Disentangling sources of trace element air pollution in complex urban areas by lichen biomonitoring. A case study in Milan (Italy)

DISENTANGLING SOURCES OF TRACE ELEMENT AIR POLLUTION IN COMPLEX URBAN AREAS BY LICHEN BIOMONITORING. A CASE STUDY IN MILAN (ITALY)

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Abstract

In this study we investigated the bioaccumulation of selected trace elements in lichen samples transplanted for three months in Milan, Italy, with the aim of assessing the main environmental contaminants and the overall pollution load, and of disentangling the main air pollution sources as well as of estimating fluxes of element deposition. The results highlighted Cu and Sb as important contaminants and suggested a common origin for these two elements from railways and non-exhaust sources of vehicular traffic such as brake abrasion. High or very high global air pollution emerged for all study sites. Source apportionment outlined three main factors, that found reliable correlation with distance from major roads and railways, an industrial plant, and soil resuspension. Ranges of estimated mean annual element deposition rates in the study area were similar to those reported for other cities.

Key Words: Bioaccumulation; Cu; PMF; Railways; Sb; Traffic

1. Introduction

Lichen biomonitoring is a technique used worldwide to assess air pollution, with applications also in environmental forensics (Loppi 2019; Contardo et al. 2018). The accumulation of persistent pollutants such as trace elements to levels far above their nutritional requirements (Bačkor and Loppi 2009) and proportionally to concentrations in bulk (wet and dry) deposition (Loppi and Paoli 2015) is the key feature for their use in the assessment of trace element deposition. The cost-effectiveness of lichen biomonitoring compared to conventional instrumental monitoring is a further crucial advantage of this technique, allowing a higher density of sampling sites with consequent higher spatial resolution of deposition patterns. A method largely used is based on the collection of lichen samples from an unpolluted area and subsequent exposure (transplantation) in the study area. In this way the sampling design can be done in the desired way, the exposure time is known, and pre-exposure values can be determined (Loppi et al 2019a). This method is especially suitable in urban areas, where native species are often missing (Loppi et al 2019b).

Particulate matter (PM) is one of the most dangerous air pollutants for human health, especially in urban areas (WHO, 2016). Its finest fraction can be easily inhaled and can reach the upper and lower respiratory system (Oberdöster and Utell, 2002), causing injuries such as asthma, inflammations, cardio-vascular diseases, neurodegenerative problems as well as lung cancer (Brunekreef and Holgate, 2002).

Trace elements are an important component of PM, of which may constitute a remarkable portion, especially for the finest fraction (Zhou et al., 2014). These contaminants have a great epidemiological concern because of their persistence in the environment and the negative effects on human health (Valko et al., 2005). In the complex reality of a urban environment, trace elements may have a wide variety of emission sources such as motor vehicles, heating systems, industrial plants, etc. Assessing the overall pollution load by toxic elements and identifying the contribution of each source to the total burden is crucial for addressing targeted abatement measurements and, thus, preserve population health (Thunis et al., 2019).

Source apportionment based on receptor modelling is a technique based on a wide range of statistical models, used to describe the main profile of pollution composition (Hopke, 2016). They are based on the chemical and mass conservation from the emission source to the receptor, thus, the elemental composition at the receptor is expected to reflect the source emissions profile (Hopke, 2010). Compared to other models, they have the great advantage to be easy to compute (few variable requested) and to provide quantitative outcomes (Belis et al., 2013). With instrumental monitoring, receptor modelling is resolved temporarily, i.e. the measures are repeated in time at one or a few sites, while with biomonitoring it is resolved spatially, i.e. the measures are carried out at many sites (Landis et al., 2012). The factors emerging from the model may represent well separated sources of pollution, and with the aid of Geographic Information Systems (GIS), they may in addition find a spatial distribution, providing contamination maps which are a key tool for land interpretation and local or regional management and planning (Badach et al., 2020).

The aim of this study was to carry out an exploratory spatial analysis of trace element air pollution in a complex urban environment, combining lichen biomonitoring and source apportionment analysis to assess the overall contamination load and to identify the main emission sources.

