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# Heavy Metal Accumulation in the Lichen *Evernia prunastri* Transplanted at Urban, Rural and Industrial Sites in Central Italy

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**Abstract.** The lichen *Evernia prunastri* has been employed for biomonitoring the atmospheric deposition of heavy metals at urban, rural and industrial sites in Central Italy. Lichen samples have been collected in a control site 1500 m a. s. l. (Parco Nazionale d'Abruzzo, Central Italy) and subsequently transplanted at urban site (Cassino city center), at rural location 7 km away from Cassino (S. Elia Fiumerapido) and at industrial location (Piedimonte S. Germano) surrounding an automobile factory. Once defined the surface of impact relevant to this work, the lichen samples were transplanted at the four cardinal points of each site. Studies of bioaccumulation of Pb, Cd, Cr, Cu and Zn in lichen samples were performed five times at regular intervals between November 2000–December 2001. Microwave digestion of the samples and graphite furnace atomic absorption spectrometry were employed for the heavy metal determinations. Suitable certified reference materials (CRM) were used for validation of the analytical methodology. Results showed the ability of *Evernia prunastri* to accumulate the heavy metals under study. As expected, the area chosen as control site showed significantly (Friedman test, cluster analysis) lower impact in comparison to the other sites and the rural site showed smaller impact than the urban and the industrial sites.

**Key words:** biomonitoring, *Evernia prunastri*, heavy metals, lichens, transplant method.

## 1. Introduction

The use of living organisms to monitor environmental quality has been notably developed during the last years. Lichens are the most studied biomonitors of air quality because of their sensitivity to various environmental pollutants. They are excellent bioaccumulators of trace elements as well, since the concentrations found in their thalli can be directly correlated with those present in the environment

(Herzig, 1993; Wolterbeek, 2002). They provide low-cost information on the nature of relative spatial and temporal deposition patterns of contaminants (Nash and Wirth, 1988).

Over the last 20 yr lichens have been widely employed for the assessment of trace metals impact in the atmosphere in urban and industrial sites (Conti and Cecchetti, 2001). In this study the lichen *Evernia prunastri* was transplanted into urban, rural and industrial sites in Central Italy. The transplant method has the great advantage of being applicable in areas where there are no suitable substrata or in areas that are unsuited to lichen survival due to high pollution levels (Conti and Cecchetti, 2001; Conti, 2002).

The present survey was designed to evaluate the ability of *Evernia prunastri* to accumulate Pb, Cd, Cr, Cu and Zn in different sites with different environmental impacts in a one-year period.

## 2. Materials and Methods

*Evernia prunastri* (L.) Ach. is a widely spread lichen with an extensive ecological amplitude and it is one of the most common epiphytic lichens. It has been used in various studies of heavy metal accumulation in transplants studies (Deruelle, 1992; Bartoli *et al.*, 1994; Caniglia *et al.*, 1994; Cercasov *et al.*, 2002) that show its good ability for bioaccumulation.

Lichen samples were collected in the National Park of Abruzzo (Central Italy) at a control site 1500 a. s. l. high and subsequently transplanted at an urban site (Cassino city center on the roof of the town hall) and at a rural location 7 km away from Cassino (S. Elia Fiumerapido) and at industrial location (Piedimonte S. Germano) surrounding an automobile factory (see Figure 1).

After collection and upon arrival at the laboratory, 22 lichen samples were dried for 48 h at 35 °C to constant weight and sorted to remove as much extraneous material (i.e. soil particles, mosses, other lichen species, etc.) with nylon tweezers under a binocular microscope. Then, the samples were pulverized by grinding in a mill with Teflon balls and then mineralized in a microwave oven (MDS 81D CEM, Cologno al Serio, Italy) after 12 h of cold premineralization with ultrapure concentrated $HNO_3$ Merck Suprapur (Darmstadt, Germany). These samples of the control site were analyzed to obtain the initial element contents, that is before exposure.

The dry weight calculation (20 replicates) was carried out by oven drying at 105 °C until constant weight. No sample washing was performed because the different strategies may cause relevant changes in metal contents (Bettinelli *et al.*, 1996; Wadleigh and Blake, 1999; Conti and Cecchetti, 2001; Conti *et al.*, 2002).

