Recognition of environmental trace metal contamination using pine needles as bioindicators. The urban area of Palermo (Italy)

M.G. Alaimo · G. Dongarrà · M.R. Melati · F. Monna · D. Varrica

Abstract A total of 43 composite samples of several years of needle growth from *Pinus pinea* L., collected in and around the city of Palermo were analysed for major and trace elements by INAA and ICP-MS. The chemical composition of pine needles suggests that the presence of Pb, Sb and Br in excess with respect to soil composition is due to anthropogenic emissions. The anomalously high values of these elements observed in the urban area decrease outside town. Lead isotope data confirm the anthropogenic origin of lead. The origin of Zn and Cu excess remains uncertain at the moment, although wholly-crustal origin appears doubtful. Morphological alterations attributable to phenol accumulation, such as modifications in the length of mesophyll cells and the appearance of lacunae in the distal portions, were observed in needles. The accumulation of phenols, linked to the presence of lead, gives rise to a specific pattern of metabolites providing mechanisms of detoxification and protection, so that the accumulation degree may be proposed as a marker of environmental pollution.

Key words Environmental geochemistry · Trace metals · Particulate matter · Biogeochemistry

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Introduction

Analysis of plant material is an effective method of prospecting, as pointed out by Goldschmidt (in the United States) and Vernadsky (in the Soviet Union) in the early 1930s, because plants may reveal anomalous metal concentrations in soils. The same principle may sometimes be used successfully to assist environmental studies regarding air quality. Nowadays lichens, mosses and various kinds of plants are frequently used for monitoring environmental pollution on local and also on larger scales (Rambaek and others 1980; Nimis and others 1990; Berg and others 1995; Watmough 1997; Kurczynska and others 1998). Whereas mosses and lichens obtain most of their chemical element supplies from dry deposition of particulate matter and wet deposition, the aerial part of some plants may collect metals from the atmosphere as well as from soils. Pine needles have succesfully been used as bioindicators to reveal the presence of metal concentrations in the environment because of their ability to retain certain trace elements (Bargagli and others 1991; Bargagli 1993; Dmuchowski and Bytnerowicz 1995). Justification for use of pine needles also derives from the fact that coniferous trees are widely distributed in many countries and may easily be identified even by people who are not experts or skilled botanists. Satisfactory results can be achieved provided that element concentrations in the substrate are sufficiently low and constant to yield a significant geochemical contrast. A sampling programme of pine needles (*Pinus pinea* L.) was carried out in 1998 in and around the city of Palermo (Italy) to study the presence of heavy metals in the atmospheric aerosol and to identify the main sources introducing these metals into the environment. The survey was also aimed at insights into the influence of air pollution on the morphological and cytological structure damage observed in pine needles. The main results are described in this paper.

Geology of the study area

The city of Palermo is situated on the north-eastern coast of Sicily along the wide bay *Piana di Palermo* overlooked by Mt. Pellegrino (606 m above sea level). It is delimited at NE by the Tyrrhenian sea and surrounded by mountains (*Monti di Palermo*) elevated 500–1000 m above sea level. The study area is entirely covered by sedimentary rocks. The *Monti di Palermo*, of Mesozoic and Tertiary age, are essentially made up of limestones (Abate and others 1978). Along the perimeter of the *Piana di Palermo* they overlay turbidite deposits, of Oligomiocene age, made up of clay, marly-clay and marls intercalated with quartz arenites (Numidian flysch). White or yellow quaternary biocalcarenites occur in the lower and plain portions. Most of the eastern sector of the *Piana di Palermo* is covered by a bright red-coloured soil rich in Fe-oxides, known as *Terra Rossa*. Palermo has almost 1 million inhabitants and is a city where major industrial installations are lacking. Potential local pollutants are thus limited to emission from vehicular traffic, house-

