Science of the Total Environment xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Influence of exposure sites on trace element enrichment in moss-bags and characterization of particles deposited on the biomonitor surface

M. Tretiach [a,*], E. Pittao [a], P. Crisafulli [a], P. Adamo [b]

[a] Dipartimento di Scienze della Vita, Università degli Studi di Trieste, Via L. Giorgieri, 10, I-34127 Trieste, Italy
[b] Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali (DISSPAPA), Università degli Studi di Napoli Federico II, Via Università 100, I-80055 Portici (NA), Italy

ARTICLE INFO

Article history:
Received 21 July 2010
Received in revised form 13 October 2010
Accepted 14 October 2010
Available online xxxx

Keywords:
Atmospheric deposition
Trace elements
Moss-bags
Exposure site
PM_{10}

ABSTRACT

The hypothesis that exposure environment and land use influence element accumulation and particulate size composition in transplants of *Hypnum cupressiforme* has been tested using moss-bags containing oven-devitalized material. The samples were exposed for three months in ten green sites and ten roadsides in two areas with different land use (A, residential; B, residential/industrial) in the Trieste conurbation (NE Italy). Observations by SEM and EDX-ray microanalysis revealed that particle density was smaller in samples exposed in A than in B, with prevalence of particles containing Al, Ca, Fe and Si, and in good accordance with the element contents measured by acid digestion and ICP-MS. Moss-bags were generally less contaminated in green sites than in roadsides, apparently due to the different enrichment in coarse particles. In both environments, however, the majority of entrapped particles (up to 98.2%) belongs in the inhalable, small size classes ($\leq PM_{10}$). The need for careful selection of the exposure sites during the phase of biomonitoring planning is discussed.

1. Introduction

Mosses and lichens, due to their unique morphological, physiological and ecological features, are among the most commonly used biomonitors of airborne pollutants. Their use allows us to make rapid surveys of trace element deposition, at low cost and with a high sampling density, and maps of pollutant deposition can readily be made (Bargagli et al., 2002). When autochthonous material is not available, both for natural and human reasons, the active technique of transplants can suitably be applied. This approach offers several advantages, because (i) the material can be exposed according to a rational scheme, (ii) it is possible to calculate enrichment rates since pre-exposure values are known, and (iii) the monitoring can be repeated (Bargagli, 1998). The use of transplants is becoming quite common, particularly after the adoption of the so-called "bags", small envelops of a variety of forms and materials containing the material to be exposed (Conti and Cecchetti, 2001; Onianwa, 2001). However, the current lack of standardization at international level, and the scarce knowledge on the factors influencing element uptake make this technique still open to criticism. Recently, an inter-university research program was specifically devoted to deepen our knowledge on the mechanisms of accumulation of elements in lichen- and moss-bags, and to identify the most effective pre-exposure treatments (Adamo et al., 2007, 2008; Giordano et al., 2009; Tretiach et al., 2007). The present contribution, carried out in the framework of the same project, was specifically aimed at describing (i) the influence of the exposure site choice on the post-exposure moss element content, and (ii) the nature (size and element composition) of particles adhering to the surface of the exposed moss, in this case the epilithic pleurocarpous *Hypnum cupressiforme* Hedw.

The influence of the exposure site has often been underestimated in the phase of planning biomonitoring studies. Whereas standard parameters are often applied to sample exposure (e.g. height above ground, time of exposure, etc.), less attention has been paid to the influence of the microenvironment of the exposure site, although this might represent an important, albeit undesirable source of variability (Gailey and Lloyd, 1986).

The second point is particularly important to understand what a moss-bag actually entraps. Previous research showed that a large fraction of the elements accumulated in exposed moss and lichen-bags can be removed by washing in distilled water (Giordano et al., 2009), and that dead samples are as efficient as, or even more efficient than living samples (Adamo et al., 2007), suggesting that intracellular uptake is negligible or too slow to be actually important when the exposure is not too long (one–three months, see Giordano et al., 2009). But are moss-bags totally passive traps, or does the moss actually act as a sort of filter, prevalently entrapping small particles ($\leq PM_{10}$), and excluding large ones (or vice versa), due to its peculiar micro-morphology? Threshold values have been established for specific PM fractions (EC Commission, 2004; EU Council, 1999; US EPA, 2006; WHO, 2006), due to the negative impact on human health (Delfino et al., 2005; Pope and Dockery, 2006), and consequently the

* Corresponding author. Tel.: +39 040 558 3886; fax: +39 040 575 079.
E-mail address: tretiach@units.it (M. Tretiach).

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doi:10.1016/j.scitotenv.2010.10.026

answer to our question is of great interest for a wider use of the moss-bag technique.

2. Materials and methods

2.1. Survey areas

The survey was carried out in two areas of c. 2 km^2 each (A, B in Fig. 1) located within the conurbation of Trieste (NE Italy), a middle sized sea town of c. 210,000 inhabitants with a sub-Mediterranean climate, and predominant dry, continental ENE winds. Area A is exclusively residential, and is characterised by a dense urban fabric with high buildings and narrow streets with intense traffic flow. Area B is residential/industrial: the urban fabric is relatively more open, buildings are often surrounded by small gardens, and traffic is less heavy, but at the southernmost margin there is an iron foundry with a coke plant, delimited by a highway; moreover, a municipal waste incinerator and an industrial zone are situated in the immediate vicinity, in a SE direction.

In each area five pairs of exposure sites were selected on the basis of aerial photographs and field surveys. The sites of each pair were located at a maximum distance of 200 m away from each other, and were located, respectively, in a green area (parks, gardens or abandoned fields; herein, "green sites"), and in a nearby road or square (herein, "roadsides"). The exposure sites had to be at a minimum distance of 10 m from the external perimeter (green sites), and at a minimum distance of 10 m from possible disturbance sources, such as refuse bins, crossroads, and bus stops (roadsides).

