.99	94.06	66.79
	max	109.29	271.39	63.54	242.23	132.03
5-ring PAHs	mean	7.48	3.56	3.53	11.35	6.12
	SD	4.84	2.4	3.35	9.31	4.9
	min	4.74	0.63	0.63	0.63	0.63
	max	20.06	9.31	6.45	23.35	15.76
6-ring PAHs	mean	0.92	1.05	0.71	0.71	1.78
	SD	0.62	0.86	0	0	2.17
	min	0.71	0.71	0.71	0.71	0.71
	max	2.58	3.74	0.71	0.71	6.42
Total PAHs	mean	95.51	85.73	62.14	151.03	107.24
	SD	18.47	47.19	7.35	61.82	18.47
	min	65.43	48.22	53.15	116.86	75.79
	max	130.07	272.73	70.66	243.57	133.37

Tab. 2. p-values (Man-Whitney *U* test) of the differences between the 4-ring PAH concentrations measured in post-exposure samples of the lichen *Pseudevernia furfuracea* exposed in the sites of Fig. 1 subdivided according to land use. (p-value considered significant <0.05).

WINTER 4-ring PAHs	forest	industrial	urban
agricultural	0.001	0.097	0
forest	-	0.016	0.002
industrial	-	-	0.040
SUMMER 4-ring PAHs	forest	industrial	urban
agricultural	0.157	0.005	0.007
forest	-	0.029	0.003
industrial	-	-	0.260

The concentrations in the pre-exposure summer samples were somewhat lower than in the winter ones, ranging from 65.43 to 130.07 ng g⁻¹ dw (Tab. 1, Tab. SD-4). The concentrations in the post-exposure summer transplants were ca. an order of magnitude lower than in winter, ranging from 48.22 to 272.73 ng g⁻¹ dw (Tab. 1, Tab. SD-4). Several PAHs were often under the LOD of the instrument: B(b+k)F in 29.7% of the samples, BaP in 75.7%, Ind in 86.5%, BghiP in 94.6% and DahA in all the samples. The post-exposure concentrations in the summer samples of both forest and agricultural strata were actually significantly lower than in the pre-exposure samples, while the post-exposure samples of the urban and industrial strata were not significantly different from the pre-exposure samples. In this season as well there were no significant differences between land-use strata in terms of concentrations of the 5-, 6-ring PAHs and BaP, whereas 95% of the total PAH content was given by 4-ring PAHs (Fig. 3). The significantly lowest concentrations of 4-ring PAHs were found in the forest and agricultural strata (which had similar values), while the significantly highest concentrations were found in the urban and industrial strata (also with similar values between them: Tab. 2, Fig. 4b).

Between the winter and the summer exposure not only a pronounced difference in PAHs concentrations of post-exposure samples was observed, but also an entirely different correlation between PAHs and land use strata (Fig. 6).

In the winter campaign (Fig. 6a,b) the urban sites were distinctly separated from all the other sites at one end of the first component and were strongly associated to the 4-ring PAHs Fth, Pyr, BaA and Chr. The 5- and 6-ring PAHs, as already mentioned, did not have a particular association with any stratum but only with 2 samples. The forest site 1B had high concentrations of 6-ring PAHs, and the urban site 4A had a high concentration of B(b+k)F, the possible emission sources remaining unidentified (Fig. 1).

In the summer campaign (Fig. 6c,d) the differences between strata were much less pronounced, although the industrial sites were those more strongly associated with 4-ring PAHs,

followed by the urban sites, while the agricultural and forest sites mostly overlapped. The 6-ring PAHs seemed to have a strong association with two urban sites (Cavasso Nuovo and Maniago; Fig. 1), due to the fact that their 6-ring PAHs content was above the LOD (actually, being the only two in the case of BghiP).

As an example of the differences between the two exposure periods, Fig. 7 shows the distributional patterns of the total PAHs load drawn with Surfer (Win32) 6.04 (Golden Software Inc.).

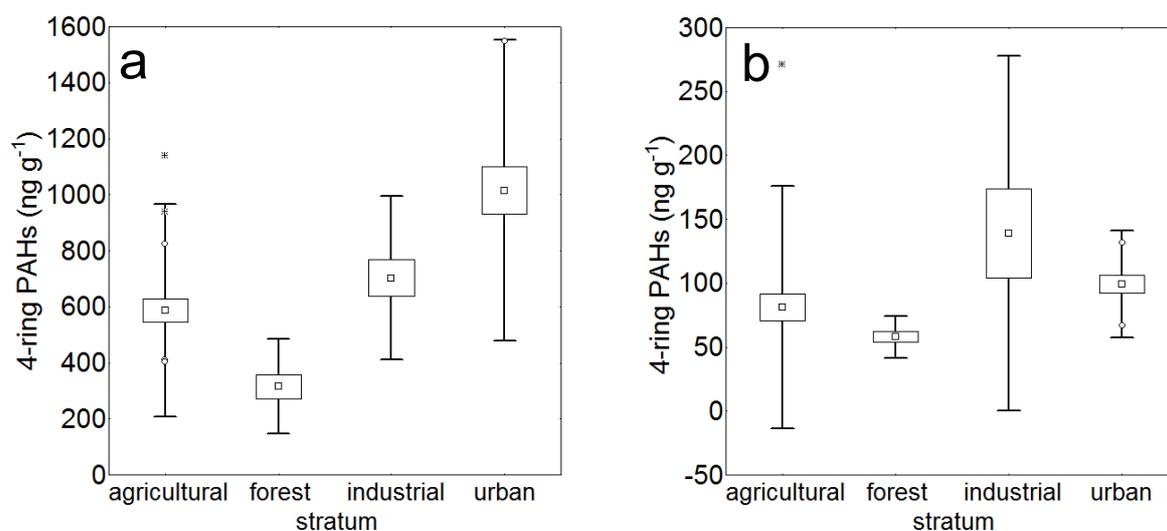


Fig. 4. 4-ring PAHs content (ng g⁻¹ dw) in the samples of the lichen *Pseudevernia furfuracea* exposed in winter (a) and in summer (b) in the 40 sites of Fig. 1 subdivided according to land use (□: mean; box: mean±SE; whiskers: mean±2×SD; ○: outliers; *: extremes).

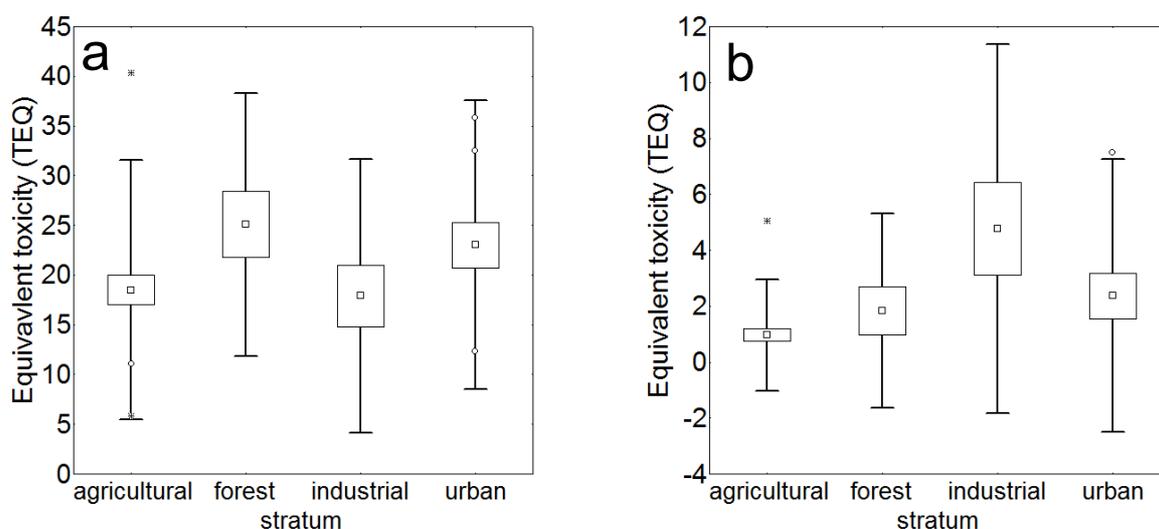


Fig 5. Equivalent Toxicity (TEQ) values of PAHs measured in the samples of the lichen *Pseudevernia furfuracea* exposed in winter (a) and in summer (b) in the 40 sites of Fig. 1 subdivided according to land use (□: mean; box: mean±SE; whiskers: mean±2×SD; ○: outliers; *: extremes).

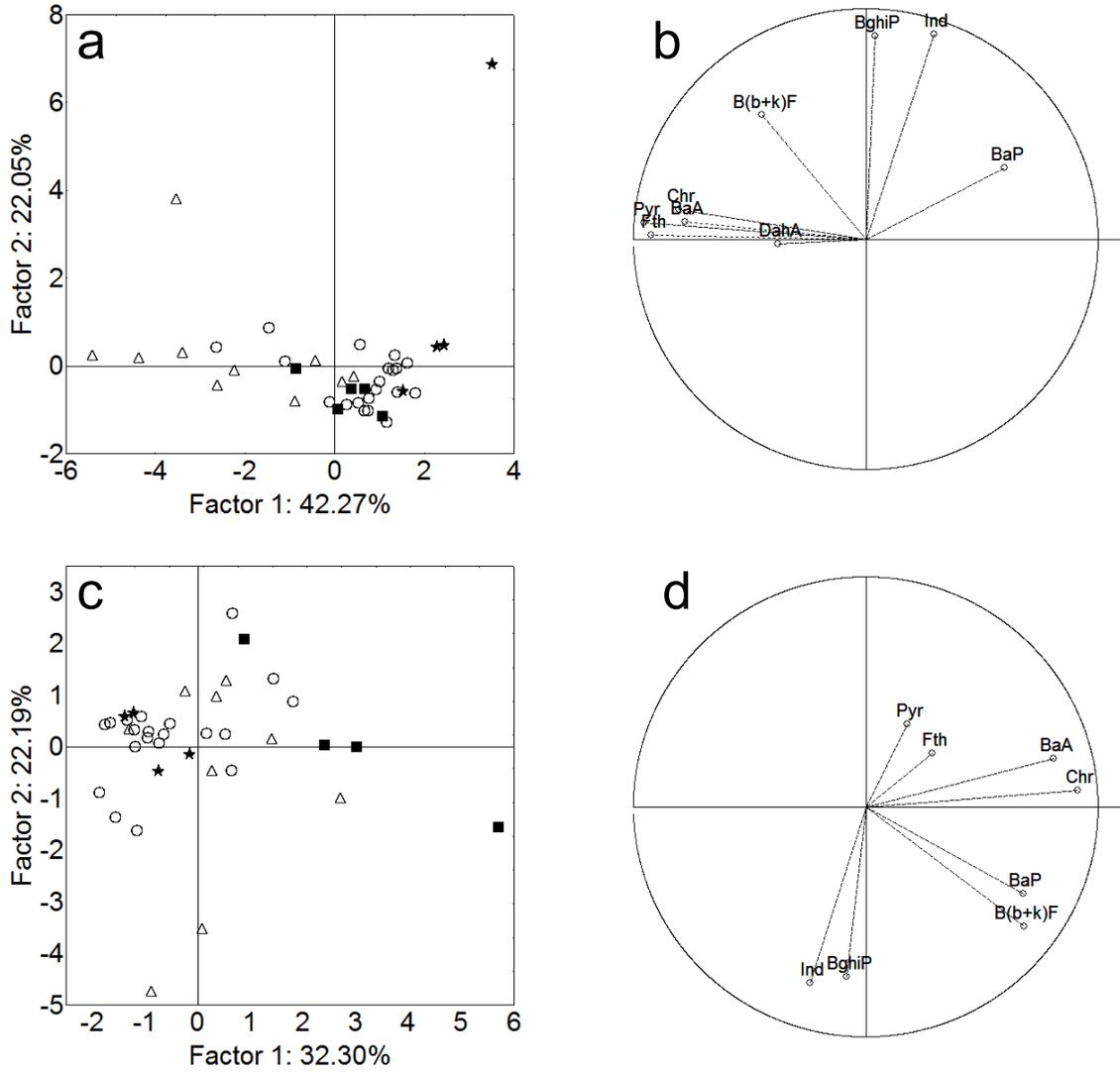


Fig. 6. Principal component analysis (PCA) of the samples of the lichen *Pseudevernia furfuracea* exposed in winter (a) and in summer (c) in the 40 sites of Fig. 1 classified according to land use (○=agricultural; ●=forest; ■=industrial; △=urban), and the corresponding loading plots of PAHs identified in the samples (b; d). DahA was eliminated from the summer analysis because it was below LOD in all the samples.

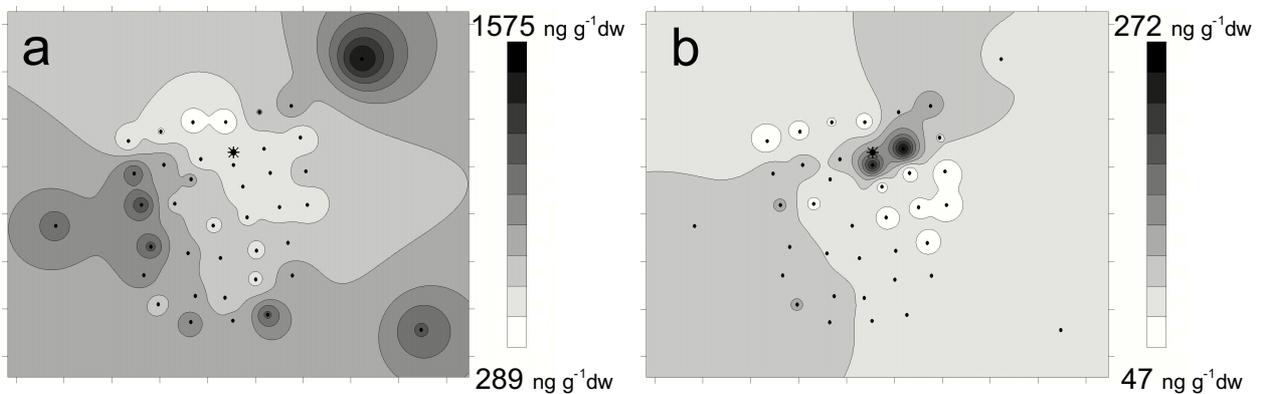


Fig. 7. Distribution patterns of the total PAHs load in the study area of Fig. 1 based on the concentrations measured in the samples of the lichen *Pseudevernia furfuracea* exposed in winter (a) and in summer (b).