2. Materials and Methods

2.1 Study area

The study was carried in the Municipal area of Milan (Italy), which is one of the most important Italian cities owing to its key role in Italian economics and is also one of the most densely populated cities, with 1.4 million inhabitants distributed over 181 km^2. During the past century Milan faced important transformations, switching from a strictly industrial economy to a dematerialized one, and now marketing and tertiary activities are the most important economic sectors, but industries and agriculture are still present (Armondi and Bruzzese, 2017). The main sources of air pollution are from vehicular traffic, heating systems, railway lines, airport, a municipal solid waste incinerator as well as some industries in operation in the area. The area is well known for being one of the most polluted in Europe (EEA, 2019), with air pollution being determined also by adverse climatic conditions such as frequent thermal inversion, stagnation of air masses and persistent fog. In fact, Milan is located in the Po plain, a huge flat region with a continental-like climate characterized by long and severe winters and high temperatures in summer; annual precipitations is 1013 mm and average annual temperature is 13.1°C.

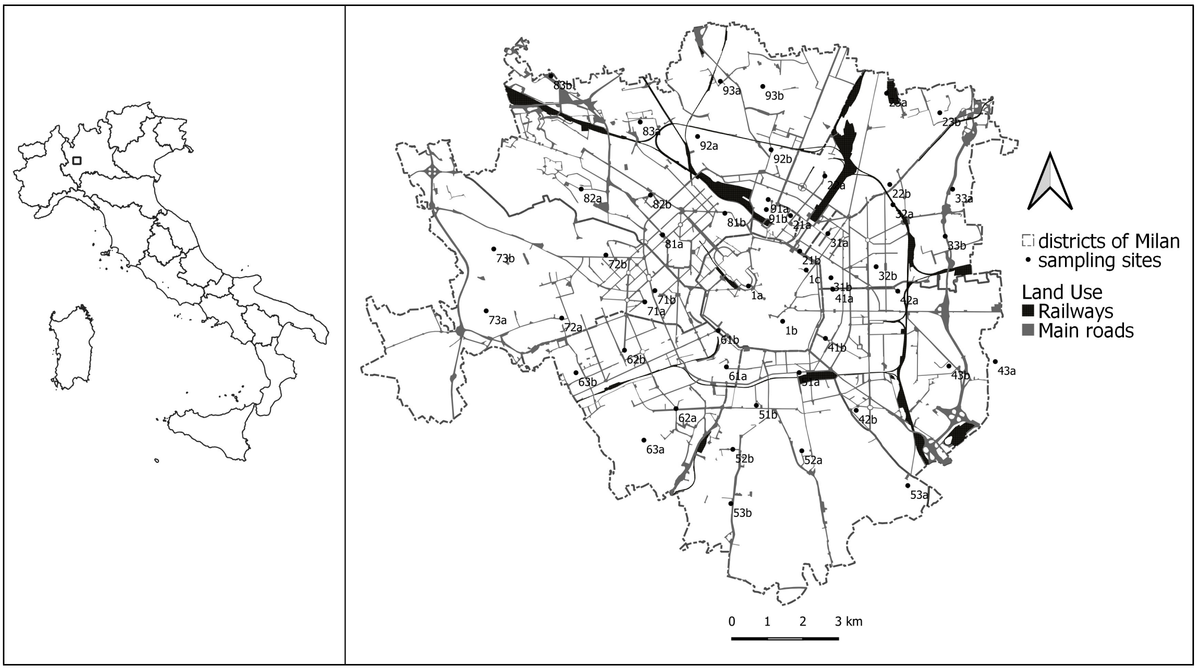

Figure 1. Study area with location of sampling sites.

2.2 Lichen biomonitoring

A biomonitoring through transplantation of lichen material was selected for this study. The fruticose lichen species *Evernia prunastri* was chosen being widely used in biomonitoring studies (Paoli et al., 2019; Vannini et al., 2017) and owing to its documented capacity to accumulate high concentrations of trace elements (Loppi et al., 1998) and to reflect atmospheric deposition (Loppi and Paoli, 2015).

The lichen material was harvested in a remote area of central Italy, far removed from any local source of air pollution. Apparently healthy thalli of similar size were selected in order to reduce natural intrinsic variability linked to age and vital status (Wolterbeek and Bode, 1995). In the laboratory, lichen material was get rid of extraneous material such as bark, insects, etc with plastic tweezers. Sample vitality was then randomly checked by analyzing the photosynthetic efficiency, which resulted always optimal, and lichen bags were prepared using a plastic net loosely wrapped around the lichens, closed at the extremities. The material was maintained in a climatic chamber at 16°C, RH= 65%, 40 μmol m^{-2} s^{-1} photons PAR with photoperiod of 12 h until transplantation, which occurred within one week from collection.

