RESEARCH ARTICLE

Assessment of trace metal air pollution in Paris using slurry-TXRF analysis on cemetery mosses

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Abstract Mosses are useful, ubiquitous accumulation biomonitors and as such can be used for biomonitoring surveys. However, the biomonitoring of atmospheric pollution can be compromised in urban contexts if the targeted biomonitors are regularly disturbed, irregularly distributed, or are difficult to access. Here, we test the hypothesis that cemeteries are appropriate moss sampling sites for the evaluation of air pollution in urban areas. We sampled mosses growing on gravestones in 21 urban and peri-urban cemeteries in the Paris metropolitan area. We focused on *Grimmia pulvinata* (Hedwig) Smith, a species abundantly found in all studied cemeteries and very common in Europe. The concentration of Al, As, Br, Ca, Ce, Cl, Cr, Cu, Fe, K, Mn, Ni, V, P, Pb, Rb, S, Sr, Ti, and Zn was determined by a total reflection X-ray fluorescence technique coupled with a slurry sampling method (slurry-TXRF). This method avoids a digestion step, reduces the risk of sample contamination, and works even at low sample quantities. Elemental markers of road traffic indicated that the highest polluted cemeteries were located near the highly frequented Parisian ring road and under the influence of prevailing winds. The sites with the lowest pollution were found not only in the peri-urban cemeteries, adjoining forest or farming landscapes, but also in the large and relatively wooded cemeteries located in the center of Paris. Our results suggest that (1) slurry-TXRF might be successfully used with moss material, (2) *G. pulvinata* might be a good biomonitor of trace metals air pollution in urban context, and (3) cemetery moss sampling could be a useful complement for monitoring urban areas.

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Highlights • Cemetery mosses were used as biomonitors of trace metal air pollution in Paris region.
• Slurry-TXRF method might be successfully used with moss material.
• Polluted cemeteries are in traffic congested areas, under main winds influence.
• Green cemeteries are less polluted than smaller cemeteries with low tree density.
• Cemetery moss sampling could be a useful complement for monitoring urban areas.

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Introduction

The monitoring of global atmospheric pollution in urban and
peri-urban areas is at a crucial phase where the expectations of
society in terms of environmental issues often exceed the ca-
pacity of integrative measures to track pollutants in the long-
term. One way to meet this demand is the use of biomonitor-
ing. It can be seen as an alternative to more conventional
measurements of pollutants from air or precipitation, through
cheaper, easier, and space- and time-integrated analyses
(Chakrabortty and Paratkar 2006). However, monitoring
using living organisms in a patchwork of urban and peri-
urban areas that can rapidly evolve and are subject to strong
anthropogenic pressure faces three major problems: (i) the
need to have spatially integrative and regularly distributed
sites with organisms that can serve as biomonitors but also
are not subject to excessive disturbances, e.g., regular
weeding; (ii) the need to have a biomonitor that is sufficiently
ubiquitous, easily harvestable (including during all seasons)
and recognizable, capable of accumulating pollutants and re-
sistant; and (iii) selection of a technique that allows fast, sim-
ple, inexpensive, and precise quantification of pollutants that
is adapted to the selected biomonitor.

For the selection of a suitable site, cemeteries in urban
contexts may represent relatively protected areas compared
to surrounding urban areas including parks. These sites are
usually easy to access and regularly distributed, and the
biomonitors may remain undisturbed during long periods.
This may, however, depend on the history and management
of the city, the spectrum of cemeteries histories, and their
structural and spatial characteristics at local (e.g., proximity
to urban road infrastructure) and regional (e.g., climate pat-
terns interacting with built environment) scales.

For the selection of a suitable urban biomonitor, the use of
mosses as biomonitors for air pollution has emerged for some
time but is presently a matter of scientific debate. Few years
ago, Aboal et al. (2010) stated that atmospheric deposition of
metals cannot be accurately estimated from the concentrations
in moss tissue. In fact, the heterogeneity of sources of elements
(atmospheric deposition from natural sources, edaphic, and
plant inputs), environmental conditions, and biological process-
es in mosses can interfere, rendering difficult the estimation of
atmospheric deposition. However, over the past few decades, a
large number of researchers have recognized mosses as valid
biomonitors in order to assess whether nitrogen (N)
bioaccumulated in mosses reflects different land uses (Pesch
et al. 2008; Boltersdorf et al. 2014), or for estimating the spatial
distribution of atmospheric nitrogen deposition in Europe

(Harmens et al. 2011), or atmospheric derived sulfur (Liu
et al. 2009) when mosses on open rocks are sampled. In a recent
review, Harmens et al. (2013) stated that mosses are suitable
organisms to monitor deposition of POPs, including polycyclic
aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs),
dioxins and furans (PCDD/Fs), and polybrominated diphenyl
ethers (PBDEs). Heavy metal pollution has also largely been
investigated using mosses as biomonitors (e.g., Shacklette
1965; Rühling and Tyler 1970; Grodzinska 1978; Shaw 1989;
Zechmeister 1994; Carpi et al. 1994; Poikolainen et al. 2004;
Schintu et al. 2005; Giordano et al. 2005; Pesh and Schröder
2006; Holy et al. 2009; Viet et al. 2010; Ares et al. 2012; Correa
Mazzoni et al. 2012; Giordano et al. 2013; Gerdol et al. 2014;
Cowden et al. 2015; Harmens et al. 2015; Maxhuni et al. 2016).
Indeed, these small, resistant, and relatively ubiquitous plants
may well play an important biomonitoring role in future,
crowded urban areas. Different protocols for active biomonitor-
ing have been recently proposed (Ares et al. 2012; Harmens
et al. 2014), and the replacement of naturally growing mosses
with cultivated clones or with a commercial fleece has even
been suggested (Giordano et al. 2013).

Mosses have several important advantages over other more
conventional techniques. First, mosses are fixed to their sub-
strate without real root system; additionally, they also exhibit a
large surface-volume ratio that promotes migration of trace
metals and other elements to the free cation-exchange sites
located on the cell walls and the external side of cell mem-
brane (Chopra and Kumra 1988; Steinnes 1995; Sucharova
and Suchara 2004; Chakrabortty and Paratkar 2006). Metals
are thus present in mosses at higher concentrations when com-
pared with those in rainfall or air samples (Tyler 1970; Chopra
and Kumra 1988; Rühling et al. 1989). Second, unlike storm-
water analysis that allows assessing air pollution at a given
time, mosses are perennial (annual growth succeeds the pre-
cedent on a single stem), are frequent and widespread, can
integrate contamination over time, and are representative of
sampling site's contamination. Third, thanks to their small size
and the fact that they are living in relative independence from
the substrate, mosses are generally easy to sample. These
properties, combined with their ability to accumulate particles
on the leaf surface and to exchange ions, are considered by
many authors to make mosses good indicators of spatial trends
and patterns of air contamination by trace metals on a regional
scale (Steinnes et al. 1997; Zechmeister et al. 2003; Sutton
et al. 2004; Cowden et al. 2015). Epilithic lichens also provide
an excellent means to monitor atmospheric deposition over
large areas in a fast, simple, cost-effective way (Matschullat
et al. 1999), but they are often less uniformly distributed in
urban sites and in particular in cemeteries.

