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# Atmospheric trace metal pollution in the Naples urban area based on results from moss and lichen bags

S. Giordano [a,*], P. Adamo [b,1], S. Sorbo [c,2], S. Vingiani [b]

[a] *Dipartimento di Biologia Strutturale e Funzionale, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cinthia - 80126 Napoli, Italy*
[b] *Dipartimento di Scienze del Suolo, della Pianta e dell'Ambiente, Università di Napoli Federico II, Via Università, 100 - 80055 Portici (NA), Italy*
[c] *Centro Interdipartimentale di Servizio per la Microscopia Elettronica CISME, Università di Napoli Federico II, Via Foria, 223 - 80139 Napoli, Italy*

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*Despite significant damage to tissue and cell integrity, moss and lichen in bags efficiently intercept airborne trace elements.*

**Abstract**

The results of trace element content analysed in *Sphagnum capillifolium* and *Pseudevernia furfuracea* exposed in bags in 1999 are reconsidered to evaluate the reliability of moss and lichen transplants to detect urban trace element atmospheric pollution, using Naples as a case example. After 4 months' exposure, trace element concentrations were at least twice as high as the pre-exposure values and in general higher in *Sphagnum* than in *Pseudevernia*. Moss samples were enriched in the following order: As = Cu > Mo > Pb > V > Co > Cr > Zn; lichen samples in the order: Mo > Cu > As = Co = Ni > V > Pb. Based on the calculation of a cumulative load factor, all sites located along the coast had higher trace element loads compared to sites in the hilly inland area. Complementary SEM, TEM and EDS observations showed, despite significant damage to tissue and cell integrity, the recurrent presence of particulate matter in moss and lichen, indicating the considerable presence of dust in the urban atmosphere which, according to chemical composition, may be due both to anthropogenic and natural sources such as volcanic rock and soil and sea salts.


*Keywords:* Biomonitoring; Trace elements; Atmospheric particulate; Electron microscopy; Microanalysis

## 1. Introduction

Trace metals are major pollutants because they are persistent in the environment and are very widely dispersed by man-made emission sources. Biomonitoring of trace metals from atmospheric deposition can be currently evaluated by environmental biomonitors such as mosses, lichens and plant leaves (Ruhling and Tyler, 1973; Sloof and Wolterbeek, 1991; Steinnes et al., 1992; Ruhling, 1994; Herpin et al., 1996; Freitas et al., 1999; Alfani et al., 2000; Bargagli et al., 2002).

Mosses and lichens have several advantages when compared to higher plants (Tyler, 1990; Bargagli, 1998). They lack a root system, so they rely on atmospheric wet and dry deposition for their mineral nutrition, especially epiphytic species; they have a high surface/volume ratio

* Corresponding author. Tel.: +39 81 2538553; fax: +39 81 2538523.
*E-mail addresses:* giordano@unina.it (S. Giordano), adamo@unina.it (P. Adamo), sersorbo@unina.it (S. Sorbo).
[1] Tel.: +39 81 2539172; fax: +39 81 2539186.
[2] Tel.: +39 81 444046; fax: +39 81 440507.

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and ion exchange properties; unlike many other plants, they lack variability in morphology throughout the growing season and they have no cuticle. Thus mosses and lichens are used for biomonitoring purposes in many ways.

Mosses and lichens also accumulate large amounts of trace metals, making them good bioaccumulators to estimate metal pollution (Steinnes et al., 1992; Bargagli, 1998; Vasconcelos and Tavares, 1998; Ceburnis and Valiulis, 1999; Reimann et al., 2001; Bargagli et al., 2002; Bettinelli et al., 2002; Carreras and Pignata, 2002; Figueira et al., 2002). The bioaccumulation efficiency of mosses and lichens comes from their substantial cation exchange capacity, which is due to cell wall negative-charged constituents (mostly carboxylic acid groups) that may establish ionic bonds with cationic elements in soluble form (Figueira et al., 2002). Elements can also be retained in particles trapped in intercellular spaces (Figueira et al., 2002) or on uneven surfaces (Jalkanen et al., 2000). Efficiency of element retention depends on the number and nature of the extracellular binding sites, tissue age and growth condition (Brown and Bates, 1990).

The coastal city of Naples (1,008,000 inhabitants in 2001) forms part of one of the most densely populated areas in the world. A previous study concerning effects of pollution on bryophyte vegetation in Naples and other sites in the region of Campania showed that the bryoflora was strongly affected by human disturbance (Giordano et al., 2004). It was clearly shown that in urban sites the number of species and IAP (Index of Air Purity) values are lower, and that acrocarpous mosses and vegetative reproduction occur more frequently. Contents of trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Ti, V, Zn) were measured in some species and results indicated a large contribution of resuspended soil particles to the chemical composition of the analysed bryophytes. All the examined species were enriched in Cd, Cu and Zn, and in some cases showed a high enrichment factor for Pb. In addition, other studies on chemical composition of *Quercus ilex* leaves indicated that trace metal pollution does affect the urban area of Naples (Alfani et al., 2000).

The absence of well-suited moss and lichen species living in urban and extraurban reference environments, and the difficulty of choosing ideal sampling conditions for convenient area distribution, encourage the use of moss and lichen exposed in bags to monitor trace metal deposition (Wegener et al., 1992; Viskari et al., 1997; Jalkanen et al., 2000) over large areas.

Although the "bag" method is not definitively standardised as regards the amount of plant material, exposure time, correlation to airborne depositions and form of uptake (essentially passive due to atmospheric particulate entrapment and cation exchange capacity of the extracellular binding sites or active, due to bio-chemical activities of plasma membrane and cytoplasm), it has the advantage of collecting information integrated over the whole exposure time, without being influenced by momentary changes in pollutants.

Previous studies carried out using moss and lichen bags in the Naples urban area (Adamo et al., 2003; Vingiani et al., 2004) clearly showed that, for the majority of the elements, the total amounts found in the moss *Sphagnum capillifolium* were higher than in the lichen *Pseudevernia furfuracea*, whether considering the whole period of exposure or the weekly uptake and, above all, that metal uptake was greatly affected in the two biomonitors by precipitation and air humidity.

The task of monitoring airborne trace element pollution over large areas is somewhat arduous, since the concentrations of pollutants are very variable in space and time; in addition, data from automatic devices are punctual and very limited in number to describe spatio-temporal trends of pollutants. Furthermore, automatic devices can generally detect a limited number of pollutants (mainly CO, $SO_x$, $N_xO_y$, PAH and dust). In this paper we reconsider the results of trace element contents analysed in moss and lichen bags exposed in the Naples urban area during 1999 and used in a previous paper in order to compare moss and lichen interception capacity in dry and wet meteorological conditions (Adamo et al., 2003) in order to evaluate the reliability of moss and lichen transplants to provide information on urban trace element atmospheric pollution, using Naples as a case example. In addition, submicroscopic (SEM/TEM) and microanalytical (EDS) observations were used complementarily to define the presence and chemical nature of particulate matter entrapped by biomonitors during the exposure, so as to assess morphological cell damage induced by exposure.

## 2. Materials and methods

### 2.1. Environmental data of the study area

The city of Naples is situated on the Tyrrhenian coast of southern Italy between two volcanic systems, the Phlegrean Fields (Monte Nuovo max height 180 m a.s.l.), to the north-west, and Vesuvius (1098 m a.s.l.), to the east. The climate is Mediterranean, with a mean annual rainfall, in the last 20 years, of 898.8 mm concentrated principally in autumn and spring. Mean annual temperature is 18.1 °C and prevailing winds are from SE and SW.

