Pact 33 - III.3 : F. Manes, A. Altieri and P. Tripodo

# Plants as Indicators of Climatic Pollution in Urban and Nonurban Areas

*Abstract*

The bioindicator studies presented here were conducted in order to evaluate the impact of some atmospheric pollutants on urban and rural ecosystems. Active bioindicator studies used the tobacco cultivar Bel-W3 to show the occurrence of ozone in both urban and rural sites, and the accumulator species *Lolium multiflorum* to demonstrate the impact of some heavy metals near a street with heavy traffic, while passive bioindicator studies showed the influence of automobile and domestic heating unit emissions on the sulphate content of pine needles. Furthermore, a significant difference was found in pine, between urban and non-urban areas, in the erosion process of epicuticular waxes. The occurrence of elements of anthropogenic origin was observed on pine needles and holm-oak leaves by means of EDS. The results reaffirm the biological monitoring role of plants in natural and anthropogenic ecosystems.

## Introduction

The bioindication of the effects of atmospheric pollutants on plants represents a good research technique for evaluating the impact of these substances on vegetation in natural and anthropogenic ecosystems. Various authors have, in fact, used plants as bioindicators both by observing visible symptoms in species sensitive to a particular pollutant (indicator species), and by using chemical analyses of the tissues of species which are capable of accumulating toxic agents (accumulator species). The term « indicator » implicitly refers to the capacity of the organism involved simply to indicate the presence or absence of a particular factor. Clements (1920) was one of the first authors to clarify and describe in detail the use of plants as indicators of environmental conditions. In his treatise entitled « Plant Indicator » he writes : « Every plant is a measure of the conditions under which it grows. To this extent it is an index of soil and climate, and consequently an indicator of the behaviour of other plants and animals in the same spot ».

« Accumulator » species, on the other hand, are generally used to monitor pollution caused by particulate and heavy metals occurring in the atmosphere. The importance of monitoring the occurrence of these substances using a biological approach is also evident from the fact that pollution caused by heavy metals envolves complex relationships, and often the organisms most at risk are those situated towards the end of the food chain. Cities are considered to be centers of accumulation for a variety of pollutants derived from the use of large quantities of fossil fuels, raw materials and food substances. For this reason it is important to know the impact these activities have on organisms located in such environments. It is, moreover, possible to distinguish between « passive » and « active » bioindicators. In the first instance reference is made to the observation and analysis of local flora (both spontaneous and cultivated) ; in the second instance reference is made to the exposure of indicator species under standardized conditions (Steubing, 1982).

Considerable discussion has recently focused on the value of bioindicators and biological markers in evaluating stress caused by atmospheric pollution in forests. A biomarker consists of any biological index which can provide information applicable to monitoring the effects of stress on plants, and can be chosen from different levels, from the cellular to the ecosystem. Biomarkers can then be subdivided into visible biomarkers, which generally provide qualitative information, and invisible biomarkers, which can yield both qualitative and quantitative information (Tab. 1). Our research has not only been devoted to exploring specific aspects of the mechanisms by which various pollutants operate, but we have also attempted to define new tools for the application of biomarkers to natural environments.

TABLE 1. — SOME IMPORTANT BIOLOGIC MARKERS OF AIR POLLUTION STRESS AND DAMAGE IN FOREST (WOODWELL, 1989)

| | |
|---|---|
| – Foliar damage | – Photosynthesis and transpiration |
| – Nutrient cycling | – Enzyme analyses |
| – Tree ring analysis | – Loss of membrane integrity |
| – Allocation of photosynthate | – Pollutant content in tissues |
| – Symbiotic rhizosphere fungi | – Foliage histology |
| – Epiphytic cryptogams | – Cuticular alterations |

## ACTIVE BIOINDICATION

### *1. Evaluation of ozone occurrence*

In order to record the presence of phytotoxic concentrations of tropospheric ozone ($O_3$) during the summer period in the area of Rome, we used potted plants of a species known for its sensitivity to $O_3$, *Nicotiana* *tabacum* L. cv. Bel-W3. The study was carried out at both an urban station (ISS : Istituto Superiore di Sanita, Rome) and a rural one (CP : Presidential Reserve at Castelporziano, 30 Km SW of Rome) for the period from July 11th to August 7th 1989. Ozone is the quantitatively most important component of photochemical smog, whose formation is principally caused by reactions which involve nitrate oxides, hydrocarbons and atmospheric oxygen under the influence of solar radiation. Urban and industrialized areas are characterized by emission of the chief precursors and by the consequent formation of photochemical smog.