For the selection of a suitable technique, a large range of
methods have been used for measuring the heavy metal con-
tent in mosses (Harmens et al. 2008): inductively coupled
plasma mass spectrometry (ICP-MS), flame atomic

absorption spectroscopy (F-AAS), inductively coupled plasma emission spectroscopy (ICP-ES), graphite furnace atomic absorption spectroscopy (GF-AAS), instrumental neutron activation analysis (INAA), cold vapor atomic absorption spectrometry (CV-AAS), and advanced mercury analyzer (AMA). Klockenkaemper (1997) showed that total X-ray fluorescence spectroscopy (TXRF) is a reliable and convenient method for the determination of trace metal concentrations as it can be applied either with or without preliminary sample digestion. A review on biological and environmental TXRF applications has recently been published (De La Calle et al. 2013). TXRF with preliminary sample digestion (i.e., mineralization) has been used in biomonitoring to determine metal concentrations in algae (Sabatini et al. 2009; Varga et al. 1999; Barkàcs et al. 1999), waterweed (Woelfl et al. 2006), lichen (Montero et al. 2009; Borgese et al. 2009), and mosses (Markert et al. 1994; Zarazúa-Ortega et al. 2013). TXRF with "no or minimal treatment" and direct analysis on powdered samples was used on atmospheric dust collected on TXRF quartz carriers used as impaction plates (Injuk and Van Grieken 1995; Schmeling and Klockow 1997; Esaka et al. 2003; Schneider 1989), collected on filters (Borgese et al. 2012) or on adhesives (Cantaluppi et al. 2013). Finally, slurry sampling for TXRF was applied for biological samples (Meyer et al. 2012; De La Calle et al. 2012; Wellenreuther et al. 2008), drug substances (Shaw et al. 2012; Borgese et al. 2010), soils (Marguí et al. 2010), rocks (Marks et al. 2012), and archeological samples (Bonizzoni et al. 2013). Overall, "slurry sampling" appears to be the third most commonly used sample preparation method for TXRF, preceded by digestion and no (or minimal) treatment methods. Slurry sample preparation can be an especially convenient alternative to digestion, as skipping the sample digestion step, the risk of sample contamination, and element loss is reduced. This also allows avoiding lengthy digestion and vessel cleaning procedures.

In the present work, we (i) sought to assess the relevance of urban cemeteries for surveying the spatial variability of metal deposition, in the context of preliminary studies or as part of a larger program (e.g., atmospheric quality monitoring networks). We took the example of cemeteries located in the Paris Region (located within Paris and in the surrounding suburban zone), which can be considered as an example of a large European urban area; (ii) have targeted mosses present in these cemeteries as possible urban biomonitor candidates. This led us to consider *Grimmia pulvinata* (Hedwig) Smith as study species; (iii) chose to analyze the metal accumulation in mosses with a TXRF slurry sampling method (slurry-TXRF), a cost-effective method in terms of consumable use and preparation time. We analyzed the concentration of the following 20 elements—Al, As, Br, Ca, Ce, Cl, Cr, Cu, Fe, K, Mn, Ni, V, P, Pb, Rb, S, Sr, Ti, and Zn—and calculated background enrichment factors to better separate the elements according to their anthropic or natural origins. While not exhaustive of all metal pollutants that can be found in urban areas, this list of elements was sufficient to identify variations of a potential air pollution across the Paris area. As the application of the slurry-TXRF method on mosses was done here for the first time, we also compared it for validation with the digestion-TXRF method that has been previously used successfully for moss analysis.

Materials and methods

Studied sites

The chosen sampling sites were urban cemeteries located within Paris and out of the city in the surrounding suburban zone. On the whole, the study was carried out on 17 Paris and 4 suburban cemeteries (20–80 km from the center of Paris). All central Paris cemeteries were sampled except a small cemetery in Montmartre, privately owned and accessible only 1 day/year. Central Paris cemeteries (Fig. 1—right panel) are distributed over the entire city area contained within the ring road and with a large variety of characteristics such as size, vegetation cover, and proximity to the ring road. As an intermediate suburban zone, we selected the cemeteries in the City of Versailles (Fig. 1—right panel), 20 km west from Paris. Finally, a cemetery considered as less polluted and having most natural background control reference was chosen in Nemours (Fig. 1—left panel), a site located at 80 km southeast of Paris, distant from the main traffic roads and partially protected by the large forest of Fontainebleau, a Biosphere Reserve since 1998. With the dominant winds direction being southwest (Fig. 1), all of the selected suburban cemeteries are only slightly influenced by air pollution from Paris. Nevertheless, even at 20 km from Paris and not under the direct influence of the pollution produced in this metropolis, Versailles remains a significant touristic and financial center and an intermediate level of pollution was expected in this area.

Moss sampling and preparation

All moss samples were collected during six consecutive days in December 2013. While mosses of different species were present in each cemetery (two to nine species), *G. pulvinata* (Hedwig) Smith was the only species abundantly present in all cemeteries. Pearson et al. (2000) showed that, in London, this species was indeed suitable for identifying different levels of exposures to metal depositions, and so, we decided to focus the sampling effort on this species for the purpose of this study. All the collected samples were exclusively epilithic mosses, that is bryophytic colonies developed on rocky substrates. In order to avoid risks of elemental transfers by water runoff, attention was paid that no overhanging tree canopy or surface was present above sampled moss cushions. As such, whenever
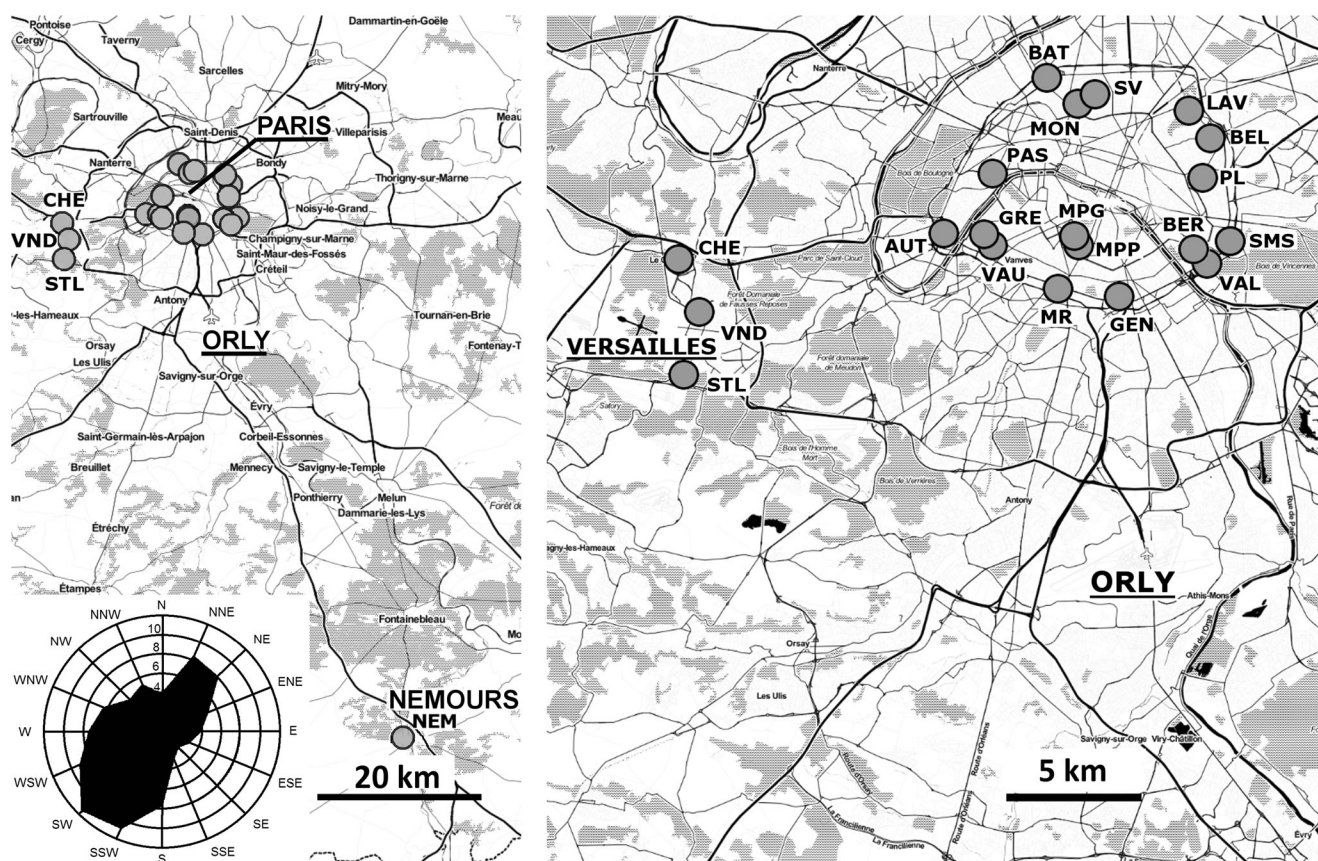
Fig. 1 Location of the sampled cemeteries. Cemeteries in the historical center of Paris (France) (city area surrounded by the Paris ring road with, in clockwise order from top (*right*) (*BAT* Batignolles, *MON* Montmartre, *SV* St. Vincent, *LAV* La Villette, *BEL* Belleville, *PL* Père-Lachaise, *SMS* St. Mandé, *BER* Bercy, *VAL* Valmy, *GEN* Gentilly, *MPP* Petit Montparnasse, *MPG* Grand Montparnasse, *MR* Montrouge, *VAU* Vaugirard, *GRE* Grenelle, *AUT* Auteuil, *PAS* Passy) and near Versailles 20 km West from Paris (*CHE* Chesnay, *VND* Versailles Notre Dame, *STL* Saint-Louis). Location of the less polluted reference site, 80 km South-East of Paris (*left*) (*NEM* Nemours). Wind rose at Orly International Airport (15 km South of Paris) recorded between 7 a.m. and 7 p.m. from 2004 to 2014 (Meteo-France ORY/LFPO station 07,149)