2.2. Sampling and pre-treatments of H. cupressiforme

Small cushions of *H. cupressiforme* were collected from calcareous rocks in a dolina of the Classic Karst (Basovizza, Trieste, NE Italy), at c. 400 m a.s.l. The material was selected and washed according to Tretiach et al. (2007), and devitalised at 120 °C for 24 h (Adamo et al., 2007), obtaining oven-dried moss, which was stored on silica gel until bag preparation. This devitalisation treatment was selected as it is easy, cheap and eco-friendly, and the material offers an excellent accumulation performance (Adamo et al., 2007). Furthermore, the oven treatment leaves virtually unaltered the peculiar morphology and leaflet arrangement of the moss, enhancing the capture of particles from the atmosphere (Giordano et al., 2009).

2.3. Moss-bag preparation, exposure and collection

Sub-spherical moss-bags were prepared using squares (c. 12× 12 cm) of plastic mosquito net (tesa© Fly Screen white, mesh: c. 2 mm) sewed with nylon fishing line, washed with distilled water, air-dried and filled with c. 400 mg of oven-dried moss. The moss-bags and 6 samples for pre-exposure analysis (c. 400 mg each) were kept in the freezer, in sealed plastic bags, until use.

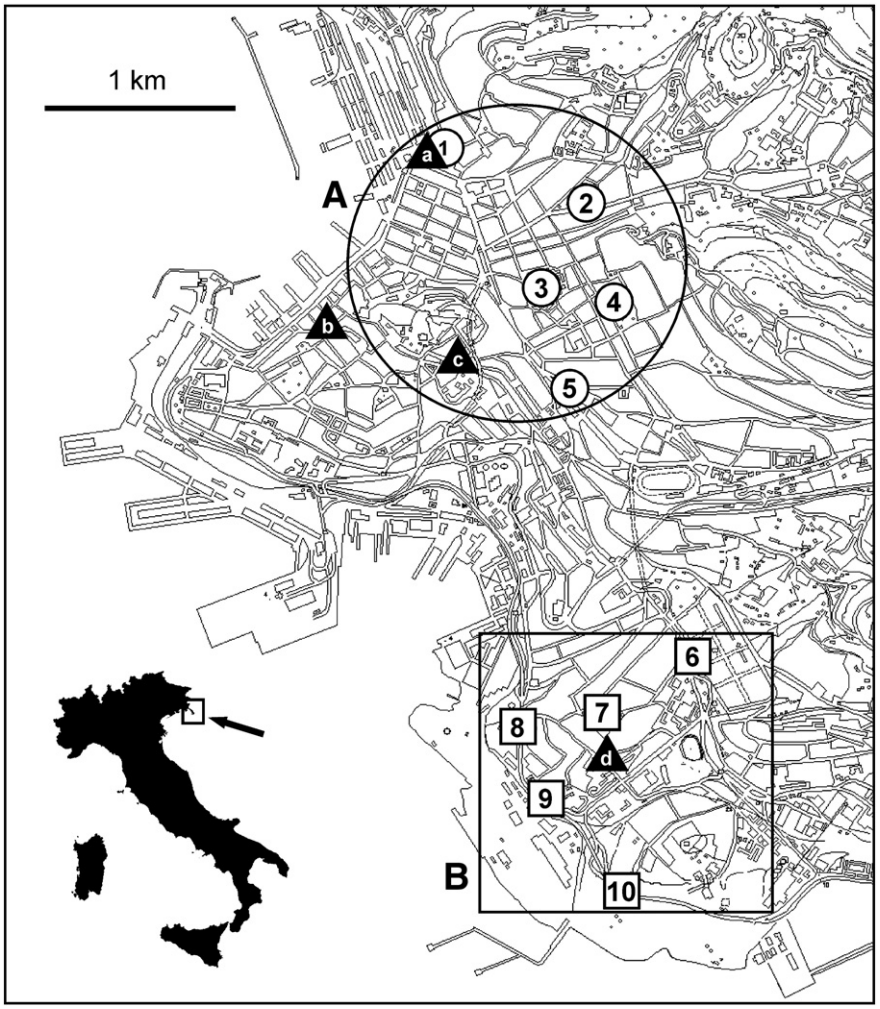

Fig. 1. Locations of the exposure site pairs in the areas A (residential, circle) and B (residential/industrial, square) within the Trieste conurbation. The distance between sites of the same pair (green sites vs. roadsides) is smaller than the symbol; a–d, full triangles: automated monitoring stations which provided the data of Table 1. Map by courtesy of Regione Autonoma Friuli–Venezia Giulia.

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For the exposure, lattice-works made with shaped stainless steel rod (4 mm in diam., c. 130 cm long) were fixed at c. 4 m above the ground on the external branches of trees in green sites, and on lighting poles (Fig. 2) in roadsides, always jutting out from tree canopies, gutter drains, roofs and electric cables (minimum distance: 50 cm).

In the laboratory, pieces of plastic hose (internal diameter: 5 mm; length: 70 cm) were washed in distilled water and air dried. Three moss-bags were fixed on each hose piece with nylon fishing line c. 5 cm long, a drop of glue (Loctite SuperAttak), and a strip of white insulating tape, at 5, 15 and 25 cm from one end. A little self-locking cable tie was loosely fastened at the other end. The hose pieces were individually closed in a plastic bag to avoid sample contamination.

Sample installation was made in a single day (17/05/2006): pipes were slipped on the lattice-works and fastened by tightening the cable ties, and plastic bags were removed. After three months (17/08/2006), each pipe with its moss-bags was enveloped in a plastic bag, and slipped off from the rod after cutting the cable tie. In the laboratory, moss-bags were left to dry out for 48 h in a closed cabinet, and then stored at room temperature in sealed plastic bags until pulverisation.

2.4. Element analysis

Pre-exposure samples, with the exclusion of a single shoot (see below), were individually coarsely cut with stainless-steel scissors, and homogeneously pulverised using a planetary ball mill RM 200 Retsch (Haan, Germany) with agate grinding jars and balls. The three post-exposure samples from each site, with the exclusion of a single shoot (see below), were carefully mixed, roughly cut, subdivided in three parts and pulverised; the pulverised material was pooled in a plastic test tube with cap, and homogenised by vigorous hand shaking. Subsamples of c. 250 mg were taken from each pre-exposure and pooled post-exposure sample (one and two subsamples, respectively) for element analysis.