In Italy, despite the occurrence of climatic conditions (intense solar radiation, high temperatures, atmospheric stability) which favour photochemical smog formation in the summer months, little information is available concerning concentrations of tropospheric $O_3$, and what is available is limited to a few urban areas (Possanzini, 1981 ; Tebaldi, 1987 ; Bertolaccini, 1988). Interest in this study thus arose from the need to integrate information derived from the monitoring stations (which, in the city of Rome, are of a totally inadequate number), and to verify if high levels of ozone are also found in the rural site, as has been demonstrated for other areas in Europe (Ashmore *et al.*, 1980 ; Ro-Poulsen *et al.*, 1981) and Italy (Schenone and Mignanego, 1988 ; Lorenzini *et al.*, 1988 ; Altieri *et al.*, 1990). The occurrence of high concentrations of this gas in remote areas is explained by the transportation of air masses containing $O_3$ and/or its precursors from zones where these pollutants are produced, and by the accumulation phenomena stemming from the lack of chemical removal caused principally by the absence of NO. Indeed, it has been calculated that in some cases of photochemical smog formation, ozone and its precursors can be transported to remote areas up to a distance of about 100 to 500 km away (Guicherit and Van Dop, 1977), but more often up to a distance of not more than 50 to 100 km (Ciccioli *et al.*, 1987).

The sensitivity of cv. Bel-W3 of tobacco to $O_3$ is manifested by the formation of small interveinal chlorotic areas (diam. 1-5 mm), which, in about 24 hours, evolve necrotic injury spots, acquiring a white-to-grey colour. Since a positive correlation has been documented between the entity of foliar injury and the concentration of environmental $O_3$ (Ro-Poulsen *et al.*, 1981), this cultivar is often used to estimate the presence of high concentrations of $O_3$ ($>$ 40 ppb).

The tobacco seedlings were divided, at the age of 8 weeks (4-6 leaf stage), into a homogeneous set of groups, and were placed in the two stations (ISS and CP ; a monitoring station for atmospheric pollutants is also located in the first station). Literature concerning ozone biomonitoring with the highly sensitive cv. Bel-W3 reports the use of the tolerant cv. Bel-B (Manning and Feder, 1980) or the moderately sensitive cv. Bel-C (Ro-

Poulsen *et al.*, 1981) as a control. In our study we introduced an antioxydant protectant chemical as a control. This method is easy to apply, but the experimental procedures adopted (concentration, application times) varies according to the antioxidant, the application technique (foliar spray, soil drench, stem injection) and the plant species (Krupa and Manning, 1988). The antioxydant we used is N-(2-(2-oxo-1-imidazolidinyl)ethy)-N'phenylurea (EDU = ethylendiurea), which is known to provide adequate protection against ozone damage in many species (Carnahan *et al.*, 1978 ; Ensing *et al.*, 1985 ; Bisessar and Palmer, 1984), but whose efficacy on the highly sensitive Bel-W3 cv. of tobacco is still unknown. In order to measure this efficacy we administered EDU (soluble powder active at 50 %) as a soil drench, using two different concentrations : 125 and 500 ppm. The applications were repeated at 12-14 day intervals.

Measurement of foliar injury was carried out weekly and the Leaf Injury Index (LII) was calculated as proposed by Ashmore *et al.*, (1980). Since sensitivity to $O_3$ varies according to the age of the leaf (the most sensitive are those which have only recently fully expanded), this index was calculated taking into account only those leaves that at the beginning of the observation week were of a length greater than 5 cm and had injury of < 10 %. The percentage of surface of each leaf which showed injury was quantified with a value of between O to 8, according to Manes *et al.* (1990).

At both stations it is possible to note an increment of LII between the first and the fourth week (Fig. 1), related to the trend of ozone occurrence. This may indicate an increasing level of ozone over the weeks for the rural station as well, as reported in a previous work (Manes *et al.*, 1990). The relationships between LII, $O_3$ concentrations and sunshine hours, for the ISS station, are reported in Tab. 2.

TABLE 2. — CORRELATION BETWEEN LII AND SOME ENVIRONMENTAL PARAMETERS FOR THE ISS STATION

| Parameter | Correlation coefficient with LII (r) | Significance level |
|---|---|---|
| + Sunshine (No. of hours) | 0.77 | P < 0.01 |
| + Average [$O_3$] 7h mean (09.00-16.00) | 0.86 | P < 0.001 |
| + Average [$O_3$] 12h mean (06.00-18.00) | 0.86 | P < 0.001 |
| + Average [$O_3$] 24h mean (00.00-24.00) | 0.82 | P < 0.001 |

+ Each parameter was calculated one day previous from the beginning of each week considered for the LII evaluation (Ashmoe *et al.*, 1980).