4. Discussion

This study shows that the exposure period of two months was sufficient for transplants of the lichen *Pseudevernia furfuracea* (i) to accumulate PAHs, and (ii) to reveal specific distribution patterns on a highly heterogeneous territory. The total PAH content measured after both exposure periods (Tab. 1) is within the ranges reported in the literature (Tab. SD-2), although this comparison is problematic, since (i) the species may differ in PAH accumulation rates (Blasco et al., 2011), which mostly depends on their surface/weight ratio (Adamo et al., 2007; Bertuzzi and Tretiach, 2013), and (ii) the analytical procedures differ from study to study, and this might deeply influence the results (Domeño et al., 2006). The most striking result, however, is the confirmation of a pronounced seasonal difference in the transplant PAHs accumulation, with winter-exposed samples one order of magnitude more heavily enriched than summer-exposed ones, and with 4-ring PAHs apparently more variable than 5- and 6-ring PAHs. This is the consequence of significant differences between the two exposure periods in terms of sources, emission levels, climatic conditions and PAHs degradation phenomena.

It is unlikely that the local industries (the most important being the two steel works and the cement plant) could be the sources of the 4-ring PAHs. In the study area the prevailing wind direction is relatively constant throughout the year, being mostly north-northwesterly (Fig. 2c), and this is in contradiction with the location of the steel works (in the southwestern corner of the study area, see Fig. 1) and the cement plant (approximately at the center of the study area, see Fig. 1), on the one side, and with the location of the most heavily polluted exposure sites, on the other side. The latter belong in fact to the urban stratum, and are all located in the north-western corner of the study area, or in the two small villages of Cavasso Nuovo and Arba, outside the grid (Fig. 1). Interestingly, the clinker production itself, that is the core of the industrial processes of a cement plant, is considered to be PAHs-free, due to the long retention times (over 2 s), the high temperatures (1,400-1,500 °C), and the strongly oxidative atmosphere of the rotary kiln (Mokrzycki and Uliasz-Bocheńczyk, 2003). These favourable conditions even allow the use of refuse-derived fuel (RDF; mainly plastics and biodegradable waste) instead of coal, methane, etc. (European Cement Association CEMBUREAU).

Vehicular traffic may also generate 4-ring PAHs due to incomplete fuel combustion. However, no evidence is available to substantiate a significant difference between the winter and summer traffic load in the study area, and therefore the contribution of this source to the overall 4-ring PAHs load must be considered as negligible. By contrast, the most probable source of 4-ring PAHs is domestic heating, and particularly wood burning. This activity, being based on a renewable source, is perceived as “green” and of low environmental impact. Actually, due to the low

combustion temperatures and the poor mixing efficiencies of oxygen and fuel, wood burning in traditional fireplaces and ovens is an important source of organic compounds released into the environment (Mastral and Callén, 2000). The wood combustion generates high levels of fluoranthene, pyrene, benzo[a]anthracene and chrysene (Boström et al., 2002; Singh et al., 2013), which are the dominant compounds of our urban winter samples (Fig. 6a,b), luckily with low carcinogenic potency (Nisbet and LaGoy, 1992). Two observations support this conclusion: (i) wood burning is quite frequent in the study area, with more than 25% of the households that use it for domestic heating, while the use of wood burning for cooking is limited to sporadic professional activities, and (ii) domestic heating is restricted by law to the winter period (data from ARPA FVG 2013a). This automatically brings down the emissions in the summer period, with the high temperatures that may further reduce the semi-volatile 4-ring PAHs eventually accumulated in the lichen samples (Beyer et al., 2003; Panther et al., 1999; Singh et al., 2013).

As far as the sources of the 5- and 6-ring PAHs are concerned, their low concentrations and uniform distribution after both exposures suggest that in the study area there are no important industrial pyrogenic pollution sources (Augusto et al., 2009; Shukla et al., 2012). However, in summer, the post-exposure concentrations of the 5- and 6-ring PAHs, scarcely volatile in comparison to the 4-ring PAHs, were even lower than the pre-exposure ones. This might be due to the short but intense thunderstorms that occurred in the study area in summer (Fig. 2a). These can have a much stronger wash effect than the prolonged but lighter rains of the winter period on the particulate matter deposited on the sample surface, and thus on the 5- and 6-ring PAHs that are associated with it (Augusto et al., 2013a, b). A second explanation – and perhaps more robust than the previous one – is certainly the photochemical and chemical degradation to which all PAHs are subjected when exposed to UV (Boström et al., 2002; Wild et al., 2005), although 5- and 6-ring PAHs less intensely than lighter PAHs (Nadal et al., 2006). Also oxidative molecules such as ozone and similarly reactive trace gases may interfere (Alves, 2008; Brown and Brown, 2012). Ozone tends to form in presence of NO_x (mostly derived from combustion processes) when there are high temperatures and intense UV radiation, conditions that at middle latitudes are typically present in summer but not in winter (Lorenzini and Nali, 2005). Schauer et al. (2003) demonstrated that filter sampling carried out without the use of a diffusion denuder that removes ozone can lead to an underestimation of the actual PAHs content in the air by up to 100% or even more due to oxidative degradation. Lichens lack the cuticle wax layer that characterizes the leaves of vascular plants. This certainly favors the accumulation of air-borne contaminants (Augusto et al., 2010; Schrlau et al., 2011), but it also permits the diffusion of ozone towards the inner layers (Pellegrini et al., 2014). We can thus assume that the degradation that occurs on aerosol filters may occur in lichens as well,

causing a potentially significant underestimation of the real PAHs emission levels in the study area during the summer season, when the ozone levels are usually the highest (Lorenzini and Nali, 2005). Interestingly, when this study was carried out, the ozone levels in the study area exceeded the limit of $120 \mu\text{g m}^{-3}$ (EU Directive 2008/50/CE) more than 25 times, with peaks that ranged between 180 and $192 \mu\text{g m}^{-3}$ (ARPA FVG 2009, 2013b).

5. Conclusions

There are important seasonal differences in the environmental PAHs intercepted by lichen transplants in the selected study area. These changes may depend on modifications in emission intensity, and on environmental conditions that interfere with both kinetics and dynamics of transport and deposition, and with the successive degradation processes of PAHs that are promoted by high temperatures, UV and oxidative conditions, consequently the entity of the changes may differ at other locations with different environmental conditions.

The seasonal differences existing in the PAHs loads should be taken into account when planning a biomonitoring survey. Studies performed during the warmest season, in fact, may correctly describe the distribution patterns of these pollutants over the territory, but they certainly underestimate the potential PAHs load, that typically occurs in the coldest periods of the year. By contrast, studies based on samples collected in different seasons are likely to over-estimate the variation in emission intensity of the purported sources, because some of the variation is actually due to differences in the climatic conditions that favor the interception/discharge of PAHs-enriched particles or the PAHs degradation processes.

At middle latitudes, the winter period might thus be favorable to detect high PAH levels, because photo- and thermal degradation is low, and more emission sources are potentially active, whereas in the summer period it might be preferable to monitor the products derived from PAHs oxidation (e.g. epoxide, endoperoxide and hydroxy species, quinones, diones etc., see Chu et al., 2010).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.atmosenv.2015.04.067>.

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Tab. SD-1. GPS coordinates (Gauss-Boaga), elevation (m a.s.l.) and Corine Land Cover classification of the 40 exposure sites of Fig. 1.

site	E	N	elevation	corine land use
1A	2343344	5116535	282	forest
1B	2344025	5116735	314	forest
1C	2344692	5116933	362	forest
1D	2345383	5116934	293	forest
1E	2346087	5117145	274	urban
1F	2346755	5117276	267	urban
2A	2343465	5115852	276	urban
2B	2344085	5116036	265	urban
2C	2344858	5116156	265	agricultural
2D	2345544	5116030	254	industrial
2E	2346186	5116374	252	agricultural
2F	2346945	5116609	251	agricultural
3A	2343618	5115191	275	urban
3B	2344315	5115216	261	agricultural
3C	2344657	5115731	252	agricultural
3D	2345739	5115575	247	agricultural
3E	2346314	5115862	241	agricultural
3F	2347057	5115899	245	agricultural
4A	2343814	5114310	272	urban
4B	2344502	5114695	260	agricultural
4C	2345117	5114753	252	agricultural
4D	2345829	5114929	238	agricultural
4E	2346505	5115145	228	agricultural
4F	2347088	5115190	226	agricultural
5A	2343667	5113708	272	industrial
5B	2344591	5114174	260	industrial
5C	2345270	5114073	248	agricultural
5D	2346026	5114227	239	agricultural
5E	2346686	5114389	228	agricultural
6A	2343969	5113097	264	industrial
6B	2344744	5113272	255	industrial
6C	2345369	5113236	245	agricultural
6D	2346009	5113620	239	agricultural
6E	2346775	5113701	228	agricultural
7B	2344650	5112723	255	agricultural
7C	2345533	5112752	242	urban
7D	2346259	5112877	233	agricultural
Arba	2349472	5112557	208	urban
Cavasso Nuovo	2348230	5118263	277	urban
Maniago	2341823	5114748	301	urban

Tab. SD-2. Literature concerning the accumulation of PAHs in autochthonous (A) and transplanted (T) lichens listed by author(s) name and publication date, species name, analytical procedure applied to sample analysis, number of PAHs analyzed and observed concentration range, and short description of each study area.

Source	Exposure/sampling period	A/T	Lichen species	Analytical procedure			n. of examined PAHs	Σ PAHs concentration range	Study area/land use
				Extr.	Purif.	Anal.			
Augusto et al. (2013b)	Each site sampled 13 times between February – September	A	<i>Parmotrema hypoleucinum</i> (J.Steiner) Hale	a	i	l	16	58 – 556 ng g ⁻¹	Highly industrialized region of Sines (Portugal)
Augusto et al. (2010)	January	A	“, <i>Xanthoria parietina</i> (L.) Th. Fr.	a	i	l	16	95.5 – 873.8 ng g ⁻¹	“
Augusto et al. (2009)	January	A	<i>P. hypoleucinum</i>	a	i	l	16	90.49 – 871.83 ng g ⁻¹	“
Bajpai et al. (2013a)	May	A	<i>Remototrachyna awasthii</i> (Hale & Patw.) Divakar & Crespo	c	k	l	16	0.29 – 62.34 μg g ⁻¹	Urban and periurban areas of Mahabaleshwar (India)
Bajpai et al. (2013b)	May	A	“	c	k	l	16	0.19 – 54.0 μg g ⁻¹	“
Blasco et al. (2011)	One sample collected at each site between Spring and Autumn	A	<i>Parmelia sulcata</i> Tayl., <i>Evernia prunastri</i> (L.) Ach., <i>Ramalina farinacea</i> (L.) Ach., <i>Pseudevernia furfuracea</i> (L.) Zopf., <i>Usnea sp.</i> , <i>Lobaria pulmonaria</i> (Schreb.) Hoffm	g	j	m	16	238 – 6,240 ng g ⁻¹	Aspe valley (France) and Aragon valley (Spain) (Pyrenees National Park)
Blasco et al. (2008)	One sample collected at each site between Spring and Autumn	A	<i>E. prunastri</i>	g	j	m	16	696 – 6,240 ng g ⁻¹	Aragon valley (Spain) with high vehicular traffic intensity
Blasco et al. (2006)	One sample collected at each site (April – October)	A	<i>P. sulcata</i>	g	-	m	16	910 – 1,920 ng g ⁻¹	Around both entrances to the Somport tunnel, Pyrenees
Cabrerizo et al. (2012)	February 2005 and January-February 2009	A	<i>Usnea antarctica</i> Du Rietz	d	k	m	18	15 – 40 ng g ⁻¹	Livingston and Deception islands (Southern Shetlands, Antarctica)
Fernández et al. (2011)	December – January	A	<i>Pyxine coralligera</i> Malme	f	k	l	16	0.24 – 9.08 μg g ⁻¹	Caracas valley (Venezuela) in areas with different traffic intensities and air pollution
Guidotti et al. (2009)	3-month exposure	T	<i>P. furfuracea</i>	e	k	m	13	168.0 – 395.0 ng g ⁻¹	City center of Viterbo (C Italy) with different vehicular traffic intensities

Guidotti et al. (2003)	November – July (5-10-15-20-25-month exposure)	T	“	e	k	m	5	33 – 375 ng g ⁻¹	In areas with different vehicular traffic intensities
Jóźwiak (2012)	3 months exposure 4 times a year between 2004 and 2007	T	<i>Hypogymnia physodes</i> (L.)Nyl.	?	?	l	?	0.26 – 2.56 µg g ⁻¹	4 crossroads in the urban area of Kielce (Poland)
Nascimbene et al. (2014)	October	A	<i>P. furfuracea</i>	b	j	m	16	186.0 – 2,129.5 ng g ⁻¹	Mountain passes in the Dolomites (N Italy) with different traffic intensities
Protano et al. (2014)	September – January (4 month exposure)	T	“	e	k	m	11	634 – 1,371 ng g ⁻¹	Malagrotta Solid-waste landfill (S Italy) with different urban and industrial contexts
Samdudin et al. (2013)	?	A	<i>Dirinaria picta</i> (Sw.) Clem. & Schear	c	k	m	10	6.89 – 2,649.70 µg g ⁻¹	In the area of the Kebangsaan Malaysia University (Bangi, Selangor, Malaysia) with different traffic intensities
Satya et al. (2012)	December	A	<i>Rinodina sophodes</i> (Ach.) Massal.	c	k	l	16	0.189 – 0.494 µg g ⁻¹	Peripheral regions of industrial and urban areas of Kanpur City (India)
Shukla et al. (2013)	March	A	<i>Dermatocarpon vellereum</i> Zschacke	c	k	l	16	0.135 – 4.96 µg g ⁻¹	Rudraprayag (India) with heavy vehicular activity as the primary source of PAHs
Shukla et al. (2012)	July	A	<i>Pyxine subcinerea</i> Stirton	c	k	l	16	1.25 – 187.3 µg g ⁻¹	Industrial, urban and periurban areas of Haridwar city (India)
Shukla & Upreti (2009)	15 th September	A	<i>Phaeophyscia hispidula</i> (Ach.) Essl.	c	k	l	16	3.38 – 25.01 µg g ⁻¹	DehraDun (India) in areas with particularly high vehicular traffic
Usenko et al. (2010)	Between 2003 and 2005	A	9 species	h	k	m	18	ca. 0.01 – 100 µg g ⁻¹ lipid (conc. normalized to lichen lipid content)	14 lake catchments in 8 Western U.S. national parks

a=soxhlet extraction with acetonitrile; b=soxhlet extraction with acetone/hexane (1:1); c=soxhlet extraction with dichloromethane; d=soxhlet extraction with dichloromethane/methanol (2:1); e=ultrasonic bath extraction with cyclohexane; f=ultrasonic bath extraction with cyclohexane/dichloromethane (4:1); g=extraction by DSASE with hexane; h=extraction by ASE with dichloromethane; i=cleanup in florisil column; j=SPE cleanup; k=cleanup in silica gel column; l=HPLC coupled with UV/visible detector and UV/fluorescence detector or chromatogram analysis; m=GC-MS

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Tab. SD-3. PAHs content (ng g⁻¹ dw) measured in pre- and post-exposure winter samples of the lichen *Pseudevernia furfuracea* transplanted in the sites of Fig. 1.

