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Use of lichens in detecting environmental risk and in geochemical prospecting

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Abstract This paper provides data on variations in the contents of As, Sb, Ni, V, Pb, Cu, Cr, Au, Zn, Sc, and Al, measured in the thalli of a saxicolous lichen species, *Xanthoria calcicola* Ochsner s.l., collected in northeastern Sicily, near an industrial zone and along a belt crossing areas of known ores containing sulfides of heavy metals. A total of 91 lichen samples were collected on roof tiles (39) and on rocks (52). In the industrial zone, analysis of lichen thalli revealed high contents of nickel and vanadium, decreasing at increasing distances from the source of contamination. The results have also revealed the versatility of *Xanthoria calcicola* in geochemical prospecting for heavy metals such as Pb, Zn, As, Au, Sb, Ni, V, and Cu. The contents of these elements in the analyzed lichens highlight the same geochemical associations observed in prospecting surveys on samples of river sediments and identify similar anomalies. Interpretation of data in terms of enrichment factors (EFs) turned out to be particularly useful.

Key words Lichen · Environmental risk · Geochemical prospecting · Environmental geochemistry

Introduction

Biogeochemistry as a scientific method of investigation was developed by Vernadsky and Goldschmidt at the beginning of the 20th century. It is based on the response of many plants to high concentrations of some elements in soils and the atmosphere. The underlying assumption is that the content of an element in plants reflects the content of that element in the environment in which the plant grows. Biogeochemical exploration differs from geobotanical exploration in that this latter is based on direct observation of plant morphology and distribution. Biogeochemical prospecting methods have mainly been used in the USSR, Fennoscandia, Canada, and the United States. Of the many species of plants used for such biogeochemical prospecting, lichens play a primary role.

Although lichens are similar to independent plants, they are in fact an extraordinary symbiotic association of fungi and algae. These two constituent parts gain from this condition of mutualism benefits that would otherwise be impossible. Together with increased resistance stress due to excessive heat or dryness, lichens are highly sensitive to polluting substances, with tolerance levels ranging from the total extinction of a species to the capacity to behave as bioaccumulators.

Their properties are therefore exploited for assessing air quality in two main ways: (1) as bioindicators, when their occurrence and external aspect are correlated with the presence of polluting substances; and (2) as bioaccumulators, i.e., as organisms capable of accumulating certain elements or substances in concentrations far higher than those present in air. Both these research strategies have been used in scientific sectors that are very varied but that are connected by the interaction between the biosphere and the superficial crust of the Earth, finding an immediate link in vegetation. Specific examples of applications may be found in Nieboer and others (1972), Tuominen and Jakkola (1973), Czehura (1977), Puckett (1980), Garty (1985), Bargagli and Barghigiani (1991), Amman and others (1992), Nimis and others (1993), and Markert (1993).

The present survey provides data on variations in the contents of As, Sb, Ni, V, Pb, Cu, Cr, Au, Zn, Sc and Al, measured in the thalli of a saxicolous lichen species, *Xanthoria calcicola* Ochsner s.l., collected in northeastern Sicily, near an industrial zone and along a belt crossing areas of known ores containing heavy-metal sulfides. The area studied for the concomitant presence of sources of heavy metals from industry and mineralized zones thus represents an interesting natural laboratory on how to

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exploit lichens to monitor air quality and to identify the presence of anomalies near ore bodies.

Notes on lichen biology

Lichens are complex organisms resulting from associations between algae and fungi, living together in a state of harmonious mutualism that allows both to grow in different environments and in conditions that are inhospitable for other forms of vegetation. The term symbiosis, coined by the German scientist Anton Heinrich De Bary, came precisely from study on the associative behaviour of lichens. Laboratory experiments have shown that lichens can survive at extremely low temperatures (Ahmadjian 1967) and even in vacuum (Becquerel 1948).

In the lichen association, the fungus (*Ascomycetes* or, more rarely, *Basidiomycetes*) is the dominant member of the pair and is the only one maintaining its reproductive capacity. The hyphae of the fungus furnish water and mineral salts and attach themselves to very diverse substrates, such as rocks, tree trunks, tiles, or cement walls, by means of rhizinae. Unicellular green or blue-green algae, with their gonidia incorporated in the tissues of the host symbiont, synthesize the organic substances necessary for nutrition. The resulting lichen possesses properties that are different from those of single fungi or algae.

Lichens have a thallous structure, i.e., unlike other vegetal species, they are not differentiated into root, stem, and leaves. Structurally, lichens are composed of four layers: an outer pseudoparenchymatous layer, covering a second layer of algal cells into which the haustoria of the fungus penetrate; a medullary layer, mainly composed of fungal hyphae; and an inner pseudoparenchymatous layer. In the medullary layer are produced lichenic acids and are deposited the sugars produced by the algal cells through photosynthesis.