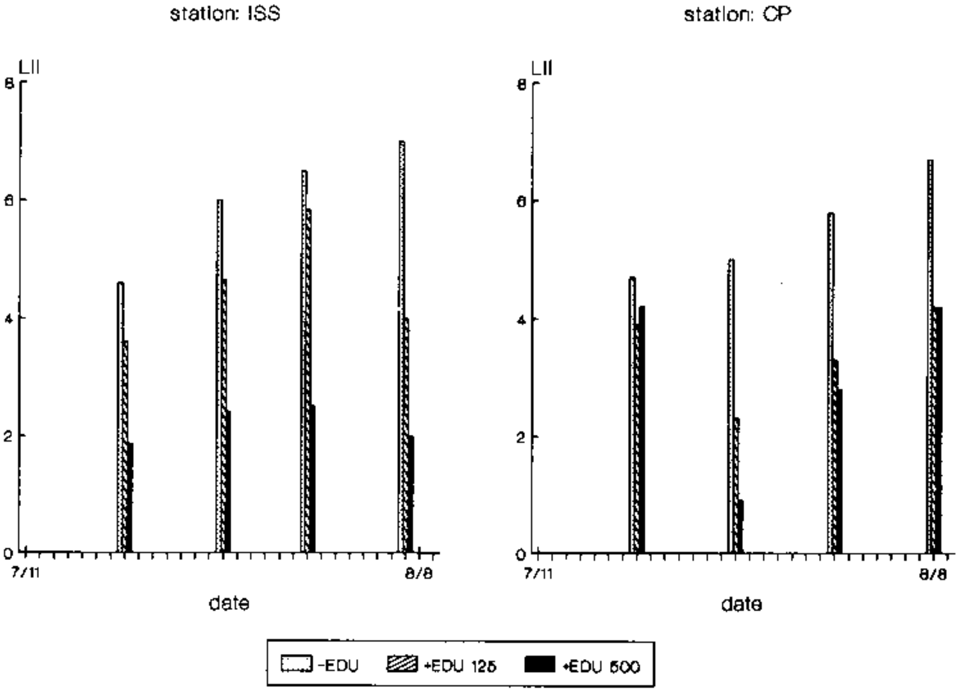


*Fig. 1. Leaf Injury Index (LII) values on tobacco plants for the two survey stations (ISS and CP), according to three different treatments (− EDU, + EDU 125, + EDU 500); differences significant at* < 0.01.

For the CP station, since environmental $O_3$ values were not available, it is only possible to compare the differences of LII between EDU-treated and untreated plants (Fig. 1). The high LII and the increasing trend observed during the period of research could be due to the transportation of $O_3$ and/or its precursors from the nearby urban areas. In fact, within the urban area of Rome, the predominant wind direction recorded during the study period between 7.00 and 8.00 a. m. is from east-north-east, thus supporting the hypothesis that the Castelporziano site is influenced by urban pollution. The protection afforded by 500 ppm EDU provides a valid control; the concentration of 125 ppm also protected leaves from visible injury, but not completely. However, as can be seen from the analysis of the variance, the difference between the three treatments was significant at < 0.01.

*2. Evaluation of the impact of some heavy metals*

A second study of active bioindication was carried out at the GB urban station: (Botanical Garden of the University « La Sapienza » of Rome), using the « accumulator » species Lolium multiflorum Lam., grown in appropriate trays. Two trays were placed close to a street with heavy traffic

(501 motor-vehicles during the 15' peak trafficperiod) and two were placed at a distance of about 50 metres from the street. As a control two other trays were placed in a greenhouse at about 200 metres from the street. The analysis was conducted using an atomic absorption spectrometer and took into consideraton (among other elements) the following heavy metals:

- Lead (Pb), which proved to be the best indicator of urban pollution, since it is a typical byproduct of vehicle exhaust.
- Copper (Cu), which is found in large quantitites in emissions from petroleum combustion from industries and heating and in emissions from incinerators.
- Zinc (Zn), which is produced by the wear of metal parts and automobile tyres, and which occurs in the proximity of the street in quantities as high as lead.

The data obtained (Tab. 3) show the direct influence of the nearby street for all three elements, when compared with the site at some distance away and the control. It should, however, be noted that the high values for zinc found in the control may derive, as already stated, from the wear of metal parts occurring within the control area.

TABLE 3. — PB, CU, ZN CONCENTRATIONS (UG/G FRESH WEIGHT) IN LOLIUM MULTIFLORUM LAM. LEAVES SAMPLED AT VARIOUS DISTANCES FROM THE STREET.

| Disance from the street | Pb | Cu | Zn |
|---|---|---|---|
| 5 meters | 44.67 | 38.22 | 103.73 |
| 50 meters | 13.4 | 24.81 | 48.64 |
| control | 9.85 | 13.64 | 76.14 |

## PASSIVE BIOINDICATION

### 1. *Analysis of sulphate content*

A study of « passive bioindication » was carried out for an entire year (March '88 — February '89). Ion chromatography was used to analyse the sulphate content of the needles of healthy and comparably aged specimens of *Pinus pinea* L. located at both an urban station (RM: Via Tiburtina, Rome) and the rural one of Castelporziano (CP). It is, indeed, known how pollutants can influence the nutrient content of leaves both directly and indirectly: absorption through the stomata and/or the cuticula; foliar leaching; modification of ionic availability in the soil.

