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Comparison of the heavy metal bioaccumulation capacity of an epiphytic moss and an epiphytic lichen

A. Basile [a,*], S. Sorbo [b], G. Aprile [c], B. Conte [a], R. Castaldo Cobianchi [a]

[a] *Dipartimento delle Scienze Biologiche, Università ''Federico II'', Plant Biology Division, via Foria 223, 80139 Napoli, Italy*
[b] *C.I.S.M.E., Università ''Federico II'', via Foria 223, 80139 Napoli, Italy*
[c] *Dipartimento di ARBOPAVE, Università ''Federico II'', Facoltà di Agraria, via Università 100, 80055 Portici (NA), Italy*

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The moss Scorpiurum circinatum has a higher capacity of accumulating heavy metals than the lichen Pseudevernia furfuracea.

Abstract

This study compared the heavy metal bioaccumulation capacity in the epiphytic moss *Scorpiurum circinatum* and the epiphytic lichen *Pseudevernia furfuracea,* exposed in bags for 3 months in the urban area of Acerra (S Italy). The content of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Ti, V, and Zn was measured by ICP-MS. The results showed that both species accumulated all the heavy metals assayed. The moss had the highest bioaccumulation capacity for all metals and showed a more constant and linear accumulation trend than the lichen. Intra-tissue heavy metal bioaccumulation was assessed by X-ray microanalysis applied to ESEM operated in high and low vacuum and ESEM modes.

Keywords: Biomonitoring; ESEM; ICP-MS; *Pseudevernia furfuracea*; *Scorpiurum circinatum*

1. Introduction

Air pollution can be studied effectively by biomonitoring techniques that estimate organism response to air pollutants. Organisms which quantify environmental quality and whose parameters change in response to pollution are called ''biomonitors'': they can be employed as ''data integrators'' since they are able to record the effects of environmental changes in time. One of the main effects of air pollution on biomonitors is bioaccumulation of elements (especially heavy metals and radio nuclides). Although lichens and mosses are completely unrelated groups of cryptogamic organisms, they have a number of features in common. They both occur in almost all terrestrial ecosystems and by virtue of their ability to tolerate long periods of desiccation may even colonize areas which have extreme environmental conditions. Lichen thalli and moss carpets lack root systems or protective waxy cuticles and are built up over a period of time, often years. Epiphytic species depend largely on the atmosphere for their nutrient supply and may show elemental composition which, in an integrated way, reflects the gaseous, dissolved or particulate elements in the atmosphere. Mosses and lichens have been widely used for more than 30 years to study element and radioactive fallout in regional and country-wide surveys. The value of mosses and lichen as trace element biomonitors is largely ascribed to their high surface/volume ratio and high cation exchange properties (Bargagli, 1998).

In polluted urban areas lichens and mosses are often absent. In such cases, transplant techniques have been used to monitor air pollution. One of these techniques consists in exposing bags containing lichen or moss in the studied area to measure concentrations of contaminants affecting the samples (Adamo et al., 2003; Giordano et al., 2005).

The aim of this study was to compare the heavy metal bioaccumulation capacity in the epiphytic moss *Scorpiurum circinatum* (Brid.) Fleisch. & Loeske and the epiphytic lichen

* Corresponding author. Tel.: +39 081 2538508; fax: +39 081 450165.
E-mail address: adbasile@unina.it (A. Basile).

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Pseudevernia furfuracea (L.) Zopf. exposed in bags in the urban area of Acerra (S Italy).

2. Materials and methods

P. furfurcea is a fruticose, epiphytic lichen, widespread in Europe, and naturally occurring near the study area. *S. circinatum* is a widespread epiphytic moss in the Mediterranean area. Lichen thalli and moss gametophytes were collected from remote sites on Mt. Faito (province of Naples) at 1200 m a.s.l. Reference samples were stored in the Herbarium of the Botanical Gardens of the University of Naples Federico II.

2.1. Study area

Acerra is a densely populated town of approximately 45,000 inhabitants located 14 km N of Naples. The area between Acerra, Marigliano and Nola has been called the ''triangle of death'' because of the dramatic increase in tumours (Mazza and Senior, 2004). Data from the meteorological station at Capodichino airport (Naples) indicate that the climate is Mediterranean, with a mean monthly rainfall of 140 mm in December and 40 mm in August, and a mean monthly temperature of 8.5 °C in January and 24.1 °C in August.

2.2. Lichen and moss bags

Homogeneous specimens were obtained by carefully mixing the collected material. Subsequently the specimens were cleaned of soil particles and underwent seven consecutive washings with distilled water (50 mL), each washing lasting 5 min; 0.75 g of lichen thalli or moss gametophytes were placed in nylon mesh bags (10 × 10 cm wide, with 1 mm^2 mesh) and closed with a nylon thread. The bags were exposed in the town centre along the eight urban streets with the highest traffic flows; further three sites were selected on the Mt. Faito as controls. Bags were exposed for 3 months at 4 m above ground, attached to plastic sticks (1 m long, 1.5 cm in diameter), far away from drain pipes.