Although primarily residential, there are some heavy industries (oil refinery, fuel depots) in the eastern zone of the city and the decommissioned ILVA iron-and-steel works operative until 1992, with the connected Eternit and Montedison chemical plants, in the western part (just outside the city; map in Fig. 1). Despite recent

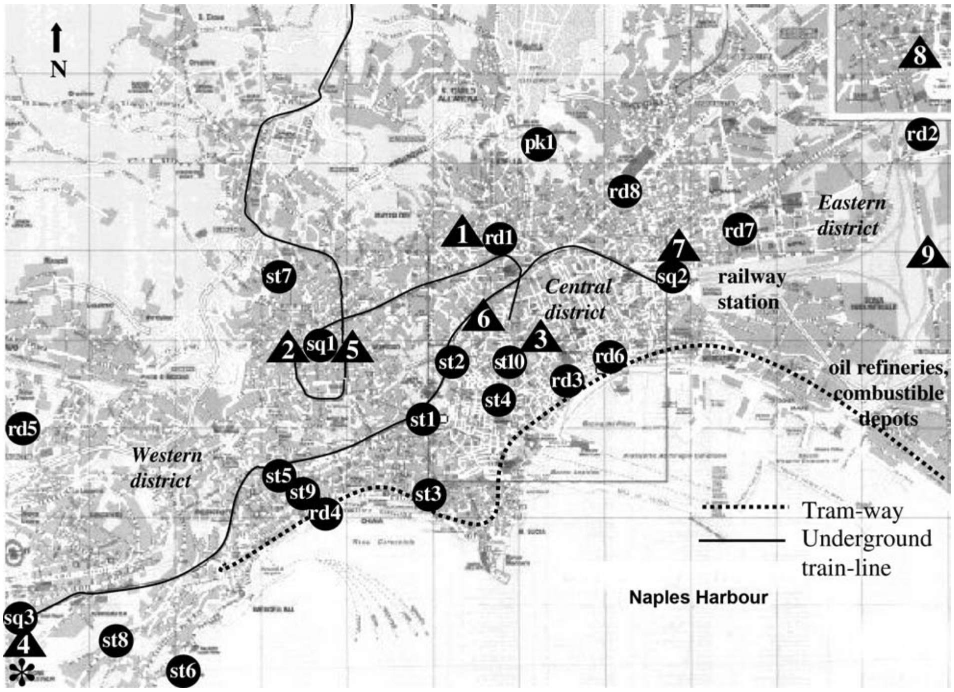


Fig. 1. Study area and location of bag exposure sites (circles) and monitoring stations (triangles). *Indicates occurrence of dismantled siderurgical plant in the plane western of Naples, just outside the map.

improvements in public transport combined with environmental protection measures, automobile and motorcycle use is still on a massive scale, with unleaded fuels introduced only in the early 1990s. Every day an average 265,000 vehicles cross the city (data from Naples City Council). Data from the Campania Air Monitoring Agency, based on physico-chemical monitoring stations located in the Naples urban area (Fig. 1), indicate that, despite restrictive measures passed to reduce private traffic, the city is still seriously affected by nitrogen, sulphur and carbon oxides, ozone, dust and hydrocarbon contamination from the atmosphere with threshold levels of many pollutants (especially $O_3$, $NO_x$) being repeatedly exceeded. Mean annual pollutant levels from different stations for the study year (1999) are presented in Table 1. More information regarding previous years can be found in Adamo et al. (2003). There is no conventional heavy metal control station in the city of Naples.

### 2.2. Experimental

Moss and lichen sampling, bag preparation and exposure, and chemical analyses were extensively described in Adamo et al. (2003). In brief, moss (*S. capillifolium* (Ehrh.) Hedw.) and lichen (*P. furfuracea* (L.) Zopf) materials were collected at Forcella Laverdet, in the Trieste Karst (NE Italy), identified as a less polluted background area (Nimis et al., 2000). Mixing, removal of soil particles and water washings were performed in the laboratory in order to homogenise and clean up the collected original materials. Between 400 and 450 mg of air dry moss or lichen samples were mounted in nylon net bags exposed in 22 (moss) and 10 (lichen) sites in the Naples urban area (Table 2). The exposure sites were randomly selected among streets (st), roads (rd), ring-road squares (sq) and urban parks (pk), ensuring that there were stations in the central, eastern and western urban districts as well as at variable altitudes and distances from the sea. Bag positioning at each site was conditioned by the presence of structures (buildings, balconies) and by the collaboration of citizens who housed sticks with bags attached. One exposure site was

Table 1
Average annual values ($\mu g\ m^{-3}$) measured by Campania Air Monitoring Agency physico-chemical monitoring stations during 1999 of sulphur ($SO_2$), carbon monoxide (CO), nitrogen ($NO_2$), ozone ($O_3$), total hydrocarbons (THC) and total dust in the atmosphere of Naples city

| Station | Type | Longitude E | Latitude N | $SO_2$ | CO | $NO_2$ | $O_3$ | THC | Dust |
|---|---|---|---|---|---|---|---|---|---|
| NA1 | A | 14°15′16″ | 40°51′46″ | 20 | 1.0 | 46 | 63 | – | 49 |
| NA2 | B | 14°13′53″ | 40°51′00″ | 13 | – | 69 | – | – | 69 |
| NA3 | B | 14°15′15″ | 40°50′59″ | 10 | – | 59 | – | – | 50 |
| NA4 | C | 14°11′54″ | 40°49′26″ | – | – | 80 | – | – | 49 |
| NA5 | C | 14°13′41″ | 40°50′40″ | – | 2.8 | 81 | – | 1465 | 54 |
| NA6 | C | 14°15′05″ | 40°51′10″ | – | 3.0 | 77 | – | – | 38 |
| NA7 | C | 14°16′18″ | 40°51′13″ | – | 2.3 | 104 | – | 1098 | 87 |
| NA8 | D | 14°16′53″ | 40°52′03″ | – | 3.0 | 21 | 42 | – | – |
| NA9 | D | 14°21′08″ | 40°51′17″ | – | – | 66 | 41 | – | – |

Type A = $SO_2$, CO, $NO_2$, $O_3$ and dust; B = $SO_2$, $NO_2$ and dust; C = CO, $NO_2$ and dust; D = CO, $NO_2$ and $O_3$ records.