Sulphur dioxide ($SO_2$) is absorbed by the pine needles principally via the stomata (Dasch, 1989); subsequent to the dissolution of $SO_2$ in extracellular fluids and in the protoplast sulphite ($SO_3$) and bisulphite ($HS_3^-$) are formed, two ionic species which have proved to be phytotoxic in their action in many biochemical and physiological processes (Ziegler, 1975). Plant organisms can convert these ions into less toxic forms; the oxidation of sulphite to sulphate ($SO_4$ =) being the most common mechanism. Plants exposed to $SO_2$ can, therefore, accumulate sulphur in their tissues (Guderian, 1977; Alfani *et al.*, 1983; Huttunen *et al.*, 1985), principally in the form of sulphates (Legge *et al.*, 1988). The sulphate content of plant tissues has thus proved to be a useful marker in the bioindication of $SO_2$ (Manes *et al.*, 1988).

The results of the sampling are presented here with the values grouped by seasonal periods (Fig. 2). The significant differences in sulphate concentrations between needle samples from RM and CP suggest possible differences in the concentration of atmospheric $SO_2$ at the two sites. These differences were found in all the seasons and were particularly evident in needles of the 1986 and '87 vegetative years, underlining the process of accumulation derived from the transformation of atmospheric $SO_2$ into $SO_4$ = in leaf tissues in the urban station (Fig. 2b, c). For the younger needles (1988 vegetative year) (Fig. 2a) the sulphate content is shown to be fairly homogeneous for the two stations during the first months of growth and development of the needles (summer-autumn '88), with a noticeable differentiation in the winter period. This can be explained both by the limited period of exposure to atmospheric pollutants, and by the fact that in urban areas $SO_2$ occurs in considerably lower quantities in the summer months (Fig. 3). The increase of the baseline concentration of suphates from about 500 ppm to more than 1000 ppm between the autumn and winter period reflects the greater capacity of young needles to convert absorbed $SO_2$ into $SO_4$ (Guderian, 1977).

The analysis of the sulphate concentration in the tissues of pine needles thus revealed the good capacity of this plant species to give information about the level of atmospheric $SO_2$

*2. Analysis of epicuticular waxes and X-ray analysis*

In another study two evergreen species were examined, *Pinus pinea* L., a conifer, and *Quercus ilex* L., a broadleaved tree; two plants having different morpho-anatomical leaf characteristics. These tree species, which are widely distributed throughout the Mediterranean area and common as ornamental plants in urban areas, were sampled at different urban stations (streets with high vehicular traffic, and urban parks) (Tab. 4a, b) and at one rural station (Presidential Reserve of Castelporziano) which formed a

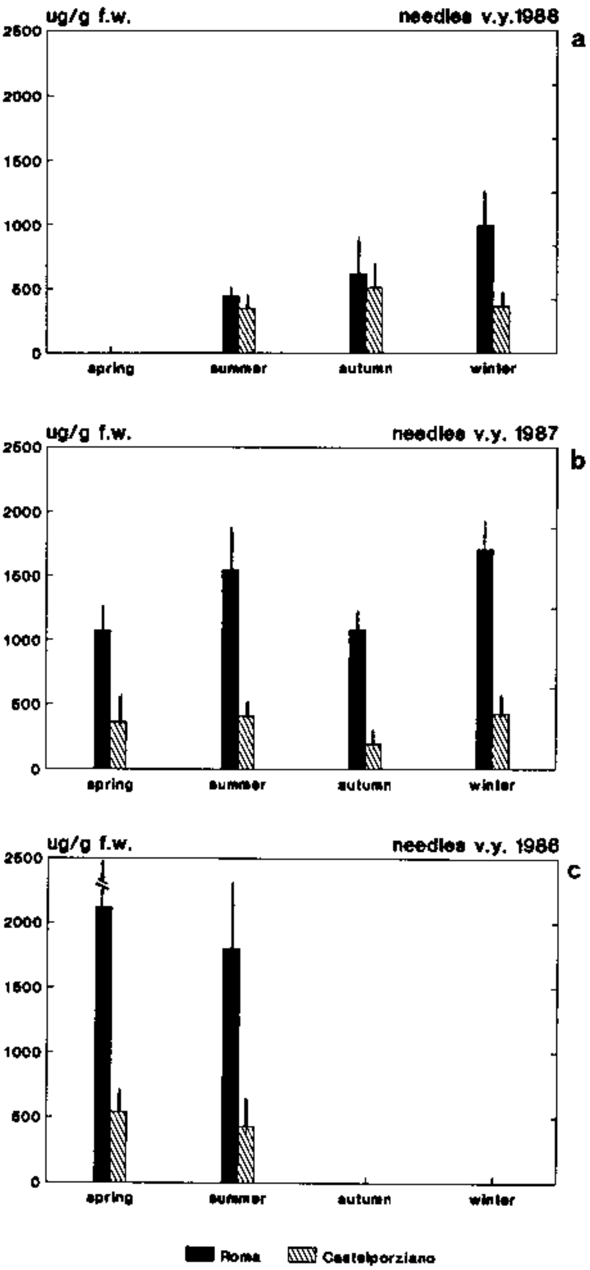


*Fig. 2. Seasonal average values of sulphate concentrations in pine needles of three different vegetative years (v. y. ; a-b-c) for the two sampling stations. Sampling period March '88 – February '89.*