possible, the sampled tombs were far (>50 m) from nearest buildings and graveyard walls. Gravestones under or near trees, or in the shade of enclosure walls were also excluded. Finally, mosses directly under headstones, or cushions that could be under the influence of metallic objects (flower pots, religious objects, photograph frames, or iron letters) were also rejected.

Five tombs were chosen in each cemetery in order to homogenously cover the largest part of the cemetery area. After the GPS coordinates of each tomb were registered, entire moss cushions (1 per tomb), with a diameter between 3 and 5 cm, were collected and immediately placed into sealed PE plastic bags. In six cemeteries, on one among the selected tombs, the diameter of moss cushions was smaller than 3 cm and we collected two moss cushions instead of one. In Batignolles cemetery (BAT), only four moss cushions showed the necessary properties. For all 21 cemeteries, the final sampling panel numbered 110 moss cushions.

The treatment of the moss samples before the process of measurement is a delicate step (Türkan et al. 1995; Markert et al. 1999; Fernández et al. 2007; Aboal et al. 2010, 2011). Mosses are not washed when information on the dust deposited on their leaves is investigated (or it is necessary to collect and analyze the washing water). However, when only metals inside moss tissues are of interest (as in the present study), mosses are washed using different liquids and techniques. Some authors wash the samples with tap, or deionized or double distilled or Millipore water (Thöni and Hertz 1992; Stryjewska et al. 1994; Türkan et al. 1995; Tsikritzis et al. 2002; Migaszewski et al. 2002). In these investigations, the washing time duration ranges from a few seconds to 10 min. The use of water displaces ions from moss tissues to the rinse water and vice versa and a washing time of more than 30 s likely causes the loss of biomaterial and minerals (Sentenac and Grignon 1981; Wells and Brown 1990).

Taking this into account, the green part of each cushion corresponding to 3–5 mm of leaved stems was cut and gently washed for less than 30 s with ultrapure water (Millipore, USA). The washed material was then collected in Petri dishes and air dried during 5 days. The dried material was then ground (Retsch MM200 grinder, 24 Hz, 2min30) and the powder produced was transferred into 2.5-ml sterile Eppendorf vials.

Determination and validation of element concentration by slurry-TXRF

Moss slurry samples were prepared in 2.5-ml sterile Eppendorf vials, first by adding 4 to 8 mg of dry moss powder, then adding 1 ml of ultrapure (Millipore) water, and finally adding 20 µl of Ga standard solution prepared at a concentration of 100 ppm. Slurries were homogenized using an electromagnetic vibrating vial shaker. One hundred microliters of slurry was pipetted from the well-homogenized slurry onto the TXRF quartz sample carrier, using sterilized metal-free Gilson 1000-µl pipette tips, immediately after shaking to avoid possible sedimentation.

To determine element concentrations, two TXRF analyses of moss slurries were carried out using a TX 2000 X-Ray Spectrometer manufactured by Ital-structures, equipped with a molybdenum/tungsten anode X-Ray tube, and operating with Mo Kα as excitation radiation. Quartz sample carriers were cleaned by soaking for 24 h in 10 % analytic grade nitric acid followed by soaking for several hours in ultrapure water (Millipore) and then rinsed with ultrapure water and finally dried in a stream of clean nitrogen. Carriers were siliconized using in-house prepared solution of dimethyl-dimethoxy-silane (DMDCS) 1 vol% in ethanol. Sample carrier blanks were analyzed for contamination prior to sample deposition. Both carrier blanks and samples were excited for 3000 s. Metal concentrations were determined by internal standardization, using a Ga standard solution. Si and other light elements such as Na or Mg were not determined since (i) the samples were placed on a quartz holder that contains Si and (ii) the energy of the fluorescence radiation for Na and Mg was too low to be detected by the instrument.

To validate the slurry-TXRF method, we compared element concentrations determined by the slurry method to those obtained by sample digestion. Digestion was done in Teflon containers (Savillex 60 ml), hermetically sealed after thoroughly cleaning according to Holy et al. (2009). Few milligrams of moss powder was weighed accurately and added into the containers. Three milliliters of ULTREX II ultrapure nitric acid (63 %) was added as well as 1 ml of ultrapure water (Millipore). Finally, about 20 µl of a Ga standard solution at 100 ppm was pipetted into the same container and weighed using an analytical balance. Hermetically closed containers were ramped up to 135 °C in an oven during 4 h and then kept at 135 °C for 16 h for digestion. After cooling down, the containers were carefully opened and some tens of microliters of the digested samples were pipetted onto TXRF siliconized quartz carriers. Three subsamples from a given moss powder were digested simultaneously in three distinct digestion vessels. The amount of moss powder digested in each container was still in the 4–8-mg range, close to the mass used for the slurry method. A fourth container, containing only the nitric acid-water mix and the Ga standard, was used as a digestion blank, to check for eventual contamination. To evaluate the accuracy of the determined metal concentrations, we analyzed National Institute of Standards and Technology (NIST, USA) pine needle standard samples (i.e., SRM 1575a) by TXRF, considering both methods (slurry and digestion).