According to how they grow, lichens are subdivided into three main morphological types (although other intermediate ones also exist): crustose, adhering so closely to the substrate that they cannot be detached from it; foliose, adhering more loosely to their growth surface, and in which their stratified structure is more easily recognizable; and fruticose, with characteristic tufted shapes, like tiny shrubs.

Unlike the higher plants, lichens have neither cuticle nor stomata. They have no forms of protection against external agents or water losses, and therefore are obliged to absorb and accumulate airborne substances in gaseous, liquid, or particulate form. Since the whole surface of the thallus is involved in the accumulation process, lichens probably also acquire substances from their substrates, following processes of erosion or chemical alteration.

As well as the above characteristics, lichens also have some peculiar features which make them excellent biomonitors: (1) Photosynthetic activity is continuous, without periods of vegetative rest; when moisture is scarce, lichens slow down their metabolism, thus increasing their resistance to polluting substances. (2) Having no excretion mechanisms, they cannot get rid of old parts in which pollutants may have accumulated. (3) Lichens are quite slow to grow and can therefore accumulate metals over long periods of time, at levels well above environmental concentrations and their own physiological needs (LeRoy and Koksoy 1962). (4) They show high tolerance to most heavy metals.

Mechanisms of metal uptake and accumulation

There are several ways in which epilithic lichens may take chemical elements from their surroundings, and the acquisition process may have both mechanical and chemicophysical characteristics. One significant method is by particulate trapping of minerals inside the medullary layer, as demonstrated by comparisons between the ratios of some elements in lichens and their ratios in the Earth's crust or in the growth environment of lichens (Puckett and others 1973; Nieboer and others 1978).

Goyal and Seaward (1981) observed that trapped metals are not removed by rainfall. Bargagli and others (1987) compared samples of *Parmelia* washed with deionized water and unwashed samples. With the exception of Cr, Pb, and Se, which show analytically important differences, other analyzed elements were not removed. Thus, there is no clear evidence of removal of metals from lichens by the leaching action of rain or dew. However, this may depend on which chemical structure metals are trapped.

Biogeochemical processes, piloted by the presence of weak acids in the thalli, and biogeophysical processes, such as repeated contraction and swelling, may alter the trapped material, which, in turn, may constitute a source of elements for the lichen. Extracellular trapping may explain the high resistance of some lichen species towards toxic metals.

Part of the metal in the thalli is bound extracellularly by chemicophysical processes of ion exchange taking place on the functional carboxyl and phenol groups bound to the cell wall (Tuominen 1967):

$$M^{2+} + 2H^{+}\text{-lichen} \rightleftharpoons M^{2+}\text{-lichen} + 2H^{+}$$

A detailed description of this ion-exchange model has been proposed by Nieboer and others (1978).

Intracellular uptake may also occur. This has been demonstrated by Brown's (1976) studies on K^{+} ion release by lichens in contact with electrolytic solutions. Metal ion uptake from a solution with significant potassium release is generally considered as evidence for intracellular trapping.

Sampling and analyses

The investigated area is located in the northeastern corner of Sicily (Fig. 1). In this study, epilithic lichens were used—a choice essentially dictated by the fact that few phor-