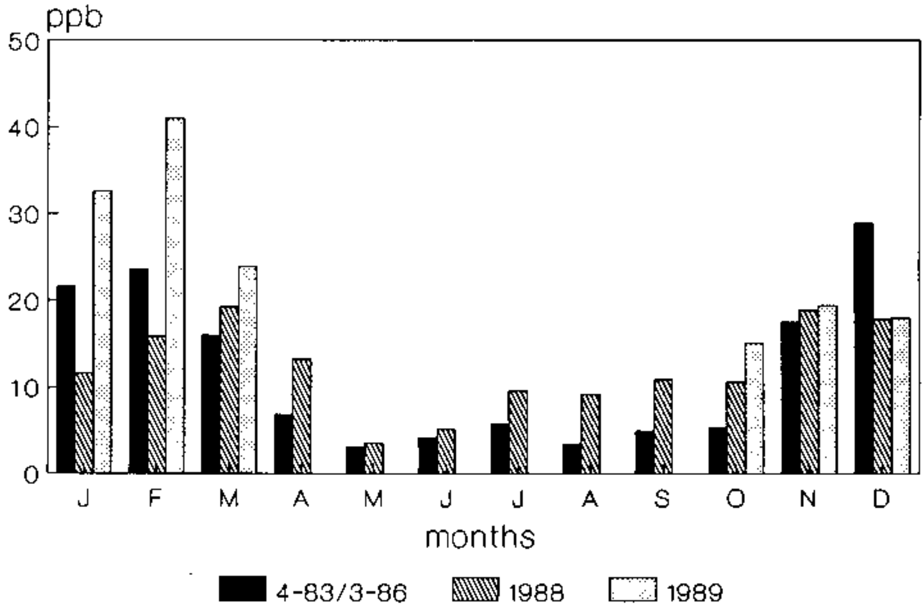


*Fig. 3. Monthly average concentrations (ppb) of atmospheric $SO_2$ in the city of Rome for different monitoring periods; the data were kindly supplied by the Instituto Superiore di Sanita — Laboratorio di Igiene Ambientale, Reparto Igiene dell'Aria, and recorded at the monitoring station located in viale Regina Elena, Roma.*

control. Sampling was carried out in the period June 24th-26th 1987 in order to evaluate, by analysis with scanning electron microscopy (SEM), the potential of some leaf surface structures in characterizing areas with different levels of pollution. Leaves and needles were sampled from two age classes (about 2 months-old and about 12 months-old).

TABLE 4A. — SAMPLING STATIONS IN ROME

| | |
|---|---|
| 1 — Villa Pamphili | 5 — Appia Nuova street |
| 2 — Villa Ada | 6 — Europa street |
| 3 — Cristoforo Colombo street | 7 — S. Bibiana street |
| 4 — Re di Roma square | 8 — Villa Borghese |

TABLE 4B. — NO. OF MOTOR-VEHICLES DURING 15' PEAK TRAFFIC PERIOD IN SOME SAMPLING STATIONS ALONG STREETS IN ROME

| station | vehicles No./15' |
|---|---|
| Re di Roma square | 930 |
| Appia Nuova | 930 |
| Europa street | 1480 |

Structural alteration of the leaf surfaces have been described in plants sampled at stations with high levels of atmospheric pollution or exposed to pollutants under controlled laboratory conditions (Cape, 1988 ; Turunen and Huttunen, 1990). The alteration can be of two main types : changes in the form, size and structure of certain characteristics specific to the cuticula (epicuticular waxes, epidermal cell surface) ; changes in the surface structure resulting from the deposition of particulate matter, but not necessarily in the characteristics of the cells of the epidermis. While the structural changes are not specific to the type of pollutant, the analysis of the particulate matter deposited on the leaf surface can yield useful information for bioindicational purposes.

With respect to the samples of *Pinus pinea* L. the needle surface exhibits a covering of structured waxes distributed in the area of the stomatic rows (Fig. 4a). Structural alterations of these components were observed both in connection with the age of the needle (Fig. 4c, d) and the physical (wind) and chemical (atmospheric pollutants) interactions (Fig. 4b) (Crossley and Fowler, 1986). The literature reports that the acceleration of the weathering process on the structure of epicuticular waxes for certain species of conifer is induced by different atmospheric pollution conditions (acid rains or fog, $O_3$, $SO_2$, exhaust fumes). In order to use these structural changes as diagnostic criteria for the evaluation of damage from atmospheric pollutants for *Pinus pinea* in a previous work we characterized the type of alteration and defined five injury classes (Manes *et al.*, 1988).