Table 2
Identification number, location name and code, altitude, geographical coordinates and traffic density of the monitoring stations in the Naples city

| Number | Location name | Location code | Altitude (m) | Latitude | Longitude | Traffic density |
|---|---|---|---|---|---|---|
| 1 | Capodimonte park | pk1 | 50 | 40°51′56″ | 14°15′06″ | low |
| 2[a] | V. Emanuele street | st1 | 45 | 40°51′10″ | 14°13′30″ | high |
| 3[a] | S. Rosa street | st2 | 48 | 40°51′10″ | 14°13′36″ | high |
| 4[a] | Chiatamone street | st3 | 12 | 40°49′55″ | 14°14′32″ | high |
| 5[a] | Toledo street | st4 | 28 | 40°50′37″ | 14°14′56″ | medium |
| 6 | V. Emanuele street | st5 | 28 | 40°50′15″ | 14°12′35″ | medium |
| 7 | Posillipo street | st6 | 18 | 40°49′13″ | 14°12′54″ | medium |
| 8 | A. Omodeo street | st7 | 180 | 40°51′08″ | 14°13′14″ | medium |
| 9 | A. Manzoni street | st8 | 185 | 40°49′06″ | 14°12′16″ | high |
| 10 | Belledonne a Chiaia street | st9 | 20 | 40°50′06″ | 14°13′14″ | low |
| 11 | V. Bellini street | st10 | 40 | 40°50′58″ | 14°15′06″ | medium |
| 12[a] | Medaglie d'Oro square | sq1 | 170 | 40°50′59″ | 14°13′49″ | high |
| 13[a] | G. Garibaldi square | sq2 | 15 | 40°51′09″ | 14°16′11″ | high |
| 14 | V. Tecchio square | sq3 | 30 | 40°49′31″ | 14°11′37″ | high |
| 15[a] | A. di Savoia road | rd1 | 50 | 40°51′34″ | 14°14′57″ | high |
| 16[a] | Provinciale delle Puglie road | rd2 | 19 | 40°52′30″ | 14°18′30″ | low |
| 17[a] | Umberto I road | rd3 | 10 | 40°50′37″ | 14°15′16″ | high |
| 18 | Chiaia road | rd4 | 5 | 40°49′58″ | 14°14′20″ | high |
| 19 | Cinthia road | rd5 | 60 | 40°50′12″ | 14°11′32″ | medium |
| 20 | Marina road | rd6 | 5 | 40°50′41″ | 14°15′42″ | high |
| 21 | G. Porzio road | rd7 | 3 | 40°51′50″ | 14°17′50″ | high |
| 22[a] | Foria road | rd8 | 25 | 40°51′34″ | 14°15′43″ | high |
| 23[a] | Castelvolturno (extraurban rural site) | ru1 | 2 | 41°03′58″ | 13°59′30″ | low |

[a] Sites where both moss and lichen were exposed.

Table 3
Trace element concentrations (μg $g^{-1}$ dry wt.) measured in *Sphagnum capillifolium* and *Pseudevernia furfuracea* after 2 and 4 month exposure in bags in 22 and 10 sites in Naples urban area and in one control extraurban site

| Elements | Exposure time (months) | Moss | | | | | | Lichen | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Median ($n = 23$) | Minimum value | Station | Maximum value | Station | Original value | Median ($n = 11$) | Minimum value | Station | Maximum value | Station | Original value |
| Al | 2 | 2922 | 1351 | sq3 | 4946 | rd6 | 1108 | 809 | 530 | rd2 | 1572 | rd3 | 415 |
| | 4 | 4525 | 2375 | rd7 | 7183 | st5 | | 2029 | 1586 | st2 | 4024 | ru1 | |
| As | 2 | 0.9 | 0.5 | st2 | 2 | rd2 | 0.11 | 0.3 | 0.2 | ru1 | 1.0 | rd2 | 0.15 |
| | 4 | 1.3 | 0.6 | st9 | 3.9 | rd2 | | 0.6 | 0.4 | rd1 | 2.0 | rd2 | |
| Cd | 2 | 0.7 | 0.5 | st3 | 5.3 | sq3 | 0.38 | 0.7 | 0.5 | ru1 | 1.1 | rd8 | 0.46 |
| | 4 | 0.7 | 0.5 | st9 | 3.4 | st8 | | 0.6 | 0.4 | sq1 | 1.4 | st1 | |
| Co | 2 | 1.5 | 0.9 | rd2 | 10.2 | sq3 | 0.43 | 0.6 | 0.3 | rd2 | 0.8 | rd3 | 0.23 |
| | 4 | 2.5 | 1.4 | rd1 | 7.7 | rd6 | | 1 | 0.6 | rd1 | 2.8 | st1 | |
| Cr | 2 | 4.4 | 2.8 | rd1 | 8.9 | rd3 | 1.60 | 2.5 | 1.9 | st4 | 4.2 | rd1 | 2.23 |
| | 4 | 7.9 | 4.3 | st9 | 15.8 | rd4 | | 4.3 | 1.4 | ru1 | 7.6 | st3 | |
| Cu | 2 | 30 | 15 | st9 | 147 | rd4 | 5.54 | 17.1 | 8.9 | rd2 | 35.9 | st3 | 5.42 |
| | 4 | 59 | 22 | ru1 | 250 | rd4 | | 41.4 | 14.8 | ru1 | 105.4 | st3 | |
| Fe | 2 | 2642 | 1278 | sq3 | 5960 | rd4 | 675 | 872 | 548 | rd2 | 1659 | st3 | 363 |
| | 4 | 5002 | 2352 | st9 | 12,182 | rd4 | | 1767 | 774 | st2 | 5999 | st3 | |
| Mo | 2 | 2.4 | 0.9 | rd2 | 13.3 | sq3 | 0.41 | 0.9 | 0.4 | rd2 | 2.1 | rd3 | 0.26 |
| | 4 | 4.5 | 0.7 | ru1 | 11.4 | rd4 | | 2.6 | 0.6 | ru1 | 7.1 | st3 | |
| Ni | 2 | 4.8 | 3.7 | st4 | 7.1 | rd4 | 2.35 | 2.2 | 1.8 | st4 | 4.7 | rd3 | 1.81 |
| | 4 | 4.2 | 2.6 | rd5 | 7.3 | st8 | | 5.6 | 2.2 | st1 | 26.8 | sq1 | |
| Pb | 2 | 76 | 32 | ru1 | 212 | sq3 | 18.87 | 32 | 18.3 | ru1 | 43.9 | st1 | 23.00 |
| | 4 | 133 | 45 | ru1 | 551 | st1 | | 55 | 15.6 | ru1 | 152.7 | st1 | |
| Ti | 2 | 39 | 14 | sq3 | 141 | rd6 | 11.46 | 12.5 | 8.3 | rd1 | 18.9 | sq2 | 8.73 |
| | 4 | 74 | 20 | rd7 | 462 | rd6 | | 25.4 | 7.5 | ru1 | 121.3 | sq1 | |
| V | 2 | 7.1 | 3.5 | sq3 | 14.5 | sq2 | 1.55 | 2.7 | 2.2 | rd2 | 55.2 | st1 | 1.49 |
| | 4 | 10.5 | 4.9 | st9 | 19.6 | sq2 | | 6.2 | 3.2 | sq1 | 10.3 | sq2 | |
| Zn | 2 | 157 | 111 | ru1 | 612 | st8 | 83 | 128 | 95 | ru1 | 182 | rd8 | 99 |
| | 4 | 239 | 113 | ru1 | 1918 | st8 | | 180 | 66 | ru1 | 243 | st4 | |

Table 4
Trace element load factors (LF) in (a) *Sphagnum capillifolium* and (b) *Pseudevernia furfuracea* after 4-month exposure in bags in Naples urban and extraurban sites