Each subsample was digested with 2 mL of HNO_3 for 1 h, then with 6 mL 2-2-2 HCl–HNO_3–H_2O at c. 95 °C for 1 h, diluted to 20 mL , and analysed by Inductively Coupled Plasma–Mass Spectrometry by ACME Inc. Analytical Laboratories (Vancouver, Canada) for quantification of 18 elements: Al, Ba, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, S, Ti, V, and Zn. The accuracy of digestion and analytical procedures was checked by routine element determination in laboratory standard reference material [V14 Mountain Hemlock (*Tsuga mertensiana*) needles]. Recovery ranged between 90% (Ti) and 114% (Cr), with the exception of Na (63%) ($n = 6$).

2.5. Scanning Electron Microscopy observations and Energy Dispersive X-ray analysis

Before grinding the material, as described above, a single, intact shoot was taken from each moss-bag for Scanning Electron Microscopy (SEM) observations and Energy Dispersive X-ray (EDX-ray) microanalysis. Due to fund constraints, this investigation was necessarily limited to the couplets (green sites/roadsides) of the two most polluted sites of each area, defined on the basis of the post-exposure element content measured in the respective moss-bags, for a total of eight triplets of moss-bags. The shoots were gently cut with a razor-blade on a microscope slide under a stereomicroscope Wild Makroscop M420 (Wild Heerbrugg Ltd, Heerbrugg, Switzerland) connected to a digital camera Leica DC 300 (Leica Microsystems AG, Heerbrugg, Switzerland). Three stubs (M4909-100EA Specimen mounts Sigma-Aldrich) were prepared for each site: a couple contained all the moss shoot portions, whereas the third one contained the residual material and the leaves which had detached during the manipulation. This material was gold-metallised and observed with a SEM Leica Stereoscan 430i (Leica, Cambridge Instruments, Cambridge, UK).

Estimation of particle (>10 and ≤10 μm in maximum diam.) coverage on the moss surface was carried out at 600× on the median portion of the adaxial face of the first sixteen leaflets identified on each couple of stubs along two perpendicular diameters, the first of which had been selected randomly. An ordinal cover estimation value (1, very low; 2, low; 3, medium; 4, high; 5, very high) was assigned to each observed portion. These estimations enlighten a critical aspect that had to be kept in mind in the following phase of particle counting: the particle coverage was always highly heterogeneous, and therefore the selection of the spot areas to be photographed was particularly problematic. From the beginning, we decided to select leaflets reflecting the overall contamination of the sample under study, which was therefore carefully examined leaflet by leaflet under different angle-shots.

For each site, single images of ten other leaflet surfaces were acquired for each couple of stubs at 600× to further characterize type and size of adhering particles. Each image was enlarged at 150% with a graphic program (Corel Paint Shop Pro Photo XI), and the discernible particles, subdivided in four size classes (<2.5 μm, 2.5–10 μm, 10–25 μm and >25 μm in maximum diameter) were counted. EDX-ray micro-analyses of the entire surface of one of these ten leaflets and thirty randomly selected particles were carried out. Ten further microanalyses were carried out on the stubs with the residual material.

2.6. Data processing

The data was processed using Microsoft Excel 97 and StatSoft STATISTICA 6.0. Basic, non-parametric statistics [Wilcoxon Matched Pairs Test, Mann–Whitney *U* Test ($p = p$-value; $p^* =$ exact p-value), Kruskal–Wallis ANOVA] and Cluster Analysis [after data standardisation; squared Euclidean distance as measure of distance, and Ward's

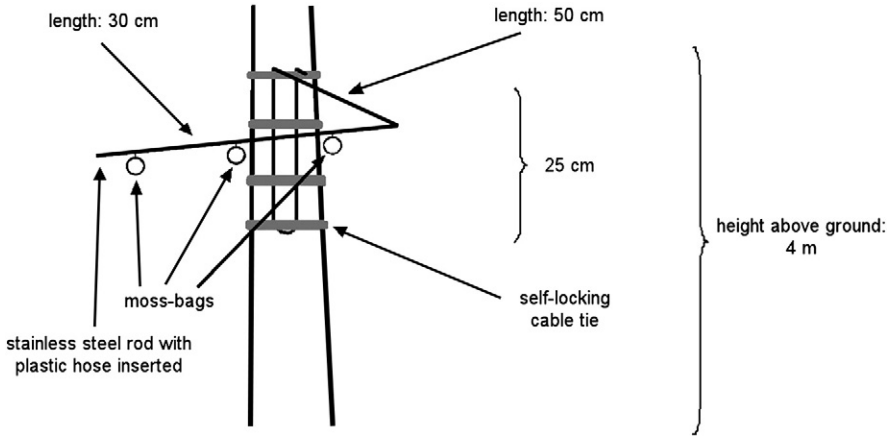

Fig. 2. Schematic drawing of the lattice used for moss-bag exposure on the roadside lighting poles. The bags were fixed on a piece of plastic hose 70 cm long which was inserted on the stainless steel rod.

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Table 1
Wind speed and air concentration of pollutants recorded by automated monitoring stations located in the areas A, B of Fig. 1 during sample exposure. Statistically significant differences are marked (SD: standard deviation).

	Daily average of hourly means				p	Daily maximum of hourly means				p
	A		B			A		B		
	Mean ± SD	Median	Mean ± SD	Median		Mean ± SD	Median	Mean ± SD	Median	
Wind speed ($m\ s^{-1}$)	2.9 ± 1.6	2.4[b]	2.1 ± 1.0	1.8[d]	**	5.3 ± 2.6	4.7	3.9 ± 1.7	3.6	**
CO ($mg\ m^{-3}$)	0.5 ± 0.1	0.6[a]	0.2 ± 0.1	0.2[d]	**	1.2 ± 0.4	1.1	0.8 ± 0.5	0.6	**
NO_2 ($\mu g\ m^{-3}$)	87.9 ± 25.4	89.1[a]	26.4 ± 14.3	26.2[d]	**	163.8 ± 50.6	160.3	62.3 ± 31.7	59.8	**
SO_2 ($\mu g\ m^{-3}$)	8.7 ± 5.9	8.0[c]	8.7 ± 6.8	6.9[d]		28.2 ± 27.2	20.9	18.6 ± 22.5	13.4	*
PM_{10} ($\mu g\ m^{-3}$)	25.8 ± 12.5	25.9[a]	26.0 ± 14.3	26.6[d]		48.0 ± 18.6	46.9	62.7 ± 37.5	56.2	**

* $p<0.01$; ** $p<0.001$ (Wilcoxon Matched Pairs Test).
[a] TS01LIBE.
[b] Meteorogical station of Trieste – Piazza Hortis.
[c] TS04VICO.
[d] TS02CARP (ARPA Friuli Venezia Giulia: http://www.arpa.fvg.it/Aria-Radia/Tutela-Qua/RETE-DIRI/CENTRALINE).

method as linkage rule (Anderberg, 1973; Podani, 2000)] were performed.