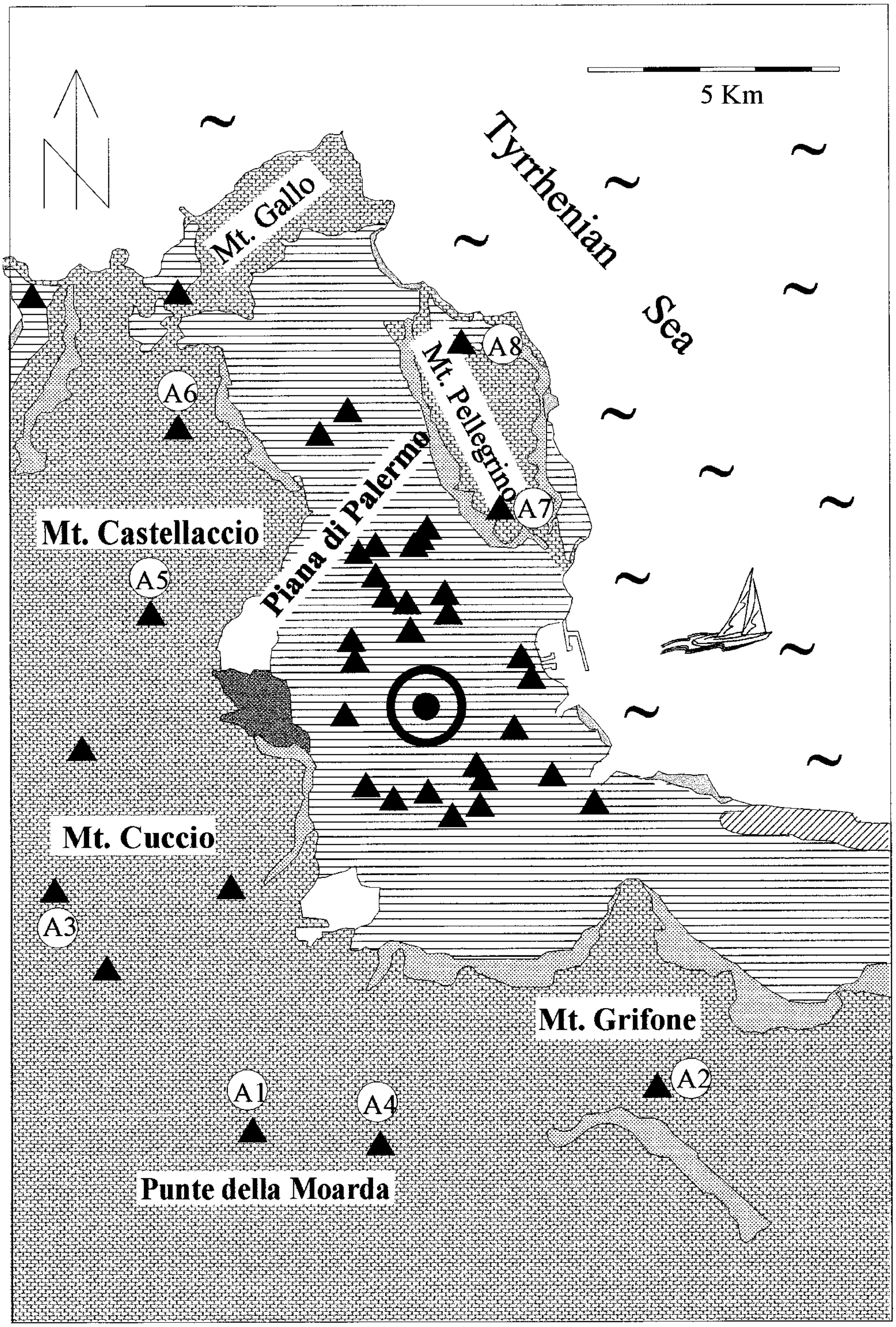

Fig. 1
Geological sketch map of the study area with the location of the sampling sites. Samples A1–A8 fall outside the urban agglomeration. ▒ Carbonate rocks belonging to different stratigraphic units. ☰ Bioclastic calcarenites. ▓ Clay, marly-clay and marl intercalate with quartz arenites (Numidian flysch). ▨ Marly-clay. ☐ Old aluvials. ░ Nappe debris. ◎ Palermo centre

heating and small manufacturing industries. A geological sketch map of the study area, together with the location of the sampling sites, is shown in Fig 1.

Materials and experimental methods

A total of 43 composite samples of several years of needle growth from *Pinus pineae* L. were collected in July 1998. Each sample, made up of several sub-samples collected from all around the tree, was stored in paper bags. The samples were dried for 48 h at 40°C and then ground to a fine powder. A portion of each sample was analysed for major and trace elements by INAA (Br, Ca, Co, Cr, Fe, K, La, Mo, Na, Sb, Sc, Sm) and ICP-MS (Al, Mg, P, Sr, Mn, Cu, Pb, Zn) using NBS 1572 and 1632 B as standard reference material. Several replicates yielded a precision in the ranges 20–30% and 5–10% for trace elements and main components respectively. A different sample portion was used to determine the Pb isotope composition. Approximately 200 mg of needles were totally dissolved with 5 ml of concentrated HNO_3 (Merk suprapure grade). Digestion was achieved with microwave assistance (MLS ETHOS) in pressurized Teflon bombs. Pb isotope ratios were measured after Pb purification by quadrupole based ICP-MS (HP 4500) following the procedure described in Monna and others (1997, 1998). Blanks were systematically measured and were always negligible in regard to the total amount of Pb in the samples. The Pb isotopic determination in soil samples followed the same procedure described above, except that the total digestion was achieved with a mixture of 2 ml of each HNO_3, HCl and HF suprapure grade. Phenols were highlighted in transversal cryosections from mature needles, using Gahan's test (Gahan 1984), with Fast Blue BB 0.08% in acetate buffer at room temperature for 30 min. Phenols were red or brown in colour.

Mineralogical composition of airborne particles

A previous study on the mineralogical composition of atmospheric particulate in the urban air of Palermo was carried out by Alaimo and Ferla (1979), who identified calcite, dolomite, quartz, talc, clay minerals and palygorskite in aerosol samples. Palygorskite was only occasionally found and appeared to be transported as dust from the Sahara. Badalamenti and others (1984), in their study on the chemical composition of rain in Palermo, confirmed the occurrence of calcite, dolomite, quartz and phyllosilicates.

Results

Inorganic elements in needles and local substrates

Table 1 shows a statistical summary of the chemical concentrations of macronutrients and trace elements determined in needle samples. It is worth noting that

Table 1
Basic statistical parameters for a total of 43 pine needles samples. Data are in ppm (dry weight). CV% is the variation coefficient

	Al	Ca	Fe	Mg	K	P	Mn	Na	Sc	La	Sm	Sr	Br	Co	Cr	Cu	Mo	Pb	Sb	Zn
Minimum	100	3300	120	1700	2320	450	5	409	3	0.09	0.01	6	4.3	0.1	0.1	2	0.04	4	0.03	11
Maximum	700	36650	2200	4600	15850	1400	81	7520	29	0.47	0.07	29	120	0.8	4.4	38	2.50	50	7	60
Average	253	9012	486	2588	5382	919	26	2057	11	0.21	0.02	12	32	0.3	1.9	12	0.66	13	1.6	23
Median	200	7950	400	2500	4810	890	25	1510	10	0.20	0.02	11	27	0.3	1.8	11	0.57	13	1.5	21
Geom. mean	212	8069	383	2532	5050	888	24	1536	10	0.20	0.02	11	24	0.3	1.5	10	0.46	11	0.9	21
Std. Dev. (1σ)	159	5399	435	569	2224	235	11	1661	5	0.09	0.01	6	25	0.2	1.0	7	0.56	9	1.5	9
CV %	63	60	89	22	41	26	44	81	50	43	55	48	80	53	54	61	85	65	93	41