The lichen samples were transplanted at 50 sites in Milan, selected following a stratified random design according to distance from the centre (three belts: central, semi-peripheral, peripheral), and the 9 administrative city districts. In addition, a control site was selected at a relatively unpolluted forested area 50 km N of Milan, far from roads and local pollution sources. At each exposure site (and at the control site) 3 lichen bags were exposed from December 2018 to February 2019 at a height of ca. 2 m above the ground, tied to the branches of trees located along roads or inside parks. The winter period was selected to assure a high metabolic activity of the samples (Vannini et al., 2017) and the duration of three months is regarded as optimal for this species (Loppi et al., 2019a) After the exposure, samples were retrieved and air-dried (residual water<10%). For the chemical analysis, the lichen material inside each lichen bag was pooled. Having a control site accounting also for the possible effect of transplantation, unexposed samples were not assayed.

2.3 Chemical analysis

The content of Al, As, Cd, Cr, Cu, Fe, Pb, Sb and Zn was assessed by acid digestion of 200 mg of lichen material using 3 mL 70% HNO_3, 0.2 mL of 60% HF and 0.5 ml H_2O_2 in a microwave digestion system (Ethos 900, Milestone) and subsequent quantification by ICP-MS (Sciex Elan 6100, Perkin-Elmer). One procedural blank and one sample of the certified material IAEA-336 "lichen" were included in each set of analysis. Recoveries were in the range of 90-113% and the precision of analysis, expressed as coefficient of variation of five replicates, was within 10% for all elements. Results are expressed on a dry weight basis.

2.4 Data Analysis

2.4.1 Exposed to Control Ratios and Contamination Index

The biomonitoring techniques carry on an intrinsic variability associated with the fluctuations of living organism in their reactions to environmental changes. To assess the effective bioaccumulation of trace elements, the effect of natural variability has to be accounted for, in order to avoid especially α type error (false positives) that can lead to a misleading overestimation of pollution phenomena, masking the real extent of their spatial patterns (Loppi et al., 2019a). Following the approach suggested by Frati et al. (2005), the bioaccumulation of trace elements in exposed samples was assessed by comparison with the concentrations in control samples, by calculating an exposed-to-control (EC) ratio. To account for the variability in control samples and for the definition of an "effect detection limit" (Klumpp et al., 2009), for each element, the reference value in control samples was taken as the mean concentration plus 10 times its standard deviation (Couto et al., 2004). Ratios exceeding 1 are indicative of bioaccumulation. Following the suggestions of Contardo et al. (2018), elements with an EC higher than 1 in at least 50% of sampling sites (i.e., elements with median >1) were considered as effective pollutants in the area. A Contamination Index (CI) was then calculated for each site as the geometric mean of EC values of effective pollutants (Contardo et al. 2018; Loppi et al., 2019b). The interpretation of EC and CI values was based on the scale suggested by Frati et al. (2005).

2.4.2 Source Apportionment

To disentangle the major sources of air pollution in the study area, a spatially resolved multivariate receptor modelling technique was used to extract information on the sources of air pollutants from the measured concentrations in lichens at the sampling sites. Specifically, positive (non-negative) matrix factorization (PMF) (Paatero and Tapper, 1994) was used to resolve the original data matrix X(m x n), where m = EC ratios and n = sites, into the product of a loading or source profile matrix G(m x f), where f = factors, and a score or contribution matrix F(f x n), X = GF+E, where E = residuals (error matrix). Factor contributions (%) were calculated as the sum of all matrix elements of a row in the F matrix, for a given factor, over the aggregate sum for all factors. Statistical elaborations were done using the free software R (R Core Team, 2020).

2.4.3 Spatial data

The CI and the factors emerged from PMF were mapped over the study area through the deterministic Inverse Weighted Distance (IDW) interpolation algorithm using QGIS 3.8, and sorted into quintiles to a suitable visualisation. The pattern of each PMF factor was used to search the reliable underlying urban elements responsible of the contamination, coherently with the profile of the factor itself. Once identified a pool of possible and reliable urban elements, a correlation between the distance to these elements and the trend of the factor was checked. As geographic support for this operation, we used the Census Units (CU) vector layer that represent the small patches in which the whole area is subdivided for administration purposes (ca. 6,000 inhab.). For each CU, the value of the factor was calculated as the mean of the pixel values inside its boundaries. Then, the minimum distance between the geometric centre of the CU and the urban elements selected as mentioned above was calculated. Finally, the distances were sorted into deciles, and the mean value of the distance for each decile was correlated with the mean value of the factor for the same decile. This operation was compute separately for each factor and each urban element selected. Statistical elaborations were done using the free software R (R Core Team, 2020).