Three moss and three lichen bags were exposed at each site to obtain monthly samplings. During the exposure period, bags were sprayed weekly with 10 mL distilled water to avoid dehydration and to favour bioaccumulation of pollutants. One bag per month was retrieved at each site for chemical analyses.

2.3. Chemical analysis

After collection, the lichen and moss material (three samples each time) was cleaned from soil particles, dried at 105 °C for 24 h and homogenized to a fine powder in an agate mortar. To measure metal concentration, 125 mg of homogenized sample were mineralized in Teflon vessels by a microwave oven (Milestone mls 1200 mega) to 500 °C using a fixed programme (250 Watt for 1′, 0 Watt for 1′, 250 Watt for 5′, 350 Watt for 5′ and 450 Watt for 5′) with 6 mL of 65% HNO_3, 2 mL of 39% H_2O_2 and 0.2 mL of HF. The digested material was diluted in distilled water and analyzed by inductively coupled plasma-mass spectrometry (ICP-MS, Perkin Elmer Elan 600) for As, Cd, Cr, Pb, V, Cu, Zn, Fe, Al and Mn content. Metal contents were assayed in triplicate and measurements were repeated three times; all concentrations were expressed on a dry weight basis. Reliability of results was checked by analyzing the Standard Reference Materials CRM 482 (*P. furfuracea*) and CTA-VTL-2 (tobacco leaves).

2.4. Light microscope observations

Light microscope observations were made on three moss gametophytes and three lichen thalli each time (just after collection, with no preparation at all) and toluidine blue stained semi thin sections from TEM prepared samples, before exposure and after 1, 2 and 3 months. Percentage of dead cells from the total number within the moss leaflet and the lichen thallus was calculated by light microscopy: highly plasmolysed, totally empty and collapsed cells were regarded as dead. In each sample 100 cells were observed.

2.5. X-ray SEM microanalysis

Qualitative X-ray microanalysis (EDS) was carried out with an energy dispersive spectrometer (EDAX Genesis 2000i) applied to a FEI Quanta 200 environmental scanning electron microscope (ESEM). EDS microanalysis was carried out in three microscope operating modes: high vacuum, low vacuum and ESEM modes, allowing a comparison of microanalysis data from prepared samples (high vacuum) (Basile et al., 1994) and unprepared ones, both as air dried (low vacuum) and hydrated (ESEM). High vacuum mode, the conventional SEM mode, was operated on samples prepared according to the following protocol. Samples were fixed with 2.5% glutaraldehyde in phosphate buffer (0.065 M, pH 7.2–7.4) for 1.5 h at room temperature, then dehydrated with ethanol, critical point dried, and mounted on carbon stubs, covered with a 15 nm thick carbon film and observed in high vacuum operating mode. ESEM mode was operated on samples with no preparation. Hydrated moss and lichen were directly mounted on carbon stubs and observed with no carbon film covering. Low vacuum mode was operated on samples air-dried and with no other preparation. After air-drying, moss and lichen were mounted on carbon stubs and observed with no carbon covering. Operating parameters were as follows: accelerating voltage 30 kV, working distance (WD) 10 mm, circular spot diameter 0.5 mm, and take off angle 35°. Spectra were collected over a time of 100 s; the mean count rate was 1000–1500 counts/s. Spectra were collected both in full frame and spot mode. Microanalysis was carried out on 3-month exposed samples at each site: 10 lichen thalli and 10 moss gametophytes per site were analyzed each time.

2.6. Statistical analysis

The raw concentration data were used to calculate exposed-to-control (EC) ratios that were interpreted according to the scale of accumulation/loss suggested by Frati et al. (2005). Significance of differences was checked by one-way analysis of variance (ANOVA).

3. Results

Control samples exposed at Mt. Faito site did not show any significant change in element content throughout the whole exposure period ($P > 0.05$), and were thus merged. After 1-month of exposure, the content of most elements was already significantly higher ($P < 0.05$) than before exposure for both biomonitors (Table 1). Element concentrations were generally higher in *S. circinatum* than in *P. furfuracea*. The increase in elements in the moss during exposure was constant and linear, while lichen showed a more discontinuous trend, especially for ions more likely to be leached (e.g. Cd). EC ratios of the moss were statistically higher ($P < 0.05$) than those of the lichen, being on the whole 65% higher (Fig. 1). Pb was the element most highly accumulated in both organisms and Fe the least. The EC ratios indicated severe accumulation for all elements in the moss, while only for Cu and Pb in the lichen, and accumulation for all remaining elements.

One-month exposed lichens showed both symbionts suffering, but algae were more damaged. Three-month exposed lichen showed increased dead algal cells (55% ± 10%) and seriously suffering live cells. Conversely, dead fungal cells were 22% ± 15%. By contrast, 1-month exposed moss showed only 1% of dead cells, reaching 5% ± 2% after 3 months.

Unexposed *P. furfuracea* and *S. circinatum* samples showed X-ray microanalysis spectra with Si, Al and Fe peaks, probably due to soil contamination, and no other heavy metal peaks in any operating mode (high vacuum, low vacuum and ESEM