WINTER	PAH									
	sample	Fth	Pyr	BaA	Chr	B(b+k)F	BaP	Ind	DahA	BghiP
Pre-exp 1	146.02	37.74	8.76	6.65	3.96	7.68	<LOD	<LOD	<LOD	210.81
Pre-exp 2	109.43	28.25	1.99	5.89	10.40	11.32	<LOD	<LOD	<LOD	167.28
Pre-exp 3	139.09	34.21	4.84	5.31	2.76	<LOD	<LOD	<LOD	<LOD	186.23
Pre-exp 4	133.79	36.47	1.73	6.82	5.35	8.56	1.06	<LOD	<LOD	193.78
Pre-exp 5	119.01	28.44	6.72	8.22	8.80	11.30	1.04	0.87	0.80	185.20
Pre-exp 6	133.94	29.25	6.47	3.36	9.91	6.52	0.90	<LOD	<LOD	190.35
Pre-exp 7	119.56	29.82	1.47	6.13	13.52	7.44	4.06	<LOD	0.54	182.54
1A	248.73	146.37	10.55	24.94	12.82	17.28	2.48	<LOD	0.78	463.95
1B	207.76	87.71	8.15	16.61	16.22	25.06	45.16	<LOD	13.16	419.83
1C	170.89	66.52	12.72	20.60	5.10	14.88	14.86	<LOD	2.20	307.76
1D	151.68	72.63	3.38	9.08	22.18	12.92	14.52	2.26	1.08	289.73
1E	412.22	233.94	17.32	27.57	9.76	14.40	8.66	<LOD	<LOD	723.88
1F	462.22	291.51	15.37	33.25	7.31	19.50	5.08	<LOD	<LOD	834.24
2A	622.73	368.39	66.84	44.44	22.50	3.36	4.44	4.32	2.02	1139.04
2B	453.47	289.49	24.83	41.79	10.50	18.32	5.04	<LOD	1.68	845.12
2C	247.01	162.05	5.58	19.53	10.02	16.04	4.84	<LOD	<LOD	465.07
2D	338.91	129.74	15.92	24.79	2.09	10.84	3.04	<LOD	<LOD	525.33
2E	240.11	138.16	9.56	23.42	9.96	15.20	3.20	4.44	1.46	445.51
2F	255.60	146.03	12.65	44.18	4.10	10.42	2.66	<LOD	<LOD	475.64
3A	681.03	443.30	48.38	59.82	10.44	12.36	7.68	1.24	1.34	1265.59
3B	394.76	211.88	14.67	36.41	3.60	11.20	4.20	<LOD	<LOD	676.73
3C	435.34	228.89	16.69	45.81	4.40	14.56	2.32	<LOD	<LOD	748.01
3D	253.88	114.43	4.56	30.76	29.30	14.46	8.32	<LOD	<LOD	455.70
3E	300.08	150.74	10.25	21.44	4.56	8.72	1.94	<LOD	<LOD	497.73
3F	341.92	184.26	18.68	46.39	3.50	13.58	19.23	<LOD	<LOD	627.56
4A	532.00	433.76	28.38	86.13	73.33	18.76	16.80	<LOD	2.90	1192.06
4C	244.41	205.97	17.83	31.35	9.87	17.52	5.81	<LOD	<LOD	532.77
4D	330.37	162.41	8.20	21.07	10.60	12.30	12.90	<LOD	<LOD	557.84
4E	297.36	138.22	9.30	21.42	12.90	14.88	12.40	<LOD	<LOD	506.49
4F	279.24	131.64	8.23	25.82	13.98	14.06	4.14	<LOD	<LOD	477.11
5A	564.15	288.37	8.79	44.30	16.52	15.53	3.78	1.96	1.35	944.75
5B	486.05	218.34	10.55	34.31	6.60	15.40	5.80	<LOD	<LOD	777.06
5C	332.92	193.87	12.15	47.14	5.12	16.86	<LOD	<LOD	0.78	608.84
5D	275.27	182.27	17.78	34.93	4.88	12.80	5.02	<LOD	<LOD	532.95
5E	387.30	165.88	9.28	26.80	5.34	13.36	14.00	<LOD	<LOD	621.97
6A	395.81	207.97	8.70	20.35	10.42	8.68	5.16	0.45	1.52	659.07
6B	423.68	249.92	9.61	34.21	8.20	10.40	1.73	<LOD	<LOD	737.74
6C	315.77	183.68	11.04	44.28	4.36	15.52	<LOD	<LOD	<LOD	574.66
6D	287.16	149.45	10.90	43.16	6.86	20.27	8.54	<LOD	<LOD	526.34
6E	549.74	236.33	7.65	31.56	21.50	2.11	5.91	2.80	2.92	860.52
7B	606.98	280.28	11.19	40.02	24.85	<LOD	8.28	<LOD	4.27	976.67
7C	433.63	226.08	10.87	33.03	19.71	3.21	3.62	2.98	<LOD	733.65

7D	729.11	350.42	13.32	45.55	23.47	2.77	5.16	2.51	3.27	1175.59
Arba	669.00	388.64	29.67	48.73	15.26	6.56	2.46	0.56	1.08	1161.96
Cav. Nuovo	890.83	536.46	42.81	76.80	17.68	2.80	5.22	1.40	1.86	1575.85
Maniago	624.81	354.94	26.33	42.82	16.70	2.42	7.38	0.68	1.50	1077.59

Tab. SD-4. PAHs content (ng g⁻¹ dw) measured in pre- and post-exposure summer samples of the lichen *Pseudevernia furfuracea* transplanted in the sites of Fig. 1.

SUMMER sample	PAH									total PAHs
	Fth	Pyr	BaA	Chr	B(b+k)F	BaP	Ind	DahA	BghiP	
Pre-exp 1	67.95	24.00	2.15	5.83	6.35	<LOD	<LOD	<LOD	<LOD	106.28
Pre-exp 2	55.70	19.38	0.84	5.06	6.75	<LOD	<LOD	<LOD	<LOD	87.72
Pre-exp 3	56.97	18.94	1.33	5.70	4.56	<LOD	<LOD	<LOD	<LOD	87.48
Pre-exp 4	68.57	24.16	6.89	9.68	12.94	7.12	<LOD	<LOD	<LOD	129.36
Pre-exp 5	67.40	21.61	1.66	7.55	6.31	<LOD	<LOD	<LOD	<LOD	104.53
Pre-exp 6	54.05	16.69	1.04	6.15	4.50	<LOD	<LOD	<LOD	<LOD	82.43
Pre-exp 7	56.60	18.07	0.95	5.37	4.37	<LOD	<LOD	<LOD	<LOD	85.35
Pre-exp 8	69.45	21.14	1.17	6.53	4.35	<LOD	<LOD	<LOD	<LOD	102.64
1A	27.16	12.95	1.14	4.74	3.13	3.32	<LOD	<LOD	<LOD	52.44
1B	41.74	14.16	2.60	4.59	<LOD	<LOD	<LOD	<LOD	<LOD	63.10
1C	39.94	14.38	2.35	6.87	4.51	1.91	<LOD	<LOD	<LOD	69.95
1D	35.91	14.20	2.82	6.01	<LOD	<LOD	<LOD	<LOD	<LOD	58.95
1F	78.07	38.56	3.69	11.71	<LOD	<LOD	<LOD	<LOD	<LOD	132.03
2A	67.12	32.93	3.58	8.18	<LOD	<LOD	<LOD	<LOD	<LOD	111.81
2B	55.53	26.41	4.17	11.48	8.26	<LOD	<LOD	<LOD	<LOD	105.85
2D	180.15	49.22	4.44	8.42	<LOD	<LOD	<LOD	<LOD	<LOD	242.23
2E	63.56	194.49	3.85	9.49	<LOD	<LOD	<LOD	<LOD	<LOD	271.39
2F	55.33	21.75	1.69	7.75	5.13	4.19	<LOD	<LOD	<LOD	95.83
3A	86.03	27.12	3.96	8.89	2.23	<LOD	<LOD	<LOD	<LOD	128.22
3B	34.62	15.44	1.93	6.66	4.37	<LOD	<LOD	<LOD	<LOD	63.03
3C	46.99	19.24	1.67	7.30	3.83	<LOD	<LOD	<LOD	<LOD	79.02
3D	36.32	14.68	1.32	5.03	4.34	<LOD	<LOD	<LOD	<LOD	61.70
3E	31.20	13.40	1.93	4.51	<LOD	<LOD	<LOD	<LOD	<LOD	51.04
3F	28.21	12.66	2.05	3.96	<LOD	<LOD	<LOD	<LOD	<LOD	46.88
4A	60.29	26.84	3.95	9.90	9.84	5.92	<LOD	<LOD	<LOD	116.75
4B	50.30	24.10	3.62	9.12	6.17	<LOD	<LOD	<LOD	<LOD	93.31
4C	54.12	25.36	2.35	9.37	5.42	<LOD	<LOD	<LOD	<LOD	96.62
4D	32.76	13.56	2.27	5.74	<LOD	<LOD	<LOD	<LOD	<LOD	54.33
4E	37.78	15.92	2.94	4.86	3.07	<LOD	<LOD	<LOD	<LOD	64.57
4F	34.67	16.32	2.39	6.95	<LOD	<LOD	<LOD	<LOD	<LOD	60.33
5A	49.80	20.33	7.98	15.95	17.37	5.98	<LOD	<LOD	<LOD	117.41
5C	47.20	18.30	2.98	6.74	2.83	<LOD	<LOD	<LOD	<LOD	78.05
5D	37.49	15.38	8.12	12.62	2.85	<LOD	<LOD	<LOD	<LOD	76.46
5E	36.18	17.52	2.79	3.99	2.56	<LOD	<LOD	<LOD	<LOD	63.04
6A	61.89	33.16	6.78	12.03	7.11	3.88	<LOD	<LOD	<LOD	124.85

6B	58.47	29.82	6.15	11.27	7.99	2.45	<LOD	<LOD	<LOD	116.15
6C	51.55	24.71	7.94	11.85	<LOD	<LOD	<LOD	<LOD	<LOD	96.06
6D	41.63	19.85	1.89	5.36	4.11	<LOD	<LOD	<LOD	<LOD	72.84
6E	50.09	16.70	1.74	3.39	5.01	<LOD	2.34	<LOD	<LOD	79.26
7B	62.48	32.50	2.37	4.83	4.84	<LOD	3.22	<LOD	<LOD	110.25
7C	57.63	24.31	1.90	3.09	3.49	<LOD	<LOD	<LOD	<LOD	90.61
7D	49.28	19.33	1.05	3.04	3.67	<LOD	1.77	<LOD	<LOD	78.14
Arba	39.57	17.46	1.22	8.54	5.17	3.11	<LOD	<LOD	<LOD	75.08
Cav. Nuovo	49.71	22.28	2.55	8.14	4.90	<LOD	3.07	<LOD	3.36	94.01
Maniago	58.46	20.92	2.15	7.70	5.87	3.42	2.82	<LOD	1.82	103.15

Tab. SD-5. Equivalent Toxicity (TEQ) values of PAHs measured in the samples of the lichen *Pseudevernia furfuracea* exposed in winter in the 40 sites of Fig. 1.