Lichen samples with a mass ranging between 30 and 40 g were filled in nylon bags and appropriately fixed 2.0 m above the ground at the four cardinal points of the defined area for each site. Care was taken to choose thalli at a similar stage of development. The standard size of the assayed samples was around 4 cm ($4 = \pm 0.4$ cm)

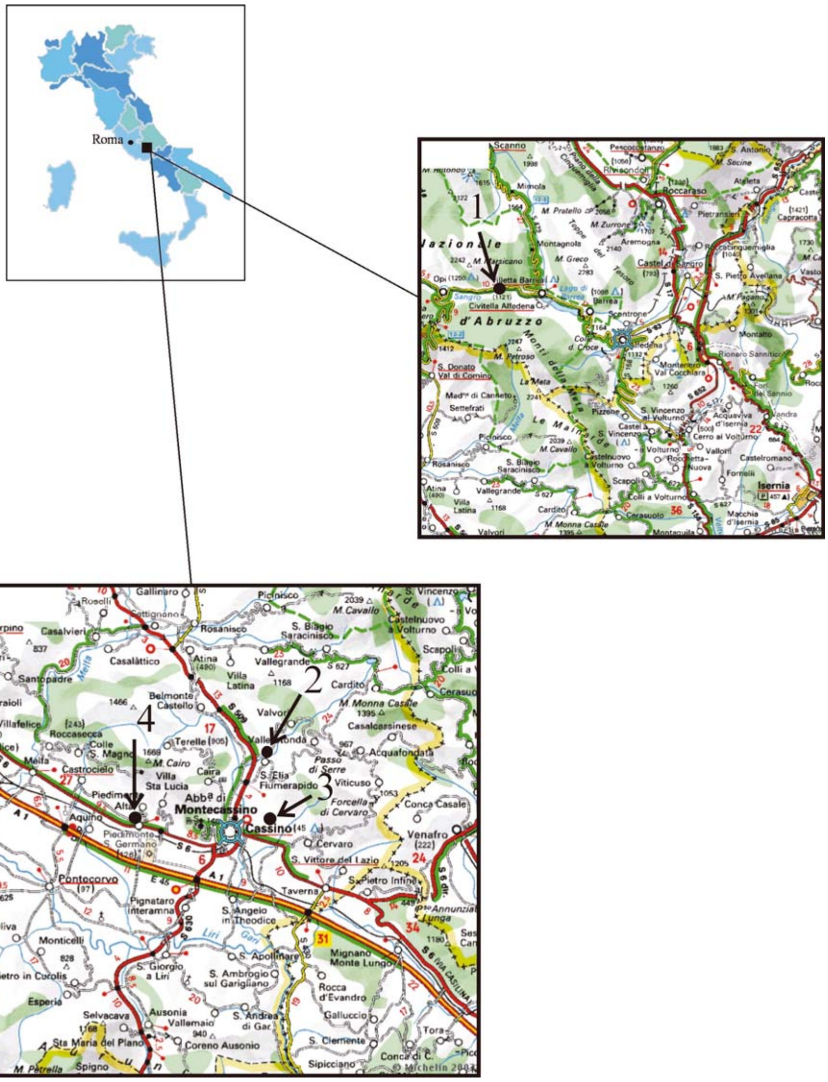



*Figure 1.* The study area: (1) National Park of Abruzzo Region; (2) Rural site (S. Elia Fiumerapido); (3) Urban site (Cassino city); (4) Industrial site (Automobile factory – Piedimonte San Germano).

as there is evidence that metal concentrations in *Evernia prunastri* increase with the lichen size (Senhou *et al.*, 2002). This indicates the importance of using biomonitors of the same size in the monitoring programs.

Each sample collected represents a pool of 4 equal subsamples originated from each cardinal point of each site. The exposure period began at December 2000. From January to December 2001 five samples of thalli were collected and subsequently treated as described above.