Table 2
Major and trace elements in three surface soils of the study area. Lead isotope ratios are also reported

	Si	Ti	Al	Fe	Mn	Ca	Na	P	K	MG	Rb	Cs
Calcarenetic soil	5424	60	1059	2098	77	384504	742	175	332	6394	<20	<0.5
Mt. Pellegrino soil	216741	4200	71720	39376	1084	20512	2522	5237	15525	5791	128	7.4
Terra Rossa soil	296220	2340	47743	32312	542	43382	1558	960	9796	5972	79	4.2

	Sr	Cr	Ni	La	Ce	Nd	Sm	Eu	Yb	Lu	Sc	As
Calcarenetic soil	660	5	2	1.7	<3	<5	0.4	0.1	0.2	<0.05	0.4	<2
Mt. Pellegrino soil	110	80	45	54.4	97	40	8.4	1.5	3.4	0.55	12.5	19
Terra Rossa soil	81	55	22	32.9	51	27	5.8	1.1	2.2	0.36	9	31

	Th	Co	Cu	Pb	Sb	Zn	Br	U
Calcarenetic soil	<0.5	2	4	<5	<0.2	5	3	2.0
Mt. Pellegrino soil	14.7	16	63	50	1.3	193	51	2.1
Terra Rossa soil	9.1	13	45	69	1.6	81	16	1.0

Lead isotope	$^{206}Pb/^{207}Pb$	$^{208}Pb/^{206}Pb$
Numidian Flysch	1.197 ± 0.006	2.081 ± 0.010
Mt. di Palermo	1.169 ± 0.005	2.096 ± 0.008
Calcarenites	1.200 ± 0.001	2.061 ± 0.007

major elements in soils are also the most abundant elements in pine needles. In particular, Al, Ca, Fe, Mg, K, Na and P generally make up 97% of the weight of the analysed elements, Ca, Mg and K being the most abundant. Geogenic and anthropogenic sources may contribute to the load of metals in needles. The formers include soil dust, sea spray and direct uptake from soil by roots. The latters are limited to emission from vehicular traffic, house-heating and small manufacturing industries.
Table 2 shows the chemical composition of the three surface soils most typical of the study area: Terra Rossa, Mt. Pellegrino and calcarenitic soils. Lead isotope compositions in both limestone and flysch of *Monti di Palermo*, and also in the calcarenite of *Piana di Palermo* are reported in Table 2.

Cluster analysis

A useful and relatively simple method of grouping variables according to their mutual correlation is cluster analysis. This procedure reveals variables with the greatest within-group correlation relative to the between-group correlation. A two-dimensional hierarchical diagram (dendrogram) is the final product of the clustering procedure. Figure 2 shows the dendrogram structure for the set of needle data. Here the result of the unweighted pair-group average method (UPGM) of clustering, in combination with coefficients of correlation, is reported. However, it was found that both unweighted (UPGM) and weighted (WPGM) methods give similar dendrogram structures and they provide better groupings than single linkage. Correlation coefficients were used as similarity coefficients because they are best interpretable in terms of geochemical associations. At 0.5r three main groups of related elements may be identified, Mn, Mg and Na not being linked to any of these. Two clusters are

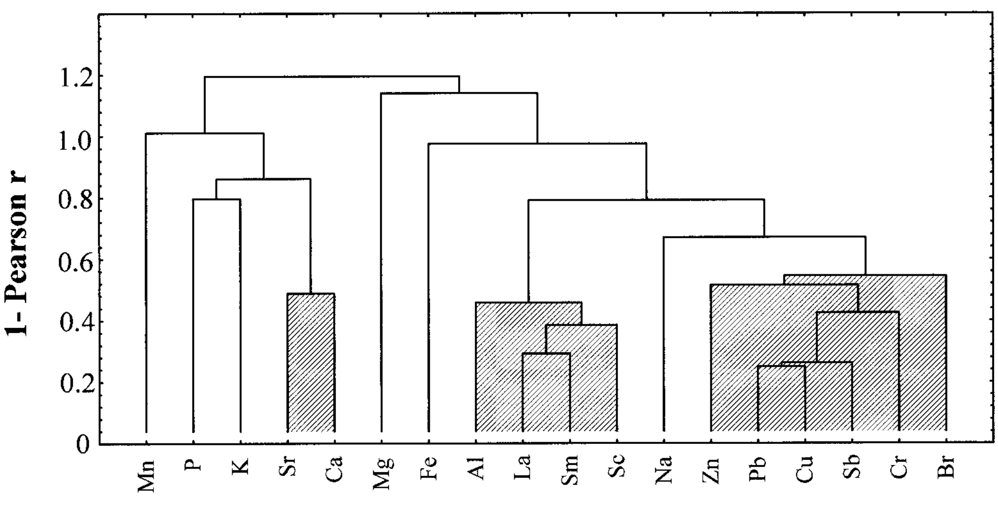

Fig. 2
Dendrogram produced by cluster analysis (UPGM) for 43 samples and 18 elements. 1-Pearson r values on the ordinant scale indicate the similarity of geochemical variables

related to the mineralogy of the main units constituting the lithological background. The first, comprising Ca and Sr, is largely influenced by the calcareous substrate of Palermo. The second cluster, consisting of Al, La, Sm and Sc, is also clearly associated with lithology, defining the clay soil component. Zn, Pb, Br, Cu, Cr and Sb are grouped together, and all of these elements may be indicators of anthropogenic sources.