WINTER	TEQ										
	sample	Fth	Pyr	BaA	Chr	B(b+k)F	BaP	Ind	DahA	BghiP	total TEQ
	1A	0.25	0.15	1.06	0.25	1.28	17.28	0.25	<LOD	0.01	20.52
	1B	0.21	0.09	0.82	0.17	1.62	25.06	4.52	<LOD	0.13	32.61
	1C	0.17	0.07	1.27	0.21	0.51	14.88	1.49	<LOD	0.02	18.61
	1D	0.15	0.07	0.34	0.09	2.22	12.92	1.45	11.30	0.01	28.55
	1E	0.41	0.23	1.73	0.28	0.98	14.40	0.87	<LOD	<LOD	18.90
	1F	0.46	0.29	1.54	0.33	0.73	19.50	0.51	<LOD	<LOD	23.36
	2A	0.62	0.37	6.68	0.44	2.25	3.36	0.44	21.60	0.02	35.79
	2B	0.45	0.29	2.48	0.42	1.05	18.32	0.50	<LOD	0.02	23.53
	2C	0.25	0.16	0.56	0.20	1.00	16.04	0.48	<LOD	<LOD	18.69
	2D	0.34	0.13	1.59	0.25	0.21	10.84	0.30	<LOD	<LOD	13.66
	2E	0.24	0.14	0.96	0.23	1.00	15.20	0.32	22.20	0.01	40.30
	2F	0.26	0.15	1.26	0.44	0.41	10.42	0.27	<LOD	<LOD	13.20
	3A	0.68	0.44	4.84	0.60	1.04	12.36	0.77	6.20	0.01	26.95
	3B	0.39	0.21	1.47	0.36	0.36	11.20	0.42	<LOD	<LOD	14.42
	3C	0.44	0.23	1.67	0.46	0.44	14.56	0.23	<LOD	<LOD	18.02
	3D	0.25	0.11	0.46	0.31	2.93	14.46	0.83	<LOD	<LOD	19.35
	3E	0.30	0.15	1.02	0.21	0.46	8.72	0.19	<LOD	<LOD	11.06
	3F	0.34	0.18	1.87	0.46	0.35	13.58	1.92	<LOD	<LOD	18.71
	4A	0.53	0.43	2.84	0.86	7.33	18.76	1.68	<LOD	0.03	32.47
	4C	0.24	0.21	1.78	0.31	0.99	17.52	0.58	<LOD	<LOD	21.63
	4D	0.33	0.16	0.82	0.21	1.06	12.30	1.29	<LOD	<LOD	16.17
	4E	0.30	0.14	0.93	0.21	1.29	14.88	1.24	<LOD	<LOD	18.99
	4F	0.28	0.13	0.82	0.26	1.40	14.06	0.41	<LOD	<LOD	17.36
	5A	0.56	0.29	0.88	0.44	1.65	15.53	0.38	9.81	0.01	29.56
	5B	0.49	0.22	1.06	0.34	0.66	15.40	0.58	<LOD	<LOD	18.74
	5C	0.33	0.19	1.22	0.47	0.51	16.86	<LOD	<LOD	0.01	19.59
	5D	0.28	0.18	1.78	0.35	0.49	12.80	0.50	<LOD	<LOD	16.38
	5E	0.39	0.17	0.93	0.27	0.53	13.36	1.40	<LOD	<LOD	17.04
	6A	0.40	0.21	0.87	0.20	1.04	8.68	0.52	2.26	0.02	14.19

6B	0.42	0.25	0.96	0.34	0.82	10.40	0.17	<LOD	<LOD	13.37
6C	0.32	0.18	1.10	0.44	0.44	15.52	<LOD	<LOD	<LOD	18.00
6D	0.29	0.15	1.09	0.43	0.69	20.27	0.85	<LOD	<LOD	23.77
6E	0.55	0.24	0.76	0.32	2.15	2.11	0.59	14.01	0.03	20.75
7B	0.61	0.28	1.12	0.40	2.48	<LOD	0.83	<LOD	0.04	5.76
7C	0.43	0.23	1.09	0.33	1.97	3.21	0.36	14.89	<LOD	22.52
7D	0.73	0.35	1.33	0.46	2.35	2.77	0.52	12.57	0.03	21.10
Arba	0.67	0.39	2.97	0.49	1.53	6.56	0.25	2.80	0.01	15.66
Cav. Nuovo	0.89	0.54	4.28	0.77	1.77	2.80	0.52	7.00	0.02	18.58
Maniago	0.62	0.35	2.63	0.43	1.67	2.42	0.74	3.41	0.02	12.29

Tab. SD-6. Equivalent Toxicity (TEQ) values of PAHs measured in the samples of the lichen *Pseudevernia furfuracea* exposed in summer in the 40 sites of Fig. 1.

SUMMER	TEQ										
	sample	Fth	Pyr	BaA	Chr	B(b+k)F	BaP	Ind	DahA	BghiP	total TEQ
	1A	0.03	0.01	0.11	0.05	0.31	3.32	<LOD	<LOD	<LOD	3.83
	1B	0.04	0.01	0.26	0.05	<LOD	<LOD	<LOD	<LOD	<LOD	0.36
	1C	0.04	0.01	0.23	0.07	0.45	1.91	<LOD	<LOD	<LOD	2.72
	1D	0.04	0.01	0.28	0.06	<LOD	<LOD	<LOD	<LOD	<LOD	0.39
	1F	0.08	0.04	0.37	0.12	<LOD	<LOD	<LOD	<LOD	<LOD	0.60
	2A	0.07	0.03	0.36	0.08	<LOD	<LOD	<LOD	<LOD	<LOD	0.54
	2B	0.06	0.03	0.42	0.11	0.83	<LOD	<LOD	<LOD	<LOD	1.44
	2D	0.18	0.05	0.44	0.08	<LOD	<LOD	<LOD	<LOD	<LOD	0.78
	2E	0.06	0.19	0.39	0.09	<LOD	<LOD	<LOD	<LOD	<LOD	0.76
	2F	0.06	0.02	0.17	0.08	0.51	4.19	<LOD	<LOD	<LOD	5.03
	3A	0.09	0.03	0.40	0.09	0.22	<LOD	<LOD	<LOD	<LOD	0.82
	3B	0.03	0.02	0.19	0.07	0.44	<LOD	<LOD	<LOD	<LOD	0.75
	3C	0.05	0.02	0.17	0.07	0.38	<LOD	<LOD	<LOD	<LOD	0.69
	3D	0.04	0.01	0.13	0.05	0.43	<LOD	<LOD	<LOD	<LOD	0.67
	3E	0.03	0.01	0.19	0.05	<LOD	<LOD	<LOD	<LOD	<LOD	0.28
	3F	0.03	0.01	0.20	0.04	<LOD	<LOD	<LOD	<LOD	<LOD	0.29
	4A	0.06	0.03	0.40	0.10	0.98	5.92	<LOD	<LOD	<LOD	7.48
	4B	0.05	0.02	0.36	0.09	0.62	<LOD	<LOD	<LOD	<LOD	1.14
	4C	0.05	0.03	0.24	0.09	0.54	<LOD	<LOD	<LOD	<LOD	0.95
	4D	0.03	0.01	0.23	0.06	<LOD	<LOD	<LOD	<LOD	<LOD	0.33
	4E	0.04	0.02	0.29	0.05	0.31	<LOD	<LOD	<LOD	<LOD	0.70
	4F	0.03	0.02	0.24	0.07	<LOD	<LOD	<LOD	<LOD	<LOD	0.36
	5A	0.05	0.02	0.80	0.16	1.74	5.98	<LOD	<LOD	<LOD	8.74
	5C	0.05	0.02	0.30	0.07	0.28	<LOD	<LOD	<LOD	<LOD	0.71
	5D	0.04	0.02	0.81	0.13	0.29	<LOD	<LOD	<LOD	<LOD	1.28
	5E	0.04	0.02	0.28	0.04	0.26	<LOD	<LOD	<LOD	<LOD	0.63
	6A	0.06	0.03	0.68	0.12	0.71	3.88	<LOD	<LOD	<LOD	5.48
	6B	0.06	0.03	0.62	0.11	0.80	2.45	<LOD	<LOD	<LOD	4.06
	6C	0.05	0.02	0.79	0.12	<LOD	<LOD	<LOD	<LOD	<LOD	0.99

6D	0.04	0.02	0.19	0.05	0.41	<LOD	<LOD	<LOD	<LOD	0.72
6E	0.05	0.02	0.17	0.03	0.50	<LOD	0.23	<LOD	<LOD	1.01
7B	0.06	0.03	0.24	0.05	0.48	<LOD	0.32	<LOD	<LOD	1.19
7C	0.06	0.02	0.19	0.03	0.35	<LOD	<LOD	<LOD	<LOD	0.65
7D	0.05	0.02	0.10	0.03	0.37	<LOD	0.18	<LOD	<LOD	0.75
Arba	0.04	0.02	0.12	0.09	0.52	3.11	<LOD	<LOD	<LOD	3.90
Cav. Nuovo	0.05	0.02	0.26	0.08	0.49	<LOD	0.31	<LOD	0.03	1.24
Maniago	0.06	0.02	0.21	0.08	0.59	3.42	0.28	<LOD	0.02	4.68

Biomagnetic monitoring with the transplanted lichen *Pseudevernia furfuracea* in a mixed land use area of NE Italy

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Highlights

- Lichen transplants allows easy and detailed data collection.
- Two-month exposed samples were enriched with low-coercivity magnetic minerals.
- Magnetic parameters and content of selected elements were correlated.
- Magnetic properties are excellent proxies also for low levels of heavy metal pollution.

Abstract

The element content and magnetic properties of the particulate matter (PM) accumulated by thalli of *Pseudevernia furfuracea* transplanted in a mixed land use area of NE Italy have been analyzed. The samples were originally collected in a pristine area of the Carnic Alps and exposed for 2 months in 40 sites located at the knots of a 700 m step grid covering ca. 40 km² of agricultural, forested, industrial and urban areas. Pre- and post-exposure samples were analyzed for elemental content and magnetic properties. The post-exposure element content and the magnetic data agreed substantially, revealing a rather modest anthropic impact on the territory, mostly limited to the industrial park. Since the magnetic mineralogy was homogeneous throughout the entire set of samples, and pseudo-single domain to multidomain magnetite resulted as the main magnetic mineral, it was not possible to discriminate between PM originating from different pollution sources. Conversely, it was possible to confirm the low environmental impact of the largest local industry, a cement plant, located outside the industrial park. *P. furfuracea* was proven an effective accumulator for biomagnetic monitoring studies, and magnetic properties of the PM are excellent proxies for heavy metal pollution even when the anthropic impact on the territory is low.

Keywords: air pollution, particulate matter, dust, environmental magnetism, magnetic properties

1. Introduction

Outdoor air-pollution is a major environmental and health problem. According to WHO (2014; 2016) fine particulate matter (PM) has the greatest effect on human health. It consists of a complex mixture of solid and liquid particles of organic and inorganic substances suspended in the air. It is associated with a broad spectrum of acute and chronic illness, such as lung cancer, chronic obstructive pulmonary disease (COPD) and cardiovascular diseases. Particles with a diameter of 10 μm or less are the most health-damaging since they can penetrate deep inside the lungs.

Instrumental monitoring of PM requires expensive equipment, power availability and continuous maintenance, so it is impossible to apply a high-density sampling design when the environmental dispersion of airborne PM is influenced by numerous variables: sources, wind direction, topography, meteorological conditions. Biomonitoring with cryptogams (in particular, lichens and mosses) is an excellent technique that has frequently been used in the past decades for revealing emission sources and distribution patterns of various airborne persistent pollutants (e.g. heavy metals, PAHs, dioxins, furans; Augusto et al., 2013; 2015; Bargagli and Mikhailova, 2002; Lucadamo et al., 2015; Nascimbene et al., 2014; Tretiach et al., 2007; 2011). Unlike plants, lichens and mosses lack a root system, and gas exchanges, absorption of nutrients and pollutants occur throughout the whole surface of the organism (Bargagli, 1998; Bargagli and Mikhailova, 2002; Garty, 2000). The use of transplants permits the coverage of those areas where autochthonous cryptogams are absent or rare (e.g. urban centers), and the low costs of the materials allow the application of a high-density sampling design (Kodnik et al., 2015). This way they can help identify not only isolated point emission sources (Carreras and Pignata, 2002; Rusu et al., 2006; Tretiach et al., 2011), but they can also allow an estimation of the effects of diffuse pollution sources (e.g. domestic heating, vehicular traffic; Kodnik et al., 2015; Yemets et al., 2014). Furthermore, they are extremely useful to determine temporal patterns of airborne pollutants (Branquinho et al., 2008; Frati et al., 2005) and even to help validate diffusion models (De Nicola et al., 2013).

However, when several anthropogenic activities are present in a relatively small area characterized by a mixed land use, it becomes difficult to determine with certainty the source of pollution relying only on the element composition of PM (Capozzi et al., 2016). Nitrogen, carbonium, sulphur and heavy metal isotope fingerprints may be useful for this purpose, since the analysis by mass spectrometer is an effective and sensitive method which needs very small quantities of material (e.g. Batts et al., 2004; Cloquet et al., 2009; Purvis et al., 2004; Spiro et al., 2004; Wadleigh, 2003; Wiseman and Wadleigh, 2002), but the analyses may have high costs and thus they are not always applicable on large sets of samples.

A further, promising method is the analyses of the magnetic properties of the airborne particulate matter. Previous studies demonstrated that aerosols have remarkable magnetic properties related to the content of magnetite-like ferrimagnetic particles (Hunt et al., 1984; Flanders, 1994; 1999), often associated to heavy metals such as Cd, Cr, Zn (Georgeaud et al., 1997; Hunt et al., 1984) and even to mutagenic organic compounds (Morris et al., 1995). Moreover, magnetic analyses are sensitive and allow discriminating between different sources of pollution, according to the magnetic grain-size, as pointed out in urban and traffic related contexts (Sagnotti et al., 2009; Revuelta et al., 2014).

In biomonitoring studies, tree leaves (Maher et al., 2008; Mitchell et al., 2010; Moreno et al., 2003; Rai, 2013), pine needles (Lehndorff et al., 2006), bark (Böhm et al., 1998), mosses (Fabian et al., 2011; Vuković et al., 2015) and also lichens (Chaparro et al., 2013; Salo et al., 2012; Paoli et al., in press) have been successfully used as passive dust collectors in magnetic monitoring, both in urban (e.g. Gautam et al., 2005; Szönyi et al., 2008; Zhang et al., 2006) and industrial areas (Hansard et al., 2011; Jordanova et al., 2010; Salo and Mäkinen, 2014; Zhang et al., 2008).

The aim of this study was to analyze both the element content and the magnetic properties of lichens transplanted in a mixed land use area of NE Italy in order to (i) characterize the magnetic fraction of the PM accumulated in lichen transplants, (ii) compare and integrate the two data sets, and finally (iii) to test the effectiveness of the methodology in attributing the origin of the PM to one or more known specific emission sources present in a study area characterized by mixed land use.