This study shows that the alteration of waxes in the peristomatal area is already found in young needles (Fig. 5a) sampled from trees located along streets in the urban environment in contrast to needles sampled in urban parks and at the rural station. In the older needles (about 12 months-old) (Fig. 5b) not only is the higher injury class (class 5 of alteration) more common but there is also a differentiation in behaviour by urban station ; indeed greater damage seems to exist in the parks. This fact may be explained by the microclimatic conditions within the urban green areas, and above all by the occurrence of hidden precipitation. Indeed, in the presence of dew, pollutants accumulated on the surface dissolve and react with the wax components (Bermadinger *et al.*, 1988).

The greater damage observed in young needles sampled from individuals located along streets as compared with those located in parks seems to provide a better indication of the differential impact of atmospheric pollutants, especially those derived from motor vehicles, between the two types of urban station. For young needles this is further supported by the quantification of particulate matter ( §5 m in size) occurring in the peristomatal area (Tab. 5 ; Fig. 4a, b). It should be noted, however, that the differences in wax alterations and the occurrence of particulate mattern

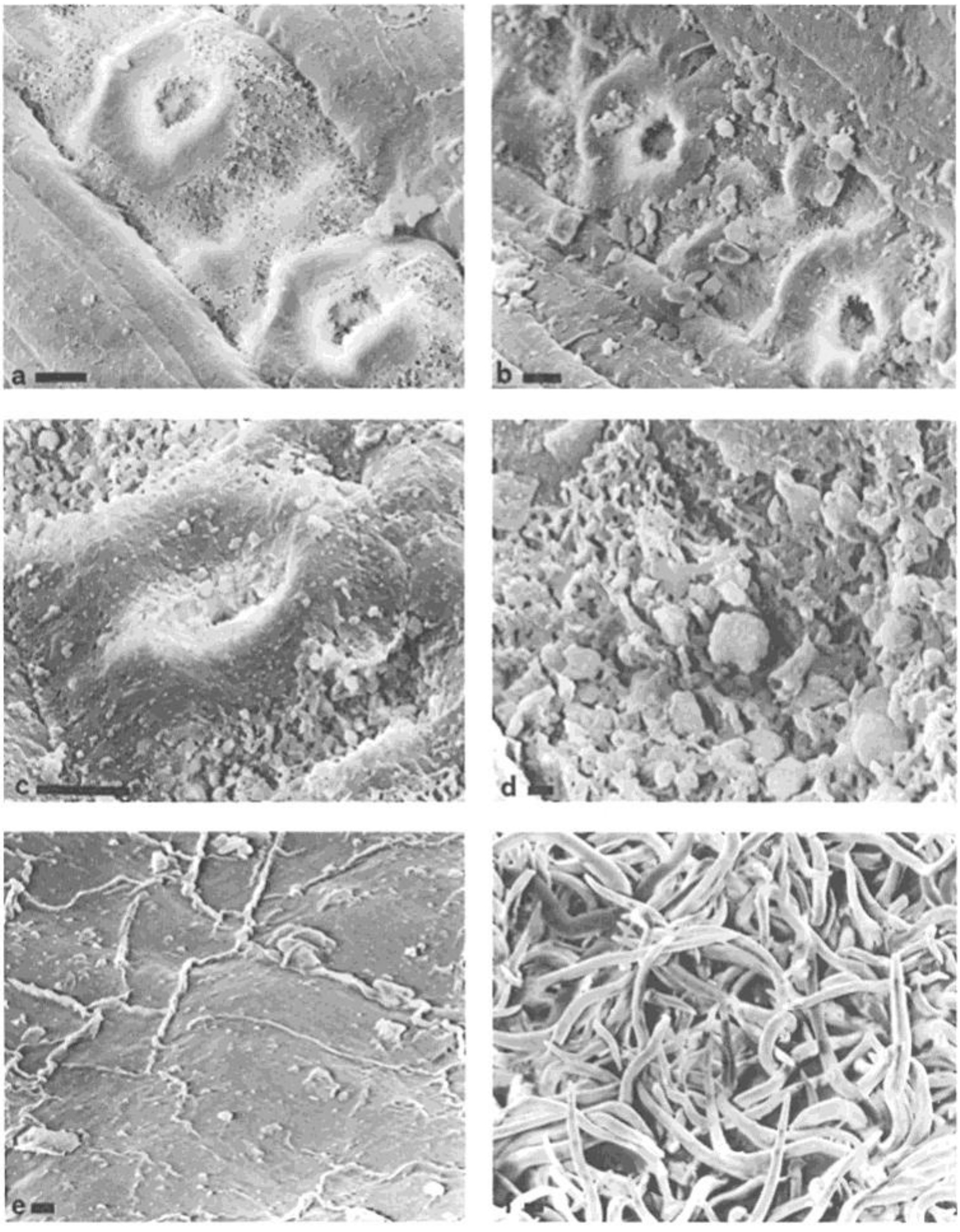

*Fig. 4. SEM micrographs of* Pinus pinea *needles and* Quercus ilex *leaves. (a-d): The peristomatal area of: a – young needle (about 2 months-old) from a rural area and an urban park, bar = 10 m; b – young needle from trees located along streets, bar = 10 m; c – old needle (12 months-old) heavily damaged, class 5 of wax alteration, bar = 10 m; d – detail of wax alteration, bar = 1 m; (e-f): Leaf of* Quercus ilex*: e – adaxial surface, bar = 1 m; f – abaxial surface, bar = 10 m.*