3. Results

3.1. Weather conditions and air pollution

During exposure, area A was characterized by slightly more intense winds, and significantly higher concentrations of CO and NO_2 with respect to area B (Table 1). Only PM_{10} daily maximum concentrations were significantly higher in B than in A (62.7 ± 37.5 $\mu g\ m^{-3}$ vs. 48.0 ± 18.6 $\mu g\ m^{-3}$, respectively). In both areas precipitations were particularly intense at the beginning and at the end of the exposure period, with 256 mm (area A) and 297 mm (area B) of total rainfall.

3.2. Exposure effects on element concentration

Accumulation (post- minus pre-exposure) mean values were significantly lower in A than in B sites for Ca, Zn ($p^*<0.05$, Mann–Whitney *U* Test), Cd, Fe, Ni, S, V ($p^*<0.01$), Al, Mn, and Ti ($p^*<0.001$), with the exception of K, higher in A than in B (Table 2).

The exposure environment strongly affected accumulation, because this was generally significantly higher in the samples exposed in roadsides than in green sites for Al, Mg, Mn, Ti ($p<0.05$, Wilcoxon Matched Pairs Test), Ba, Ca, Cr, Cu, Fe, Ni, Pb, and Zn ($p<0.001$). Statistically significant correlations (*R*, Spearman rank correlation coefficient) between samples exposed along roadsides and those exposed in the nearby green sites were observed for 10 elements at $p<0.01$ and for a further six elements at $p<0.05$ (Fig. 3).

The average accumulation data of the moss-bag triplets were subjected to Cluster Analysis to verify whether a classification might reflect the origin of samples from the two exposure areas (A, B) and/or the two exposure environments (roadsides vs. green sites). The resulting dendrogram (Fig. 4) shows four distinct main clusters (a–d): the first two (a, b) essentially group together the exposure sites of the residential area A, the remaining two (c, d) those of the residential/industrial area B, although cluster a and b are farther apart than b and c. The two exposure environments are less clearly partitioned: with the exception of group d, consisting of only two roadside sites, no other group contains sites belonging exclusively to a single environment, although the assembling is congruent with some characteristics of the exposure sites. Cluster a groups together the less contaminated samples. They had been exposed in four of the five green A sites. The mean element increase observed in this cluster is generally lower than in clusters b and c, with significant differences for Ba, Ca, Cr, Cu, Fe, Mn, Ni, Pb, Ti, and Zn (Mann–Whitney *U* Test, $p^*<0.05$, 0.01 or 0.001). The fifth green A site (a wooded square surrounded by busy roads) is grouped in cluster b with three roadside sites A characterized by heavy traffic. The average increase of most elements observed in this cluster is generally intermediate compared to clusters a and c (Al, Ca, Cd, Fe, Mn, Ni, S, and Ti) or higher (Ba, Cr, Cu, Hg, K, Mg, Na, and Pb), but differences are often not statistically significant. Cluster c groups together the majority of the B sites: two green sites with their roadside homologous, a further roadside site, and the green sites 9 and 10, whose homologous are relegated in cluster d, as they have the highest post-exposure element concentrations recorded in this study.

Table 2
Mean, standard deviation (SD), and median concentration values (in $\mu g\ g^{-1}$ dry wt. except where otherwise specified) of major and trace elements in oven-dried samples of the moss *Hypnum cupressiforme* before and after three-month-exposure in the ten site pairs of Fig. 1 (roadsides: regular; green sites: italics), subdivided according to the area of exposure (A,B). For details, see the supplementary information.

Exposure site			n	Al %	Ba	Ca %	Cd	Cr	Cu	Fe %	Hg	K %	Mg %	Mn	Na %	Ni	Pb	S %	Ti	V	Zn
Pre-exposure		Mean	6	0.03	8.9	0.73	0.26	5.4	8.20	0.040	0.082	0.44	0.114	67	0.002	1.8	5.43	0.11	9	5	20.5
		SD		0	0.6	0.02	0.02	4.2	0.29	0.002	0.011	0.01	0.004	3	0.002	0.3	0.13	0.02	0	1	1.9
		Median		0.03	9.2	0.73	0.26	4.1	8.14	0.040	0.084	0.44	0.114	67	0.002	1.7	5.39	0.11	9	5	20.8
A	Roadsides	Mean	10	0.05	30.9	0.99	0.33	5.6	43.86	0.096	0.121	0.07	0.102	62	0.014	3.0	18.71	0.06	13	3	85.0
		SD		0.01	16.5	0.12	0.05	1.7	16.94	0.024	0.019	0.01	0.011	4	0.005	0.6	13.33	0.03	2	1	34.4
		Median		0.05	27.5	0.97	0.32	6.0	45.06	0.102	0.117	0.07	0.097	63	0.015	3.0	11.59	0.06	13	3	85.7
	Green sites	Mean	"	*0.05*	*14.7*	*0.83*	*0.37*	*3.2*	*18.91*	*0.065*	*0.128*	*0.06*	*0.098*	*57*	*0.011*	*2.3*	*8.76*	*0.05*	*11*	*3*	*43.4*
		SD		*0.01*	*4.2*	*0.09*	*0.03*	*1.1*	*11.59*	*0.016*	*0.016*	*0.02*	*0.013*	*5*	*0.006*	*0.4*	*2.21*	*0.03*	*1*	*1*	*14.1*
		Median		*0.05*	*14.4*	*0.81*	*0.37*	*2.9*	*15.58*	*0.059*	*0.132*	*0.06*	*0.097*	*57*	*0.012*	*2.2*	*8.32*	*0.06*	*11*	*2*	*39.1*
B	Roadsides	Mean	10	0.09	30.7	1.28	0.49	6.8	46.05	0.447	0.122	0.04	0.110	118	0.011	5.6	21.51	0.10	25	5	167.2
		SD		0.03	12.1	0.30	0.16	2.7	16.88	0.355	0.010	0.01	0.016	64	0.002	2.5	8.11	0.02	11	2	75.7
		Median		0.07	22.6	1.10	0.44	5.4	44.81	0.249	0.122	0.04	0.103	80	0.012	3.9	20.28	0.10	19	5	136.8
	Green sites	Mean	"	*0.08*	*19.2*	*1.11*	*0.47*	*4.6*	*22.80*	*0.329*	*0.124*	*0.05*	*0.098*	*98*	*0.011*	*4.2*	*15.25*	*0.09*	*20*	*5*	*75.5*
		SD		*0.02*	*5.3*	*0.28*	*0.07*	*1.7*	*5.94*	*0.257*	*0.009*	*0.01*	*0.008*	*41*	*0.004*	*1.7*	*5.85*	*0.03*	*7*	*2*	*33.0*
		Median		*0.08*	*18.3*	*1.06*	*0.47*	*4.0*	*21.48*	*0.212*	*0.121*	*0.05*	*0.101*	*81*	*0.009*	*3.7*	*13.47*	*0.10*	*20*	*5*	*59.8*