Lead isotope composition in needles and airborne particles

In environmental studies, lead isotope composition is a powerful tool to identify the sources of emission of this metal into the atmosphere, or to study the dispersion processes. Lead has four naturally occurring isotopes. Three of these: ^{206}Pb ^{207}Pb and ^{208}Pb, derive from the decay of long-lived ^{238}U, ^{235}U and ^{232}Th respectively, whereas the fourth is the nonradiogenic ^{204}Pb. In Western Europe, Pb used for human activities totally derives from remote allochtoneous ore bodies, which are characterised by their own isotopic compositions (Doe and Stacey 1974; Kramers and Tolstikhin 1997). As Pb retains the signature of the source from which it was extracted, it may be possible to discriminate the anthropogenic Pb from the geogenic and local-derived Pb. Results from an earlier survey (Monna and others 1999) showed that two types of anthropogenic Pb can be distinguished in Sicily: Pb added as anti-knock compound in gasoline ($^{206}Pb/^{207}Pb$: 1.066–1.137; Table 3), and Pb generally used by industry ($^{206}Pb/^{207}Pb$: 1.141–1.165). These lead sources are isotopically different from the terrains constituting the area in and around Palermo ($^{206}Pb/^{207}Pb$: 1.169–1.200; Table 2). Some needle samples were analysed for their lead isotope compositions yielding $^{206}Pb/^{207}Pb$ ratios which vary in the range 1.132–1.154 (Table 3). Considering that ‘industrial’ Pb is, with respect to lead from gasoline, of minor importance, as Palermo lacks any significant industrial installations, these values should be the result of a simple binary mixing between automotive exhausts and soil minerals (Fig. 3). It is interesting to note that the needles appear to have received a greater contribution from soil dust (more radiogenic) than the atmospheric filters previously collected in the centre of Palermo ($^{206}Pb/^{207}Pb$ ~ 1.106–1.123, typical of leaded gasoline), probably due to the longer exposure time of the needles or even to the uptake of a significant part of geogenic Pb by the tree roots.

Table 3
Lead isotope ratios in some selected pine needles samples from the study area. Isotope ratios in gasoline (from Monna and others 1999) are also reported for comparative purposes. The errors are given at 95% confidence level

		$^{206}Pb/^{207}Pb$	$^{208}Pb/^{207}Pb$
Pine needles	A1	1.154 ± 0.006	2.108 ± 0.014
	A3	1.132 ± 0.004	2.125 ± 0.008
	A6	1.148 ± 0.005	2.123 ± 0.009
	A7	1.138 ± 0.004	2.125 ± 0.009
	A9	1.132 ± 0.004	2.121 ± 0.008
	A10	1.144 ± 0.006	2.114 ± 0.008
	A11	1.143 ± 0.004	2.111 ± 0.008
	A13	1.143 ± 0.004	2.106 ± 0.008
Gasoline	Agip	1.066 ± 0.004	2.205 ± 0.010
	Shell	1.137 ± 0.006	2.112 ± 0.008
	IP	1.083 ± 0.004	2.166 ± 0.006

Discussion

A serious problem in the interpretation of biogeochemical data is being able to distinguish between the origin of elements which are derived from soil and those arising from anthropogenic sources. The element contents in pine needles are mainly related to the local soils and