*Table I.* Results obtained for Zn, Cu, Pb, Cd and Cr in certified environmental reference materials ($n = 4$, mean ± S.D.), all values in $\mu$g/g dry weight

| | *Ulva lactuca* (BCR-279) | | *Apple leaves* (SRM 1515, NIST) | |
|---|---|---|---|---|
| Element[a] | Certified value | Obtained value | Certified value | Obtained value |
| Zn | 51.3 ± 3.9 | 52.4 ± 2.9 | 12.5 ± 0.3 | 11.73 ± 0.77 |
| Cu | 13.14 ± 1.28 | 13.28 ± 0.23 | 5.64 ± 0.24 | 5.34 ± 0.60 |
| Pb | 13.48 ± 0.93 | 12.60 ± 0.60 | 0.470 ± 0.024 | 0.460 ± 0.070 |
| Cd | 0.274 ± 0.065 | 0.256 ± 0.070 | 0.013 ± 0.002 | <0.020 |
| Cr | 10.7[b] | 9.9 ± 0.5 | 0.3[b] | 0.35 ± 0.05 |

[a]LODs in solutions after digestion: Zn (FAAS) 0.010 mg/l; Cu (FAAS) 0.020 mg/l; Cd (GFAAS) 0.0002 mg/l; Pb (GFAAS) 0.001 mg/l; Cr (GFAAS) 0.0005 mg/l. FAAS: Flame atomic absorption spectrometry; GFAAS: Graphite furnace atomic absorption spectrometry.
[b]Not certified, only as indicative value.

Heavy metal levels were determined by atomic absorption spectrometry (Shimadzu AA 6800, Japan) with electrothermal atomization employing graphite furnace atomizers (GFAAS) or flame atomizers (FAAS) depending on the analyte concentration in each sample. Mostly Cu and Zn determinations were performed by FAAS.

The accuracy of measurements was tested through the employment of suitable certified reference materials. All results were in very good agreement with certified values (95% confidence level) and their standard deviations values showed that good precision was attained for all the measurements (see Table I).

## 3. Results and Discussion

Table II reports the heavy metals contents of 22 samples of *Evernia prunastri* (mean ± S.D.), which were tested for comparative purposes before exposure. These results, with the exception of Pb, are in the same order of magnitude as those reported by Cercasov *et al*. (2002) in *E. prunastri* from a mountain site used as control (we assume these data are reported in $\mu$g/g wet weight). The differences found between both set of data are probably because the lichens studied by Cercasov *et al*. (2002) had been washed previously.

Once verified the lack of conditions needed for performing the ANOVA test (normality of the distribution and homogeneity of variances), the nonparametric Friedman test was employed. This test compares three or more paired groups of data and is equivalent to the variance analysis for repeated measurements. It can be used, for instance, when each subject undergoes several treatments. Since it is a nonparametric test, no hypothesis regarding the form of the underlying distribution is required.

*Table II.* Heavy metal concentrations ($\mu$g/g dry weight) in the lichen *Evernia prunastri* before exposure[a]

| Sample number | Cd | Cr | Cu | Pb | Zn |
|---|---|---|---|---|---|
| 1 | 0.096 | 1.73 | 3.84 | 2.99 | 44.8 |
| 2 | 0.074 | 1.59 | 3.85 | 2.81 | 28.1 |
| 3 | 0.083 | 1.58 | 3.90 | 1.99 | 41.6 |
| 4 | 0.082 | 1.33 | 4.45 | 2.22 | 41.7 |
| 5 | 0.081 | 1.39 | 4.25 | 2.60 | 34.7 |
| 6 | 0.083 | 1.41 | 4.21 | 2.61 | 30.5 |
| 7 | 0.072 | 1.09 | 2.28 | 1.61 | 23.5 |
| 8 | 0.067 | 1.91 | 3.67 | 1.44 | 37.6 |
| 9 | 0.073 | 1.27 | 2.81 | 1.90 | 26.3 |
| 10 | 0.073 | 1.60 | 3.04 | 1.52 | 53.2 |
| 11 | 0.083 | 1.85 | 3.03 | 2.02 | 27.5 |
| 12 | 0.096 | 2.34 | 3.67 | 2.71 | 33.8 |
| 13 | 0.056 | 1.67 | 3.12 | 1.43 | 25.5 |
| 14 | 0.071 | 1.76 | 3.22 | 1.91 | 25.3 |
| 15 | 0.094 | 1.96 | 4.07 | 2.06 | 32.5 |
| 16 | 0.087 | 1.87 | 3.91 | 2.18 | 30.7 |
| 17 | 0.083 | 2.81 | 3.68 | 3.62 | 31.1 |
| 18 | 0.080 | 2.04 | 3.98 | 3.52 | 28.1 |
| 19 | 0.063 | 1.63 | 2.40 | 2.65 | 24.8 |
| 20 | 0.053 | 1.69 | 2.30 | 2.19 | 22.2 |
| 21 | 0.053 | 1.04 | 2.01 | 1.05 | 25.3 |
| 22 | 0.066 | 1.54 | 1.94 | 2.25 | 20.3 |
| Mean | 0.080 | 1.69 | 3.35 | 2.24 | 31.3 |
| s.d. | 0.010 | 0.40 | 0.77 | 0.66 | 8.2 |