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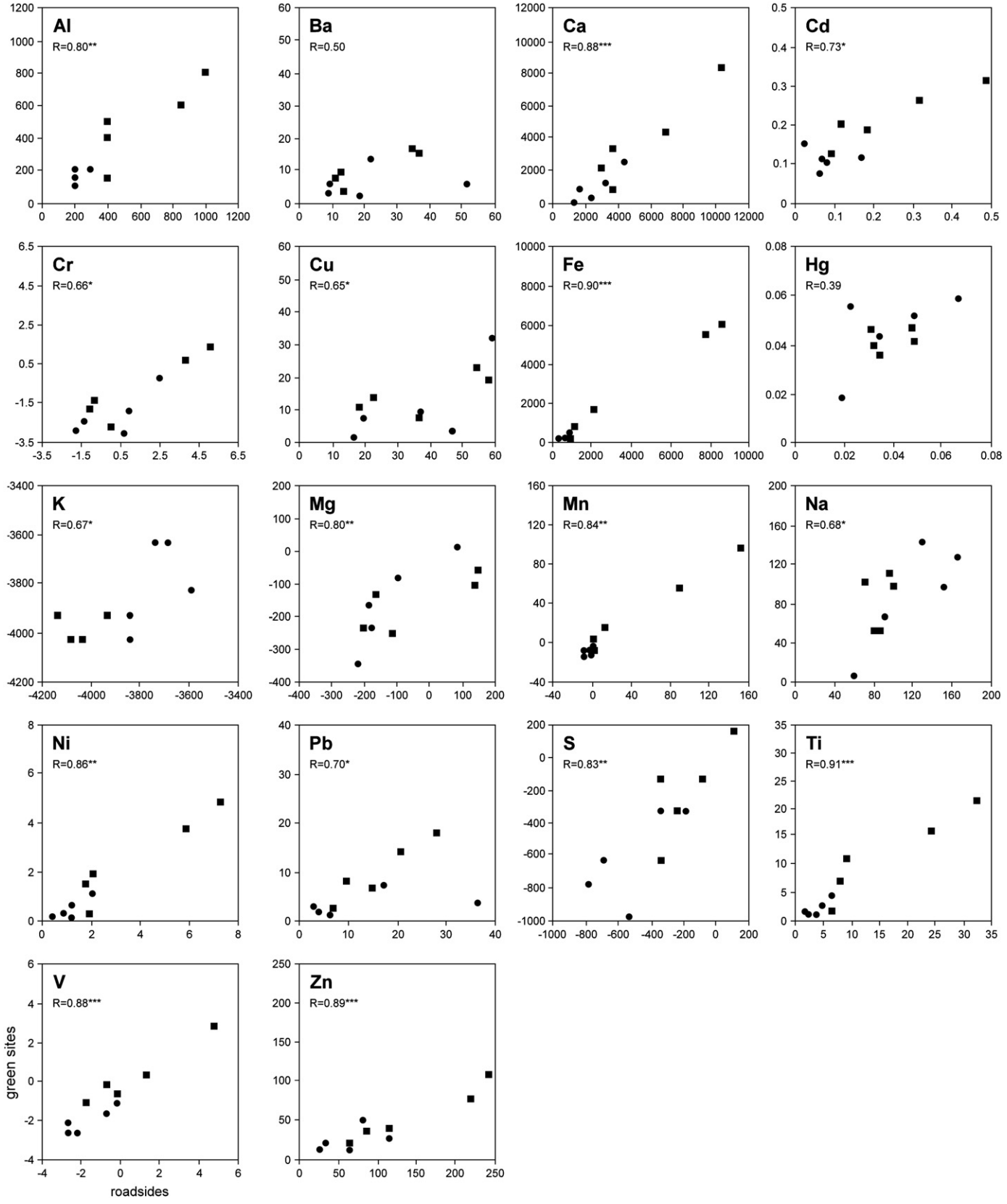

Fig. 3. Scatter plots of element increase (post- minus pre-exposure mean values; μg g^{-1} dry wt.) measured in moss-bags exposed in roadsides (*x*-axis) and green sites (*y*-axis) (*R*: Spearman rank correlation coefficient; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$). ●: site pairs of area A; ■: site pairs of area B.