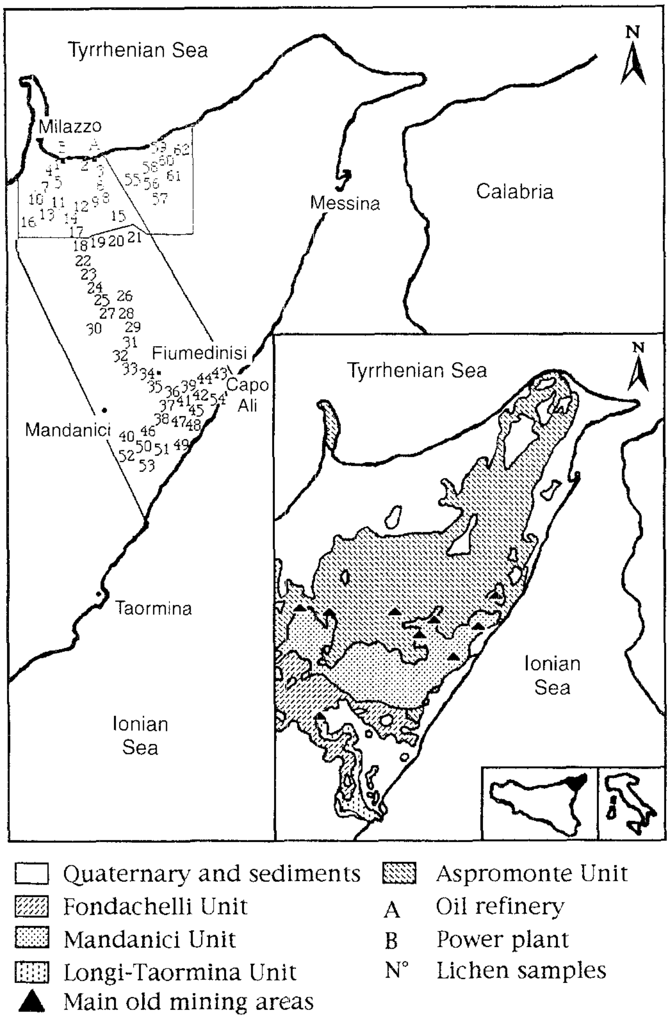

Fig. 1 Geological sketch map of the area studied with indication of the sampling points.

ophytes containing suitable lichen species could be found in the area, tree species being mainly conifer, eucalyptus, or citrus fruit. More or less natural woodland only occurs in the higher areas. It was therefore necessary to use lichen species amply distributed from low-lying areas to higher ones, at the same time resistant to the dryness of the air in cities and relatively tolerant to pollution. A preliminary study of the flora showed that the most frequent lichen species was *Xanthoria calcicola* Ochsner s.l. It has an orange to reddish orange thallus formed of lobes not arranged in regular rosettes. This species is very frequent on basic silicates, carbonatic rocks, and acidic silicates with a eutrophized surface, almost always well exposed to the sun, and on subhorizontal or only slightly inclined surfaces. A total of 91 lichen samples were collected on roof tiles (39) and on rocks (52).

The sampled lichens were carefully cleaned of fragments of substrate, and oven-dried at 40°C. Nonmetallic objects were always used during the collection and cleaning phases. Many thalli variously exposed to atmospheric agents were collected at each sampling station. Au, Sb, Sc, Zn, and Ni were chemically analyzed using INAA techniques; As, Al, Ti, Cu, Pb, and V were analyzed by ICAP. The composition of the substrate was also analyzed using the same techniques.

Similarities in the way of accumulation, with respect to *Xanthoria*, were observed in two *Parmelia* species, *P. pulla* and *P. conspersa*. In some cases, samples of both *Xanthoria* and *Parmelia* were collected at the same station, and their analytical data show how they accumulate comparable quantities of metals. Therefore, in a few cases, in the absence of *Xanthoria* thalli, *Parmelia* ones were collected. Figure 1 shows the general geological setting of the area investigated, together with sample locations.

General geological setting

The Peloritani Mountains, in northeast Sicily, are a complex overthrusting structure, considered as the lower portion of the Calabrian–Peloritan Arc, geographically divided into two sections by the Straits of Messina. Pre-Hercynian magmatic, metamorphic, and tectonic events are well represented throughout the arc.

The Sicilian portion of this arc is a Hercynian chain affected by alpine tectonics and consisting of a pre-Hercynian metamorphic basement, overthrust and overturned on Devonian metamorphic terrains. Most of the basement metamorphites originate from sedimentary sequences interlayered with both sialic and basic synsedimentary volcanites, with minor intrusive magmatites represented by amphibolites, augengneisses, and Ca felses. These metamorphites are divided into two units, Aspromonte (upper) and Mandanici (lower). The Aspromonte Unit is mainly composed of paragneisses, amphibolites, migmatites, and marbles, while the Mandanici Unit is mainly composed of phyllites, quartzites, metabasites, and crystalline limestones (Ferla 1982a,b; Omenetto and others 1988; Atzori and Ferla 1992).

The Peloritani Mountains contain ore deposits that have been mined in the past, mainly for Pb, Zn, and Cu (Ag, Sb). These deposits are considered strata-bound ores of simple Pb–Zn sulfides reworked by late tectonic events and hydrothermal activity by means of which discordant veins and bodies of complex Sb–Cu–Pb sulfosalts were deposited. (Oteri and others 1986; Triscari and Saccà 1982; Locardi and Triscardi 1991). The main ore deposits occur around the villages of Fiumedinisi and Alì on the Ionian coast, where terrains are typical of the low-grade metamorphites of the Mandanici Unit. The Milazzo area represents a typical sedimentary plane with no evidence of mineralization. New industrial installations, such as an oil refinery and a thermoelectric power station, have recently been built in the area.