[a]Relative standard deviation (RSD) ($n = 5$) (%): Cu = 2.1; Zn = 1.5; Cd = 5.0; Pb = 4.3 and Cr = 6.1.

The test is a rank based procedure and it is based on the order number of the observations rather than on the actual observations. Table III reports, as an example, the ranks for each one of the five periods taken into consideration with relation to the copper industrial site. The Friedman test is used to determine whether the differences between these ranks are statistically significant.

In the example reported (Cu industrial site) Table III shows that the value of the chi-square statistic is 20.0 with a significance of 0.00. Therefore, it can reject the null hypothesis which assumes that there is no significant difference among the concentration of copper over the months under study.

*Table III.* Friedman test and ranked measures for the Cu in the industrial site

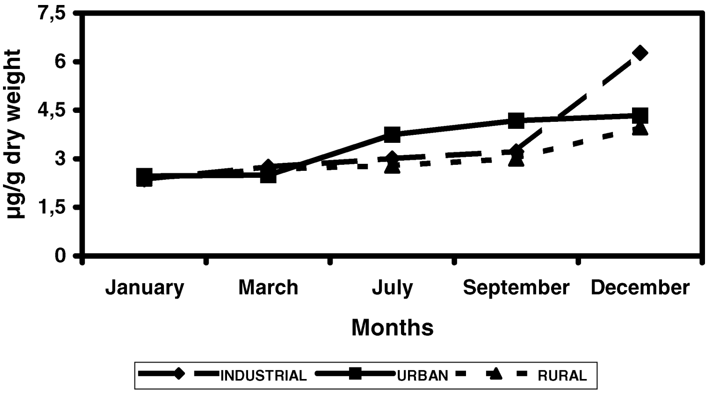
| | |
|---|---|
| Friedman test: Industrial site—Cu | |
| *N* | 5 |
| Chi-square | 20.000 |
| df | 4 |
| Sig. Asint. | 0.000 |
| Ranked measures for industrial site—Cu | |
| | Rank mean |
| January | 3.00 |
| March | 2.00 |
| July | 4.00 |
| September | 5.00 |
| December | 1.00 |

The results of the Friedman test for all sites and periods under study indicate statistically significant differences among the metal levels detected in *Evernia prunastri*. The mean values calculated can therefore be effectively compared and their rate over time can be analyzed since the results obtained are not just the outcome of random chance.

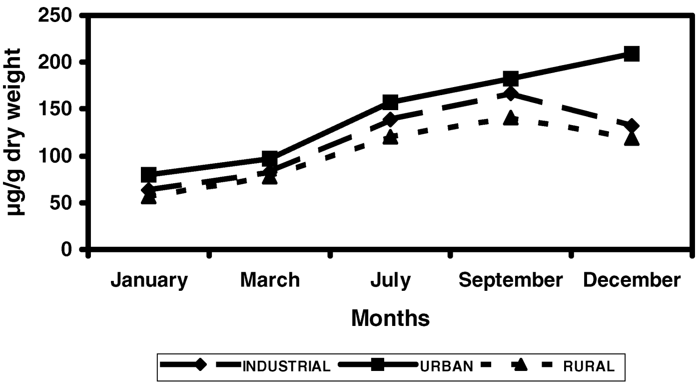
Figure 2 shows the accumulation values ($\mu$g/g dry weight) vs elapsed time (months) for each one of the heavy metals studied in each location. Table IV shows the percentage of variation of the mean metal concentrations in *Evernia prunastri* in the sites and sampling periods of analysis.