2.4.4 Estimation of element deposition rates

The concentrations of trace elements accumulated by lichens can be converted into estimates of heavy metal deposition rates (Loppi, 2014; Loppi et al., 2003; Loppi et al., 2019b). To do this, a weight/area ratio for *E. prunastri* of ~160 g/m^2 was calculated by cutting several thallus pieces and measuring their surface area and dry weight. Based on the known exposure time of 3 months and assuming that the final concentrations represent an equilibrium with the new environment, it was possible to convert element concentrations in lichens into estimates of average annual element deposition rates, according to the formula:

deposition (kg/km^2/y) = concentration (µg/g) x 160 (g/m^2) x 4 (months) / 1000

3. Results

Table 1 summarizes, for each element, mean concentration in control samples, effect detection limit, mean concentration in exposed samples, median values of exposed-to-control (EC) ratios. Bioccumulation was not detected for Al, As, Cd and Zn, and the contribution of these elements to the overall contamination of the study area was regarded as negligible (Frati et al., 2005). Elements with a median EC ratio higher than 1 were Cr (1.34), Cu (3.76), Fe (1.49), Pb (1.75) and Sb (4.00). A situation of contamination (EC ratio in the range 1.25-175, Frati et al., 2005) emerged for Cr, Fe and Pb, while severe contamination (EC ratio >1.75) was evident for Cu and Sb, that are thus the main air pollutants in the area, with all sampling sites (but 3 for Cu and 1 for Sb) being concerned by a severe pollution by these elements.

	Control	EDL	Exposed	EC
Al	496 ± 20	698	619 ± 209	0.84
As	0.21 ± 0.01	0.35	0.32 ± 0.06	0.88
Cd	0.06 ± 0.01	0.11	0.02 ± 0.01	0.64
Cr	1.67 ± 0.09	2.53	3.63 ± 0.90	1.34
Cu	3.79 ± 0.03	4.06	16.6 ± 5.6	3.76
Fe	442 ± 10	543	837 ± 214	1.49
Pb	1.96 ± 0.08	2.78	5.84 ± 2.39	1.75
Sb	0.11 ± 0.01	0.17	0.76 ± 0.32	4.00
Zn	27.1 ± 1.55	42.6	38.2 ± 13.1	0.90

Table 1. Mean (± standard deviation) values (μg/g dw) of control samples, effect detection limit (EDL), mean (± standard deviation) values (μg/g dw) of exposed samples, Exposed-to-Control ratios (EC).

The CI values ranged from 1.36 to 4.50, indicating that the contamination is high over the whole study area (Fig. 2), with the vast majority of study sites (80%) being concerned by a severe overall contamination. Noteworthy, in the central part of the study area, the lowest contamination was found inside or in proximity of the main urban forested parks of the city.

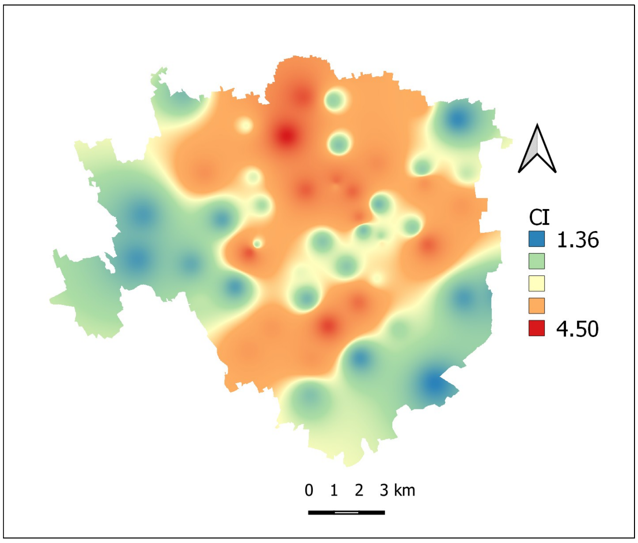

Figure 2. Distribution map of the contamination index (CI)