Assessment of pollution levels by calculation of enrichment factors (EFs)

To assess which elements principally contributed to atmospheric pollution in the Paris area, a first approach determined the relative enrichment factors (EFs) with respect to the cemetery of Nemours (NEM) considered as the background, less polluted site, out of the city and protected by the large forest of Fontainebleau. Taking Al as a commonly used normalization element (Gombert et al. 2004) and comparing the element ratios in a site of interest to the same element ratios in NEM, the background EFs were defined using the following equation:

$$BEF_{element} = [c(element)sample \ / \ c(Al)sample] \ / \ [c(element)background \ / \ c(Al)background].$$

As soil dust also plays an important role in moss accumulation, a common way to evaluate the soil-dust contribution is to compare element ratios in a site of interest, typically computed with respect to Al (Gombert et al. 2004), with the corresponding ratios in the upper continental crust. The average composition of the upper continental crust (Wedepohl 1995; Taylor and McLennan 1995) is often taken as a reference for the composition of soil dust and the corresponding crustal enrichment factors (CEFs) calculated as follows:

$$CEF_{element} = [c(element)sample \ / \ c(Al)sample \ / \ c(element)crust \ / \ c(Al)crust].$$

The use of limited sites to calculate background concentrations of each element for EF calculation may be insufficient for obtaining a representative general reference on the subcontinental-scale (Reimann and Garret 2005). It is nevertheless appropriate for standardizing a set of data collected in a confined region when the aim is to identify eventual variations of values within this area. Moreover, a recent study in Canada (Cowden et al. 2015) suggests that while heavy metal concentrations may be variable between species of mosses, similar trends in the same area can be observed.

Statistical analyses

All statistical analyses were performed by using R software (R Development Core Team 2014). The scope of the present survey was to use mosses as biomonitors of air quality and to associate a pollution score to each site rather than mapping single element concentrations—as concentrations of trace metal in biomonitors tissues may change with the measurement methodology. This may be done by computing a "multimetal contamination index" as a weighted average of element

concentrations as reported by Thévenot et al. (2007) and Holy et al. (2009). However, when different elements are correlated (soil dust elements are usually correlated), a set of "multi-element" scores can be computed by performing principal component analysis (PCA), attributing to each moss cushion the PCA component scores and averaging PCA scores for each site. These average scores then represent the contribution of a given pollution source to the sites corresponding to an element fingerprint stated by the PCA component loadings. The element fingerprint or component loadings represent the typical element ratios in the pollution source associated with the component. Furthermore, the standard deviation of the PCA scores at a given site represents the homogeneity of the site with respect to the given pollution source.

Since the large dynamic range of element concentrations (from less than 1 to more than 10,000 ppm) did not allow performing a PCA analysis on a rough element concentration-sample data matrix, we reduced and centered the data with respect to arithmetic standard deviation and arithmetic mean of the log concentrations before applying PCA. As suggested in Brumelis et al. (2000), we performed a Varimax rotation (Varimax function) of the principal components of the analysis in order to maximize the sum of the variances of the squared loadings.

To verify the log-normal distribution of the measured element concentrations, a set of normality tests were performed. After log-transforming the element concentration data, Shapiro-Wilk test of normality gave p values >0.05 for all elements except As, Ca, Ce, Cl, P, S, Sr, and V. A Kolmogorov-Smirnov test (comparing the set of log-transformed element concentrations to a set of normally distributed random numbers having the same mean and standard deviation) confirmed the log normal distribution for all elements except P, Ce, Cl, and As. Finally, a Wilcoxon test (two series of data compared two by two, chosen in the same way as for the Kolmogorov-Smirnov test) gave all p values above 0.05.

To clarify the site effect on pollutant distribution, cemeteries were classified into three groups: lowly, moderately, and highly polluted cemeteries based on both the ranking of their average PCA component score and estimated environmental condition (green space, number of trees, distance from roads, and exposition to main winds), nonparametric rank tests were performed on the element concentrations to prove the effectiveness of this classification, Wilcoxon test for groups of cemeteries taken two by two and Kruskal–Wallis for all groups together.

Results

Slurry-TXRF method validation

In Table 1, element concentration values and corresponding measurement errors (standard deviation of values) are compared for slurry-TXRF and digestion-TXRF methods for a pine needle standard reference material (SRM 1575a) and for a selected moss sample. In the case of pine needle samples, the results are also compared with certified values reported in Markert et al. (1994), which summarized the statements of 12 different authors. Results were obtained by analyzing three subsamples of the same slurry sample or three powder sub-samples digested in separate vessels.

Pairwise agreements were found between slurry-TXRF, TXRF-digested, and certified values for the pine needle standard reference material, within error of measurements. In moss samples, agreement within errors was obtained for all elements except Cl, Pb, and Ti. The relatively high errors are due to the intrinsic inhomogeneity of moss samples at the small quantities used for the analysis. Since sufficient agreement was obtained between digestion-TXRF and slurry-TXRF concentration values, suggesting that sedimentation errors were avoided by our procedure to pipette slurries immediately after homogenization (as also suggested in De La Calle et al. 2013), we decided not to use surfactants. Results are also expressed in Fig. 2, where the spectra of digestion-TXRF and slurry-TXRF processes are represented, directly showing how element relative peak heights match closely in the two spectra.

Trace element concentrations and enrichment factors

When grouping together the 110 samples, the regional trace element concentrations in urban and suburban mosses decreased according to the following order Ca>Fe>K>S>Al>Cr>P>Cl>Ti>Zn>Cu>Mn>Pb>Sr>Ce>Ni > Rb > V > Br > As (Fig. 3a). All these elements are known to be associated to different natural or anthropogenic sources, which can sometimes interfere at regional scale.

To separate anthropogenic from natural soil dust contributions, we compared the enrichment factors (EFs) of urban and suburban moss samples computed with respect to Al taking the average composition of the upper continental crust as reference. Local variations of soil compositions can obviously occur, but these variations did not play a significant role in previous analysis involving French soils and mosses (Hernandez et al. 2003; Gombert et al. 2004; Reimann and Garret 2005; Reimann and de Caritat 2005; Reimann et al. 2005; Galuszka and Migaszewski 2011). When grouping together the 110 samples, the regional trace element crustal enrichment factors (CEFs) in mosses decreased according to the following order Cr > Br > S > Cu > Pb > As > Zn>Ni>Cl>P>Ca>Ce>V>Fe>K>Rb>Mn>Sr>Ti>Al=1 (Fig. 3b). The nearer the CEFs values are to 1 (value assigned to Al), the more the corresponding elements are likely to have soil dust as a source. Three groups stand out from these ordered CEF values: low (<10: Ti, Sr, Mn, Rb, K, Fe, and V),

Table 1 Comparisons of concentrations and measurement errors (standard deviation) for the slurry-TXRF and digestion-TXRF methods

	NIST 1575 pine-needle SRM				Moss sample		
	Certified [Maerkert 1994] (ppm)	Slurry-TXRF (ppm)	Digestion-TXRF (ppm)			Slurry-TXRF (ppm)	Digestion-TXRF (ppm)
As	0.21 ± 0.02	0.94 ± 0.67	0.12 ± 0.21		Al	2015 ± 179	2497 ± 1230
Ba	7.2 ± 0.8	8.5 ± 4	12 ± 3		As	2.2 ± 1.5	3 ± 1.6
Ca	4200 ± 360	4383 ± 551	4716 ± 521		Br	4 ± 0.2	3 ± 1
Cr	2.6 ± 0.2	1.4 ± 0.9	2.1 ± 1.5		Ca	25,116 ± 3245	25,924 ± 8455
Cu	3 ± 0.4	2.7 ± 0.7	6.1 ± 3.1		Ce	31 ± 8	14 ± 23
Fe	185 ± 26	151 ± 68	194 ± 35		Cl	180 ± 20	23 ± 39
K	3670 ± 310	3135 ± 567	3740 ± 367		Cr	510 ± 21	644 ± 211
Mn	650 ± 70	568 ± 68	645 ± 57		Cu	77 ± 6	91 ± 29
Ni	2.5 ± 0.3	2.7 ± 0.7	3.7 ± 0.8		Fe	6929 ± 177	7314 ± 1153
Pb	10.7 ± 0.5	7.3 ± 2.3	10.4 ± 2.7		K	6103 ± 515	5833 ± 1659
Rb	11.7 ± 1	10.6 ± 1.8	11.7 ± 1.1		Mn	102 ± 12	102 ± 24
S	1320 ± 110	938 ± 447	1723 ± 338		Ni	13 ± 0.5	18 ± 5
Sr	5 ± 0.4	4.6 ± 2.8	4.95 ± 0.5		P	775 ± 32	822 ± 125
V	0.39 ± 0.07	1.3 ± 2.1	0.63 ± 0.73		Pb	24 ± 2.3	28 ± 0.6
Zn	67 ± 9	54 ± 14	62.8 ± 4.9		Rb	18 ± 1.8	17 ± 0.4
					S	2191 ± 300	2659 ± 730
					Sr	48 ± 6	44 ± 11
					Ti	301 ± 23	205 ± 18
					V	9 ± 2.6	14 ± 8
					Zn	179 ± 8	205 ± 6