Results and discussion

Table 1 gives a statistical picture of the metal contents in the lichen thalli of the area studied, along with the met-

Table 1 Minimum, maximum, average and standard deviation of the analyzed trace elements[a]

	Minimum	Maximum	Mean	SD
Elements				
Al	2400	64,400	18,000	14,000
Au	1.4	125	13	16
As	1.0	180	12	25
Cr	5.6	54	21	10
Cu	9	104	25	14
Ni	1.0	120	24	19
Pb	1.0	1,032	68	125
Sb	0.1	14	2	2.8
Sc	0.8	10	3	1.8
V	1.0	139	41	26
Zn	41	1,575	148	174
Ratios				
Au/Al $* 10^3$	0.28	160	12	20
As/Al	0.3	230	8	25
Cr/Al	3.1	44	16	10
Cu/Al	3.6	81	22	18
Ni/Al	1.0	68	18	14
Pb/Al	1.0	884	55	103
Sb/Al	0.1	16	1	2.0
Sc/Al	0.7	7	2	1.5
V/Al	1.0	112	31	20
Zn/Al	9	632	121	112

[a] Data are reported in mg/kg of dry weight, except gold concentrations reported in µg/kg. Ratios are computed as X/Al%, where X is the concentration of metal X. Number of samples 91.

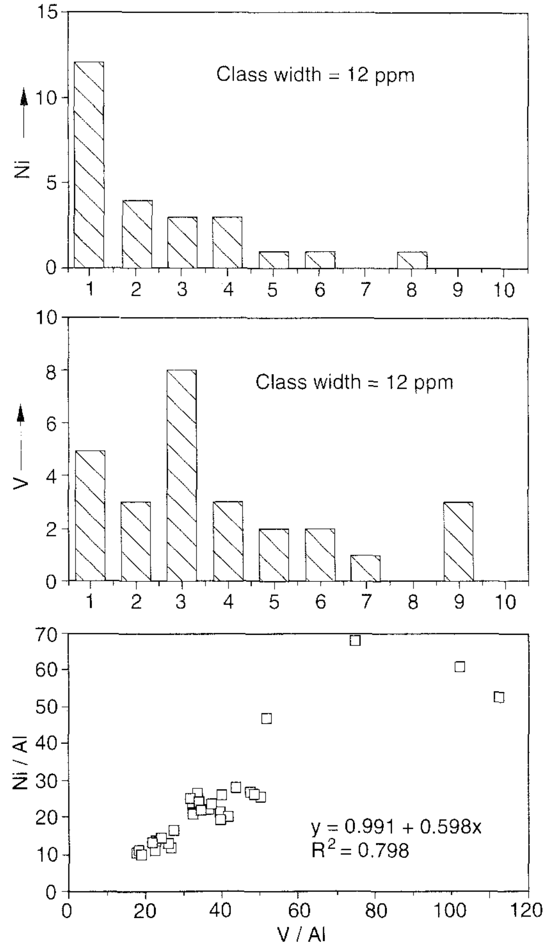

Fig. 2 Frequency distributions of nickel and vanadium and their correlation

al/aluminum ratios. It was necessary to compute these ratios in order to link the variability of metal concentrations to the quantity of solid crustal material trapped in the lichens. Aluminum as a normalizing factor was chosen since, in the area in question, it is of definitely terrigenous origin, as shown by the high correlation coefficients for Al/Sc and Al/Ti (r^2 = 0.832 and 0.723, respectively).

Milazzo industrial zone

In the Milazzo industrial zone, analysis of lichen thalli revealed high contents of nickel, corresponding to high contents of vanadium (Fig. 2), with a correlation coefficient of the metal/aluminum ratios of 0.89. In the same figure the frequency distributions of the two metals are also shown. In the case of a study on air quality in an area with different lithological outcrops, enrichment factor (EF) should be calculated, defined as:

$$EF = (X/\mathrm{Al})_{\mathrm{lich}}/(X/\mathrm{Al})_{\mathrm{subst}}$$

where X and Al are, respectively, the concentrations of metal X and Al in lichen and substrate.

This enrichment factor takes account of any crustal material that is trapped in the medulla or that adhers mechanically to the lichen and cannot easily be removed during sample preparation. Enrichment factors are thus useful in suppressing background disturbances, in stressing higher-contrast anomalies, and in defining models of dispersion from sources of contamination. The EFs of nickel and vanadium, shown on the maps of Figs. 3 and 4, show that higher contents are found near industrial plants and that, at increasing distances, they tend to a value of about 1. Damage to plants, with necrotic forms on leaves and fruit due to the emission of V- and Ni-rich ashes has already been reported in the Milazzo industrial zone (Vaccarino and others 1983). Chemical analyses of oil ashes sampled at the hoppers of the electrostatic precipitators of the power station were reported by Corigliano and others (1992), who found significant quantities of V (7%), Ni, Fe, and Mg.