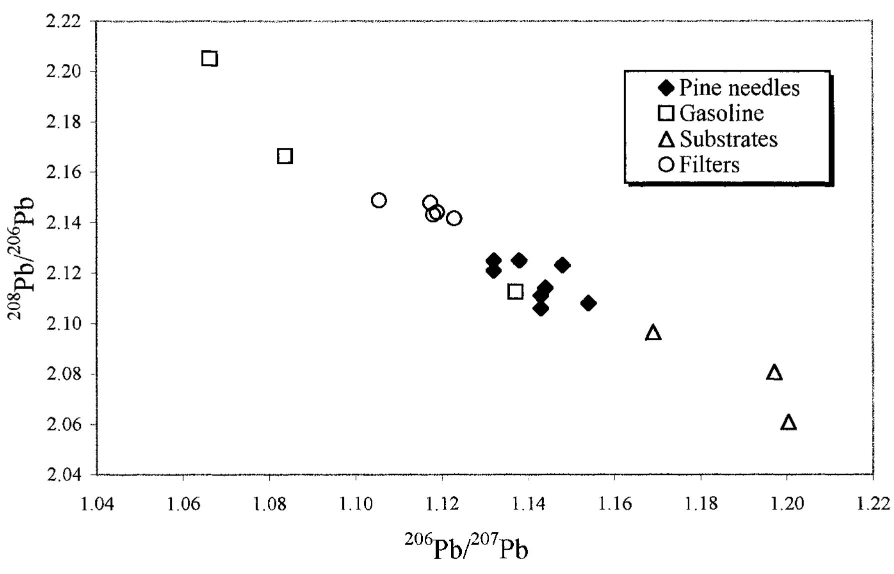

Fig. 3
$^{208}Pb/^{206}Pb$ vs. $^{206}Pb/^{207}Pb$ scatter diagram

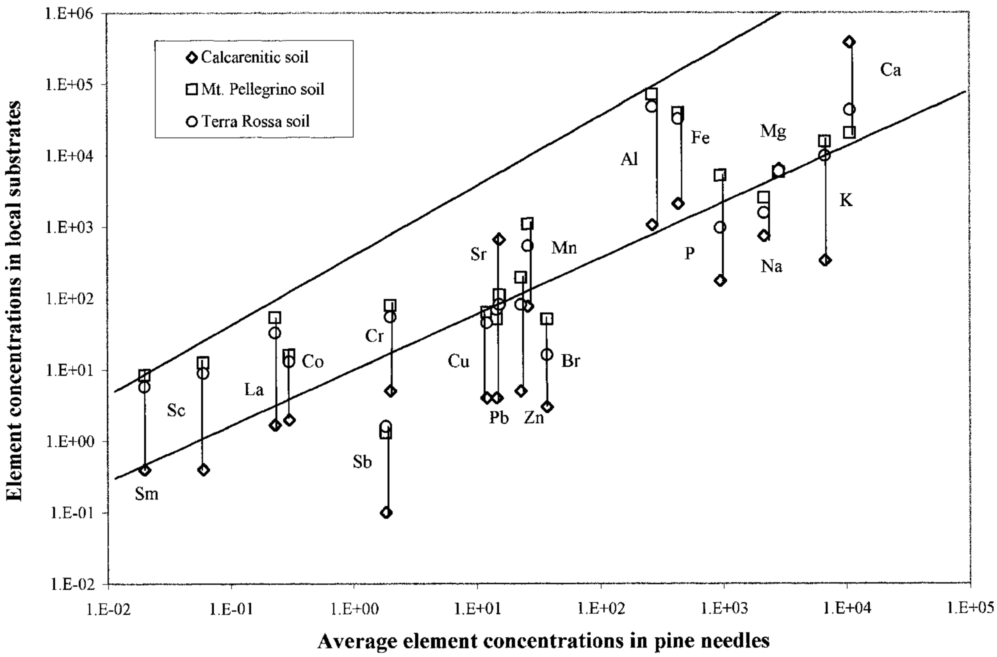

Fig. 4
Comparison of the mean concentrations of each single element in pine needles with those in three main lithotypes composing the soil of Palermo. The two *solid straight lines* delimit the field which includes the elements of definitely terrigeneous origin

particularly to the abundance of mineral species in which they are present. Therefore, in order for the contents of trace elements in needles to be used as indicators of the presence of these elements in air, the distribution of the elements in local soil must be taken into account.

Figure 4 shows the mean concentration of each single element in pine needles compared with the distribution of the same elements in the three main lithotypes composing the soil of Palermo. Each element is thus associated with three points on the graph. Considering the elements of definitely terrigenous origin, it is possible to delimit the field which includes all the elements whose origin is attributable to natural processes of assimilation by plants or to deposition of soil dust on pine needles. Sb, Pb, Br, Zn and Cu plot outside this field. These elements are thus enriched in the needles with respect to the local substrate and there is a definite correspondence between this group of elements and those indicated by cluster analysis as being of anthropogenic nature.

Na-enrichment is likely related to sea spray contribution. Allen and others (1974) indicated the normal natural concentration of lead in plants as about 3 ppm, whereas Alloway (1968) gave a higher value of 10 ppm. The concentrations of Pb in needles for 58% of the analysed samples were above 10 ppm, a level higher than normal for this element with respect to less urbanized areas. Eight needle samples (A1–8 in Fig. 1) collected outside Palermo yielded a mean concentration of 5 ppm. Therefore, despite its declining use in gasoline, lead is still a significant urban air pollutant. Bromine, beside being a characteristic of marine aerosols, is also emitted in significant amounts by non-catalyst vehicles, as leaded gasoline contains brominated compounds added to reduce the formation of lead oxides inside the engine. After correction for sea spray contribution (Dongarrà and Varrica 1998), an excess of Br still remains, confirming the importance of automobile combustion in the dispersion of lead and bromine into the atmosphere. The Pb/Sc and Sb/Sc ratios observed in pine needles (~ 262 and 31 respectively), when compared to those observed in the substrates (ranges 4–10 and 0.1–0.3 respectively), indicate that Pb and Sb are anthropic in origin.

Although Zn and Cu appear to be slightly enriched with respect to soil, it must be borne in mind that both these elements are essential for plants. Their concentration in needles are within the normal natural range (2–20 ppm, with most plants in the range 4–12 ppm) indicated by Kabata-Pendias and Piotrowska (1984). Only 4 samples had Cu content above 20 ppm and 16 above 15 ppm. However, it should be noted that the order of abundance of these elements in pine needles corresponds to the observation of Monna and others (1999) in aerosol samples. Figure 5 shows the areal distribution patterns of Pb, Cu, Zn and Sb. The element distribution shown on the maps exhibits the geochemical anomalies of anthropic origin within the study area. Metal concentrations are higher in the urban area and decrease immediately farther inland. The anomalies occupy the whole urban agglomeration indicating that local sources play a great role in the heavy metals pollution.

Morphological and cytological characteristics of needles

As the excess of certain elements in soils and air can affect plant morphology, the pine needles were subjected to microstructural analysis and the accumulation of secondary metabolites (phenols), considered as endoprotective products and bound to cell walls, was also evaluated (Strack and others 1998; Zobel and Nighswander 1991; Bussotti and others 1998). Figure 6 shows the cyto-