From the analysis of data it is evident that in relation to cadmium, a not really homogeneous rate occurs in the three sites considered since the maximum concentration values were reached in September 2000. The increase of Cd concentration in the industrial site is detectable only during the hottest months of the year, that is March–September. On the other hand, a general decrease of Cd concentration is detected over the September–December period in the three sites under scrutiny. However, the levels of Cd in the rural site are always lower than in the other sites (with the exception of the control site).

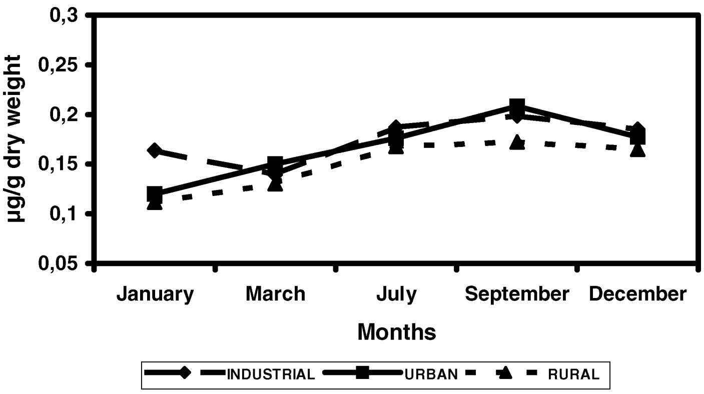
Regarding lead, a notably high concentration peak can be detected in the industrial site in September with a significant increase between March–July (+79%) and July–September (+22%) (see Table IV). In the urban site, the rate of mean concentrations of lead turns out to be growing, with an increase rate greater than in the first trimester (+38%). The constant increase of Pb concentrations in this site (Cassino city) is probably justified by the great amount of metal deriving from exhaust gases. On the other hand, the rural site shows a constantly growing rate of lead levels detected in the lichens, especially over the March–July period (+33%). Mean levels are lower in this site (see Figure 2(d)) than in the other sites, which




(a)

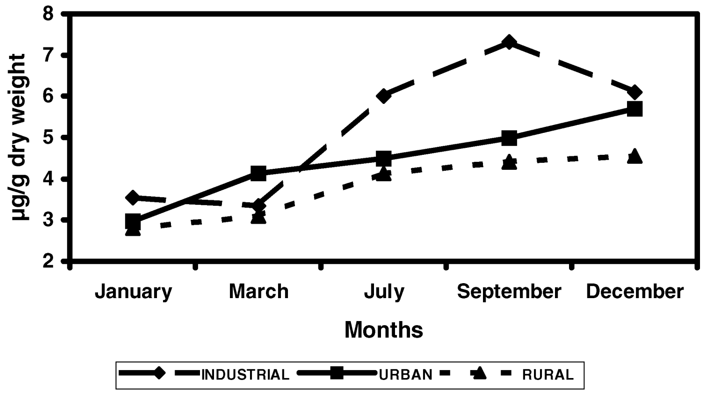



(b)

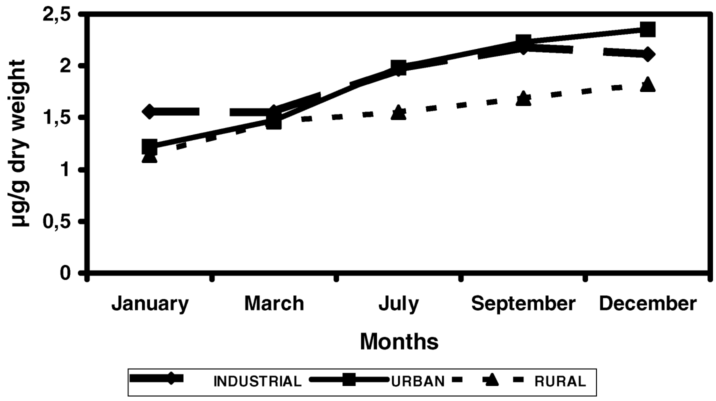



(c)

*Figure 2.* (a) Cu concentrations in the lichen *Evernia prunastri* at the sites studied; (b) Zn concentrations in the lichen *Evernia prunastri* at the sites studied; (c) Cd concentrations in the lichen *Evernia prunastri* at the sites studied; (d) Pb concentrations in the lichen *Evernia prunastri* at the sites studied; (e) Cr concentrations in the lichen *Evernia prunastri* at the sites studied. (*Continued on next page*).