A three-factor solution was found to be optimal for the PMF model, with no factor resulting uncorrelated. Predicted data were highly correlated with observed data (R^2>0.6) for all elements except Al and Cd (R^2 = 0.16 and 0.21, respectively). Residual analysis showed that all elements were well modelled, with standardized residuals in the range ±2. Figure 3 shows the map of each factor along with its elemental profile. The first Factor (F1) accounted for 45.2% of total variability and was clearly dominated by Cu (50.4%) and Sb (30.1%) that are well known tracers of abrasion of brake systems, and thus vehicles and railways are likely sources for this factor. The second factor (F2) accounted for 25.4% of total variability and resulted dominated by Pb (53.9%) and Sb (34.3%). This factor was mainly distributed in the northern part of the study area, where the presence of a hotspot suggested the existence of a point source. Territorial inspection in the hotspot area revealed the past presence of a large glasswork and crystal factory, where Pb and Sb were used in some steps of glass production. The profile of the third Factor (F3), which accounted for 29.3% of variability, showed the main contribution of Fe (20.2%), Cr (17.0%) and Al (16.1%). The composition and distribution of F3 suggested that soil dust re-suspension may play an important role, consistently with the presence of agricultural areas in the southern part of the city, where F3 was distributed.

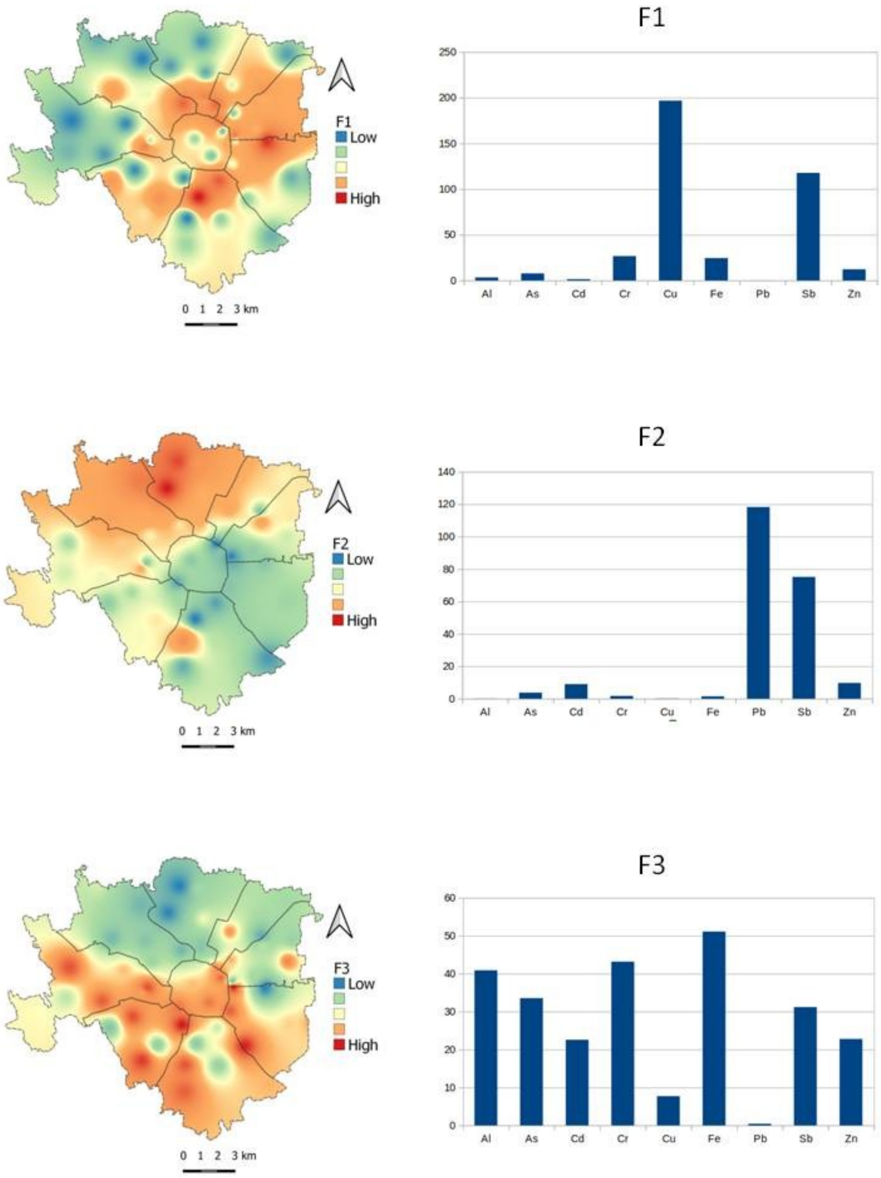

Figure 3. Spatial distribution of the three factors emerged from the PMF analysis, along with their elemental profile.