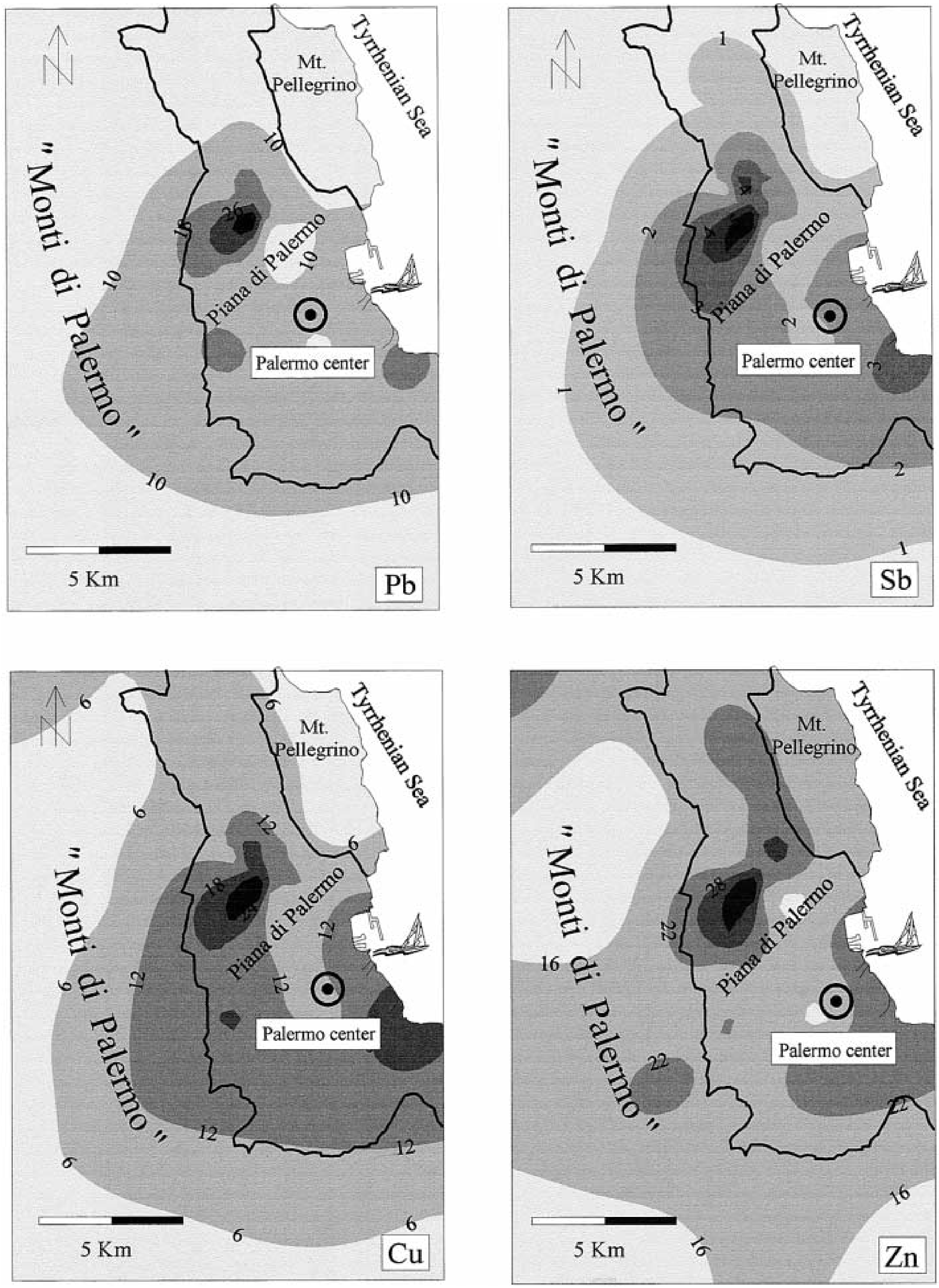

Fig. 5
Areal distribution maps of Pb, Sb, Cu and Zn in pine needles. Two solid curve lines delimit the urban agglomeration

histological characteristics of control samples that can be compared with pine needles coming from polluted stations (Fig. 7). Although the cytohistological characteristics cannot, alone, be considered specific, they do take on significance as indicators of environmental pollution when correlated with other morphological and biochemi-