The main anthropogenic emission sources of Ni and V in the atmosphere are oil and coal combustion. Of trace elements in mineral oils, Ni and V are the most abundant, sometimes being found at levels two orders of magnitude higher than others. Vanadium occurs in fuel oils in concentrations of up to 0.07% and even more in some oils from Venezuela. It reaches them through the *Ascidia*, marine organisms that contain large quantities of V in their blood in the form of hemovanadin, a vanadium protein, up to one million times the amount occurring in seawater. Ni and V are found in mineral oils in the form of organometallic compounds with petroporphyrins or as salts of organic acids. Although it has not been ascertained

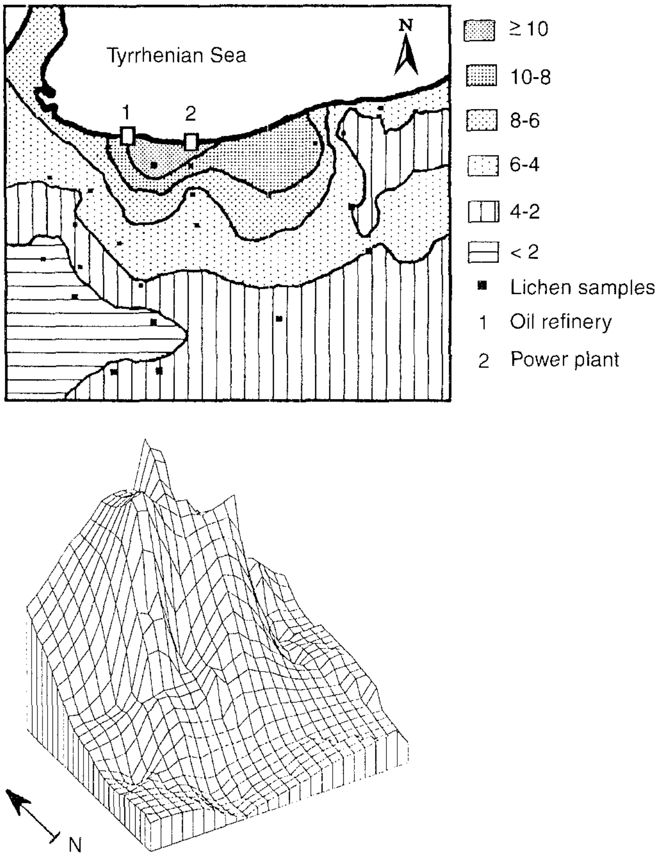

Fig. 3 Areal distribution of the enrichment factors of nickel. A 3-D image of the same distribution is also shown

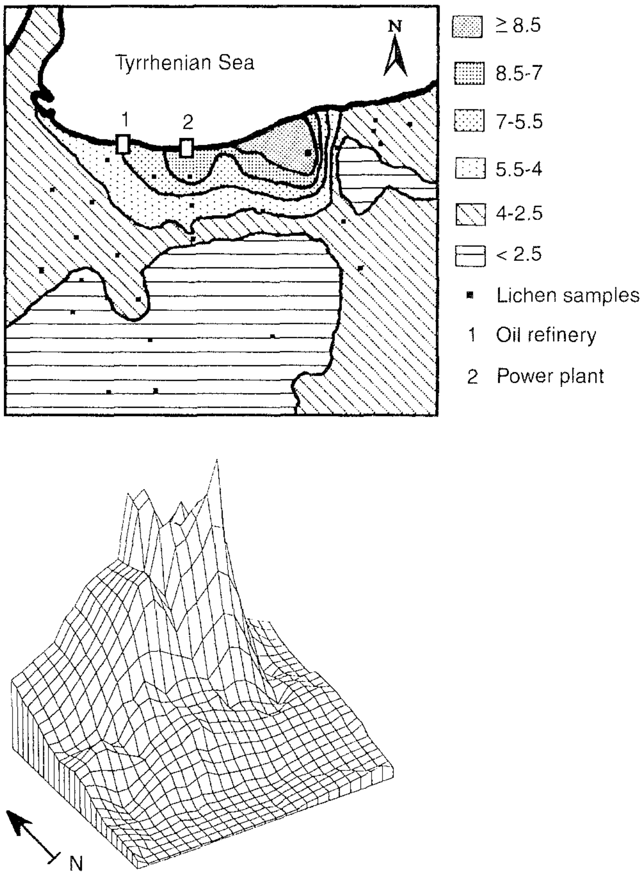

Fig. 4 Areal distribution of the enrichment factors of vanadium. A 3-D image of the same distribution is also shown

whether V is particularly toxic for man, according to Athanassiadis (1962), permanent exposure to vanadium compounds is responsible for cardiovascular diseases and certain forms of cancer. Studies on the toxicity of Ni have clearly shown that it is hazardous for man, causing forms of cancer.