(d)




(e)

*Figure 2.* (Continued.)

confirms that the rural site is that with the smallest impact (exception made of the control site).

Chromium shows an ever-growing rate in the urban and rural sites; in the urban site the highest increase is in March–July (+34%). In the industrial site, chromium levels increase over the March–July (+27%) and July–September periods (+10%) (Figure 2(e) and Table IV). As expected, the rural site shows the lowest metal levels in the biomonitor.

Mean levels of copper show a constant increase rate in all sites. Over the period March–July, the urban site shows a significant increase (+50%) (Figure 2(a) and Table IV). During the following two periods (March–July and July–September), mean levels in the urban and industrial sites are higher than in the rural site, moreover the urban site shows greater levels than the industrial one. Finally, in the industrial site a definitely high peak is detectable during the last period (+94%).

In the three sites under study, zinc shows a homogeneous and growing rate up to the third period (industrial > urban > rural) (Figure 2(b) and Table IV). On the

*Table IV.* Percentage variation on metal concentrations in *Evernia prunastri* in the periods and sites studied (year 2001)

| | Industrial | Urban | Rural |
|---|---|---|---|
| | Cu | | |
| January–March | 16.3 | 1.2 | 11.8 |
| March–July | 9.4 | 50.2 | 4.6 |
| July–September | 7.1 | 11.4 | 7.6 |
| September–December | 94.4 | 3.7 | 32.0 |
| | Zn | | |
| January–March | 30.5 | 21.7 | 37.0 |
| March–July | 67.5 | 61.2 | 54.0 |
| July–September | 20.1 | 16.3 | 17.5 |
| September–December | −20.7 | 14.5 | −15.7 |
| | Cd | | |
| January–March | −14.6 | 25.0 | 16.6 |
| March–July | 33.9 | 17.5 | 28.5 |
| July–September | 6.1 | 18.0 | 2.5 |
| September–December | −6.8 | −14.9 | −4.0 |
| | Pb | | |
| January–March | −5.4 | 38.8 | 10.7 |
| March–July | 79.1 | 8.4 | 33.4 |
| July–September | 22.0 | 11.2 | 6.5 |
| September–December | −16.7 | 14.3 | 3.3 |
| | Cr | | |
| January–March | −0.6 | 20.5 | 28.0 |
| March–July | 27.1 | 34.7 | 6.2 |
| July–September | 10.7 | 12.6 | 9.0 |
| September–December | −3.2 | 5.4 | 7.7 |

other hand, during the last period (September–December) there is a decrease of the mean levels in the industrial site as well as in the rural site. Also for Zn the rural site shows the lowest metal levels when compared to the other two sites.

Since no specific data on the background level of these atmospheric contaminants in the sites under scrutiny are available, concentration factors (CFs) were evaluated: They were calculated as the ratio between the mean concentration of metals in the biomonitor at the end of this study (December 2001) and the mean concentration of contaminants found at the control site (before exposure, see Table II).

Percentage variations in CFs over time are reported in Table V. Data underline the outstanding bioaccumulation ability of *E. prunastri*, and, as a consequence,

*Table V.* Percentage variation on metal concentrations stated as the end of this study (December 2001) vs Control site (December 2000)

| Sites | Cu | Zn | Cd | Pb | Cr |
|---|---|---|---|---|---|
| Industrial | 84.9 | 376 | 153 | 187 | 45.1 |
| Urban | 27.7 | 654 | 142 | 168 | 61.6 |
| Rural | 17.2 | 329 | 126 | 114 | 25.1 |

its aptitude to be used in biomonitoring studies. It is generally observable that the rural site shows smaller increases in comparison to the industrial and the urban sites. These data confirm the hypothesis that the rural site is that with the lowest impact. Zn concentrations turn out to have a higher increase rate in the urban site than in the control site.