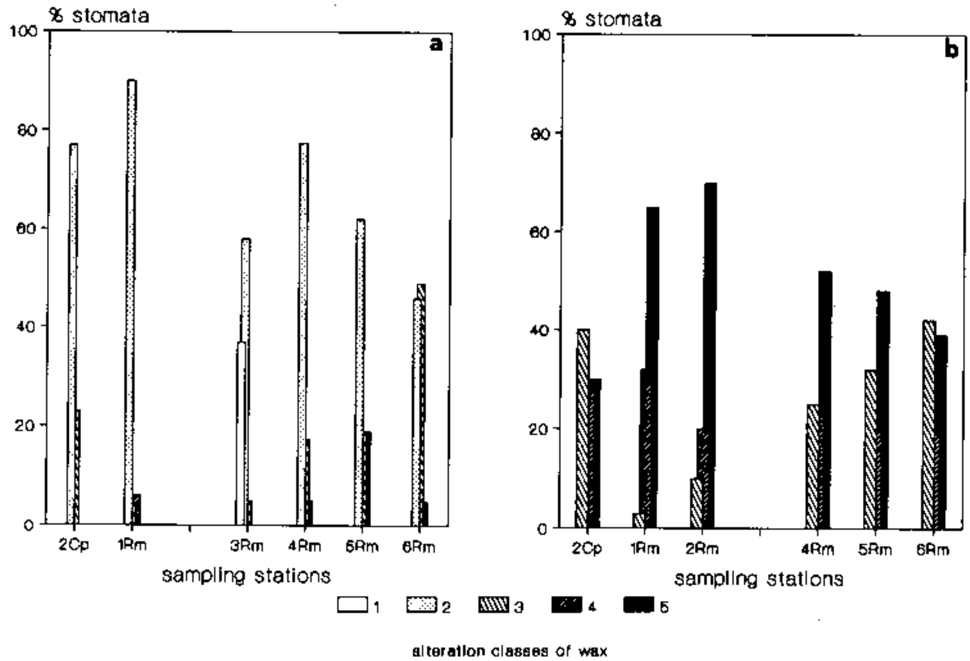


*Fig. 5. Frequency distribution of the alteration classes of epicuticular wax, evaluated by SEM analysis, in* Pinus pinea *needles of different ages (a : about 2 months-old, b : 12 months-old).*

observed between the stations tends to diminish in older needles in parallel with the natural aging process of needles exposed to environmental factors.

Another confirmation of these results is obtained with the quantification of *Quercus ilex* L. leaves, which emphasises the differential response between the urban street stations and those in parks and in the rural site (Tab. 5b).

In holm-oak the upper leaf surface appears, under the SEM, to be amorphous with a wax component (soluble with chloroform washes) which forms refracting crests (Fig. 4e). Sporadic pluricellular and unicellular hairs also occur ; the lower surface, on the other hand, has a thick layer of hairs (Fig. 4f). The analysis of particulate matter thus appears to be the major parameter when using this species as a biomonitor. A qualitative identification was carried out on selected scanned areas on the leaf surface using X-ray microanalysis with an energy dispersive spectrometer (EDS). The size of the areas scanned (about 5-7 $mm^2$ for the *Pinus pinea* needle and 10 $mm^2$ for *Quercus ilex* leaf) and the scanning time were approximately constant, which made it feasible to compare the relative concentration, based on peak height, of the elements in the different scanned samples, from the different sampling stations.

TABLE 5. — DISTRIBUTION PERCENTAGES OF THE PARTICULATE DENSITY CLASSES (A > 60 % ; B > 30 - < 60 % ; C < 30 %) ON LEAVES SAMPLED IN THE DIFFERENT STATIONS. THE EVALUATION WAS CARRIED OUT BY SEM ANALYSIS ON SCANNED SURFACES OF ABOUT 20 $MM^2$.

| Species | Quercus ilex L. | | | Pinus pinea L. | | |
|---|---|---|---|---|---|---|
| Station/Classes | A | B | C | A | B | C |
| *Urban streets (RM)* | | | | | | |
| Re di Roma Square | 16.5 | 16.5 | 67 | | 23 | 77 |
| Appia street | — | — | — | | 100 | |
| C. Colombo street | — | — | — | 63 | 37 | |
| Europa street | — | — | — | 95 | 5 | |
| S. Bibiana street | 100 | | | — | — | — |
| *Urban parks (RM)* | | | | | | |
| Villa Pamphili | | 17 | 83 | | 10 | 90 |
| Villa Ada | | 14 | 86 | — | — | — |
| Villa Borghese | | 14 | 86 | — | — | — |
| *Rural station (CP)* | | | | | | |
| 1 | | 17 | 83 | — | — | — |
| 2 | — | — | — | | 23 | 77 |