NNW–SSE belt from Milazzo (Tyrrhenian coast) to Alì (Ionian coast)

Mechanical dispersion haloes may be found around ore bodies. Biogeochemical prospecting may complement and in some cases replace more conventional methods of geochemical exploration. Lichens, for example, become enriched with chemical elements in quantities that reflect concentrations in surrounding soils and rocks, whose small particles, produced by weathering process, can be picked up and spread by winds and lastly on lichens.

The Milazzo–Alì belt was chosen because it allows analysis of variations in the contents of some metals in *Xanthoria calcicola* samples in the presence of an industrial zone and of nearby ore bodies. The belt also contains an area definitely free from contamination, which is useful as a reference for background values. Figures 5–7 show the contents of Pb, Zn, Sb, Cu, As, Au, Ni, and V, normalized with respect to Al, in single lichen samples along the belt that links the Tyrrhenian and Ionian coasts (see Fig. 1). The strong analogy of behavior between V and Ni in the industrial zone is still evident (Fig. 5), with an exponential reduction in content at increasing distances from the source of contamination. Similar exponential trends

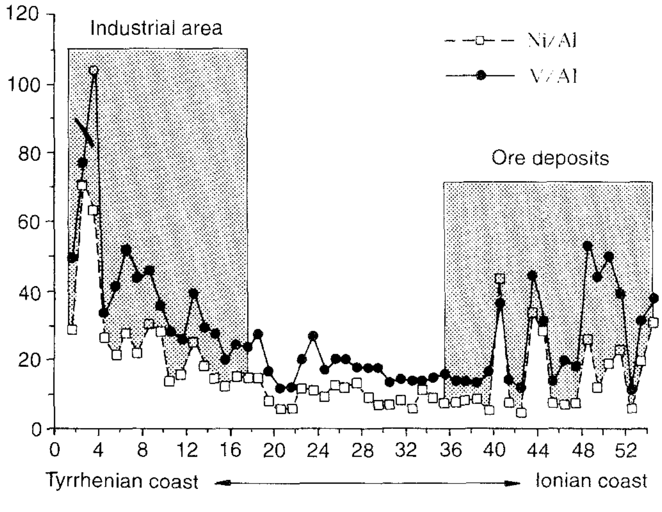

Fig. 5 Variations of Ni/Al and V/Al ratios along the NNW–SSE belt. Sampling points on the horizontal axes are numbered as in Fig. 1

were also observed by Bargagli and others (1985) in their study on trace metals around the Rosignano Solvay industrial zone. V and Ni contents also increase at the end of the traverse, near the mineralized areas, revealing the presence of basic rocks in the Mandanici and Aspromonte units. Enrichments in Pb, Zn, As, Sb, Cu, and Au are also very evident here.

Pb (Fig. 6) shows more or less constant contents all along the belt, with peaks for samples collected in areas of old excavations for galena and blende in a quartz–fluorite matrix. Anomalous values occur in the 39–54 interval, with a peak of 884 and a background value for the whole traverse of about 29 (referring to the Pb/Al ratio). A more careful look at Fig. 6 also shows that, if background values are assumed to be those of the uncontaminated area (samples 21–38 with an average Pb/Al ratio of 22), there are also lichens with Pb contents two or three times higher than the background found in samples taken near traffic-laden roads or in town centers.

Zinc, commonly considered to be a potential indicator of sulfide deposits, shows similar peaks in the mineralized area, highlighting its common origin with Pb, but it also shows significant enrichment in the industrial zone on the Tyrrhenian coast, where its origin may be anthropic.

Antimony and arsenic (Fig. 7) show trends that are in part similar to those of Pb, with enrichments particularly at the end of the belt near Alì. However, there is also an evident second mineralized area before that of Alì, although of different mineralogical type, characterized by complex As and Sb sulfosalts in a quartz–carbonate matrix.

De Vivo and others (1993) reported the results of geochemical prospecting in the Peloritani Mountains on about 1200 river sediments. Their data on the western sector of their study area show metal associations of the type As-Sb-Hg-Au and Zn-Cu-Pb-B, with geochemical anomalies connected to the old mines nearby. In order to verify the compatibility of results using the biogeochemical method with those of sediment analysis, we carried out factorial analysis on data from 34 lichen samples of the Milazzo–Alì belt, regarding the following eight variables: Au/Al, As/Al, Cu/Al, Ni/Al, Pb/Al, Sb/Al, V/Al, and Zn/Al. Of the three factors extracted, rotated according to the Varimax procedure (Table 2), the first—accounting for 41% of total variance—is relevant as regards mining prospecting, since it reflects the As-Au-Sb association. The geochemical relation between gold and arsenic in hydrothermal deposits is well known (Cavender 1964). Arsenic and antimony are considered to be pathfinders for gold. While the presence of As and Sb in the mineralized area of the traverse appears evident, the distribution of Au (Fig. 7) is not easy to interpret. Ongoing research aims at more careful examination of Au contents in *Xanthoria* samples, with extension of the study area to the mining district.