2. Material and methods

2.1 Study area

The study area is located in NE Italy (46°10'N 12°44'E; Fig. 1), at an elevation of 200-380 m a.s.l. It covers nearly 40 km², hosts a resident population of over 16,000 inhabitants (2015), and consists of the typical mixed land use patchwork that extends over large areas of N Italy, with natural, agricultural, urban, and industrial parks of different extension intermingled all together. The main potential pollution sources of the study area are: (i) an isolated, middle-sized cement plant (clinker production: 556,000 ton year⁻¹ in 2012; M. Vicenzetto, personal reference); (ii) a large industrial park; (iii) vehicular traffic, concentrated in the main urban centers and along a national road (“464 - Spilimbergo”) that crosses the north part of the study area; (iv) agricultural activities, mostly related by ploughing (in late summer and in winter) and threshing (in summer); (v) domestic heating (in winter).

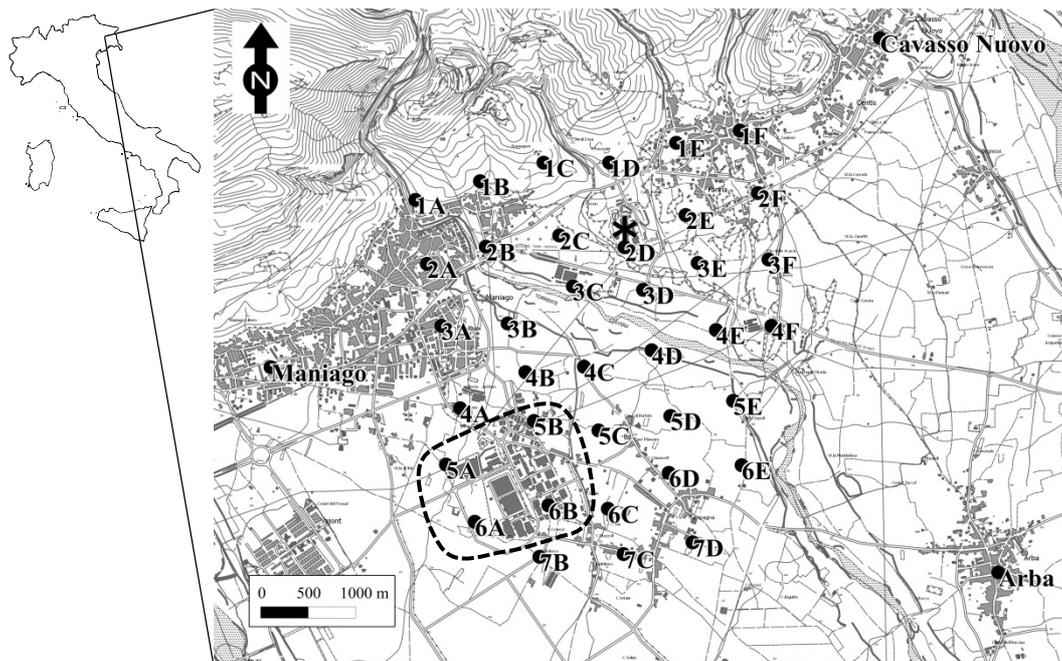


Fig. 1. Study area and localization of the 40 exposure sites. The cement plant is indicated by an asterisk, the industrial park by a closed dashed line.

The selected sampling method was systematic, with 37 exposure sites located at the knots of a 700 m step grid, and 3 further sites located in the nearby urban centers of Arba, Cavasso Nuovo and Maniago (Fig. 1).

The data on the meteorological conditions during the exposure period were retrieved from the closest weather station, located near Arba (Fig. 1).

2.2 Lichen sampling, exposure and recovery

The epiphytic lichen *Pseudevernia furfuracea* (L.) Zopf. var. *furfuracea* was selected as a frequently used biomonitor of trace elements and PAHs (Gallo et al., 2014; Kodnik et al., 2015; Nascimbene et al., 2014; Tretiach et al., 2007, 2011). This lichen is relatively common, easy to identify, stress-tolerant, with a good resistance to transplantation (Tretiach et al., 2007), and morphologically prone to intercept particulate matter due to the development of a myriad of finger-like vegetative propagules (“isidia”) (Tretiach et al., 2005). The pre-transplant samples were collected in the Carnic Alps (46°25’N 12°44’E) at ca. 1,700 m a.s.l., far from any local pollution sources (Adamo et al., 2008; Tretiach et al., 2011), from branches of solitary larch (*Larix decidua* Mill.) trees at 2 to 4 m above the ground. The material was collected along with ca. 20 cm long piece of the supporting twigs, transported to the laboratory in paper bags and left to dry out at room temperature. Moderately isidiate samples were selected avoiding those over-isidiate or with

apothecia (because these structures may alter the surface/weight ratio of the thallus), infected by lichenicolous fungi or covered by epiphytic algae (Kodnik et al., 2015; Tretiach et al., 2007, 2011).

Each twig carrying 1-3 thalli was attached to a 120 cm long bamboo stick using plastic bonds (both first rinsed with distilled water) and sealed in plastic bags until exposure to avoid contamination.

The exposure was done on June 19th 2012, within 12 hours of field work. In each site, two bamboo sticks were attached to the external branches of deciduous trees at approximately 4 m above the ground using plastic bonds. After two months, the samples were retrieved with a piece of the bamboo stick, sealed in individual plastic bags, and transported in a coolbag within 6 hours to the laboratory, where the bags were opened, and the samples were left to dry out at room temperature (Kodnik et al., 2015). Samples of the sites 1E, 2C and 5B were lost during the exposure. Samples 4B and from Cavasso Nuovo had insufficient material for the measurement of the magnetic properties and were thus excluded from the statistical analyses as well.

2.3 Chemical analyses of the element content

A part of the most external lobe portions of each thallus was cut with porcelain scissors (ca. 1 g), pulverized in an agate mortar with liquid nitrogen, dried out over silica for 24 h and then sealed in Eppendorf containers until analysis (Tretiach et al., 2011). The samples were then digested with concentrated HNO₃, first cold then hot. After cooling, 6 ml of aqua regia were added per 1 g of sample and kept at 95 °C for 2 h, then the suspension was brought to volume (20 ml g⁻¹) by adding a 5% solution of HCl and filtered. The solution was then analyzed by ICP-MS for the following elements: Al, As, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mn, Ni, Pb, Sb, Sn, V, Zn. The accuracy of the analysis was verified with the “Certified Reference Material BCR-482 (*Pseudevernia furfuracea* (L.) Zopf.)”.

2.4 Magnetic Measurements

The remaining parts of the most external lobe portions of each lichen were cut with porcelain scissors and placed in pharmaceutical gel caps #4 (~ 0.15 ml) for the hysteresis measurements and in standard 8 cm³ plastic cubes for the magnetic susceptibility analyses. Gel cap samples were inserted into the carbon fiber probe for vibrating in the Princeton Measurement Corporation Micromag 3900 Vibrating Sample Magnetometer (VSM). Lichen fragments were carefully pressed inside the caps to reduce the interstices and to maximize the mass content. The coercive force (B_C), the saturation remanent magnetization (M_{RS}) and its 100 s decay, as well as the saturation magnetization (M_S) were determined using the VSM under cycling in a maximum field

of 1.0 T after subtracting the high field paramagnetic linear trend. The mass specific magnetization values for the concentration dependent parameters (M_S and M_{RS}) were calculated dividing the magnetic moments by the dry weight of the samples. The coercivity of remanence (B_{CR}) was extrapolated from backfield remagnetization curves up to -1 T, after being magnetized in a positive 1 T field.

First order reversal curves (FORCs; see Pike et al., 1999; Roberts et al., 2000) were measured using the Micromag operating software, and processed, smoothed and drawn with the FORCINEL Igor Pro routine (Harrison and Feinberg, 2008). FORCs were measured in steps of 2.0 mT with an averaging time of 100 ms, the maximum applied field being 1.0 T. The percentage decay of M_{RS} after 100 s was calculated as:

$$M_{RS} \text{ (SP)\%} = 100 \times (M_{RS0} - M_{RS100})/M_{RS0};$$

where SP refers to the superparamagnetic fraction, M_{RS0} is the remanent magnetization measured as soon as the magnetic field is reduced to noise levels after the application of a 1 T field; M_{RS100} is the remanence measured 100 s later. The values of $M_{RS} \text{ (SP)\%}$ are indicative of the contribution of the rapidly decaying viscous components of magnetization with respect to the overall remanent magnetization of the samples. The viscous components are usually carried by ultrafine magnetic particles, dimensionally in the superparamagnetic/stable single domain boundary, which is around 20-35 nm for magnetite (Sagnotti and Winkler, 2012; Wang et al., 2010).

The magnetic grain-size of the PM accumulated by the lichens was described according to the hysteresis ratios M_{RS}/M_S vs. B_{CR}/B_C in the “Day plot” (Day et al., 1977; Dunlop, 2002a, 2002b).

The magnetic susceptibility (k), as well as its temperature dependency in the range 40 - 700 °C were measured in an AGICO MFK-1 Kappabridge equipped with a CS-3 furnace and corrected for the susceptibility of the empty furnace; the mass normalized values of the magnetic susceptibility are indicated with χ .

The magnetic properties of a cement sample produced by the plant located at the center of the study area (Fig. 1) and of a dust sample directly collected from the filters located above the main furnace were also analyzed to test the magnetic similarity between the final products and the dusts bioaccumulated by the lichen transplants.

All the magnetic measurements were carried out at the palaeomagnetism laboratory of Istituto Nazionale di Geofisica e Vulcanologia, Rome (Italy).

2.5 Statistics

Descriptive statistics were performed using Microsoft Excel (Microsoft Office Professional Plus 2010). Principal component analysis (PCA), cluster analysis (tree clustering with Complete

Linkage as the clustering method and 1-Pearson r as the linkage measure), Mann-Whitney *U* Test and Spearman's Rank correlation (p-value significant <0.05) were performed to verify differences between groups using STATISTICA 8.0 StatSoft Inc. (2007). The localization of the exposure sites in the study area was graphically represented using Quantum GIS 1.8.0.-Lisboa (2012).

3. Results

3.1 Meteorological conditions

The total rainfall during the exposure period was 213.8 mm, given mostly by brief, intense summer storms. The mean air temperature was 24.6 °C, ranging from 19.3 to 30.2 °C (mean daily minimum and maximum, respectively). The winds were mainly north-northwesterly.

3.2 Element content of the lichen transplants

Pre-exposure element concentrations in *P. furfuracea* thalli were within the ranges of background values of epiphytic lichens (Bargagli, 1998), being very similar to those of previous samples collected in the same pristine area (Adamo et al., 2008). The exposure modified significantly the content of most elements (67%; Tab. 1).

The tree diagram of the elements (Fig. 2) consists of 5 main groups. Mn, V and K (group 1 of Fig. 2) were partially leached from the exposed thalli, i.e. their post-exposure content was lower than the pre-exposure value. Cr, Ni, Co, and Sn (group 2) showed a slight increment (statistically significant for Ni and Sn), often with a maximum in site 6A (industrial zone; Fig. 1). As and Al

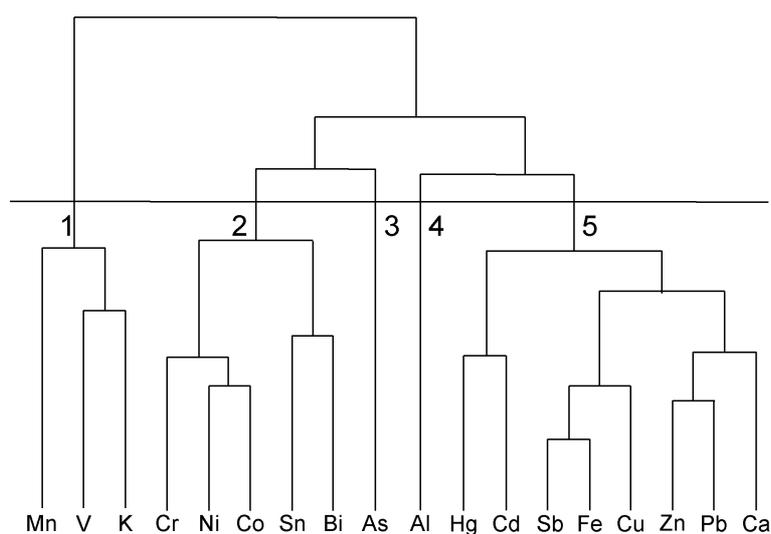


Fig. 2. Tree diagram of the elements measured in the samples of the lichen *Pseudevernia furfuracea* after the two-month exposure in the 40 sites of Fig. 1.

(groups 3 and 4, respectively) had peculiar distribution patterns in the study area, which differ from those of all the other elements. Group 5 gathers elements with heterogeneous distribution on the territory, but with a significant increase in post-exposure concentration, particularly high for Cd, Sb, Fe, Cu, Pb and Ca, with maxima frequently located in 6B (industrial park, e.g. Sb, Fe, Cu).