(a) *Sphagnum capillifolium*

| | Al | As | Cd | Co | Cr | Cu | Fe | Mo | Ni | Pb | Ti | V | Zn | ∑LF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pk1 | 2.6 | 7.3 | 0.4 | 4.5 | 1.9 | 4.5 | 4.7 | 3.5 | 0.6 | 3.0 | 6.0 | 4.3 | 0.7 | 44.0 |
| st1 | 2.8 | 9.7 | 0.8 | 4.9 | 3.2 | 7.6 | 5.0 | 6.2 | 0.8 | 28.2 | 5.5 | 4.9 | 5.3 | 85.0 |
| st2 | 2.2 | 6.6 | 0.5 | 2.9 | 4.0 | 12.3 | 4.9 | 12.2 | 0.8 | 4.3 | 4.1 | 3.8 | 1.3 | 60.0 |
| st3 | 2.4 | 15.8 | 0.8 | 6.1 | 8.2 | 40.8 | 16.0 | 25.6 | 1.7 | 11.1 | 5.1 | 5.8 | 2.5 | 141.9 |
| st4 | 4.4 | 28.2 | 2.6 | 7.8 | 5.3 | 10.5 | 7.9 | 9.5 | 0.5 | 14.6 | 8.8 | 7.7 | 4.7 | 112.4 |
| st5 | 5.5 | 14.1 | 1.1 | 7.0 | 3.9 | 6.1 | 7.8 | 5.6 | 0.8 | 11.1 | 7.3 | 7.9 | 1.9 | 80.2 |
| st6 | 3.5 | 9.5 | 0.5 | 3.6 | 4.3 | 9.6 | 6.5 | 11.1 | 0.8 | 5.7 | 4.6 | 6.3 | 1.7 | 67.9 |
| st7 | 2.4 | 7.2 | 0.5 | 4.5 | 1.9 | 4.6 | 4.2 | 4.5 | 0.6 | 3.3 | 4.7 | 4.0 | 1.2 | 43.6 |
| st8 | 4.5 | 10.8 | 7.9 | 8.2 | 5.0 | 9.7 | 8.3 | 9.9 | 2.1 | 23.9 | 7.6 | 7.1 | 22.1 | 127.0 |
| st9 | 1.3 | 4.5 | 0.3 | 2.3 | 1.7 | 3.5 | 2.5 | 2.5 | 0.8 | 3.2 | 3.5 | 2.2 | 1.2 | 29.3 |
| st10 | 3.8 | 11.6 | 0.6 | 4.9 | 3.3 | 8.6 | 6.4 | 8.1 | 0.7 | 5.6 | 6.5 | 6.3 | 1.9 | 68.4 |
| sq1 | 2.6 | 8.8 | 0.8 | 5.1 | 4.3 | 15.0 | 5.6 | 16.1 | 0.7 | 5.8 | 4.8 | 4.6 | 2.0 | 76.1 |
| sq2 | 3.9 | 15.5 | 1.2 | 6.5 | 6.6 | 21.5 | 10.5 | 15.6 | 2.0 | 10.6 | 6.2 | 11.6 | 3.3 | 115.0 |
| sq3 | 1.8 | 6.3 | 1.0 | 3.5 | 2.6 | 4.9 | 4.7 | 17.5 | 0.7 | 12.6 | 3.4 | 3.6 | 1.1 | 63.6 |
| rd1 | 1.7 | 5.2 | 0.4 | 2.3 | 1.7 | 4.5 | 3.6 | 4.1 | 0.4 | 2.8 | 3.6 | 3.4 | 1.0 | 34.7 |
| rd2 | 2.0 | 34.2 | 1.3 | 3.4 | 2.3 | 6.0 | 4.0 | 2.6 | 0.9 | 2.9 | 4.1 | 4.6 | 1.4 | 69.6 |
| rd3 | 4.7 | 13.0 | 1.1 | 6.3 | 7.0 | 18.4 | 9.6 | 16.1 | 1.2 | 6.1 | 7.5 | 8.5 | 3.4 | 102.8 |
| rd4 | 4.9 | 18.5 | 0.9 | 7.4 | 8.9 | 44.2 | 17.0 | 26.7 | 1.4 | 10.7 | 8.7 | 8.9 | 2.5 | 160.8 |
| rd5 | 4.4 | 11.7 | 0.3 | 2.7 | 1.8 | 3.8 | 5.2 | 4.4 | 0.1 | 2.2 | 5.5 | 5.0 | 0.8 | 48.0 |
| rd6 | 3.9 | 20.7 | 1.6 | 16.9 | 6.3 | 20.4 | 10.1 | 10.8 | 1.4 | 18.5 | 39.3 | 10.5 | 7.6 | 168.0 |
| rd7 | 1.1 | 22.5 | 1.1 | 4.5 | 3.9 | 25.2 | 7.5 | 20.3 | 1.3 | 10.8 | 0.8 | 8.0 | 3.5 | 110.6 |
| rd8 | 4.1 | 10.9 | 0.8 | 4.8 | 4.0 | 11.7 | 7.1 | 10.4 | 0.7 | 6.7 | 6.9 | 7.1 | 1.4 | 76.5 |
| ru1 | 3.1 | 5.8 | 0.5 | 3.7 | 2.1 | 2.9 | 4.2 | 0.8 | 0.7 | 1.4 | 5.1 | 4.3 | 0.4 | 34.9 |

(b) *Pseudevernia furfuracea*

| | Al | As | Cd | Co | Cr | Cu | Fe | Mo | Ni | Pb | Ti | V | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| st1 | 3.7 | 3.0 | 2.0 | 11.0 | 1.0 | 4.5 | 4.8 | 6.3 | 0.2 | 5.6 | 3.5 | 2.6 | 1.5 |
| st2 | 2.8 | 2.1 | 0.4 | 2.7 | 1.1 | 6.6 | 1.1 | 18.8 | 12.2 | 2.0 | 10.4 | 1.2 | −0.3 |
| st3 | 6.2 | 5.3 | 0.3 | 5.4 | 2.4 | 18.4 | 15.5 | 26.2 | 4.6 | 1.4 | 1.7 | 3.4 | 1.3 |
| st4 | 5.2 | 4.7 | 0.5 | 6.4 | 0.7 | 4.8 | 3.9 | 9.2 | 2.1 | 1.4 | 1.9 | 3.5 | 1.5 |
| sq1 | 3.1 | 2.3 | −0.2 | 2.0 | 0.6 | 7.8 | 1.4 | 7.2 | 13.8 | 2.7 | 12.9 | 1.1 | −0.2 |
| sq2 | 3.6 | 2.3 | 0.3 | 4.0 | 1.1 | 9.2 | 6.0 | 11.3 | 3.2 | 1.1 | 1.6 | 5.9 | 1.2 |
| rd1 | 3.9 | 1.8 | 0.2 | 1.7 | 0.3 | 2.5 | 3.3 | 5.4 | 1.6 | 0.2 | 1.4 | 2.6 | 0.5 |
| rd2 | 4.1 | 12.5 | 0.3 | 2.0 | 0.3 | 2.7 | 2.9 | 3.4 | 1.9 | 0.2 | 1.0 | 3.1 | 0.6 |
| rd3 | 3.0 | 2.2 | 0.1 | 3.2 | 1.1 | 10.2 | 5.3 | 10.3 | 0.5 | 2.1 | 3.4 | 3.2 | 0.9 |
| rd8 | 4.1 | 2.8 | 0.5 | 3.3 | 0.9 | 7.3 | 6.1 | 13.9 | 2.1 | 1.3 | 2.2 | 4.4 | 0.8 |
| ru1 | 8.7 | 3.8 | 0.1 | 2.3 | −0.4 | 1.7 | 1.9 | 1.4 | 2.0 | −0.3 | −0.1 | 1.6 | 0.3 |

25 km from Naples in a rural area (ru) in the lowlands of the Volturno river basin. Bags were exposed for 2 and 4 months starting from 3 July 1999. The 2-month exposure corresponds to the dry season and the 4 months include both dry and wet periods. At each exposure site, four moss and/or lichen bags were positioned ~4 m above ground surface. Higher suspension height was adopted at rd1 (8 m) and rd8 (6 m), according to exposure facilities found at both sites. After exposure moss and lichen material was taken off the nylon bags, ground in an agate mill, digested with $HNO_3$, HF, $H_2O_2$ and analysed for Al, As, Cd, Cr, Co, Cu, Fe, Mo, Ni, Pb, Ti, V and Zn by ICP-MS. Reference standard materials (CRM 482 and CTA-VTL-2) were used to check accuracy and precision of the digestion and ICP-MS analysis procedure.