However, it must be noted that climatic factors, which can be highly changeable (especially rain), might have a bearing on the ability of lichens to bioaccumulate metals (Conti and Cecchetti, 2003).

To find common characteristics in the studied sites, a cluster analysis was carried out. Through this statistic technique it was tried to classify the sites according to the mean levels of the metals considered simultaneously. The aim was to verify if there are homogenous site groups with respect to the mean level of the metals under study. Several hierarchical methods of analysis of the groups were employed, particularly, dendrograms related to the Single Linkage method and to the Ward method (Figure 3).

From Figure 3 we can observe the existence of three site groups. The first is composed by the industrial and the urban sites and shows the highest impact. In the second group we find the rural site, which can therefore be considered the lowest impact site when compared to the industrial and the urban ones. Finally, the third group is composed by the control site which, as it is evident from the dendrograms, is totally separated from the other groups.

This validates that

- as expected, the area chosen as control site has a significantly lower impact in comparison to the other sites;
- the rural site shows a smaller impact than the urban and the industrial sites;
- the urban and the industrial sites show a relatively similar impact and therefore there is no dominance of one over the other with respect to the analyzed metals.

Despite the strong dematerialization occurring in the automobile sector for the benefit of environmental impact and despite the fact that metallic materials are being replaced in many industries, it is still necessary to pay more attention to the environmental impact that can be brought about by the use of materials or the production of wastes containing heavy metals. Data reported by Senhou *et al.* (2002) for *E. prunastri* for Cu and Pb (we assume these data are reported in $\mu$g/g wet

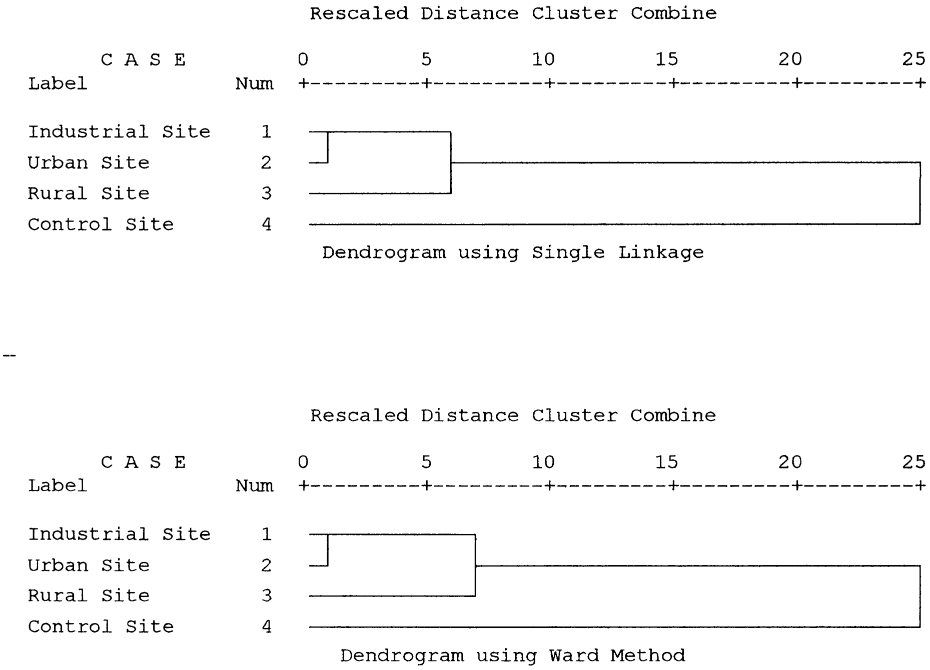



*Figure 3.* Classification (cluster analysis) of the sites studied.

weight) in an industrial and highly contaminated site are higher than data reported in this study. Our Zn levels in the industrial site are similar or higher than that reported by the same author.

Moreover, remarkable is the increase of Pb amounts in lichens transplanted into the urban site in comparison to the control site (+168%); this can be attributed to the impact of exhaust gas in Cassino city center.