medium (20–30: Ce, Ca, P, Cl, and Ni), and high (>100: Cr, Br, S, Cu, Pb, As, and Zn) CEFs.

Finally, in order to identify the main contributors to atmospheric pollution in the Paris Region, we compared the EFs of urban and suburban moss samples computed with respect to Al taking the average composition of our background site (Nemours). When grouping together the 110 samples, the regional trace element background enrichment factors (BEFs) in mosses decreased according to the following order Cu > Ni > Fe > Ti > Cr > V > Mn > Ce > Pb > Al = 1 > As > Br > Sr > K > S > Ca > Zn > Cl > P > Rb (Fig. 3c). There are therefore two groups constituted of trace element concentrations in mosses that are lower or higher with respect to the background site. We observed particularly higher values for Cu.

Correlations between trace elements at a regional scale

Correlations between trace elements can help to discern whether they share common natural or anthropogenic sources as well as providing a potential identification of the origin of the

Fig. 2 NIST SRM 1575 pine needle sample spectra from digestion-TXRF and slurry-TXRF processes
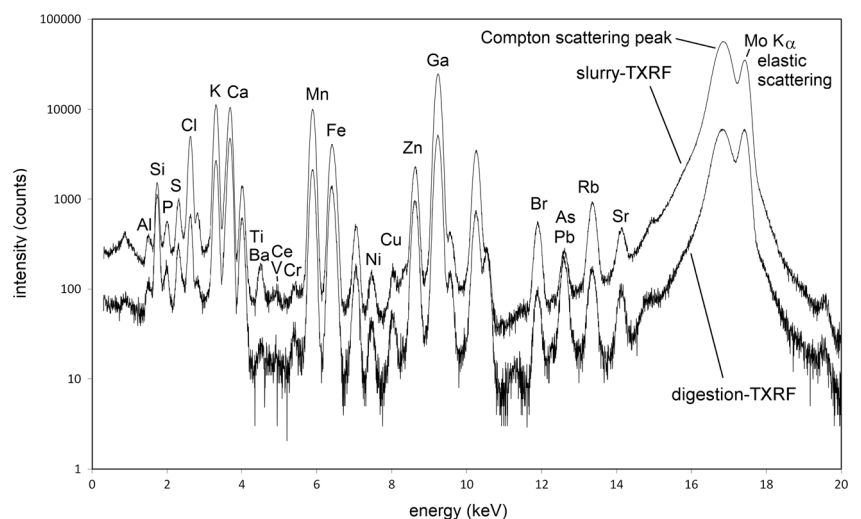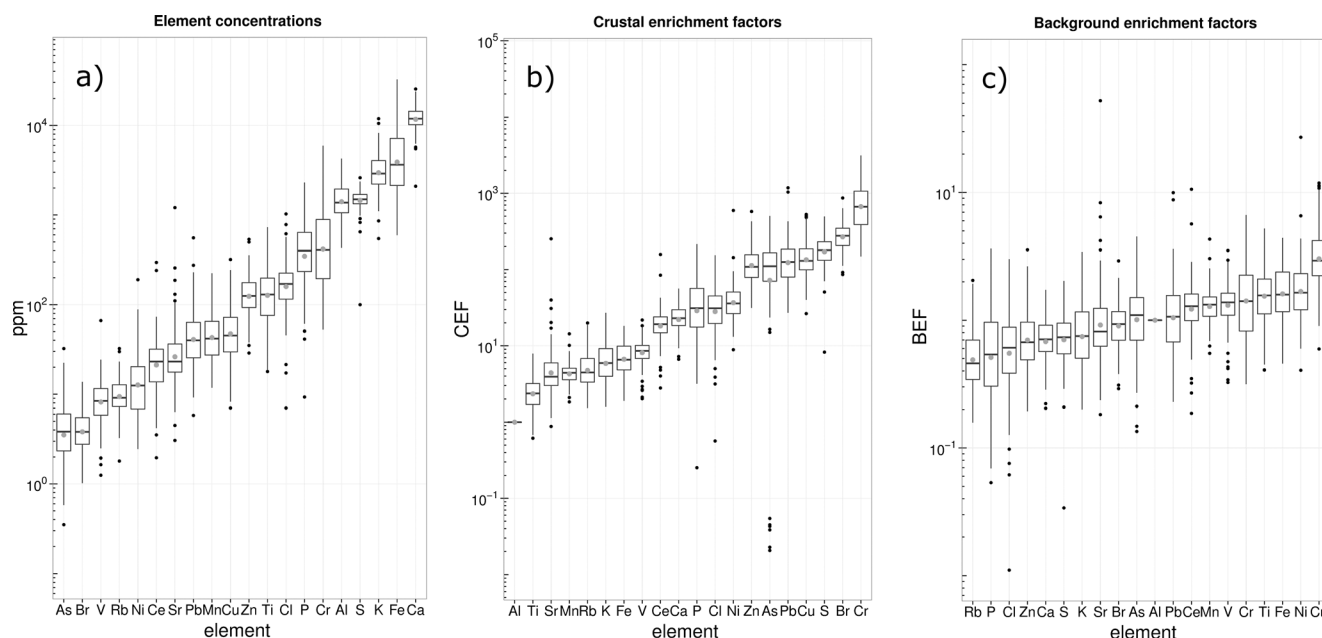
Fig. 3 *Box and whisker plots* of **a)** ordered elements concentration (ppm) in all moss samples, **b)** ordered crustal enrichment factors in all moss samples computed with respect to Al (*left end*) taking the average composition of the upper continental crust as reference, and **c)** ordered background enrichment factors in all moss samples taking Al (at *center*) as normalization element and comparing the element ratios to those of the background site NEM. The range and 25th percentile/median/75th percentile box are shown for all plots. Extreme values are given and plotted as *full diamonds*

contamination. Varimax rotation applied on the PCA of all element concentrations showed that the first four rotated components (RC) explained 68 % of total variance (Table 2). In order of decreasing explained variance, the first component RC1 had high loadings on Fe > Cr > Ni > Mn > Al > Cu = Ti > V and explained nearly one third (28 %) of the variance. In particular, Cr, Ni, and Mn showed very high correlations (0.94, 0.90, and 0.82, respectively), while Pb was weakly correlated with the dominant elements of the component RC1. The third component RC3 had high loadings on Br > Zn > Ti > Ca > Pb > Cu > V and explained nearly one fifth (18 %) of the variance. The second component RC2 had high loadings on K > Rb > Sr and explained 13 % of the variance. Finally, the fourth component RC4 had high loadings on Cl > S and explained 9 % of the variance.