The trace element concentrations in *S. capillifolium* and *P. furfuracea* corresponding to each exposure site were used for the computation of a load factor (LF) defined as the ratio: $LF = (C_{exposed} - C_{original})/C_{original}$, where $C_{exposed}$ is the content of an element in the moss and lichen samples after exposure, while $C_{original}$ is the content of the same element determined in the moss and lichen samples before exposure.

## 2.3. Statistics

Summary statistics were used to obtain the median, minimum and maximum values of data sets. To detect groups of exposure sites with similar concentration patterns, the sites were classified using the software "Syntax 2000" (Podani, 2001). After standardisation, a data matrix of 13 selected metals × 23 monitoring stations and original contents was subjected to hierarchical clustering, using average link (UPGMA) as

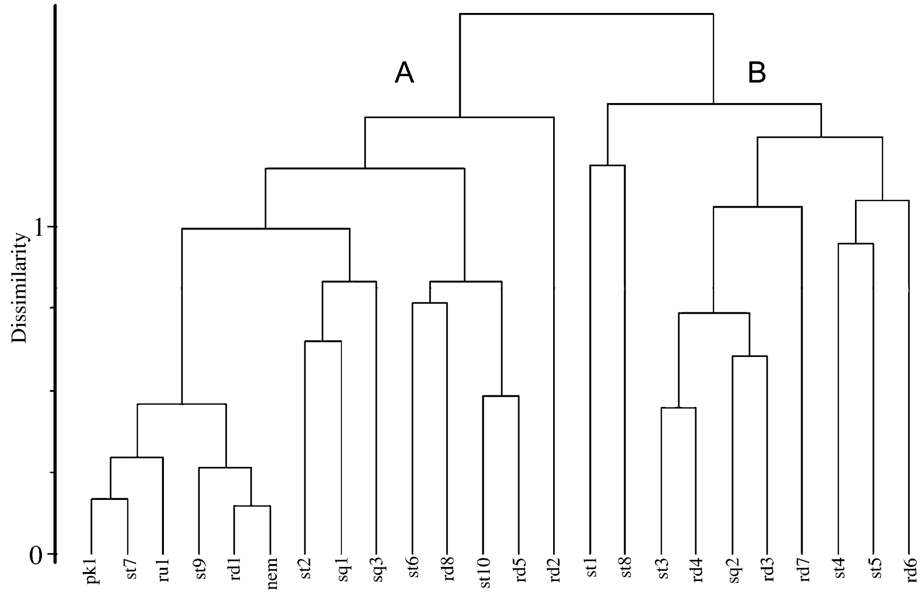


Fig. 2. Classification of exposure sites using average link (UPGMA) as clustering algorithm and Chord Distance as dissimilarity coefficient.

clustering algorithm and Chord Distance as dissimilarity coefficient.

### 2.4. SEM, TEM and EDS analysis

Due to their high trace element content moss and lichen samples from exposure sites st2, st3 and sq2 were selected for analysis by scanning and transmission electron microscopy equipped with energy dispersive spectroscopy (SEM, TEM/EDS). For SEM analysis, samples were cut into small pieces and fixed in 3% glutaraldehyde in 0.065 M phosphate buffer (pH 7.4) for 2 h at room temperature and then dehydrated in an ethanol series. Specimens were subsequently critical-point dried in order to avoid cell collapse. Samples were attached to carbon stubs, coated with carbon film in a sputter-coater and observed at 20 kV with a Cambridge 250 Mk3 scanning electron microscope (SEM) equipped with a Link System AN10000 energy dispersive spectrometer (EDS).

For TEM analysis, after fixation with glutaraldehyde, some samples were post-fixed in 2% $OsO_4$ in 0.1 M phosphate buffer (pH 6.8) at 4 °C, before being dehydrated with ethanol, while others were not post-fixed. After dehydration, samples were embedded in Spurr's epoxy resin. Ultrathin sections (60 nm) were cut with a diamond knife on a Supernova microtome and for osmicated samples sequentially stained at room temperature with 2% uranyl acetate (aqueous) for 10 min and lead citrate (2%) for 10 min. Ultrastructural studies were made using a Philips CM12 transmission electron microscope (TEM) operated at 80 kV and equipped with the above-mentioned EDS. EDS spectra are all from non-osmicated samples and, unless otherwise specified, refer to selected 0.1 $\mu m^2$ acquiring areas.

## 3. Results and discussion

### 3.1. Trace element concentrations

Concentrations of 13 elements were determined in *S. capillifolium* and *P. furfuracea* samples before and after exposure in the Naples urban area. Data presented integrally in a previous paper (Adamo et al., 2003) are reconsidered here with reference to site location in the city environment (Table 3, Fig. 1). In agreement with the literature, Mn and Zn in *S. capillifolium* and Pb in *P. furfuracea* were already high in original material (Bargagli, 1998). After 2 and 4 months of exposure, both biomonitors had significantly higher contents of the majority of the metals than before exposure. Concentrations were generally higher in *Sphagnum* than in *Pseudevernia* indicating the higher interception capacity of the moss for trace elements.

In the city of Naples vehicular traffic is generally considered the main source of trace elements found in exposed biomonitors (Alfani et al., 2000). However, according to recent urban soil surveys, the contribution to the trace element load of abrasion processes of tram- and rail-ways crossing the urban area as well as of the activity of industrial units and oil refinery plants cannot be disregarded (Imperato et al., 2003). In both moss and lichen, values for Pb and Zn, elements typically associated with vehicle traffic, as well for As, Cd, Cr, Cu, Mo and Ti, reach their minimum or show low values at the Castelvolturno extraurban rural site (ru1). Lowest values of many elements also characterise the urban exposure sites rd1 and rd2 (Al, Co, Cr, Cu, Mo, Ti, V) and, only for moss, st9 (As, Cd, Cr, Cu, Fe, V), probably as a result of better air circulation due to the higher position of bag exposure (8 m above street surface) for rd1, and to low vehicular pollution for sites rd2 and st9. Considering only concentrations measured in moss, exposed in all 22 selected urban sites, the largest number of maxima was found in samples exposed in roads (54%), followed by samples hung in squares (23%) and streets (23%). The highest values of Cu and Fe, but also of Cr, Mo and Ni, occur in moss samples from site rd4. This suggests for these elements common emission sources, likely automobile exhausts, but also tyre and tram- or rail-way line abrasion processes. A similar consideration applies to lichen samples exposed in site st3, with a heavy traffic load, but also crossed by tramway, showing highest values of Cu and Fe, along with Cr and Mo. Zinc concentrations show a peak at site st8, where high values of Ni and Cd also occur. The Pb maxima were measured in moss samples at sites sq3 and st1, the second showing highest Pb content also in lichen. High traffic flows and, especially, frequent traffic queues at these sites together with the so far still widespread use in Naples of leaded gasoline could explain these results. Both biomonitors accumulate the highest concentration of As at peripheral urban site rd2, where cultivated lands occur.