Tab. 1. Element content (mg g⁻¹), magnetic susceptibility (χ ; 10⁻⁸ m³ kg⁻¹), saturation remanent magnetization (M_{RS}; mA m² kg⁻¹), saturation magnetization (M_S; mA m² kg⁻¹), coercivity of remanence (B_{CR}; T) and coercive force (B_C; T) measured in pre- and post-exposure samples of the lichen *Pseudevernia furfuracea* transplanted for two months in the sites of Fig. 1.

	pre-exposure (n=8)					post-exposure (n=35)				
	mean	S.D.	C.V.	min	max	mean	S.D.	C.V.	min	max
Al	130.00	48.30	37%	100	200	213.51	34.66	16%	200	300
As	0.10	0.00	0%	0.1	0.1	0.22	0.14	64%	0.1	0.5
Bi	0.09	0.06	67%	0.04	0.21	0.05	0.04	88%	0.02	0.27
Ca	3,330.00	326.77	10%	2,900	3,800	4,494.59	1,754.67	39%	2,300	11,400
Cd	0.12	0.01	10%	0.1	0.14	0.15	0.03	23%	0.1	0.27
Co	0.14	0.04	26%	0.09	0.21	0.16	0.04	22%	0.08	0.24
Cr	2.33	0.51	22%	1.9	3.3	2.44	0.55	23%	1	4.3
Cu	4.45	0.63	14%	3.93	5.51	6.02	1.10	18%	4.48	8.29
Fe	169.00	43.58	26%	130	250	253.24	63.29	25%	170	440
Hg	0.13	0.02	18%	0.102	0.165	0.13	0.03	21%	0.09	0.202
K	2,830.00	605.62	21%	2,400	3,800	2,272.97	359.51	16%	1,800	3,300
Mn	96.90	7.05	7%	90	112	88.16	27.08	31%	47	161
Ni	0.83	0.26	32%	0.5	1.3	1.07	0.34	32%	0.7	2.3
Pb	3.00	0.41	14%	2.27	3.41	3.51	0.63	18%	2.46	5.14
Sb	0.08	0.01	9%	0.07	0.09	0.11	0.02	20%	0.07	0.19
Sn	0.28	0.03	12%	0.24	0.33	0.35	0.07	21%	0.23	0.55
V	4.10	1.73	42%	2	6	3.84	1.17	30%	2	7
Zn	33.38	1.45	4%	31.2	36.6	35.00	4.94	14%	25.6	42.6
χ	0.467	0.444	95%	0.118	1.490	1.798	1.355	75%	0.350	7.409
M_{RS}	0.151	0.055	36%	0.071	0.243	0.269	0.131	49%	0.068	0.797
M_S	0.754	0.102	14%	0.660	0.972	2.234	1.168	52%	0.948	6.548
B_{CR}	0.041	0.008	20%	0.031	0.055	0.039	0.004	11%	0.031	0.048
B_C	0.017	0.004	23%	0.012	0.025	0.012	0.003	25%	0.006	0.020

3.3 Magnetic properties of the lichen transplants

The values of χ , M_S and M_{RS} (Tab. 1) have been normalized on the mean pre-exposure values as:

$$[(\text{post-exposure} - \text{pre-exposure})/\text{pre-exposure}];$$

and they are shown in Fig. 3a. B_C and B_{CR} are shown in Fig. 3b.

The pre-exposure samples had very weak but measurable concentration dependent magnetic properties, always lower than the post-exposure samples. The highest post-exposure values were found for the samples 6A and 6B, from the industrial park.

B_C and B_{CR} are homogeneous over the whole set; B_{CR} is less than 0.050 T for most of the samples, indicating the prevalence of low-coercivity magnetic minerals.

The shapes of the hysteresis loops (Fig 4a, d), of the IRM acquisition and of the back-field curves (Fig. 4b, e) of the samples 2D (cement plant) and 6A (industrial park) are similar, suggesting

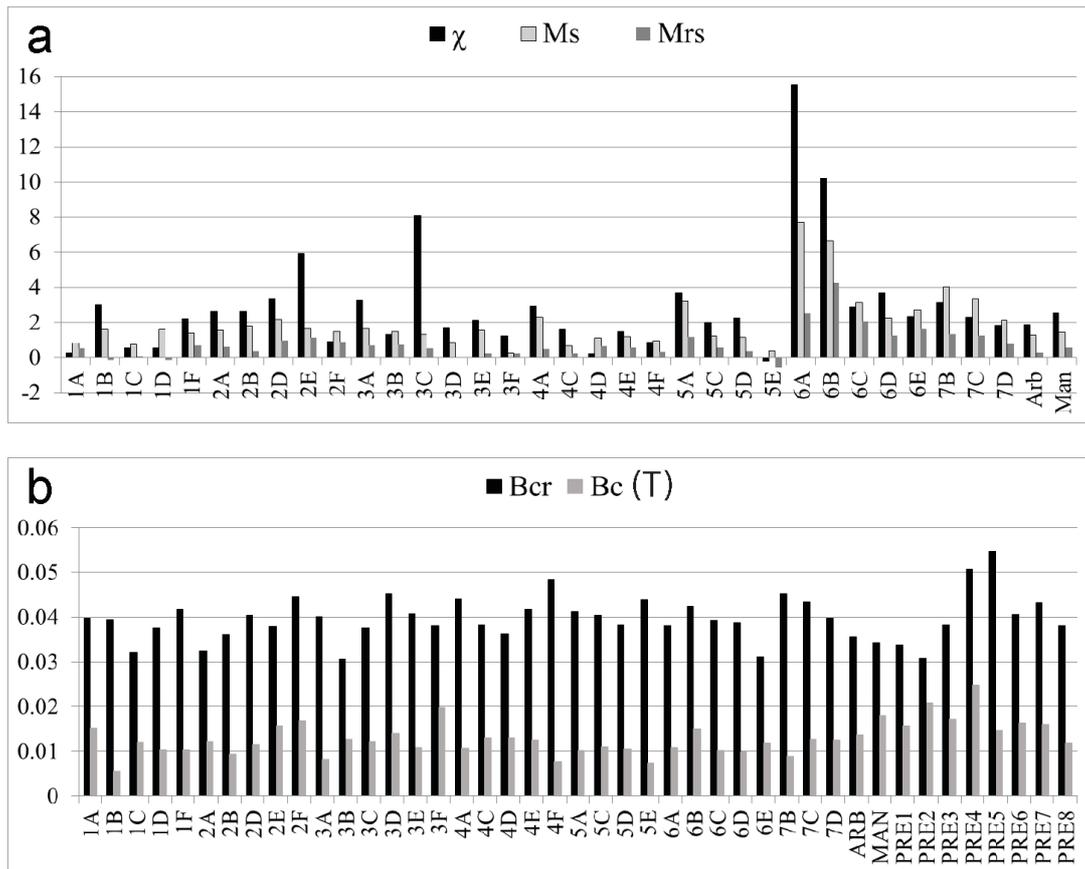


Fig. 3. Histograms of χ , M_S , M_{RS} (a) measured in post-exposure samples of the lichen *Pseudevernia furfuracea* transplanted for two months in the sites of Fig. 1., and normalized on the mean pre-exposure values as follows: [(post-exposure – pre-exposure)/pre-exposure]. Histograms of B_{CR} (T) and B_C (T) measured in the pre and post-exposure samples are in (b).

a common magnetic mineralogy. Higher values of the concentration dependent parameters are probably given by higher concentrations of analogous magnetic particles accumulated by the lichens exposed in the industrial park. It is difficult to estimate the M_{RS} decay in 100 seconds because of the large measurement errors due to the low remanent magnetization values. However, a clear decay is not evident after 100 seconds (Fig. 4c, f), indicating that in both samples the viscous components of magnetization should be negligible. The variation of the magnetic susceptibility vs. temperature is shown in Fig. 5a, b for the samples 6B (industrial park) and 2D (cement plant), respectively; black circles represents the heating, white circles the following cooling to room temperature. The curves are noisy and somewhat difficult to read, because of the weak susceptibility and the prevailing lichen matrix on the overall volume of the sample. For both samples, the Hopkinson peak precedes the abrupt decrease of susceptibility at around 580 °C, indicating that the main magnetic mineral is magnetite.

The “Day plot” (Fig. 6) shows that the magnetic grain-size of the samples is mostly in the central part of the plot, which can depend on a broad mixture of single domain (SD) to multidomain

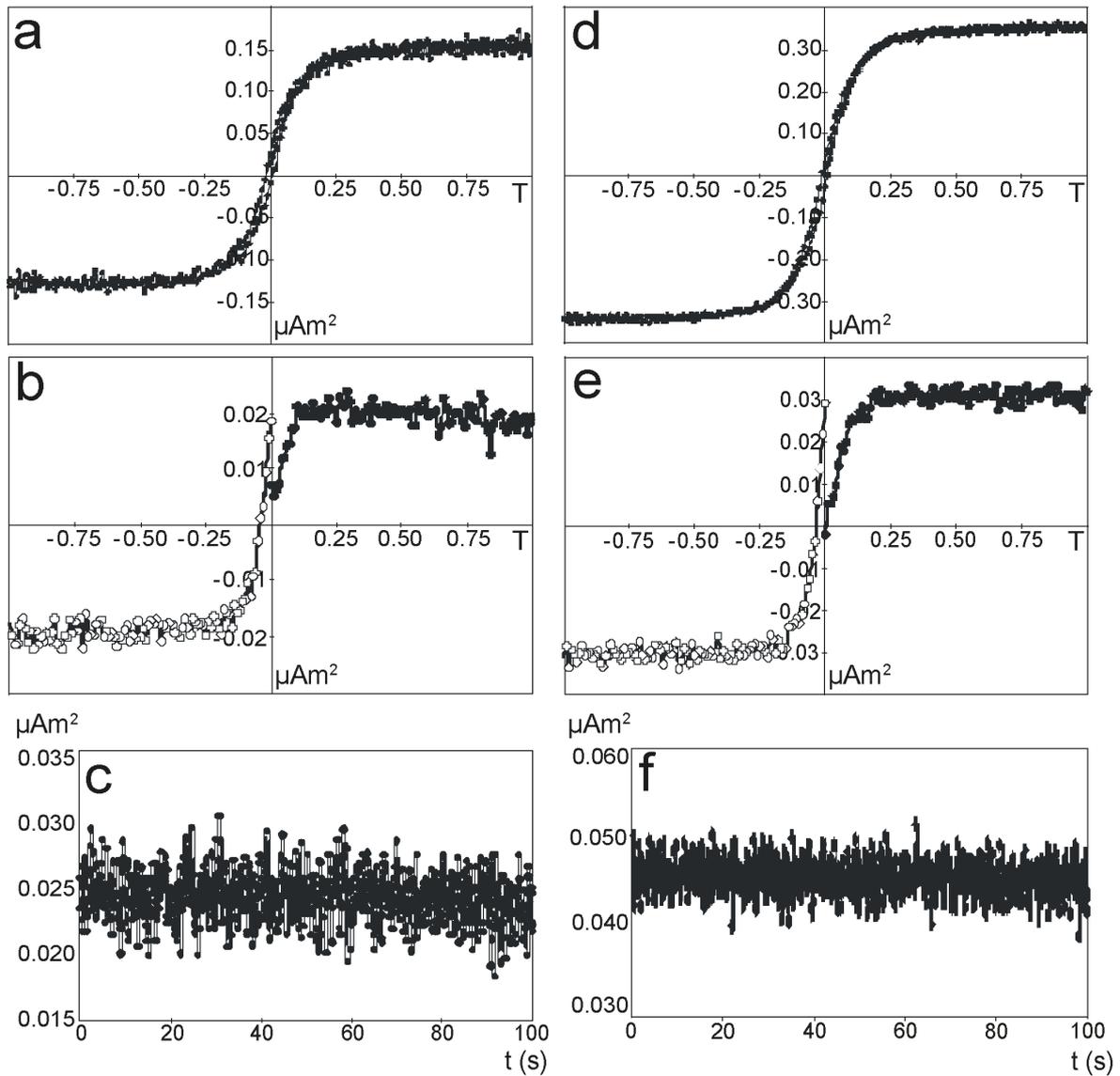


Fig 4. Hysteresis loops (a, d), IRM acquisition and back-field application curves (b, e; black circles for IRM acquisition) of the samples 2D (cement plant, left column) and 6A (industrial park, right column) of the lichen *Pseudevernia furfuracea* transplanted for two months in the sites of Fig. 1., and 100 seconds MRS decay for samples 2D and 6A, respectively (c, f).

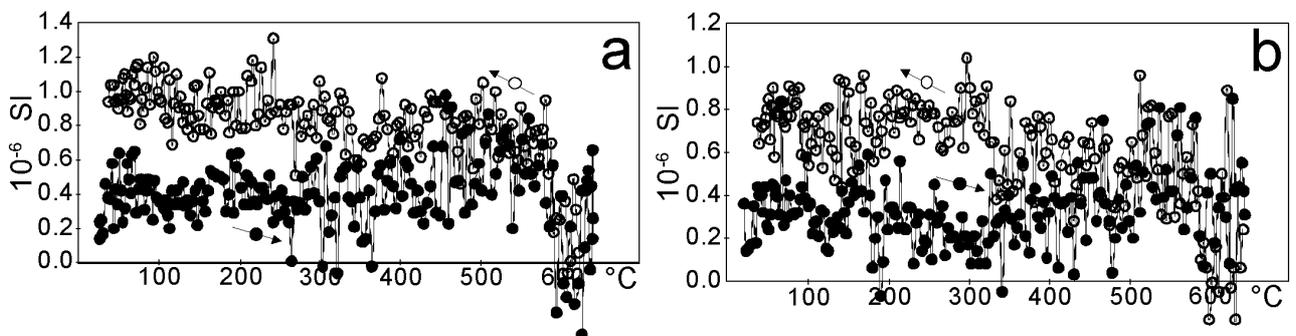


Fig. 5. Temperature variation of the magnetic susceptibility for the samples 6B (a) and 2D (b) of the lichen *Pseudevernia furfuracea* transplanted for two months in the sites of Fig. 1. Black circles are for the heating process, open circles for the following cooling. Data are corrected for the empty furnace.

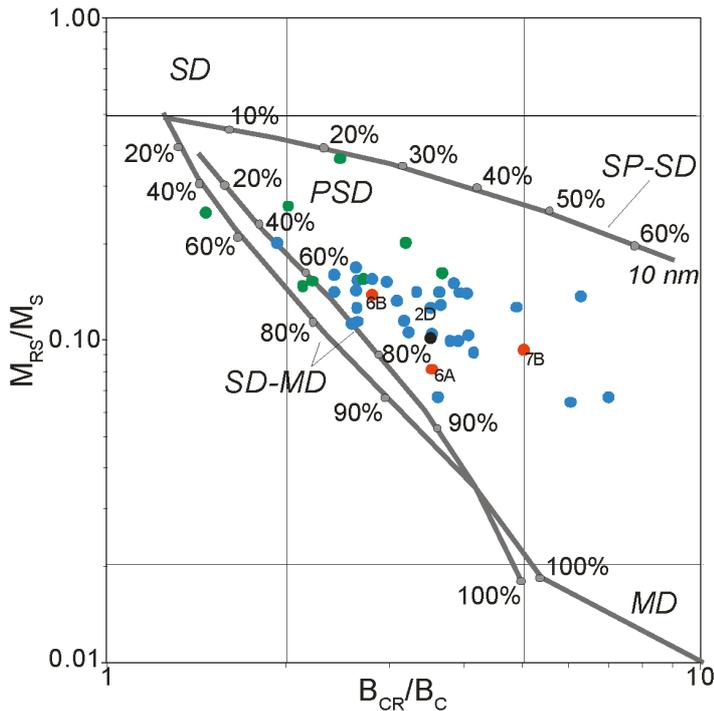


Fig. 6. Bi-logarithmic “Day plot” of the hysteresis ratios M_{RS}/M_S vs. B_{CR}/B_C for samples of the lichen *Pseudevernia furfuracea* transplanted for two months in the sites of Fig. 1., and of the pre-exposure samples (red symbols – industrial park; green – pre-exposure; black – cement; light-blue – all the other samples). The SD, PSD and MD fields and the theoretical mixing trends for SD-MD and SP-SD grains are from Dunlop (2002) and refer to magnetite.