Figure 4 shows the relationship of F1 and F2 with distance from main roads and railways, as well as the glasswork, respectively. The shape of the trendline was exponential for the former and linear for the latter, with very high values of the determination coefficient (R^2=0.93-0.99) in all cases.

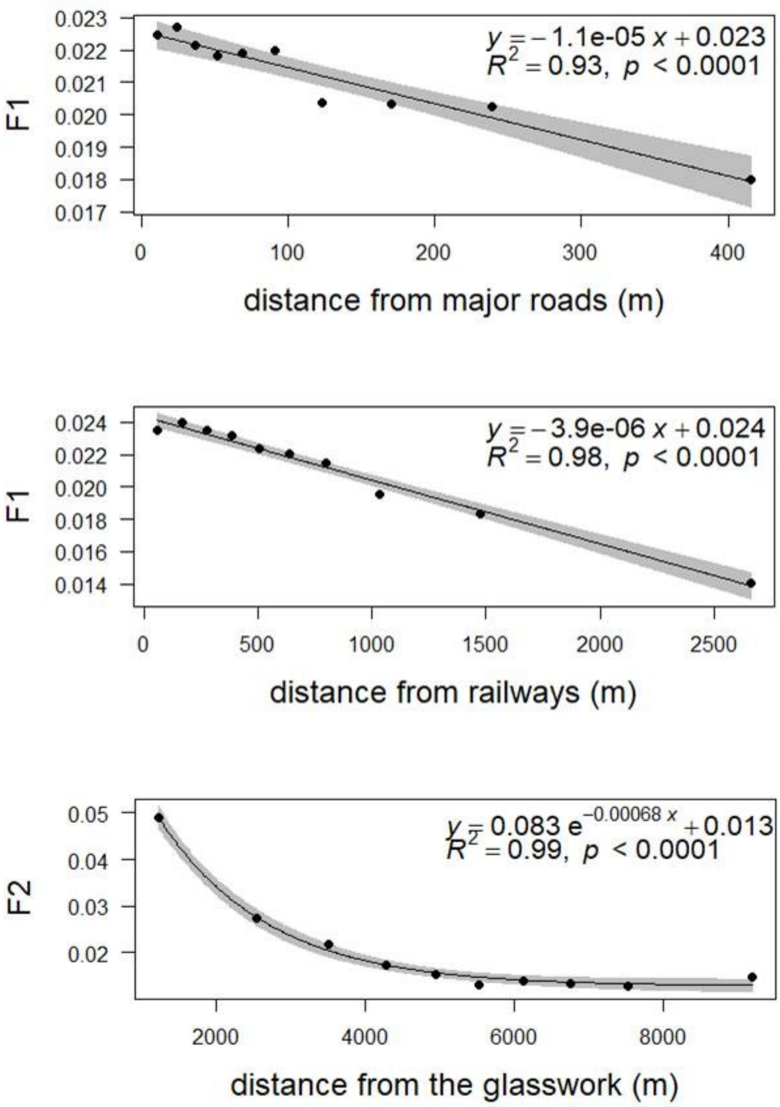

Figure 4. Relationship of F1 with main roads and railways (see text) and of F2 with distance from the glasswork.

Table 2 summarizes the ranges (min-max) of the 95% confidence interval for the estimated average annual element deposition rates ($kg/km^2/y$) based on lichen bioaccumulation data.

Element	min	max
Al	359	433
As	0.19	0.21
Cd	0.04	0.05
Cr	2.2	2.5
Cu	9.1	12.1
Fe	498	574
Pb	3.3	4.2
Sb	0.43	0.54
Zn	22.1	26.7

Table 2. Ranges (95% confidence intervals) of estimated average annual element deposition rates (kg/km^2/y).