Contribution of windblown soil dust to trace element content of exposed biomonitors is suggested by increments of Al, Fe and Ti. These elements have high concentrations downtown in comparison with values in rural areas. Differences between soil nature (volcanic in Naples urban area and sedimentary in the Volturno river basin) and contribution of traffic to iron values, might explain this result. Moreover, at the exposure sites settled at higher altitudes biomonitor trace element accumulation (especially for Pb, Cd and Zn) might be influenced by deposition of long range transported soil and rock dust (Ruhling et al., 1992, 1996).

The amounts of trace elements intercepted by biomonitors during the entire exposure time (post-exposure–pre-exposure content) were compared after normalisation (as LF) by dividing their absolute values by the respective pre-exposure content. The trace element LF values at the exposure sites are reported in Table 4a, for *S. capillifolium,* and Table 4b, for *P. furfuracea*. It may be noted that, with the exception of Al and Ni, LF values are higher in moss than in lichen, which is why most values of Cd, Cr and Zn are below unity. All the elements probably due to human activities, i.e., As, Co, Cr, Pb, V and Zn (only in moss), Cu, Fe, Mo, Ni (only in lichen), have values above unity (i.e., they have contents at least twice as high as the original value). On the basis of the mean LF, moss samples are enriched in trace elements in the following order: As = Cu > Mo > Pb > V > Co > Cr > Zn, lichen samples in the order: Mo > Cu > As = Co = Ni > V > Pb.

In this work data are used as a case example to compute a cumulative LF value ($\sum$LF) in order to estimate the trace element load of the exposure sites. Due to the limited number of exposure sites and to the lower capacity of interception of trace elements by the lichen, only the moss data were employed for this calculation (Table 4a). Based on cumulative LF value, all sites in the southern part of the city, at sea level along the coastline, appear to be characterised by higher trace element loads, compared with sites in the hilly inland area.

Classification of sites with different agglomerative multivariate methods gives similar results, producing dendrograms with two main clusters, A and B (Fig. 2). Cluster A includes st, rd and sq sites located far away from the coastline, the urban park (pk1), the rural site (ru1) and the non-exposed moss material (nem).

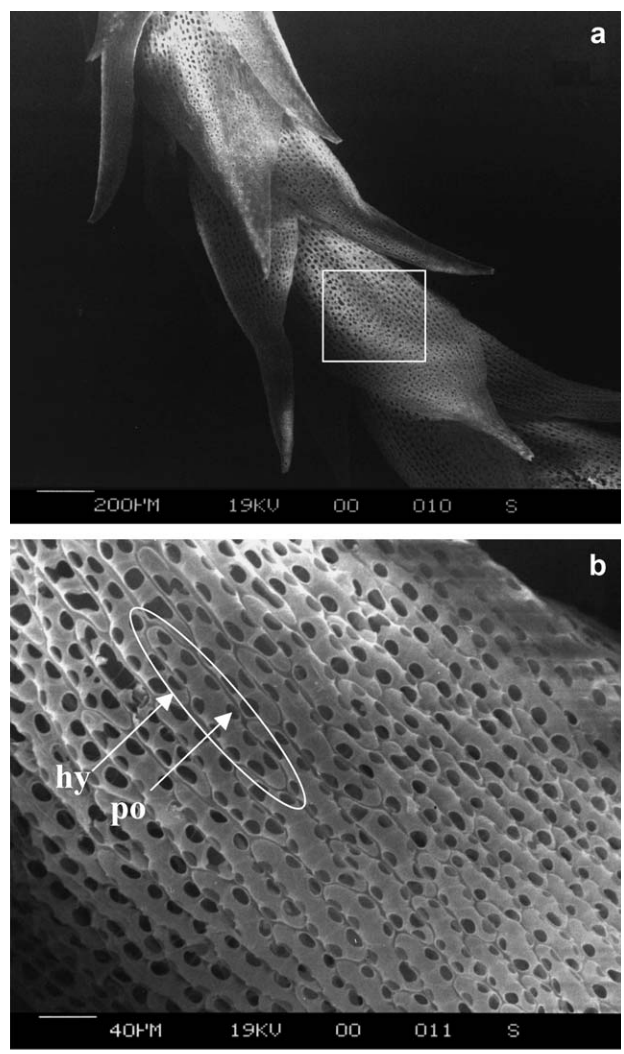


Fig. 3. Scanning electron micrographs of non-exposed *Sphagnum capillifolium* shoot (a) and leaf (b) showing hyalocysts (hy) with large pores (po).

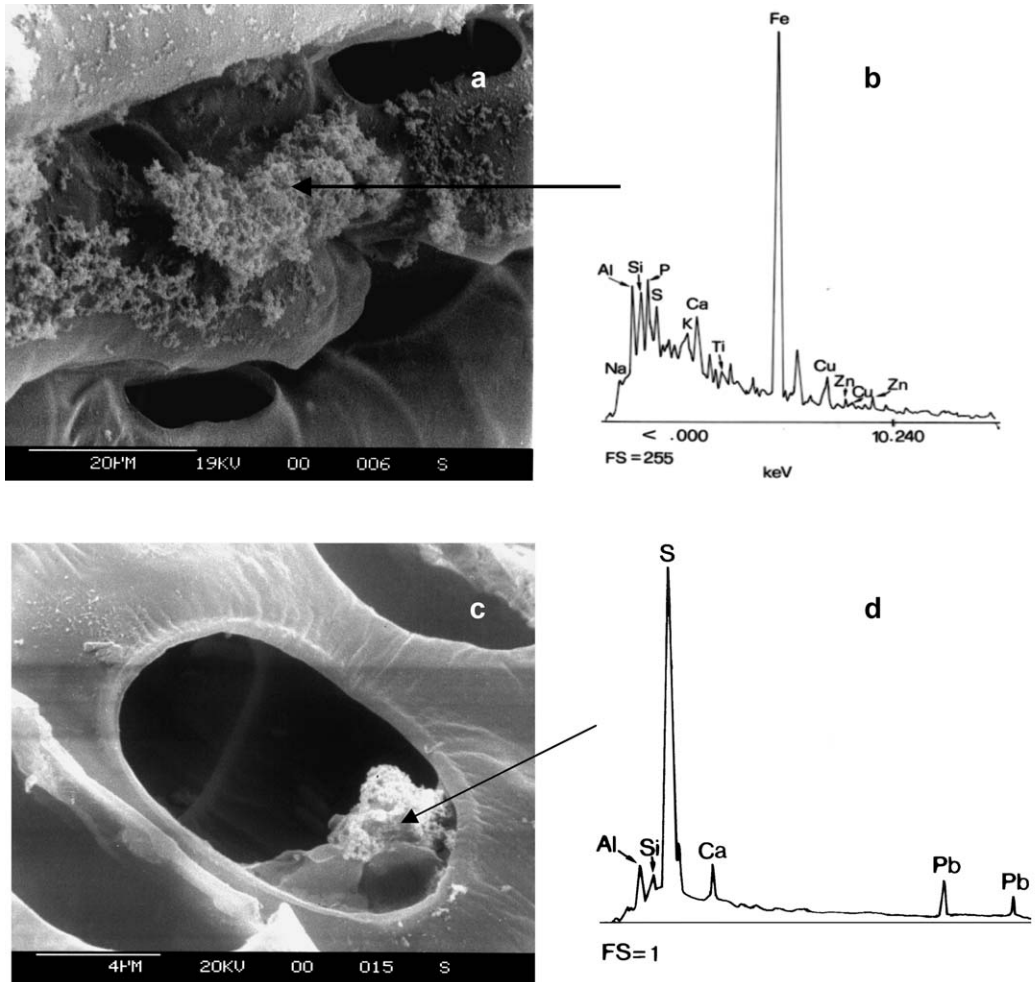


Fig. 4. Scanning electron micrographs (a and c) and EDS spectra (b and d) of particulate matter in 4 month exposed samples of *Sphagnum capillifolium* from site st3.