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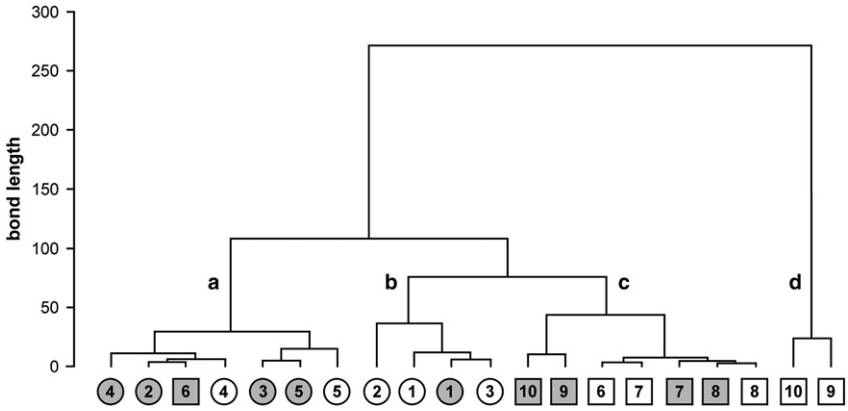

Fig. 4. Cluster analysis classification of the exposure sites of Fig. 1, based on the post-minus pre-exposure mean values of all analysed elements; 1–5, circles: area A; 6–10, squares: area B; white symbols: roadsides; grey symbols: green sites.

The enrichment observed in cluster c is intermediate with respect to those of clusters a and b for Ba, Cr, Cu, Mg, Na, and Pb, but it is higher (p^*<0.05, 0.01 or 0.001) for Al, Cd, Fe, Mn, Ti, and V.

3.3. SEM observations and EDX-ray microanalyses

Based on the previous results, the characterization of particles adhering to the moss leaflet surface was limited to samples exposed in sites 1, 2 (area A), and 9, 10 (area B).

Leaflets of non-exposed samples showed a particle-free surface (Fig. 5a), which contrasts with that of exposed samples (Fig. 5b,c), as the latter were always covered by particles of different size and shape (Fig. 5d–f), although heterogeneously. Particles were generally poor on the abaxial, convex surface of moss leaflets, rather copious on the adaxial, concave surface, particularly near the area of attachment of the leaflet to the stem. Most of the 320 particles analyzed by EDX contained Al, Ca, Fe and Si (Fig. 6a), indicating a possible soil origin. Iron was detected in two types of particles: irregular in shape, with Ca, Ti or Cr as co-occurring elements (Fig. 5d), and sub-spherical, c. 2–10 μm in diam. (Fig. 5e), with Al, Cu and Si in traces (Fig. 6b). The particles of the latter type were particularly abundant in the sites B. Sporadically, traces of K, Mg, Ba and Mn and Cr could be detected in samples exposed in sites A. In some cases the leaflet surfaces hosted unidentified spores, fungal hyphae, pollen grains (Fig. 5f), or fragments of plant materials, but these components were never abundant.

The particle count carried out on samples exposed in the two most polluted pairs of both areas confirmed the absolute, and certainly underestimated, prevalence of particles ≤10 μm in diam. in comparison to larger ones (>10 μm) (Fig. 7). The smallest particle classes (<2.5 and 2.5–10 μm) were largely predominant (78.8% and 19.4%, respectively), with only 0.2% of particles greater than 25 μm in diam., although it is important to underline that some of the largest particles were lost during

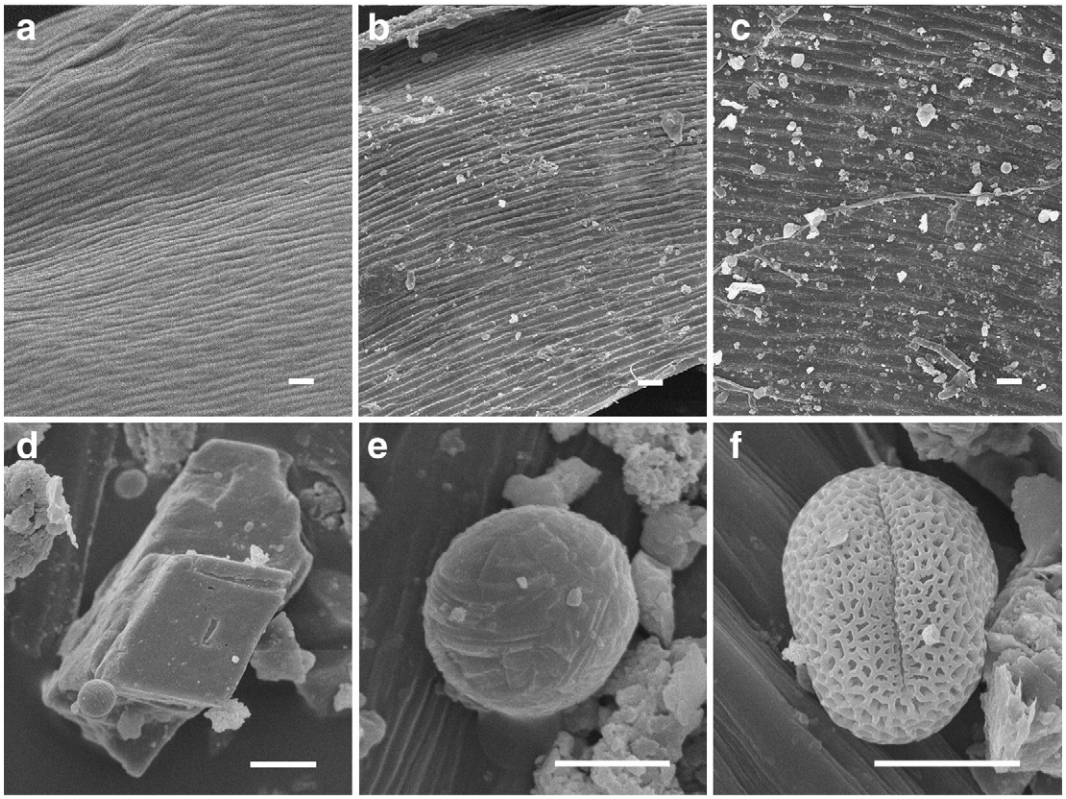

Fig. 5. SEM micrographs of moss leaflets before (a) and after exposure (b,c) in the green (b) and roadside (c) site pair nr. 9 of Fig. 1, and enlargement of particulate matter (d,e) and pollen grain (f). Bar = 10 μm (a–d, f); 3 μm (e).