In the second extracted factor, nickel and vanadium highlight sources of industrial contamination, as shown by their high frequency in samples taken near the power station and oil refinery at Milazzo.

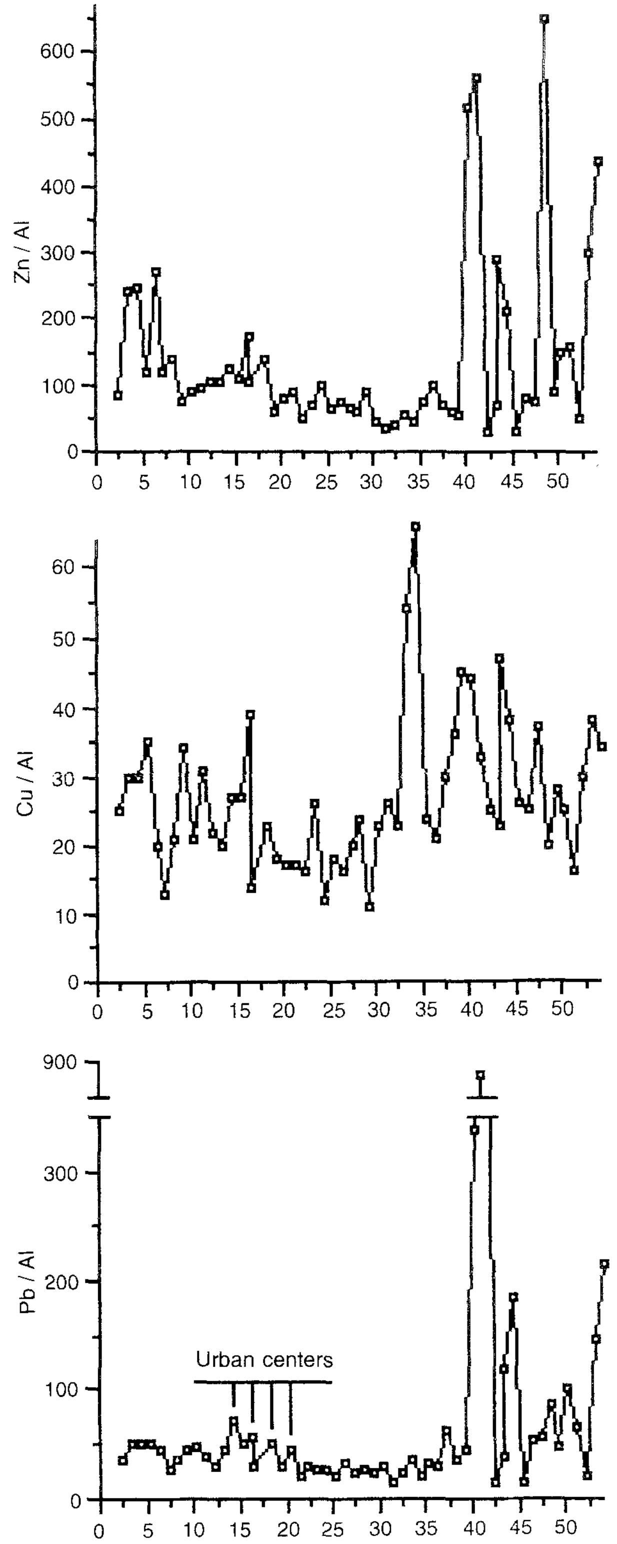

Fig. 6 Contents of zinc, copper and lead, normalized with respect to Al, in the lichen samples collected along the belt. Sampling points on the horizontal axes are numbered as in Fig. 1

The third factor reflects the Pb-Zn-Cu association, due to the presence of blende and galena at the end of the traverse. Because of the high loading of Pb with respect to Zn and Cu, along the whole traverse, part of the variance