The analysis carried out on the pine needles made it possible to document conspicuous peaks of Al, Si, K, Ca and Fe. Fig. 6a shows the lower occurrence, in the urban environment, of such elements as Si and Al which originate mostly in the soil, and an increase in Fe and Ca. A similar trend for the elements Al, Si and Fe is described both for the airborne urban dust in the downtown area of Rome (Paoletti *et al.*, 1989) and for the surface of *Pinus virginiana* needles sampled in areas with high vehicular traffic (Elias and Croxdale, 1980). Thus, it seems possible to attribute the origin of particules occurring on the surface of needles in urban areas principally to vehicular traffic.

The analysis carried out on holm-oak leaves shows analytical spectra with peaks of Al, Si, S, K, Ca and Fe (Fig. 6b). The difference in the trends of the detected elements, between the urban and non-urban stations, are less evident than those observed in the pine needles. In both species no differences were observed between the different ages of the leaf/needle, as is the case in the occurence of the elements.

Although numerous individual particles (of different size) were also scanned, no differences in elemental composition, in contrast to the X-ray

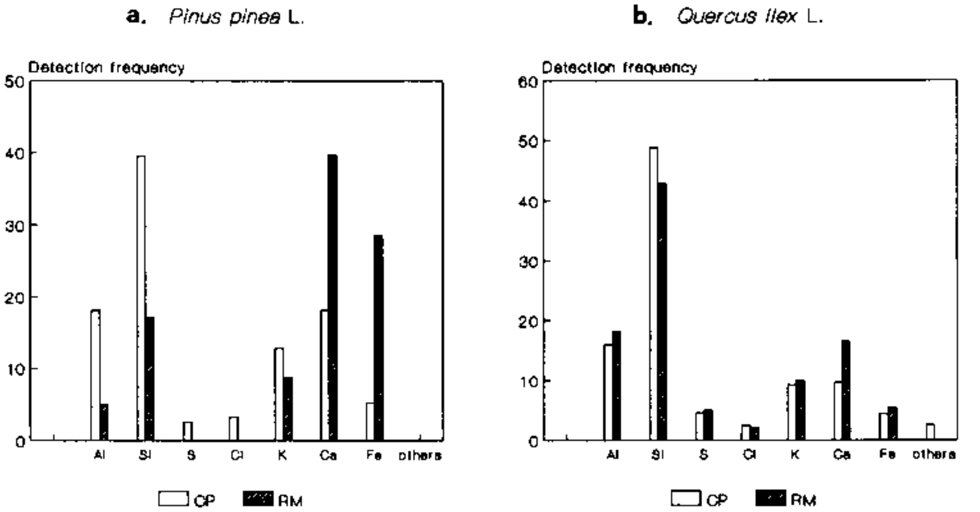


*Fig. 6. Frequencies of elements observed on the leaf surfaces from the rural (CP) and urban (RM) sites ; a :* Pinus pinea *L. needles, b :* Quercus ilex *leaves.*

microanalysis of the selected scanned areas, were found and none of the particles contained lead, at the detection limits of the instrument, as has already been shown by Elias and Croxdale (1980).

When comparing the potential of the two species for characterizing the urban and non-urban sites by analysising the elemental spectrum, *Pinus pinea* seems to be more effective, probably owing to its greater surface roughness. Indeed, it is known that surface roughness can increase efficacy in the capture of particulate matter (approximately 5 μm in diameter) even in relation to a diminished stability of the boundary layer (Smith, 1981).

## Conclusions

The studies of bioindication we have carried out make it possible to document certain significant differences in the response to atmospheric pollutants of the plant species studied in the two urban and nonurban sites. Using different plant species and various type of biomarkers we have been able to establish the occurrence of phytotoxic concentrations of $O_3$ and a sizable load of $SO_2$, heavy metals and particulate matter of anthropogenic origin. In particular, although, for ozone there does not seem to be any notable difference in concentration between the two sites, for the primary pollutants derived chiefly from emissions from automobile and heating units, the difference in impact between the two sites is more easily identified.

These results thus make it possible to reaffirm the role of biological monitors played by plants in natural and anthropogenic ecosystems and, once more, to emphasise, apart from the need for greater control on the emission of atmospheric pollutants, the need for correct planning and management in the development of metropolitan areas. This should entail appropriate « reforestation » efforts in the streets and in the urban green areas.

We can thus conclude by stressing the value of plants in the urban environment, not only from the historical and landscape points of view, but also in « functional » terms, their being useful both in the evaluation of environmental quality and in constituting a removal « sink » for gaseous and particulate pollutants.

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