4. Discussion

The results of lichen biomonitoring pinpointed at Cu and Sb as the major contributors to the atmospheric burden in the study area among the measured elements, and are in agreement with the findings of several other similar studies in urban areas, confirming the great capacity of lichens to accumulate Cu and Sb released from vehicular traffic (Loppi and Paoli, 2015; Paoli et al., 2013; Vannini et al., 2019; Loppi et al., 2019b), even when exposed inside car cabins (Paoli et al., 2019). These results are consistent with the fact that in urban areas PM is especially enriched in Cu and Sb, which are mostly apportioned to the finest fraction (Zereini et al., 2005). It is well known that traffic is presently the major source of PM in European cities (Karagulian et al., 2015), with non-exhaust emissions accounting up to 90% of the total (Omstedt et al., 2005; Forsberg et al., 2005). Although exhaust emission has greatly reduced in spite of improved abatement and control systems, the emission of particles from the wearing of brakes and tires, as well as from road dust resuspension is still high (EEA, 2018). Moreover, owing to the reduced height of the emission source, released contaminants are of high concern for human health, being readily available and concentrated at ground level (Wu et al., 2002). Copper emission from brake wear may be the main source of this metal along European roads, accounting up to 75% of total Cu emissions (Van der Gon et al., 2007), and Cu/Pb ratios in the range 2.1–3.9, as verified for ca. 50% of sites in the study area, with a mean value of 2.8, have been shown to be a good tracer of traffic emission (Mazzei, et al., 2008). Natural inputs of Sb are negligible if compared with anthropogenic ones (Nriagu, 1996). The main Sb atmospheric contribution is from non-exhaust sources of vehicle traffic such as brake wear and engine corrosion (Wåhlin et al., 2006; Jeong et al., 2019), and Sb is now widely used as tracer of vehicle traffic (Dietl et al., 1997).

The Contamination Index delineated a reliable picture, with an overall uncertainty <10% at the 95% confidence interval, of the global air pollution in the study area, and indicated that all sites are subjected to high or very high pollution loads.

By analyzing the concentrations (normalized as EC ratios) measured at each sampling site (receptors), PMF identified three factors which were taken to represent the major emission sources in the study area; the scores on these factors were then regressed against the concentrations to estimate the contributions from each source.

The first factor, strongly characterized by Cu and Sb, was clearly referable to non-exhaust emissions from vehicles (Sanders et al., 2003, Sternbeck et al., 2002). The diffuse presence of these elements in the study area is consistent with the trend the modern cities are presently facing (Massimi et al., 2019). Exhaust emissions were abated during the years, with technological improvements like catalytic converters, the removal of lead as anti-knocking additive in fuel, as well as non-technological behaviour e.g. car sharing, resulting in a well documented decrease of exhaust-related trace elements in monitoring studies (Thorpe and Harrison, 2008). On the other hand, non-exhaust emissions faced minor abatement plans, gaining more and more relevance in air pollution studies. Abrasion of brakes, tires and road wear as well as mechanical parts are the main responsible of non-exhaust emissions (Thorpe and Harrison, 2008). The strong linear correlation emerged for this factor with distance from the main roads is clear evidence of the relevance of this source, and indicated that the influence of major roads is clearly detectable at distances up to 100 m. Moreover, such non-exhaust emissions may arise also from railways, which are another important source for these elements (Timmers and Achten, 2016). Several studies reported the impact of railways on air and soil quality of modern cities (e.g. Wierzbicka et al., 2015), and it has been suggested that railways may be the most important contributor of these trace elements in lichens (Massimi et al., 2019). The strong linear decrease of F1 with distance from railways is a further confirmation of this evidence, and suggested that the influence of railways can be detected up to ca. 1000 m, consistently with the findings of Wiłkomirski et al. (2011).

An industrial origin was hypothesized for the second factor, having a profile dominated by Pb and Sb and showing a distribution centred on the northern part of the study area, with a hotspot in the surrounding of a past glasswork and crystal factory, and a very clear and exponential decreasing trend from this plant. Lead has long been used as tracer of vehicle exhaust emissions, owing to its use as anti-knocking agent in fuel engine, but a remarkable decline has been reported in urban lichens from Italy after its ban in 1992 (Loppi et al., 2003, 2004). Presently, Pb originates mainly from road dust resuspension and industrial activities (Dong et al., 2017). The glasswork and crystal factory was in operation from 1964 to 2004, and both Pb and Sb were commonly used during productive steps (Apostoli, 1998). High Pb concentrations around this kind of plants are well documented (Helmfrid et al., 2019). Moreover, the low mobility and high persistence of Pb in the environment (Young et al., 2002) may further explain the relatively high concentrations found in the study area. Consistently, Monaci et al. (2000), analysing PM_{10} and tree leaves, reported that Pb contamination still occurs along busy roads as a consequence of street dust re-suspension.