Cluster B encompasses st, rd and sq sites located close to the coastline.

### 3.2. Submicroscopic and microanalytical analysis

SEM and TEM micrographs and EDS spectra of original and selected exposed samples of *S. capillifolium* and *P. furfuracea* are reported in Figs. 3–9.

Before exposure *Sphagnum* leaves, typically composed of a regular mosaic of chlorocysts and hyalocysts, have externally well-preserved tissues (Fig. 3). Scanning electron micrographs show the peculiar structure of hyalocysts whose large pores can act as a trapping system for airborne particulate. After the 4-month exposure, damage is visible in the external cell walls and abundant heterogeneous particles of some square nanometers are found in aggregates on the surface and inside hyalocyst pores (Fig. 4a–c). X-ray microanalysis shows that chemical composition of the particles is mostly defined by the occurrence of elements such as Fe,

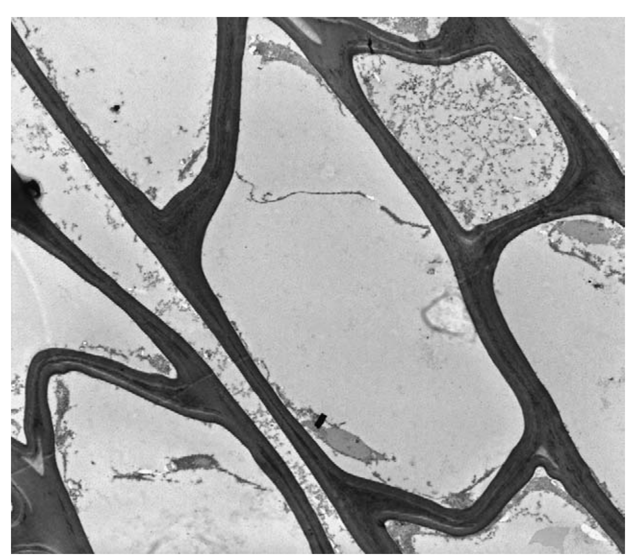

Fig. 5. Transmission electron micrograph of non-exposed *Sphagnum capillifolium* shoot showing dead and almost completely empty cells, ×5000.

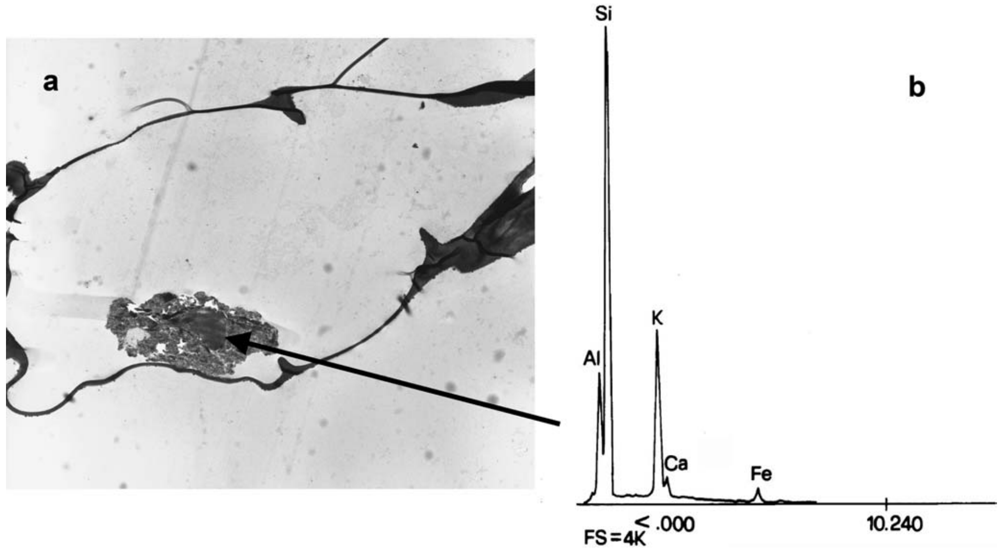


Fig. 6. Transmission electron micrograph of ultrathin section (a), ×3200 and EDS spectrum (b) of mineral particles embedded in hyalocyst of 4-month exposed samples of *Sphagnum capillifolium* from site st2.

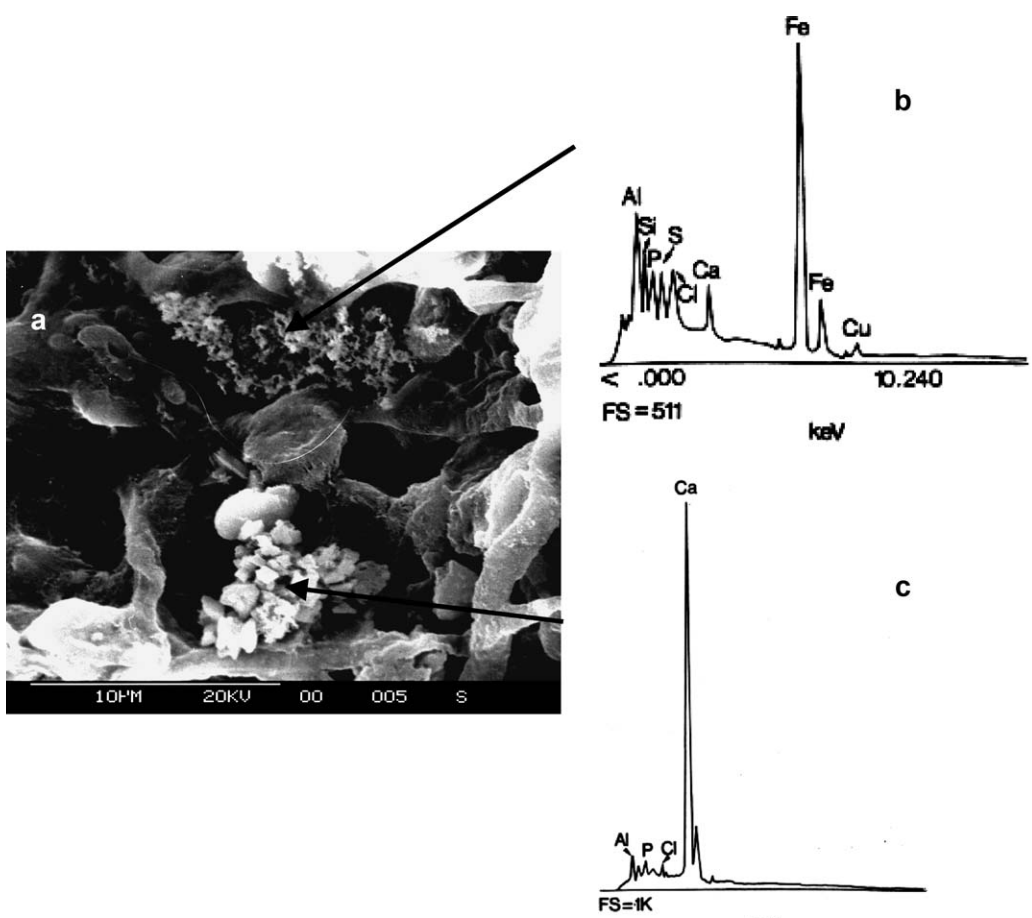


Fig. 7. Scanning electron micrograph (a) and EDS spectra of particulate matter (b) and calcium oxalate crystals (c) in 2 month exposed samples of *Pseudevernia furfuracea* from site st3.