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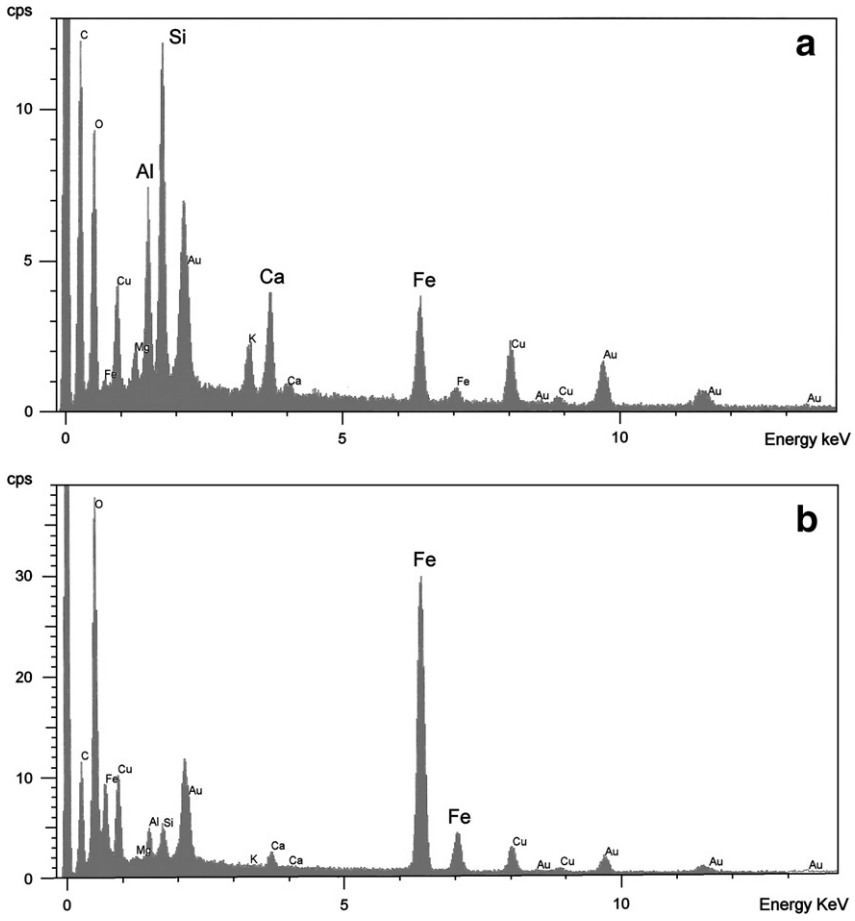

Fig. 6. Element composition of the two particles of Fig. 5(d,e) (respectively, a and b) determined by EDX-ray microanalysis.

sample manipulation, as they easily detach. The samples exposed in green sites and roadsides did not differ statistically, with site 10 having an unusual high data spread (Fig. 8). Particles were less numerous per area unit in sites A than in sites B (Mann–Whitney *U* Test, $p<0.001$). The estimation of particle coverage on an ordinal scale had already suggested the greater importance of geographic location (A vs. B) with respect to exposure environment (green sites vs. roadsides) in determining statistically significant differences among sites (data not shown).

4. Discussion

The moss-bag technique has proved to be an effective tool for the characterization of environmental contamination by airborne particulate matter. The element analysis suggests that the particulate fallout differs in the two areas. The post-exposure values of element content demonstrate that the area A is subjected to a lower level of particulate matter deposition than the area B (Table 2), in good agreement with the different land use of the two areas (residential the former,

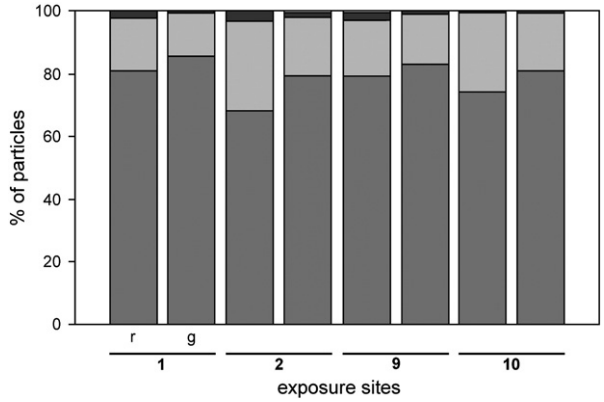

Fig. 7. Mean percentage of particle size classes counted on the adaxial surface of the moss leaflets exposed for three months in the most polluted site couplets, roadside (r) vs. greensites (g), of the areas A, B of Fig. 1. ■: <2.5 μm; ■: 2.5–10 μm; ■: 10–25 μm; □: >25 μm ($n=10$).

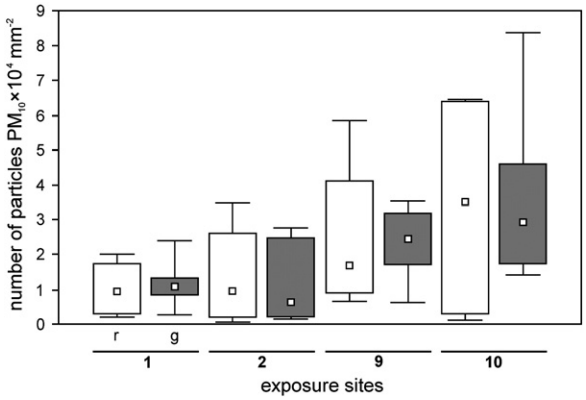

Fig. 8. Density of PM_{10} particles (number $\times 10^4$ mm^{-2}; median, 25–75%, minimum and maximum values) counted on the adaxial surface of the moss leaflets exposed for three months in the most polluted site couplets, roadside (r) vs. greensites (g), of the areas A, B of Fig. 1.