Lithogenic contribution to the elemental composition of samples was suggested for the interpretation of the third factor, with elements usually associated with soil resuspension such as Al, Fe and Cr being representative here. As opposite and complimentary to the second factor, the mainly southern distribution in the study area, mostly overlapping with agricultural areas, where a high production of airborne dust is typical, would confirm this hypothesis.

It is of paramount interest that our results indicated the lowest overall contamination in the city centre at urban forested parks, confirming that green areas and urban forests are very important barriers against air pollution (Baraldi et al., 2019). It has been shown that urban trees provide remarkable ecosystem services that contribute to improve air quality by consistently reducing PM levels owing to leaf surface adsorption as a consequence of dry deposition processes (Manes et al., 2016). Consistently, our results clearly indicate that when the central part (see sampling design) is analyzed separately for sites in green areas and other sites and compared with semi-peripheral and peripheral sites, EC ratios of Cu and Sb in parks are the lowest (Fig. 5).

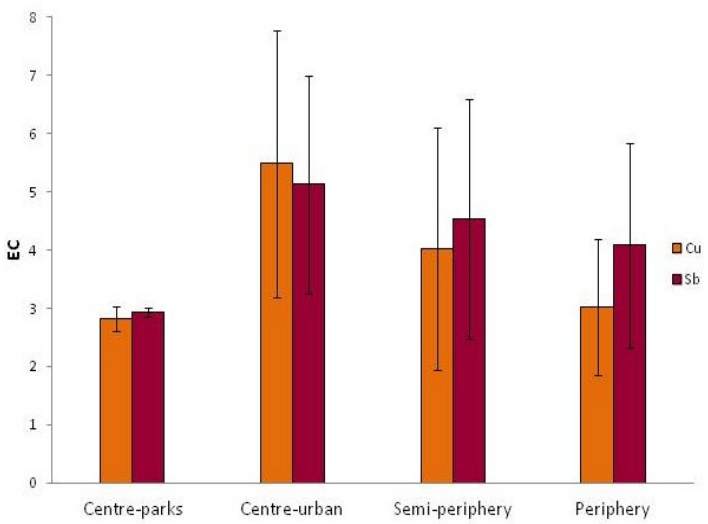

Figure 5. EC ratios (mean ± standard deviation) of Cu and Sb in green areas and other urban areas of the centre, semi-periphery and periphery.

Knowledge of element deposition fluxes is extremely important since it is known that chronic exposure to ambient PM is associated with adverse health effects (Churg et al., 2003), but this kind of data is usually very limited. As a consequence, the possibility offered by such estimation by means of lichen biomonitoring is invaluable. Moreover, since urban horticulture has become popular, knowing element deposition fluxes in cities is mandatory to assess possible impacts on these agricultural products and the possible consequences for human health through the food chain (Saümel et al., 2012). Ranges of estimated mean annual element deposition rates in the study area are consistent with those measured in other large cities such as Los Angeles (Sabin et al., 2006), Chicago (Shahin et al., 2000), Munich (Dietl et al., 1997), Paris (Ayrault et al., 2013), or the whole China (Ni and Ma, 2018).

5. Conclusions

In this work we confirmed the great versatility and usefulness of lichen biomonitoring in complex urban areas. The transplant technique allowed for a fully planned sampling design and the high number of sampling sites allowed the drawing of detailed maps, useful for land interpretation and planning. The results highlighted Cu and Sb as the main contaminants in the study area and suggested a common origin for these two elements from railways and non-exhaust sources of vehicular traffic such as brake abrasion. High or very high global air pollution emerged for all study sites, with an uncertainty <10%. The use of positive matrix factorisation, a spatially resolved multivariate receptor modelling technique, allowed to extract information on the sources of air pollutants from the measured concentrations in lichens at the sampling sites. Three major factors were disentangled, which suggested brake abrasion from vehicles, industry and soil resuspension as the main air pollution sources in the area. Ranges of estimated mean annual element deposition rates in the study area were similar to those reported for other big cities.

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