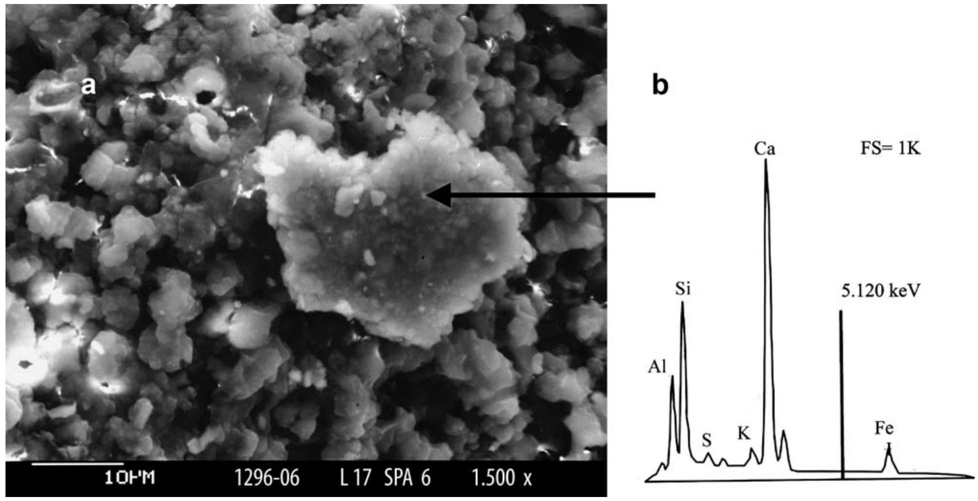


Fig. 8. Scanning electron micrograph (a) and EDS spectrum of particulate matter (b) in 4 month exposed samples of *Pseudevernia furfuracea* from site st2.

Cu, Zn (Fig. 4b) or S and Pb (Fig. 4d). It must be stressed that in light of their small size, particles other than from local sources might also originate far away. TEM observations of pre-exposure *Sphagnum* shoots show the occurrence of numerous dead and empty cells, indicating that moss samples in bags consist mostly of dead material (Fig. 5). In the 4-month exposed samples, mineral particles, mainly made up by Al, Si and K with small amounts of Ca and Fe, are deposited on external cell walls or are embedded in hyalocysts (Fig. 6).

Particulate matter, as well as calcium oxalates, is observed in exposed samples of *P. furfuracea* (Figs. 7a and 8a). Microanalysis of particulate material between the lichen hyphae shows distinct peaks for Fe with which Cu is associated (Fig. 7b) and peaks for Ca, Si and Al with small amounts of S, K and Fe (Fig. 8b). Although the occurrence of Ca oxalate crystals in lichens is typical and even found in organisms living in natural, rural areas, their presence in many exposed lichen samples (Fig. 7a,c) may well be related to oxidative stress induced by the combined effect of pollution, high irradiance and water stress. Indeed, it has been suggested (Modenesi et al., 1998; Caviglia and Modenesi, 1999) that the production of heavy crystalline deposits of calcium oxalate at the surface of lichens *Parmotrema reticulatum* and *Parmelia sulcata* can be linked to exposure of oxidative stress induced by $SO_2$, paraquat and high light intensity. Other particles may be soil dust or originate from exhaust fumes.

Under TEM, in the 4-month exposed lichen samples, besides a well preserved majority of algal and fungal cells, a certain number of cells showing damage referable to prolonged exposure stress can be observed (Fig. 9).

## 4. Conclusions

The significantly increased content of trace elements in *S. capillifolium* and *P. furfuracea* after exposure in bags for 2 and 4 months clearly indicates that the urban area of Naples is affected by atmospheric inputs of elements of both geochemical and anthropogenic origin.

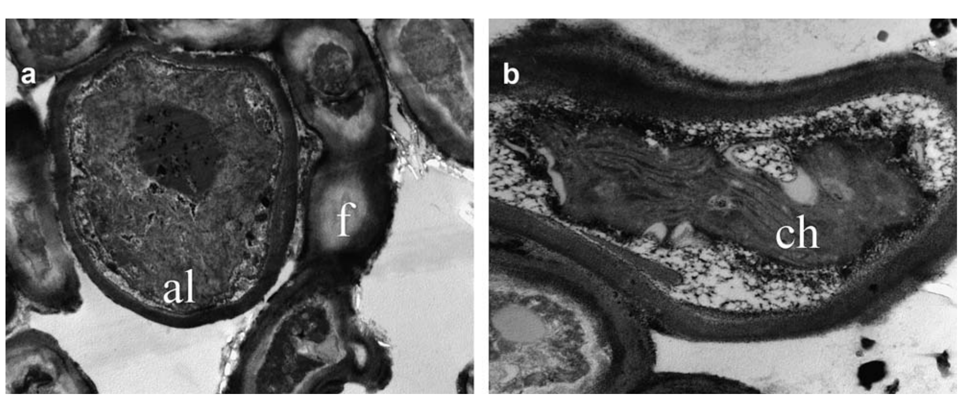


Fig. 9. Transmission electron micrographs of ultrathin sections of 4 month exposed *Pseudevernia furfuracea* from site sq2, showing (a), ×10,000 well preserved algal (al) and fungal (f) cells and (b), ×25,000 cells with altered chloroplast (ch).

In addition, despite the limited number of exposure sites and their random distribution, data from moss and lichen bags discriminate between city areas with different trace element loads. Enrichment in trace metal content found in biomonitors at exposure sites near the coastline is in agreement with what was observed by Imperato et al. (2003) in urban soils of Naples and suggests the use of a biomonitoring network to detect high-risk sites where automatic devices should be located. Presently none of the nine automatic devices placed in the urban area is equipped to measure atmospheric trace element load and is located near the coastline.

Both its south-facing coastline and its proximity to the sea make Naples particularly exposed to the effects of southerly and southwesterly winds. In addition, the hills to the north of the city and Vesuvius to the south-east channel the winds, which blow from and towards the sea, between the mountains. Combined with the presence of local trace element sources near the sea (i.e., high traffic flows, tramway, sea-salt and biogenic emissions from the sea, industry and heavy traffic connected to the harbour), this might explain the enrichment in trace metal content found in biomonitors located in urban sites near the coastline.

Finally, SEM/TEM and EDS observations have added further valuable information concerning the form of metal intercepted by biomonitors. Despite significant damage to moss and lichen tissue and cell integrity after exposure, both organisms are able to efficiently intercept and accumulate airborne trace elements. The recurring presence of particulate matter in moss and lichen indicates that the urban atmosphere is affected by a considerable presence of dust which, according to chemical composition, may be due not only to anthropogenic sources but also to natural sources such as volcanic rock and soil and sea salts.

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