3.4 Magnetic analyses of the cement and dust sample

The hysteresis loop, the IRM acquisition and the back-field application curves of the cement sample are shown in Fig. 8a and b, respectively. The decay of MRS in 100 seconds (Fig. 8c) suggests the presence of viscous components of magnetization, contributing for about 10% of the overall remanent magnetization.

The thermomagnetic curve (Fig. 8d) is much better defined, with respect to those of the samples 6B and 2D (Fig 5), due to the remarkably higher values of k . The main decrease of the magnetic susceptibility occurs from about 400 °C up to 580 °C, which can be ascribed to the presence of magnetite and maghemite, and continues up to 680 °C, the Néel temperature for hematite. Overall, the presence of a heterogeneous mix of iron oxides is suggested. The magnetic grain-size of the cement sample (Fig. 6) does not differ from the lichen sample data and falls into the central region of the “Day plot” as well. The dust samples collected from the filters located above the main furnace, have noticeably high M_S and M_{RS} values ($0.11 \text{ Am}^2 \text{ kg}^{-1}$ and $0.02 \text{ Am}^2 \text{ kg}^{-1}$, respectively). The thermomagnetic curve and the analysis of $1/k$ values points out that the main magnetic minerals are magnetite and hematite.

(MD) magnetite, or on a prevailing pseudo-single domain (PSD) grain-size range. The samples 6A and 6B (industrial park) are within the cluster of points in the central PSD region, together with the sample 2D (cement plant) and most of the pre-exposure samples.

FORC diagrams of samples 2D, 6B and of a pre-exposure sample are shown in Fig. 7. PSD/MD low-coercivity features emerge for both of the transplants, whereas no relevant magnetic component is present in the pre-exposure sample. These results confirm the features already shown by the “Day Plot” and point out the enrichment of the transplants in magnetic particles after the exposure.

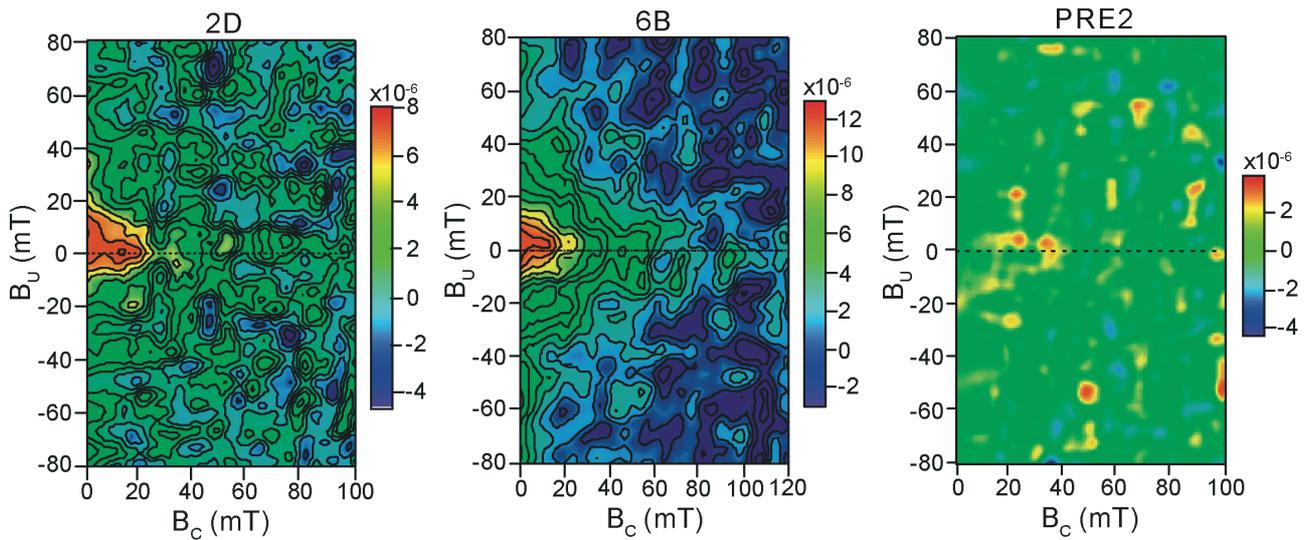


Fig. 7. FORC (First Order Reversal Curve) diagrams for samples 2D and 6B of the lichen *Pseudevernia furfuracea* transplanted for two months in the sites of Fig. 1., and of a pre-exposure sample (PRE2).

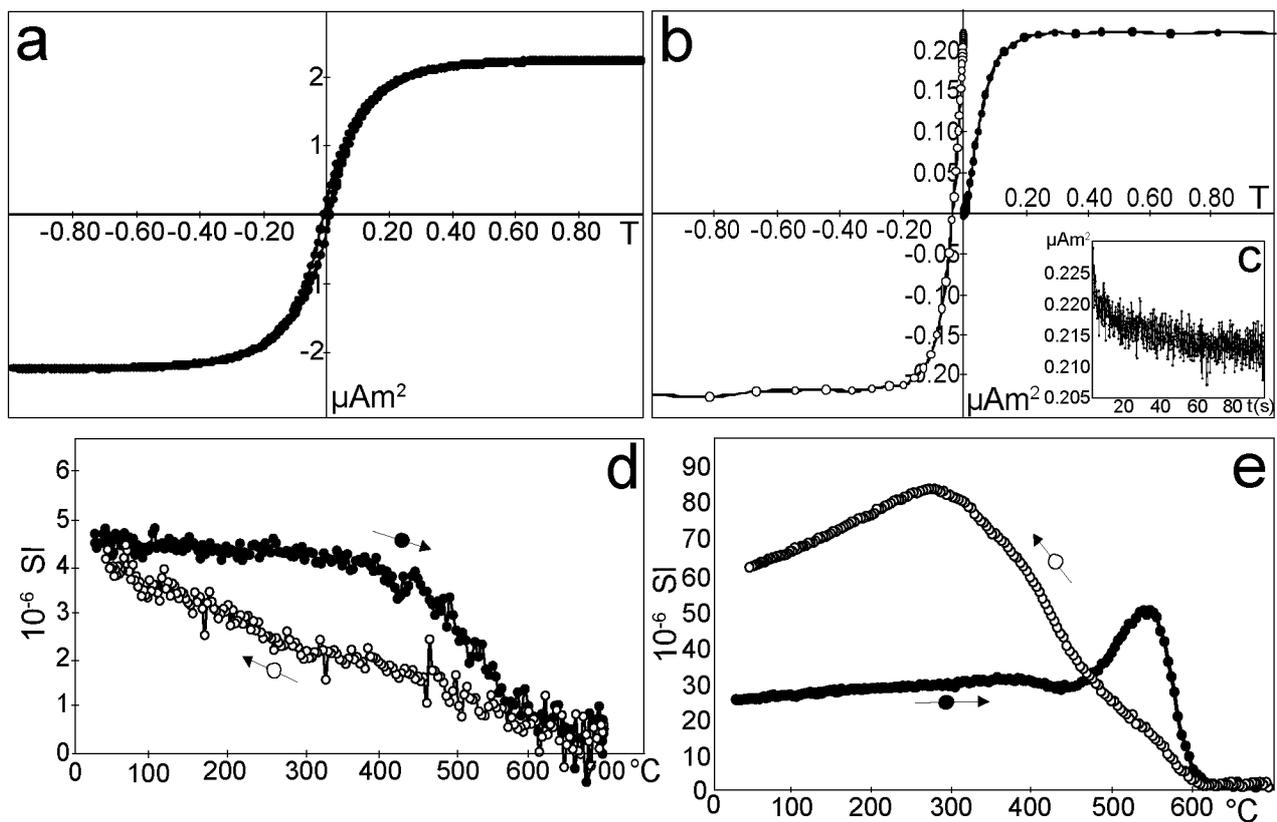


Fig. 8 Magnetic mineralogy of the cement sample and of the dusts from the cement plant filters: (a) hysteresis loop; (b) IRM acquisition/back-field application curves; (c) decay curve of M_{RS} in 100 seconds; (d, e) thermomagnetic curves after correction for the empty furnace (black circles are for the heating process, white circles for the following cooling) for the cement and the filter dust, respectively.

3.4 Data Statistics

The principal component analysis (PCA) of Fig. 9a, b is based on the variables χ , M_{RS} , M_S , and post-exposure element content normalized with respect to the mean pre-exposure values as follows:

$$(\text{post-exposure} - \text{pre-exposure})/\text{pre-exposure}.$$

Pronounced differences are evident among the samples. The sample 1D, located at one end of the first component, segregates from all the other samples; it was exposed in a forested area and has low levels of contamination. On the contrary, the samples 6A and 6B, from the industrial park, are located at the opposite end of the first component of Fig. 9a; they had the highest levels of contamination for several elements (Cr, Cu, Fe, Ni, Sb) and the highest values of concentration dependent magnetic parameters.

Spearman's Rank correlation between χ , M_S , M_{RS} and element concentration was tested (Tab. 2); significant positive correlations have been found between the concentrations of Cu, Fe, Ni, Sn and χ , M_S and M_{RS} ; the elements Cd and Co correlated only to χ , while Bi, Ca, Sb and Zn to M_S and/or M_{RS} . These deviations in the significance of the correlations between elemental concentration data and concentration dependent magnetic parameters may depend on the generally low and sometimes barely measurable values of the latter.

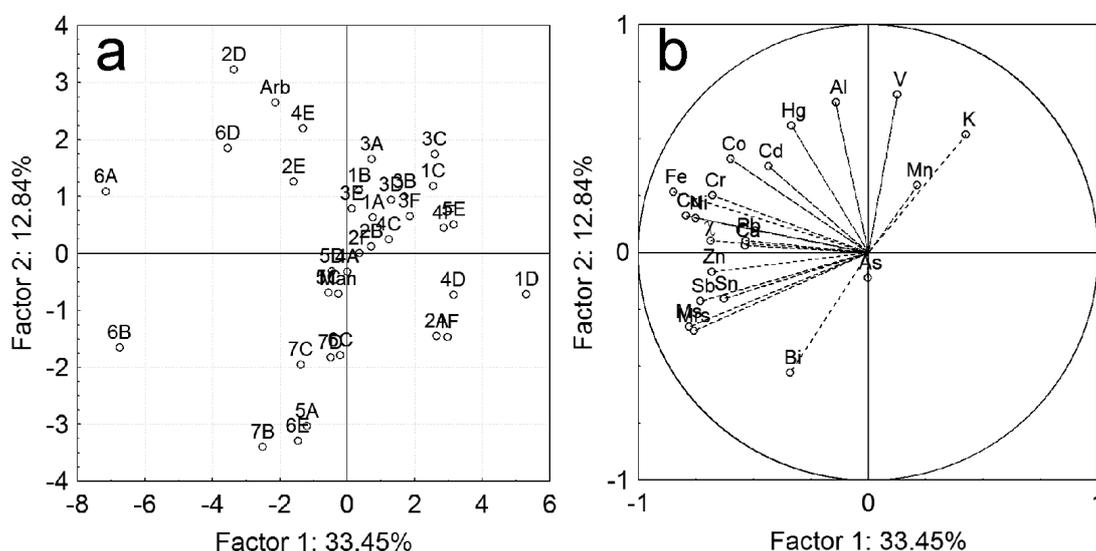


Fig. 9. Principal component analysis of the samples of *Pseudevernia furfuracea* after the two-month exposure in the 40 sites of Fig. 1. based on the values of χ , M_S , M_{RS} and the element content normalized on the mean pre-exposure values as $[(\text{post-exposure} - \text{pre-exposure})/\text{pre-exposure}]$ (a), and the corresponding loading plot (b).

Tab. 2. Spearman’s Rank correlation between χ , M_S , M_{RS} and element concentration measured in the post-exposure samples of the lichen *Pseudevernia furfuracea* transplanted for two months in the sites of Fig. 1. (correlations considered significant for $p < 0.05$ are marked in bold).

	χ	M_S	M_{RS}
Al	0.079243	-0.231308	-0.210043
As	-0.073530	-0.178698	-0.173718
Bi	0.247147	0.386490	0.469923
Ca	0.278346	0.355711	0.262088
Cd	0.367551	0.101379	0.189400
Co	0.338681	0.172294	0.074122
Cr	0.267275	0.271247	0.266849
Cu	0.470020	0.347296	0.363827
Fe	0.447875	0.414997	0.341198
Hg	0.187014	-0.066541	0.063599
K	-0.209411	-0.364811	-0.516546
Mn	-0.348112	-0.233663	-0.394761
Ni	0.412132	0.469807	0.397891
Pb	0.013307	0.016809	0.131671
Sb	0.329672	0.471809	0.398690
Sn	0.564233	0.548096	0.528311
V	-0.159548	-0.341375	-0.260595
Zn	0.315170	0.336041	0.347248

4. Discussion

Most of the works dealing with the magnetic properties of PM intercepted by biomonitors have been carried out on leaves of seed plants, generally trees or shrubs, more rarely on autochthonous cryptogams (mosses and/or lichens) (e.g. Chaparro et al., 2013; Fabian et al., 2011; Jordanova et al., 2010; Szönyi et al., 2007; Szönyi et al., 2008). In the intercomparison, lichens and mosses were found by Jordanova et al. (2010), to have the strongest contrast between clean and polluted environment, a further proof – if needed – that these cryptogams are very good biomonitors of PM. The lack of sufficient biological material in the most polluted sites, characterized by a true “lichen and moss desert”, can request the application of the transplant technique: suitable species are collected from pristine sites and are exposed in the target areas for assessing air pollutant depositions. This technique offers two main advantages compared to the use of autochthonous species (Bargagli, 1998; Ayrault et al., 2007): (i) the material can be repeatedly exposed for known time periods and according to a rational design, and (ii) enrichment rates of the elemental composition during exposure can be calculated on the basis of pre-exposure values (Frati et al., 2005). The reliability of this approach has been widely investigated in the last decade with particular focus on methodological and operational issues (Frati et al., 2005; Adamo et al., 2007; 2008; Tretiach et al., 2007), whose results have also been applied in this study. Transplants have

only sporadically been used in connection to the application of magnetic measurements (Salo et al., 2012; Salo, 2014; Salo and Mäkinen, 2014) and actually only Salo (2014) tested the performance of a lichen, *Hypogymnia physodes*, used in parallel to a moss, *Sphagnum papillosum*, and a filter fabric, concluding that this species can intercept air pollutants in sufficient quantity to carry out a detailed enviromagnetic survey, and can do it better than the moss when the pollution load is low. On the other side, the information given by the analysis of the magnetic signature of the exposed thalli allowed the author to distinguish among the different pollution sources present in the survey area.