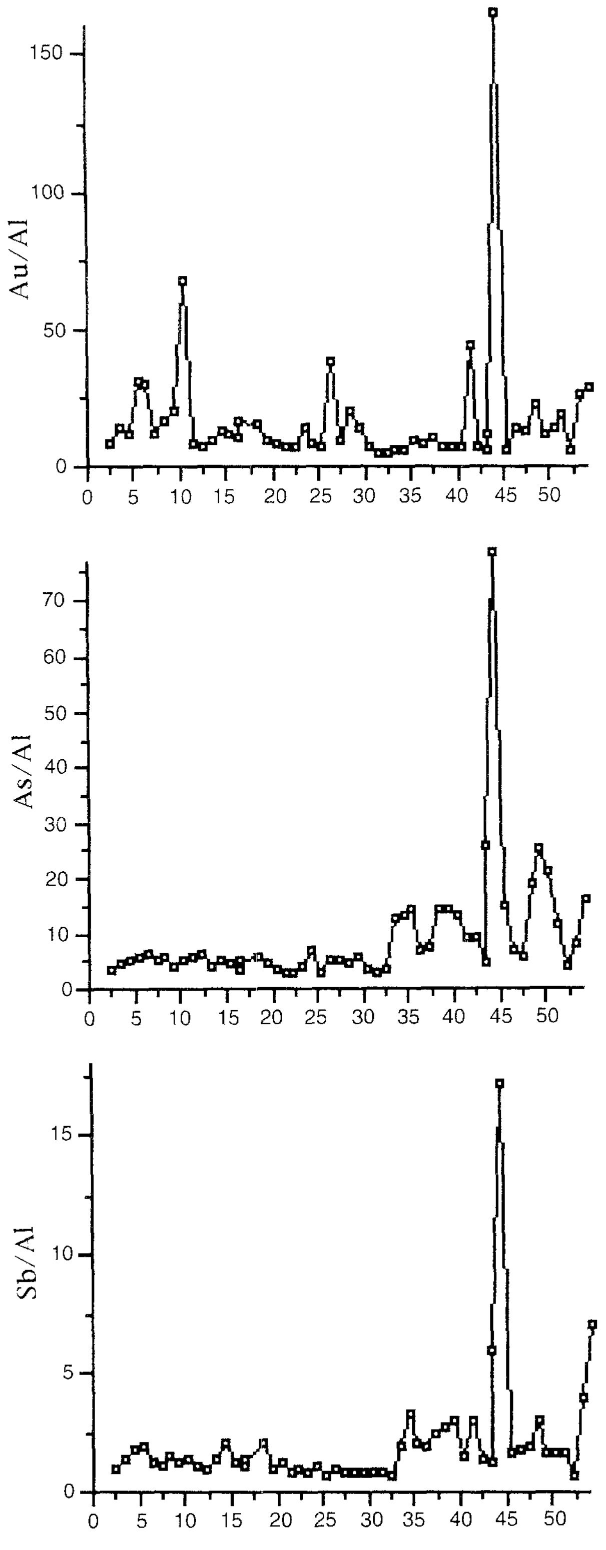

Fig. 7 Contents of gold, arsenic, and antimony, normalized with respect to Al, in the lichen samples collected along the belt. Sampling points on the horizontal axes are numbered as in Fig. 1

Table 2 Varimax rotated factor matrix

	Factor 1	Factor 2	Factor 3
Au/Al	0.87	0.06	0.03
As/Al	0.96	−0.04	−0.08
Cu/Al	3.10E-01	0.33	0.45
Ni/Al	0.04	0.86	0.08
Pb/Al	−4.86E-04	0.15	0.88
Sb/Al	0.89	2.64E-3	0.11
V/Al	−0.05	0.94	−0.05
Zn/Al	−0.03	−0.19	−0.77

explained by factor three is probably associated only to the variability in values of Pb of anthropic origin.

The correspondence between the results from analyses on lichens and on soil prospecting suggests that metal contents in lichens are mainly caused by concentrations in soils and partly by other factors.

Conclusions

Variations in the metal contents of any one lichen species depend on several factors, such as the geological and geographical context of the growth environment and the presence of anthropic sources of contamination. Of the biogeochemical methods of prospecting, the examination (analysis and occurrence) of lichens is of particular importance in monitoring air quality and in the search for ore bodies.

The method adopted here is innovative in that, until now, mainly epiphytic lichens have been used to for biomonitoring of polluting substances and, in particular, the bioaccumulation of heavy metals. The results of this study reveal the versatility of the lichen species *Xanthoria calcicola* Ochsner s.l. in prospecting for heavy metals such as Pb, Zn, As, Au, Sb, Ni, V, and Cu. The contents of these elements in the lichens analyzed highlight the same geochemical associations observed in prospecting surveys on samples of river sediments and identify similar anomalies. This means that lichens behave as collectors of the fine fractions of soils that are representative of areas clearly far larger than the sampling site. Our results also show that, in areas of intense motor traffic, Pb contents are clearly higher.

In the study on air quality, interpretation of data in terms of enrichment factors (EFs) turned out to be particularly useful. Although it impossible, to data, to establish a quantitative relationship between the concentration of a metal in the lichen thalli and its concentration in the atmosphere, the data obtained clearly show that the area around the industrial zone of Milazzo is particularly at risk, because of emissions of nickel and vanadium into the air.

The close relation between Al, Ti, and Sc is further proof that these terrigenous elements occur in lichens as trapped material.

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