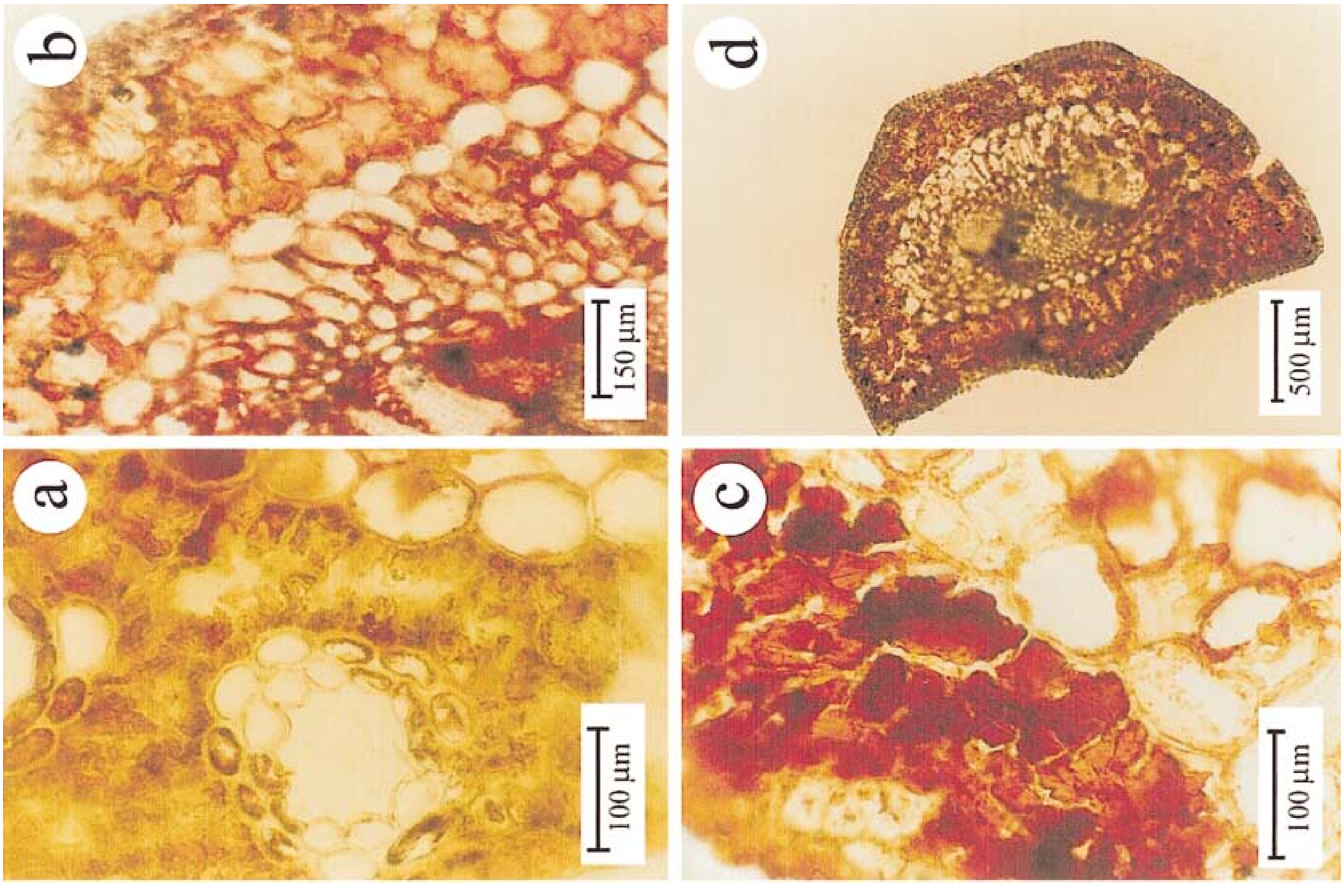

Fig. 7
Microscopy image of needle samples from a polluted site. Dense red droplets from phenols located around the resin duct (*a*) and in the vascular bundles (*b*) may be observed. Mesophyll abnormal cellular enlargement (*c*). Phenols fill the mesophyll and epidermis cells and also the vascular and transfusion tissues (*d*)

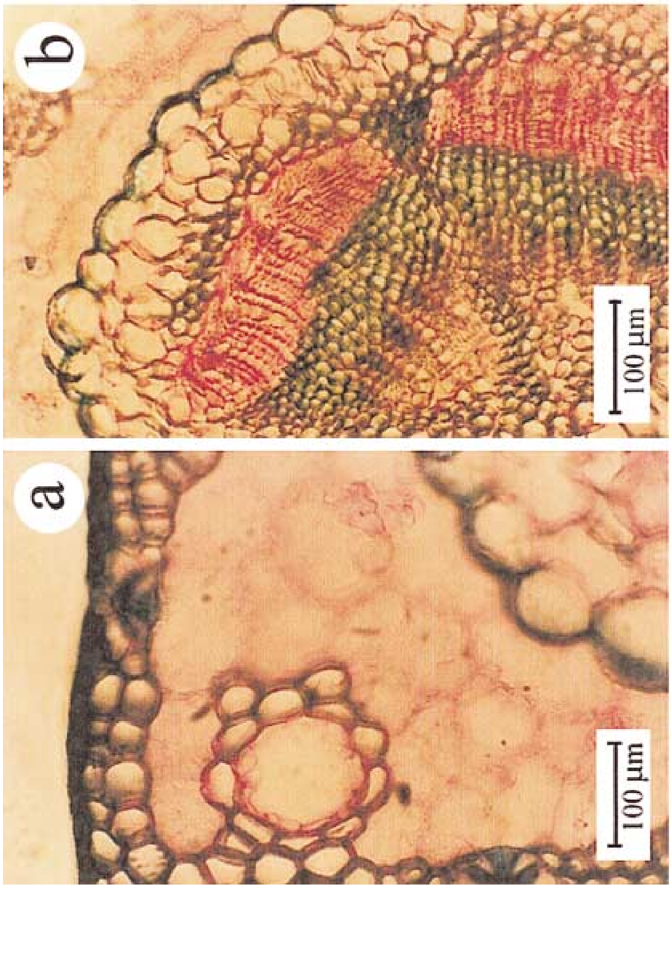

Fig. 6
Cross section of needle sample from a control site showing the absence of phenols (*a*). Mesophyll cells and vascular tissue exhibit a normal enlargement (*b*)

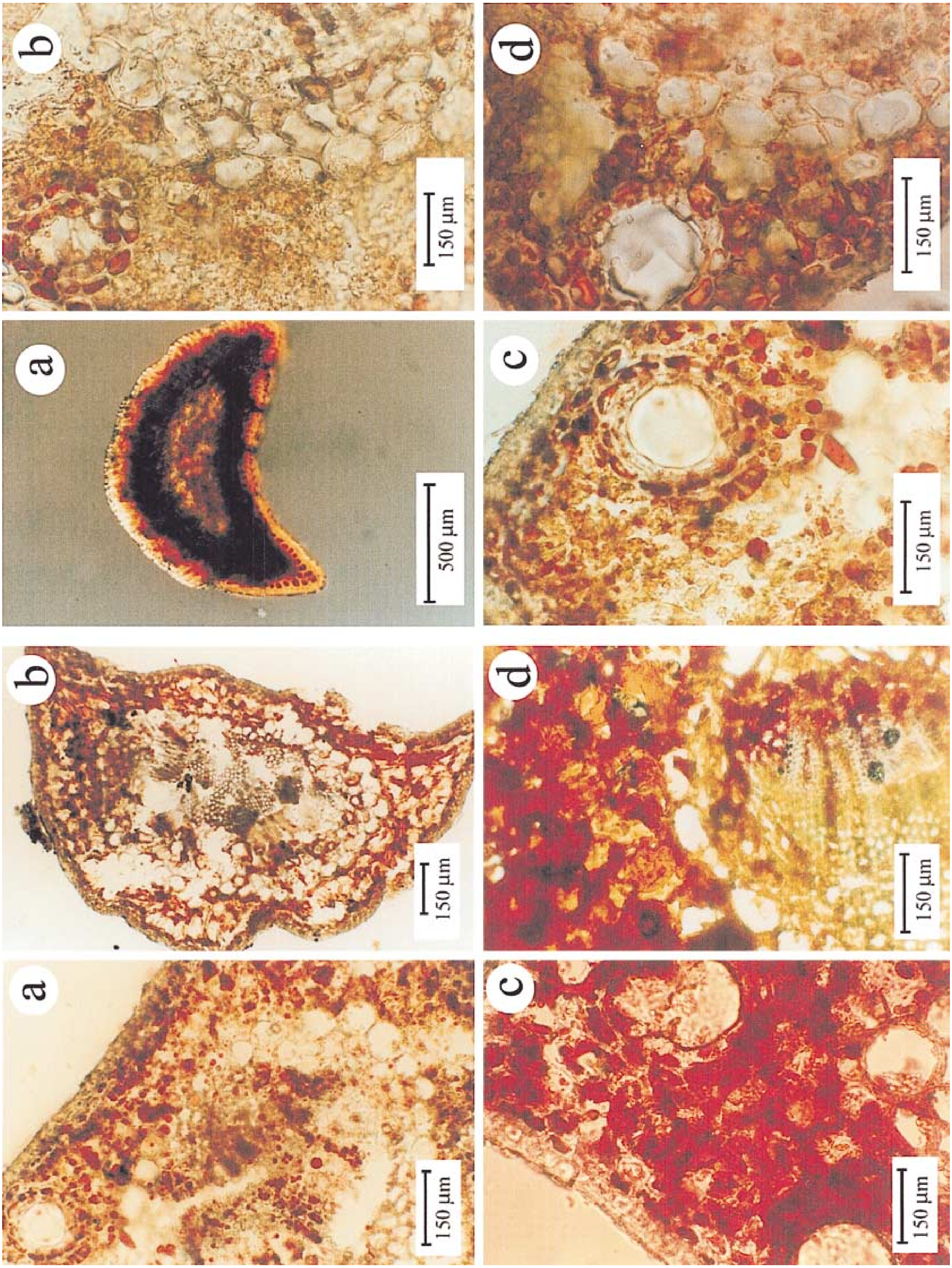
a
b
c
d
500 µm
150 µm
150 µm
150 µm
150 µm
150 µm
150 µm
150 µm