Site effect on pollution loads and site clustering according to their structural and spatial characteristics

In order to clarify the weight of the site effect on pollutant distribution, cemeteries were ranged regarding to the increasing mean standardized values of element concentrations in mosses and arranged by PCA on the two most important components (RC1 and RC3, cumulative variance =47 %), i.e., a gradient of cemetery sites ordered from less to highly polluted (Fig. 4). A large dispersion of values has been registered in the cemetery of Grenelle. This cemetery was the only one with a very large unpaved earthen recreation area that may have contributed a large amount of dust on tombs.

Differences were observed between the cemetery gradients of RC1 and RC3 due to the correlations between elements and components. However, a general trend could be observed when clustering sites. Since the group of less polluted cemeteries (which lie towards the bottom of the graphics) appeared to correspond to large and wooded cemeteries in the city center or to cemeteries surrounded by suburban gardens, farming, or less built-up environments, we decided to test the existence of three distinct groups of cemeteries following their geographical and environmental distribution.

The factors considered in grouping the cemeteries were total area (ha), amount of green space (i.e., lawns, hedges and shrub beds (ha)), number of planted trees, distance from the principal roads (the heavily frequented ring road of Paris and its main departing exit roads), and exposition to the prevailing winds from southwest, which are likely to sweep and deposit particles of traffic pollution on the cemeteries that lie immediately downwind of the ring road (Fig. 1). Even in cemeteries in the center of Paris, a high variability was observed since, e.g., total area ranged from 0.6 to 43.2 ha, or percentage of green space ranged from 0 to 15 %. Groups were:

1. Group 1, with expected relatively low polluted (LP) cemeteries: Montparnasse Grand (MPG), Montparnasse Petit (MPP), Nemours (NEM), Montmartre (MON), Père Lachaise (PL), and Chesnay (CHE)
2. Group 2, with expected moderately polluted (MP) cemeteries, i.e., cemeteries in the center of Paris or in the northern part of the city, and a large cemetery

Table 2 Correlation coefficients of element concentrations with the first four components of the Varimax PCA rotation, listed in sequence of decreasing explained variance

Elements	RC1	RC3	RC2	RC4
SS-loadings	*5.64*	*3.69*	*2.59*	*1.72*
Proportion-Var	*0.28*	*0.18*	*0.13*	*0.09*
Cumulative-Var	*0.28*	*0.47*	*0.6*	*0.68*
Al	**0.78**	0.44	0.27	−0.06
As	0.14	0.26	0.12	−0.25
Br	0.36	**0.77**	0.02	−0.12
Ca	0.04	**0.64**	0.44	0.05
Ce	0.4	0.28	0.32	−0.01
Cl	0.03	−0.13	−0.01	**0.85**
Cr	**0.94**	0.01	0.02	−0.07
Cu	**0.63**	**0.55**	0.04	0.13
Fe	**0.96**	0.2	0.03	−0.06
K	−0.03	−0.04	**0.89**	0.11
Mn	**0.82**	0.41	0.27	0.03
Ni	**0.9**	0.19	−0.01	−0.02
P	−0.1	−0.36	0.38	−0.01
Pb	0.22	**0.6**	−0.12	−0.49
Rb	0.2	0.11	**0.8**	0.13
S	0.02	0.22	0.29	**0.78**
Sr	0.26	0.15	**0.62**	−0.02
Ti	**0.63**	**0.65**	0.15	−0.02
V	**0.62**	**0.5**	0.23	−0.08
Zn	0.32	**0.75**	−0.04	0.06

The *first three lines* provide the total of sum of squared loadings and the proportional and cumulative variances, respectively. Correlation values ≥0.5 are highlighted in bold

in Versailles that is in the middle of a residential part of the city: Batignolles (BAT), Belleville (BEL), La Villette (LAV), Saint-Vincent (SV), and Notre Dame de Versailles (VND)

3. Group 3, with expected highly polluted (HP) cemeteries, i.e., cemeteries bordering the ring road to the north, under the influence of the southwest prevailing winds, and a cemetery limiting Versailles toward the southwest and nearing main roads and railways: Auteuil (AUT), Bercy (BER), Gentilly (GEN), Grenelle (GRE), Montrouge (MR), Passy (PAS), Saint-Mandé (SMS), Valmy (VAL), Vaugirard (VAU), and Saint-Louis (STL)

Wilcoxon and Kruskal–Wallis tests on the element concentrations were used to identify the differences between these three groups (columns 1-2-3 and 4, respectively, in Table 3). They showed that the HP group was significantly more polluted than the LP group for all elements except P, Cl, and K, and was significantly more polluted than the intermediate MP group for Cu, Ce, Mn, V, and Rb. The MP group was more polluted than the LP group for 13 elements out of 20 except P,

K, Cl, S, As, Rb, and Ca. When grouped all together, LP, MP, and HP were globally significantly different for almost the same 13 elements out of 20 than the MP vs. LP comparison, except for Rb instead of Pb.

Discussion

Is the slurry-TXRF method suited for mosses?

The agreement between slurry-TXRF and digestion-TXRF methods for element concentrations against the NIST 1575 pine needle standard reference material is comparable to that found by Markert et al. (1994) for TXRF-digested moss samples (Table 1). This suggests that instrumental setup and sample preparation methods worked correctly and were not affected by major contamination (spectra in Fig. 2). It also shows that the slurry-TXRF method gives sufficiently accurate values. It is worth noting that for the selected moss sample in Table 1, the error is lower for slurry-TXRF than digestion-TXRF, except for Pb, Rb, Ti, and Zn. This lower error for slurry-TXRF is probably because the results are obtained in three subsamples of the same slurry, while in the case of digestion-TXRF, we worked with three separate moss powder subsamples digested in three different vials. Since the mass of powder (ca. 4 mg) used to prepare a slurry sample was the same as that used for each of the three single digestion vessels, the powder digested separately in three distinct vessels revealed its inhomogeneity.

The relative smallness of the dispersion errors of slurry-TXRF method when pipetting from the same slurry indicates that very reproducible results can be obtained when slurries are sufficiently homogeneous. In agreement with Meyer et al. (2012), we also observed that using surfactants (such as Triton X-100 to obtain slightly more homogeneous dried sample spots on TXRF holders) significantly increased the background noise in TXRF spectra, making the quantification of low concentration elements more difficult. Surfactants were therefore not added to slurry samples. We also concluded that sedimentation errors were avoided by our procedure to pipette slurries immediately after homogenization as already proposed for instance by De La Calle et al. (2013).