With the transplant technique, a critical point is the characteristics of the pre-exposure material. Typically, the material is collected in pristine areas, far from known pollution sources, and therefore it should have a baseline content for most of the elements. In prevision of an enviromagnetic survey, the material should have very low magnetic susceptibility (χ) values; χ is directly measurable even in the field and can be interpreted as an overall indicator of the concentration of magnetic minerals. Those reported in the literature range between $3.4 - 3.9 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$ for *Hypogymnia physodes* (Salo, 2014; Salo et al., 2012), between $14.3 - 41.0 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$ for other foliose and microfoliose lichens (Chaparro et al., 2013) and between $1.0 - 1.0 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$ for mosses (Salo, 2014; Salo et al., 2012; Salo and Mäkinen, 2014). In Jordanova et al. (2010) lichens and mosses were considered together, and much higher and more variable χ background levels were observed ($9.18 - 86.8 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$), but it should be kept in mind that in the latter study sampling was extended to epilithic and epigaeic species, and therefore the wider range of values is not a surprise, being related to a stronger contamination by soil particles. In comparison to these works, our pre-exposure samples had very low levels of χ ($0.118 - 1.490 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$; Tab. 1) and, according to the FORC diagram (Fig. 7), the magnetic fraction was not relevant. This confirms that the Alpine area we repeatedly visited for sampling (Adamo et al., 2008; Kodnik et al., 2015; Tretiach et al., 2007, 2011) has a very low anthropic or natural impact, in accordance to the very low element concentrations (Tab. 1), that are fully in line with the results of a recent sampling extended to whole Italy, aimed at pinpointing true “background” areas (Capozzi et al., 2015).

If the pre-exposure values were low, both in term of element content and enviromagnetic properties, our post-exposure data sets suggest a modest anthropic impact on the environment. In comparison to other surveys carried out with the same technique throughout Italy (see e.g. Gallo et al., 2014; Giordano et al., 2005; Sorbo et al., 2008), but even in the same area, in a different period of the year (unpubl. results), the element concentration enrichment observed after the two-month exposure was certainly low, and this was also reflected by the post-exposure values of the magnetic susceptibility, which ranged between 0.350 and $7.409 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$ (Tab. 1). On the contrary, after

a six-month exposure of *H. physodes* transplants around a cluster of heavy metal and chemical industries, including a Cu-Ni smelter complex, Salo (2014) observed χ values ranging between $0.7 - 77.2 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$, whereas moss bags exposed in the same area reached higher values already after only two months ($0.2 - 186.7 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$) (Salo et al., 2012), and after a six-month exposure reached a maximum of $127.3 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$ (Salo, 2014) and $408.5 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$ (Salo and Mäkinen, 2014).

One reason for the modest increase observed in this study could be the meteorological conditions during the exposure period. Although the total rainfall was not particularly abundant, it occurred in form of several brief and intense thunderstorms that might have caused a wash effect of the PM accumulated on the surface of the lichens. This is partially confirmed by the leakage of Mn, K and V observed in the exposed transplants. However, the slight, but statistically significant increase of some elements and of magnetic susceptibility observed in 97.5% of exposed lichen samples also indicates that there was actually an enrichment, mostly of anthropic origin. This conclusion is supported by the fact that the soil-related elements (e.g. aluminum) remained very low, and maxima were never observed in exposure sites belonging to the agricultural stratum, whereas the highest increase was concentrated in the sites within or immediately near the industrial park. According to the data made available by the local authorities, the activities potentially consistent with the observed high levels of Cr, Cu, Fe, Ni and Sb, are various: one electroplating factory which works brass, bronze, copper, nickel and zinc; two gray cast iron and steel smelters; several forging factories; several knife manufacturing factories.

Although the post-exposure enrichment in PM was generally low, the magnetic analysis revealed some interesting traits that complemented the information derived from the elemental analysis. The magnetic mineralogy was reasonably homogenous in the whole set of samples and, crossing FORC and “Day Plot” data, PSD to MD magnetite is the main magnetic carrier. This type of magnetic PM can be associated to industrial, domestic heating or vehicle emissions (Hunt et al., 1984; Flanders, 1994), abrasion products from asphalt and vehicles brakes (Hoffmann et al., 1999; Sagnotti et al., 2009) or industrial activities such as smelters (Salo and Mäkinen, 2014). In the study area vehicle traffic is not particularly intense since there are no large urban centers present, however domestic heating by wood burning during the winter period can be an important source of airborne particulate matter in the city centers that is not detectable during summer, when this study was conducted (Kodnik et al., 2015; WHO, 2005).

Clinker production, that is the core of the industrial processes of the cement plant present at the center of our study area, far from the industrial park, occurs at high temperatures (1,400-1,500 °C) when a mixture of magnetite and hematite is generated (Hansard et al., 2011; 2012; Flanders,

1994; Petrovský and Ellwood, 1999; Magiera et al., 2011). Hematite was not found in the lichen transplants, while it was present in the cement sample and in the dust sample collected from the filters located above the main furnace. Moreover, no particular metal enrichment, both from the chemical and magnetic point of view, was found in the samples exposed in and around the cement plant, so that it might be concluded that it has a very low environmental fingerprint.

From the methodological point of view, it can be underlined that the significant correlations observed between the concentration dependent magnetic parameters and the elements Bi, Ca, Cd, Co, Cu, Fe, Ni, Sn, Sb and Zn support the concentration dependent magnetic properties as excellent proxies for heavy metal pollution even at low contamination levels.

5. Conclusions

In this survey, the main pollution source resulted to be the industrial park located in the SW corner of the study area. The concentrations of selected elements and the concentration dependent magnetic properties were, in that sense, in substantial agreement. The magnetic mineralogy was homogeneous throughout the entire set of samples and low-coercivity, pseudo-single domain to multidomain magnetite resulted as the main magnetic mineral. Unfortunately, it was not possible to attribute the origin of the PM to the different industrial activities, but it was possible to exclude the cement plant as the main source, as confirmed by the different magnetic mineralogy of the cement and dust sample therein produced with respect to the post-exposure PM intercepted by the lichens. From this point of view, the transplants of *Pseudevernia furfuracea* have proven to be effective for carrying out biomagnetic measurements, and the two-month exposure was sufficient for them to accumulate enough PM for a detailed magnetic characterization. Finally, the magnetic properties were confirmed as excellent proxies for heavy metal pollution even when the anthropic impact on the territory is rather modest. Their measurements are very sensitive, rapid and relatively cheap and they can allow to identify areas of interest for further, more detailed monitoring.

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Conclusions

The lichen *Pseudevernia furfuracea* (L.) Zopf var. *furfuracea* has proven to be an excellent PAH bioaccumulator. It was effective in revealing both local and large scale patterns of PAH pollution in semi-natural environments, and in industrial and urban areas.

Mountain environments are generally perceived by the public as natural and pristine but may actually suffer rather high levels of PAH pollution due to vehicular traffic, especially in summer and winter. Two main patterns were found: the first was among roads and reflected the gradient of touristic impact on the environment, whereas the second was within roads and reflected this impact in relation to the distance from the road margin. Differences among the sites were evident mainly for samples collected nearest to the road showing a filtering function of the forest vegetation (McLachlan and Horstmann, 1998) which had an effect in reducing PAH loads with the distance. Local patterns were more evident in the most impacted sites where PAH pollution had a steeper gradient with distance from the road. Furthermore, the fact that PAH concentrations similar to those of unpolluted sites were found in only one pass at 300 m from the road, indicates that traffic PAH pollution impacts natural ecosystems even at relatively long distances from the source.

Lichen transplants, after a two-month exposure, were able to accumulate a sufficient concentration of PAHs to reveal distribution patterns and attribute their origin to specific emission sources in a highly heterogeneous territory. They confirmed that the environmental levels of PAHs are an order of magnitude higher in the winter period compared to the summer levels. These changes may depend on modifications in emission intensity, since domestic heating by wood burning is quite frequent in the study area (ARPA FVG, 2013) and it is restricted by law to the winter season which automatically brings down the emissions in the summer period. The photochemical and chemical degradation to which PAHs are subjected when exposed to intense UV radiation (Boström et al., 2002; Wild et al., 2005) and oxidative molecules such as ozone (Alves, 2008; Brown and Brown, 2012), may also strongly influence the PAH loads on the territory. These are all conditions that at middle latitudes typically occur during the summer period (Lorenzini and Nali, 2005), causing a potentially significant underestimation of the real PAHs emission levels. Seasonal differences of the PAHs loads have to be considered when planning a biomonitoring survey. Studies performed during the warmest season may correctly describe the distribution patterns of these pollutants over the territory, but they certainly underestimate their potential environmental load, that typically occurs in the coldest periods of the year.

The same set of samples exposed during the summer period was analyzed for its element content and magnetic properties. The lichen transplants of *Pseudevernia furfuracea* have proven to be effective for carrying out biomagnetic measurements, and the two-month exposure was sufficient for them to accumulate enough PM for a detailed magnetic characterization. The magnetic analyses complemented the information derived from the element content and both suggest that there is a modest anthropic impact on the environment, concentrated in the industrial park at the SW corner of the study area. High levels of Cr, Cu, Fe, Ni and Sb found in the samples are potentially consistent with the activities present in the industrial park (one electroplating factory, two smelters, several forging and knife manufacturing factories). The magnetic mineralogy was homogeneous throughout the entire set of samples and low-coercivity, pseudo-single domain to multidomain magnetite resulted as the main magnetic carrier. This type of magnetic PM can be associated to domestic heating or vehicle emissions (Hunt et al., 1984; Flanders, 1994), abrasion products from asphalt and vehicle brakes (Hoffmann et al., 1999; Sagnotti et al., 2009) or industrial activities such as smelters (Salo and Mäkinen, 2014). Considering that domestic heating is absent during the summer period and vehicle traffic is not particularly intense in the area, it was possible to identify the industrial park as the primary heavy metal pollution source. However it was not possible to discriminate between PM originating from different pollution sources because of the very modest emissions from the other potential sources thus confirming their low environmental impact. The magnetic characterization of the lichen samples, integrated with the element content analyses, has proven to be a very innovative and promising method that could permit to attribute the origin of the PM to a specific anthropic activity. The measurements are very sensitive, rapid and relatively cheap and they can allow to identify areas of interest for further, more detailed monitoring.

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Appendix

Conference contributions

- D. Kodnik**, F. Candotto Carniel, S. Licen, A. Tolloi, P. Barbieri, M. Tretiach, 2013. Biomonitoraggio della deposizione di elementi in traccia e IPA mediante trapianti di *Pseudevernia furfuracea*: effetti della stagionalità. Notiziario della Società Lichenologica Italiana 26, 14 (platform presentation).
- A. Winkler, F. Candotto Carniel, **D. Kodnik**, M. Tretiach, 2014. Proprietà magnetiche di trapianti del lichene *Pseudevernia furfuracea* esposti intorno al cementificio di Fanna (PN). Notiziario della Società Lichenologica Italiana 27, 19 (platform presentation).
- D. Kodnik**, S. Bertuzzi, F. Bove, C. Nali, G. Lorenzini, F. Panepinto, E. Pellegrini, M. Tretiach, 2014. Effetti combinati del mesoclima e degli inquinanti aerodiffusi sul lichene epifita *Flavoparmelia caperata* (L.) Hale. Notiziario della Società Lichenologica Italiana 27, 20 (platform presentation).
- F. Candotto Carniel, T. Craighero, M. Crosera, **D. Kodnik**, G. Adami, M. Tretiach, 2014. Sono davvero innocui i lavaggi in acetone per la rimozione delle sostanze licheniche? Notiziario della Società Lichenologica Italiana 27, 40 (poster presentation).
- A. Winkler, **D. Kodnik**, F. Candotto Carniel, M. Tretiach, 2015. Magnetic properties of the lichen *Pseudevernia furfuracea* transplanted near a cement plant in NE Italy. Geophysical Research Abstracts 17, EGU2015-2787 (platform presentation).
- D. Kodnik**, A. Winkler, F. Candotto Carniel, M. Tretiach, 2015. Magnetic properties of the lichen *Pseudevernia furfuracea* transplanted near a cement plant in NE Italy. SETAC Europe 25th Annual Meeting, Barcelona, Spain. WE232 (poster presentation).
- A. Winkler, L. Paoli, **D. Kodnik**, F. Candotto Carniel, A. Guttová, S. Loppi, L. Sagnotti, M. Tretiach, 2015. Biomonitoring of air pollution by magnetic measurements of native and transplanted lichens; two case studies around cement plants. AGU Fall meeting 2015, GP42A-03 (platform presentation).

A. Winkler, **D. Kodnik**, F. Candotto Carniel, M. Tretiach, 2015. Magnetic properties of lichen samples transplanted near a cement plant in NE Italy. 101 Convegno Società Italiana Fisica (platform presentation)