Fig. 8
Cross section of leaf's lower (basal) portion: dense red droplets of phenols are visible in the mesophyll, into vacuoles (*a*). Leaf lacunae and red phenols (*b*) contouring the cell walls. Cross section of leaf's upper (distal) portion reveals red phenols entirely filling vacuoles of epidermis and mesophyll cells (*c, d*)

cal alterations, such as the accumulation of phenols (Fig. 7a,b) (Zobel and Nighswander 1991; Melati and others 1997; Alaimo and others 1997, 1998, Bussotti and others 1998; Melati and others 1998). Experimentally, the distal portions of the needles were separated from the proximal ones, and transversal cryosections of the two parts were made. The cytochemical test was applied to highlight the phenols, which accumulated first in the vacuoles of epidermic cells and of the mesophyll (Fig. 8a). The mesophyll cells of polluted samples appear displasic (Fig. 7c,d), full of abundant phenols and interdigitations between evident intercellular spaces, with final concentrations of cytoplasm along the membranes (Fig. 8b). The phenols, later solubilized, are distributed in the cytoplasm (Fig. 8c,d) and may thus undergo retranslocation towards the external walls of epidermic cells, forming layers between walls and cuticle. The external walls of epidermic cells become thickened. Next, phenols are also observed in the cell components of transfusion tissue and in the two conducting bundles. They were never observed in endodermic cells (Fig. 8d). Morphological alterations attributable to phenol accumulation were observed, such as modifications in the length of mesophyll cells and the appearance of lacunae in the distal portions where, as a subpathological event, phenols accumulate to a greater extent, just before apical necrosis of the needles (Fig. 9a). Vice versa, the damage is not so great in the proximal portions where phenols accumulate in small drops and cells appear only slightly displasic and poorer in phenol metabolites (Figs. 9b,c,d). These latter gradually accumulate also around resin-bearing canals and in surrounding tissues, which become increasingly enriched in phenols (Fig. 9c,d). Phenol accumulation is directly linked to the presence of lead. Samples from heavily polluted stations were randomly subdivided into two groups: lead was assayed in one series of samples, and phenols were cytochemically identified in the other, all tests being replicated. The constancy of the results suggests that a direct correlation could be made.

Fig. 9
Necrosis of leaf tip (*a*). Increased production of phenols around the resine duct (*b–d*) in the proximal portions

Conclusion

Heavy metal contents in the pine needles analysed here are due partly to direct assimilation from the soil and partly to wet and dry deposition of atmospheric particulate on the aerial parts of pine trees. For some elements, normally emitted into the atmosphere in the gaseous state, gas-solid conversion on vegetal surfaces may constitute and important uptake process. Statistical analysis of data allowed elements in needles to be subdivided into three groups: (a) elements associated with clay minerals (Al, La, Sm, Sc), (b) elements associated with the calcarous substrate (Ca and Sr) and (c) elements associated with anthropogenic activities (Pb, Br, Sb, Cu, Zn). Comparisons with the chemical composition of the main lithotypes composing the soil of Palermo confirmed that the excess of Pb, Sb and some Br in the needles is due to anthropogenic emissions. The origin of Zn and Cu remains uncertain, although wholly crustal origin appears doubtful. As lead isotope data indicate that some anthropogenic lead becomes attached to the surface of needles, the same process may be hypothesized for the other metals, especially Sb.
Our observations show that in the species of *Pinus pinea* L. examined here, the accumulation of phenols gives rise to a specific pattern of metabolites providing mechanisms of detoxification and protection, so that the degree of accumulation may be proposed as a marker of environmental pollution. It does depend on secondary stress phenomena, i.e. on the presence of heavy metals in urban aerosols. The contribution of other gaseous pollutants (SO_2, O_3, NO_x) cannot be excluded, since they are emitted by the same sources identified for heavy metals. In any case, the results presented here confirm the presence of such metals in the urban air of Palermo and highlight their danger to vegetation. Judging from the obtained results, the same danger risk may be transferred to human beings. Lastly, this paper represents a further example of how the use of a wide variety of techniques is a modern approach to environmental control, improving the reliability of geochemical methods.

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