However, for the selected moss sample, we observed some discrepancies among methods for a few elements: (i) Pb—the partial overlap of Pb and As peaks in TXRF spectra is a well-known interference. The underestimation of Pb might be accompanied by an overestimation of As and vice versa, as observed for the pine needle sample. The above-reported moss powder inhomogeneity might greatly contribute to such differences; therefore, a finer grinding of moss samples would probably improve the results; (ii) Ti—titanium and barium form another spectral interference in TXRF so that their exact relative values can be difficult to determine. We analyzed
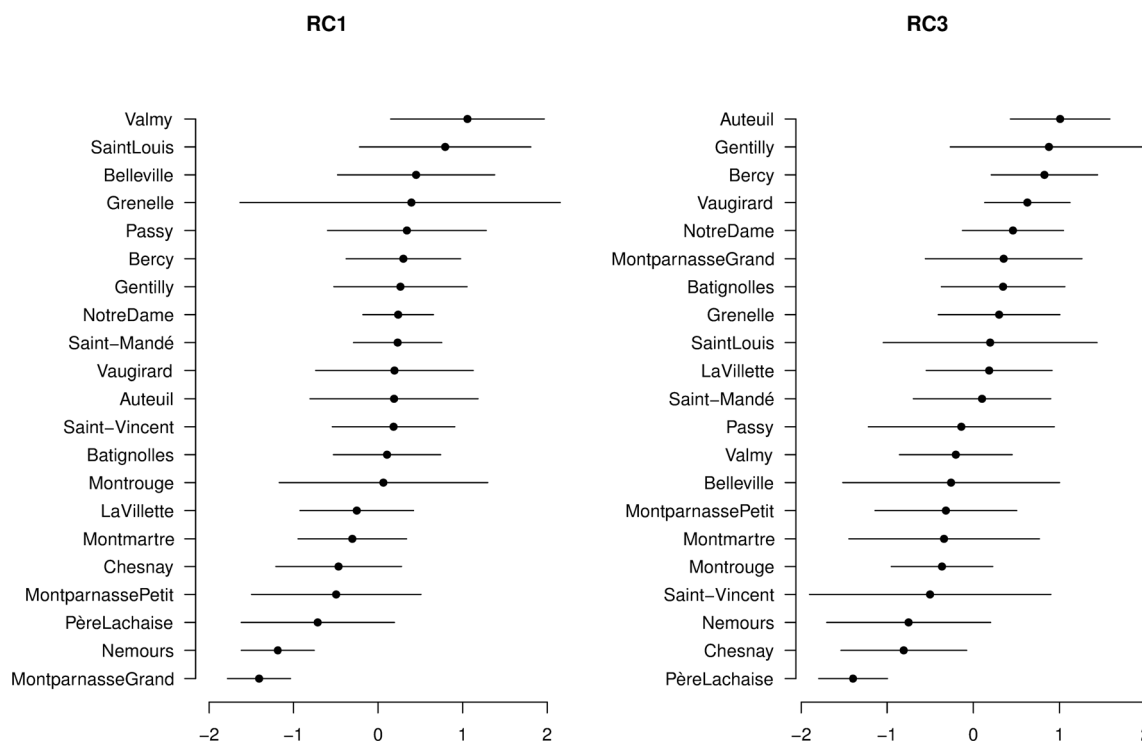
Fig. 4 Site effect on pollution loads. Scores (reduced and centered data, with respect to arithmetic standard deviation and arithmetic mean of the log concentrations of the elements in mosses) on the two most important Varimax rotated PCA components RC1 and RC3

Table 3 Wilcoxon (columns 1-2-3) and Kruskal-Wallis (column 4) tests on the concentrations of elements in moss from Parisian cemeteries grouped in highly polluted (HP), moderately polluted (MP), and lowly polluted (LP) clusters

Elements	HP > MP	HP > LP	MP > LP	HP, MP, LP different
Al	0.126	**0.000**	**0.000**	**0.000**
As	0.233	**0.012**	0.072	0.068
Br	0.506	**0.000**	**0.000**	**0.000**
Ca	0.305	**0.005**	0.052	0.036
Ce	**0.037**	**0.000**	**0.010**	**0.000**
Cl	0.398	0.469	0.594	0.960
Cr	0.598	**0.000**	**0.000**	**0.000**
Cu	**0.000**	**0.000**	**0.000**	**0.000**
Fe	0.219	**0.000**	**0.000**	**0.000**
K	0.386	0.319	0.591	0.891
Mn	**0.041**	**0.000**	**0.000**	**0.000**
Ni	0.086	**0.000**	**0.000**	**0.000**
P	0.181	0.968	0.986	0.057
Pb	0.492	**0.020**	**0.037**	0.085
Rb	**0.023**	**0.000**	0.071	**0.001**
S	0.175	**0.032**	0.283	0.174
Sr	0.156	**0.000**	**0.006**	**0.000**
Ti	0.052	**0.000**	**0.000**	**0.000**
V	**0.030**	**0.000**	**0.000**	**0.000**
Zn	0.077	**0.000**	**0.002**	**0.000**

Four hypotheses were tested (headline of the table). *p* values <0.05 are highlighted in bold

barium in selected moss samples and found that it was always present in very low concentrations compared to Ti. As a consequence of this spectral interference with Ti, we considered the Ba concentrations unreliable, and they were not presented in this paper; (iii) Cl and Br—lower concentrations of Cl and Br (particularly Cl) were observed in the digested samples. This is likely to be due to the evaporation of volatile Cl and Br compounds after the opening of digestion vessels (Naozuka et al. 2003). This underlines one of the advantages of the slurry-TXRF method, i.e., its capacity to reduce the risk of sample loss, which always occurs in digestion procedures when volatile species are generated during the process.

Can *G. pulvinata* (Hedwig) smith be used as biomonitor for detecting air pollution?

Despite its ubiquitous distribution, its ability to grow in hemispherical dense cushions as an adaptation to dry periods, and its tolerance to pollution, *G. pulvinata* has not yet been widely used in moss studies. Indeed, in over a hundred articles (published between 1972 and 2014) on the use of mosses for monitoring the atmospheric deposition of pollutants, only four include data of this species: Sawidis et al. (1999), Pearson et al. (2000), and Uğur et al. (2003, 2004). None of them are specifically dedicated to *G. pulvinata*. *G. pulvinata* was the only species present in all the Paris cemeteries and in a large number of colonies. Relatively easy to recognize, with colonies shaped in cushions that are convenient to sample, this

species was also found to be practical to manipulate during every phase of the research and as well as being a good air pollution biomonitor, at least for the elements measured in this study (Al, As, Br, Ca, Ce, Cl, Cr, Cu, Fe, K, Mn, Ni, P, Pb, Rb, S, Sr, Ti, and Zn).

Is *G. pulvinata* a good biomonitor of regional and local sources of anthropogenic pollution?

The assessment of trace element contamination at regional and subregional levels needs to clearly distinguish between natural or anthropogenic sources. This is challenging for several reasons: Elevated concentrations or deposition are not always the result of human activities; indeed, human activities can increase not only trace elements of anthropogenic but also those of natural origin, as soil dust generated by agricultural practices. First, plants concentrate essential elements for their growth and metabolism, such as the macro-nutrient elements Ca, K, P, and S (i.e., elements with concentrations >0.1 % in plants) and the micro-nutrient elements Fe, Cu, Mn, Zn, Ni, and Cl. Second, natural sources include elements of marine origin such as Cl and S as well as soil dust elements. In the upper earth crust, these latter have concentrations that decrease as follows: Si > Al > Fe > Ca > K > Ti (Wedepohl 1995; Taylor and McLennan 1995). Soil dust elements are likely to be important contributors to the metal content of mosses, especially if unwashed, as they constitute up to 5 to 20 % of the mass of atmospheric particulate matter <10 μm (PM$_{10}$—van der Gon et al. 2010), depending on regional conditions. Third and last, anthropogenic pollutants include Pb, V, Zn, Cr, As, Ni, Ti, Cu, Mn, S, or Br. Among these, Cu, Pb, and Cr are known as traffic markers (Stechmann and Dannecker 1990; Sternbeck et al. 2002), Cu and Pb from brake emissions, Pb and Zn from tyre wear emissions (Napier et al. 2008; Thorpe and Harrison 2008), and others like Ni and V are from heating emission related to fuel combustion. Ti, V, Cr, Mn, Ni, Cu, Zn, Br, Zr, and Pb were identified as anthropogenic sources in road dust (Sampson Atiemo et al. 2011); natural crustal, vehicular emissions, and corroded vehicular parts were identified as sources of these elements. The metal concentrations of Cd, Cr, Cu, Ni, Pb, and Zn are greatly toxic and highly abundant in road traffic environments (Kumar Pal 2012). S is likely to arise from fuel and coal combustion plants (European Commission 2006). Lastly, volatile Br can be produced by incineration plants when they burn plastics containing Br as flame retardant (European Chemicals Bureau 2006).