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residential/industrial the latter). These results are congruent with the information given by both coverage estimation and direct counts of particles on the moss leaflets (Figs. 7 and 8), on the one hand, and the pattern of pollutant distribution described by a diffusion model recently published by the local Atmospheric Pollution Protection Service (Mattassi and Daris, 2009), on the other hand. However, they also disprove the direct PM_{10} data gathered during exposure by two automatic monitoring stations. According to the data of Table 1, only mean maximum daily values of PM_{10} (expressed as $\mu g\ m^{-3}\ h^{-1}$) were higher in the area B with respect to the area A, whereas the mean daily values were practically identical, partially confirming that measurements at a specific site do not necessarily represent the concentrations in a wider urban area (Puustinen et al., 2007).

Two site couplets of the area B, namely n. 9 and 10 of Fig. 1, were singled out as particularly atypical due to the high post-exposure content of almost all elements analyzed. They are located near the industrial plants of area B, particularly the cast iron foundry, and in all analysed samples spherical Fe-rich particles, most probably derived by combustion processes at high temperatures, were observed with greater frequency than in all other site couplets. The emitting sources, however, seem to have a rather limited range of action, because the element concentrations in the other B sites were much lower and directly comparable with those of the A sites. This accords well with the position of the exposure sites with respect to the emitting sources and the regime of prevalent winds, which, during the exposure, blew mainly from NE and NNE. Also a recent dispersion model showed that most of the emissions from the steel smelter, the municipality waste incinerator and other industries located nearby are carried to the sea, reaching the southwest coast of the Gulf of Trieste (Mattassi and Daris, 2009).

In both areas moss-bags exposed in green sites had lower post-exposure content than those exposed in the counterpart roadsides for 15 elements (i.e. 83% of those analysed), and generally a good correlation was detected between the concentration values measured in the site pairs (Fig. 3). The re-suspension of soil particles by air flows generated by wind and motorized traffic (particularly diffuse within the area A), and the large intercepting surfaces of trees growing in green sites are most probably the first cause of the observed greater enrichment in material exposed in the roadsides. These data, however, do not perfectly fit that derived from the qualitative and quantitative assessments of particle cover. The number of PM_{10} particles was similar in samples exposed in green sites and in roadsides (Fig. 8), therefore both variables do not allow for statistical distinction between the two exposure environments. The samples exposed in green sites were less contaminated (Table 2). If we make the hypothesis that the chemical composition of PM_{10} was similar in the two nearby areas, the observed difference must be attributed to the large-size-class (>10 μm in diam.) particles entrapped by the samples. The number of these particles has most probably been underestimated, because most likely they detached in some amount during the manipulation of the moss when preparing the stubs. This hypothesis is compatible with our knowledge on the fall-out dynamics of large-sized particulates, on one side, and with the notoriously high persistence in the atmosphere, and long distance dispersion pattern of PM_{10}, on the other side (Puustinen et al., 2007). Several biomonitoring studies with higher plants, mosses and lichens highlighted a clear decrease in the concentration of several trace elements with increasing distance from roads, depending on various factors such as traffic intensity, chemical element, etc. (see e.g. Naszradi et al., 2004; Tuba and Csintalan, 1993; Zechmeister et al., 2005), and all of them explained the observed patterns as the result of a more rapid deposition rate of large sized particles in comparison to PM_{10}. Garty and Ammann (1987) also suggested that samples contaminated by coarse particles give analytical measurements with higher data spread than samples with only small particles. Unfortunately, the low number of analyses carried out on our multiple samples did not allow checking that assumption.

Our data revealed that most of the particles on the moss surface are potentially inhalable or breathable, although we certainly underestimated their number. This is certainly due to the favourable morphology of *H. cupressiforme*, because the devitalization process eliminates the metabolic contribution to element uptake (Giordano et al., 2009). This moss has actually high surface to mass ratio (from 80.6 to 204.5 $m^2\ kg^{-1}$, see Adamo et al., 2007), having a highly dissected external surface, with hundreds of small, thin leaflets, partially folded toward the stems, on which light particles can adhere for electrostatic attraction on a relatively smooth surface (Adamo et al., 2008).

The difference in element content observed in this study between samples exposed only a few dozen meters from each other, but in different environments, emphasizes the need of a careful choice of exposure sites during the phase of biomonitoring planning. An appropriate exposure strategy should be adopted in order to avoid heterogeneous results and, thus, inevitable wrong conclusions. Point sources of disturbance should be avoided as much as possible, in order to provide accumulation data representative of the whole study area. The stratification of the territory on a land use basis, already introduced among the strategies adopted in recent studies (e.g. Tretiach et al., in press; Weindorf and Zhu, 2010), is probably adequate if the information is available at small scale size (1:2000–1:5000). At higher scale size it would seem preferable to select specific environmental types, and a number of conditions that each site must satisfy a priori for being selected.

Further methodological work is certainly still needed for the optimization and harmonization of the moss bag technique (Castello, 2007; Culicov and Yurukova, 2006). In our opinion, part of the efforts should verify the effects derived from the bag cover, when a rapid survey of the large body of literature available on the bag-technique revealed that practically all the authors use bags of different size, shape, and material. Preliminary data concerning lichen thalli exposed without bags and in bags of the same type used in this study suggests that the net can actually increase the concentration of most elements in that plant material (Pittao, 2007). Much more work, however, is necessary to describe the complex interplay between wet and dry depositions, nets, and biological materials in terms of interception and/or protection capability.

5. Conclusions

This study confirmed that the moss-bag technique with oven-devitalized *H. cupressiforme* is a valid aid to study trace element deposition in urban environments. However, the enrichment may be influenced by local environmental situations, because we found that moss-bags exposed in green sites and roadsides differed in contamination by coarse particles. We also found that a moss-bag entraps a prevalence of potentially inhalable or breathable particles ($\leq PM_{10}$), which are now regarded as a health relevant characteristic of ambient particulate matter.

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2010.10.026.

Acknowledgements

We thank I. Pellegrini (ARPA FVG) and F. Stravisi and M. Berro (University of Trieste) and D. Lorenzutti (Ecothema, Trieste) for their valuable technical assistance. This study was partly financed by MIUR (PRIN 2002; nat. resp. Prof. R. Bargagli, Siena).

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