*Evernia prunastri* has a good ability for biomonitoring purposes using transplant method. A very important step, as cited above, is to eliminate the effect of the biomonitor size on the elements concentrations. In fact, for most elements, the levels increase with the lichen size (Senhou *et al.,* 2002). It must be observed that when trace metal levels of biomonitors from different areas are compared, one must be quite wary, as data are often reported in fresh weight or not declared. This fact can modify the data interpretation of metal contents in the biomonitors, even though the water content in lichens is not high. We think however that data concerning dry weight are susceptible to a smaller variability as opposed to fresh weight (Conti and Cecchetti, 2003).

## 4. Conclusions

The use of *E. prunastri* turned out to be useful for transplant studies in the areas considered. It fulfills the prerequisites to be employed as bioindicator: It is easy to identify and easy to be sampled, is available and shows a good ability to accumulate metals. Caution must be required in the data interpretation; it is important to consider that lichens present data on availability of metals in the atmospheric environment already biased by biological effects. Although *E. prunastri* present numerous advantages, it is also important to have more information on regulating and/or competition mechanisms of metals inside tissues.

## References

Bartoli, A., Cardarelli, E., Achilli, M., Campanella, L., and Massari, G., 1994: Biomonitoraggio dell'aria di Roma: accumulo di metalli pesanti in trapianti di licheni, *Ann. Bot.* **LII**(11), 239–266.

Betinelli, M., Spezia, S., and Bizzarri, G., 1996: Trace element determination in lichens by ICP-MS, *At. Spectrosc.* **17**, 133–141.

Caniglia, G., Calliari, I., Celin, L., and Tollardo, A. M., 1994: Metal determination by EDXFR in lichens. A contribution to pollutants monitoring, *Biol. Trace Elem. Res.* **43–45**, 213–221.

Cercasov, V., Pantelică, A., Sălăgean, M., Caniglia, G., and Scarlat, A., 2002: Comparative study of the suitability of three lichen species to trace-element air monitoring, *Environ. Pollut.* **119**, 129–139.

Conti, M. E., 2002: *Il monitoraggio biologico della qualità ambientale*, SEAM Editor, Rome, 181 pp.

Conti, M. E. and Cecchetti G., 2001: Biological monitoring: Lichens as bioindicators of air pollution assessment—A review, *Environ. Pollut.* **114**, 471–492.

Conti, M. E. and Cecchetti, G., 2003: A biomonitoring study: Trace metals in algae and molluscs from Tyrrhenian coastal areas, *Environ. Res.* **93**, 99–112.

Conti, M. E., Tudino, M. B., Muse, J. O., and Cecchetti, G., 2002: Biomonitoring of heavy metals and their species in the marine environment: The contribution of atomic absorption spectroscopy and inductively coupled plasma spectroscopy, *Res. Trends Appl. Spectrosc.* **4**, 295–323.

Deruelle, S., 1992: Accumulation du plomb par les lichens, *Bull. Soc. Bot. Fr.* 1139; *Actual Bot.* **1**, 99–109.

Herzig, R., 1993: Multi-residue analysis with passive biomonitoring: a new approach for volatile multi-element contents, heavy metals and polycyclic aromatic hydrocarbons with lichens in Switzerland and the Principality of Liechtenstein. In B. Markert (ed.), VCH Publishers, Weinheim, pp. 286–328.

Nash, T. H., III and Wirth, V. (eds.), 1988: Lichens, bryophytes and air quality, *Bibl. Lichenol.* **30**, 297.

Senhou, A., Chouak, A., Cherkaoui, R., Moutia, Z., Lferde, M., Elyahyaoui, A., Elkhoukhi, T., Bounakhla, M., Embarche, K., Gaudry, A., Ayrault, S., and Moskura, M., 2002: Sensitivity of biomonitors and local variations of element concentrations in air pollution biomonitoring, *J. Radioanal. Nucl. Chem.* **254**(2), 343–349.

Wadleigh, M. A. and Blake, D. M., 1999: Tracing sources of atmospheric sulphur using epiphytic lichens, *Environ. Pollut.* **106**, 265–271.

Wolterbeek, B., 2002: Biomonitoring of trace element air pollution: Principles, possibilities and perspectives, *Environ. Pollut.* **120**, 11–21.