On the regional level (Fig. 3b), the group of relatively low CEFs (<10; Ti, Sr, Mn, Rb, K, Fe, and V) are likely to have local soils as probable source. On the other hand, the group of very high CEFs (>100; Cr, Br, S, Cu, Pb, As, Zn, and Ni) contains elements mainly generated by anthropogenic sources, as traffic or organic matter combustion, including waste incineration (e.g., Br from flame retardants in plastics but also pesticides) or metal smelter industries. Finally, the elements within the group of medium CEFs (20–30) contain plant macro- and micro-nutrients such as not only Ca, P, Ni, and Cl but also cerium. The concentration of cerium (major component of mischmetal alloy, also present in catalysts, pigments, flat-screen TVs, low-energy light bulbs, and floodlights) is probably due to industrial activities.

When comparing the overall regional level to our background site at Nemours (Fig. 3c), the elevated trace elements, besides Ce, match the series of anthropogenic pollutants (Pb, V, Zn, Cr, As, Ni, Cu, Mn, and S) with high values of the traffic marker Cu. Not surprisingly in a Paris Region influenced by a subatlantic climate (Crippa et al. 2013), among the elements showing a lower background enrichment factor in Fig. 3c, we find K, S, Ca, Zn, Cl and P, which could be derived not only from plants and soils but also from marine aerosols. It is to be noted that Br (flame retardant, gasoline additive, pesticide, medical, and veterinary use) and Rb (working fluid or getter (remover of trace gases) in vapor turbines, vacuum tubes, photocell, and glass industries) were relatively high in the Nemours samples. The elevated values of these elements are probably due to the proximity of a quartz and glass factory for the transport, electrical, and solar industries (Saint Gobain Quartz) and to the presence of intensive crop fields. CEFs are globally higher than BEFs calculated with respect to Nemours (Fig. 3), indicating that even in Nemours, the moss composition is not determined only by soil dust elements.

Finally, the correlations between trace elements using Varimax rotation on the PCA showed that the major component RC1 grouped high loadings on well-known soil dust elements as Fe, Ti, and Al, and classic anthropogenic pollutants (Stechmann and Dannecker 1990; Sternbeck et al. 2002) with particularly high correlations for Cr, Ni, and Mn (0.94, 0.9, 0.82, respectively). This suggests that airborne soil dust acts as accumulator and transport medium also for anthropogenic pollutants. Soil dust represents a fraction of 5 to 20 % of the mass of fine suspended particulates less than 10 μm in diameter (PM10). Despite this apparent importance (van der Gon et al. 2010), PM10 sources are still poorly understood and not well represented in emission inventories. This is partly because some sources can be defined as natural ones (e.g., wind erosion), while others, such as resuspension, are not recognized as primary emission sources, but reemissions of already present particles.

In tunnel (Sternbeck et al. 2002) and more generally urban (Kulshrestha et al. 2009) dusts, the Cu-Pb-V association is known as to be typical of resuspended traffic dust. Specifically in the Ile-de-France Region, Jaecker-Voirol and Pelt (2000) showed that road traffic was a very significant source of PM10. The average Pb/Zn concentration ratio in our mapped area is 3.73 with a standard deviation of 2.66, which is relatively close to the one (2.8) previously observed in dry depositions in Paris by Garnaud et al. (1999). From

1990, unleaded petrol progressively replaced the leaded petrol and Pb was definitively banned in France in January 2000. This fact may explain the weak correlation of Pb with the dominant soil dust elements of component RC1. However, a high loading on Pb can be found in RC3 together with other anthropogenic pollutants such as Br (liberated by incineration of plastics), Zn, Ti, and Cu (e. g. alloys, batteries, anti-corrosions, fuels, paints, electrical wires). Lastly, two other sources can be highlighted by RC2 aggregating high loadings on K > Rb > Sr as natural sources (e.g., plant debris), and RC4 with only high loadings of Cl and S and suggesting a marine origin.

Can urban cemeteries be used as proxies of indicators and buffers of anthropogenic pollution?

Available pollution maps in the Paris area (Airparif 2013) suggest an accumulation of PM10 and PM2.5 along major traffic routes. In Paris, away from streets, these maps suppose that pollution is homogeneously distributed over the city. Our results show more spatial variability even in densely urbanized areas, and that this variability seems to be linked to local landscape structure (e.g., cemetery size, vegetation cover, etc.) and exposure to emissions (e.g., point source pollutions, density of nearby traffic, etc.). Indeed, the largest and most wooded cemeteries in the center of Paris are as "clean" (or less polluted) as cemeteries far from Paris and near forests or farming environments such in Nemours or Chesnay. This is typically the case for the historical and tourist cemeteries of Père Lachaise and Montmartre, which together possess 5378 planted trees, i.e., 78 % of all trees in the cemeteries within the Paris beltway. On the contrary, cemeteries that border the south part of the city beltway to their north (AUT, GRE, VAU, MR, GEN, VAL, BER, SMS) are more polluted than the others (the only elements not significant being from marine aerosols or plant debris Cl, P, K). Leaching from the beltway, but above all, winds coming from the southwest of the Paris region are likely to pick up and then depose pollutants on mosses growing in these cemeteries. Interestingly, the expected moderately polluted cemeteries in the center of Paris or in the residential suburbs that had a lower percentage of green spaces are more polluted than "woodiest" cemeteries for part of the elements. Among the elements that do not confirm this trend are the plant macronutrients (Ca, K, S, P) and some of the elements (Cl, As, Rb) associated with marine aerosol or anthropogenic sources. In fact, recent results have suggested that these nutrients should not be used for air pollution biomonitoring (Boquete et al. 2011), as their source is considered as natural and related to plant metabolism rather than anthropic sources.

Conclusions

Monitoring long-term global atmospheric pollution in a patchwork of urban and peri-urban areas that can rapidly evolve

calls for systematic, integrative, and simple measures of trace element contamination. In this context, moss sampling in urban cemeteries provides a useful complement to measurement stations because contrary to other biomonitor sampling sites, cemeteries provide environments that are usually easy to access, regularly distributed and where moss cushions may remain undisturbed during long periods. Cemeteries also have different histories, structural and spatial characteristics that are linked to their local (proximity to traffic routes) and regional (main winds direction) environment. Both of which can help in deciphering the loads and sources of pollutants. Overall, our results also suggest that using TXRF measurements with slurry sample preparation is a suitable and fast technique to determine subtle variations in elemental concentrations and enrichments factors in mosses both at the regional and on fine site scales. This approach could therefore be useful for both local and regional PM10 risk assessments as it is particularly suited to being implemented alongside real-time measurements.

For the evaluation of the ecosystem services provided by urban and peri-urban cemeteries, intra-cemeteries transects of trace element concentrations in mosses from outside to inside cemeteries of differing characteristics should be done, when possible. An estimation of the age variability in mosses (and consequently the time they accumulate trace elements) under different environmental pressures (e.g., drought) should also be targeted. We sampled mosses of about the same size which may be important if age is linked to the size, as *G. pulvinata* functioning is closely related to the cushion size (Zotz et al. 2000). The use of mosses for biomonitoring is under development (Izquieta-Rojano et al. 2016), and the results of this work suggest that *G. pulvinata* is a good candidate for the survey of urbanized areas.

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