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Trace Metal Biomonitoring in the Soil and the Leaves of *Quercus Ilex* in the Urban Area of Naples

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ABSTRACT

The concentrations of Pb, Cu, Fe, and Mn were analyzed in surface deposit and tissue of *Quercus ilex* leaves from several sites of the urban area of Naples, exposed to different degrees of air pollution. These included some major roads with heavy traffic loads, squares, and three urban parks. The soil from the trunk base area of *Q. ilex* trees in the same sites was also analyzed for total and available metal contents. Pb, Cu, and Fe contents in the surface deposit and leaf tissue were significantly higher ($p < 0.01$) in leaves from roadside sites than in leaves from parks; significant correlations were found between deposit- and tissue-contents of Pb, Cu, and Fe. Mn content in leaves from roadside sites and in leaves from parks were similar and Mn content in the leaf deposit was irrelevant. Significant differences ($p < 0.001$) in both total and available Pb and Cu soil content were found between sampling sites. Also for available Fe and Mn soil content differences among sites were relevant, although the highest values were measured in soil from urban parks. A positive correlation between leaf and soil metal content was found only for Pb, thus suggesting that trace metal contents of leaves directly depend on atmospheric depositions. Seasonal variations of Pb, Cu, and Fe were pronounced at a polluted site, whereas no relevant seasonal variation was observed at a control site; moreover, metal accumulation was high at the polluted site. Mn content and seasonal dynamics were comparable at control and polluted sites.

Index Entries: Lead; copper; iron; manganese; urban area pollution; soil; leaves; biomonitoring; seasonal dynamics; Holly Oak.

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INTRODUCTION

Trace metals released in the environment may be a hazard to natural biological systems and to human health. Plants and soil surface are the major sink for airborne metals *(1–9)*; moreover, plants form the basis of food chains by which biotoxic trace metals are transmitted to man *(10–12)*. Accumulation of trace metals in forest soil has been frequently used as an index of aerial metal deposition in nonurban areas *(7,13,14)*, and recent investigations have shown that atmospheric deposition is a major source of Cu and Pb to agricultural soils *(12,15,16)*.

Contamination of soil and vegetation by airborne trace metals is particularly massive in urban areas, notably along highways and near smelters and foundries *(2,7,9)*. A positive correlation between traffic flow and trace metal (Pb, V, Cu, Fe, Zn, Ni) contents has been found in unwashed leaves of *Quercus ilex* trees growing in the urban area of Naples *(17)*. This is a densely populated city with major industrial plants within the urban area, and presents heavy vehicular traffic and high concentrations of trace metals in the air *(18)*.

The present paper reports a monitoring study of Pb, Cu, Fe, and Mn in *Q. ilex* leaves and underlying soil from several sites in the urban area of Naples. Pb is a toxic element closely related to pollution by vehicular traffic as, in urban areas, it derives principally from automobile exhaust. Cu, Fe, and Mn are essential micronutrients for plants but, when released in excess by anthropogenic activity, they may be toxic for plants as well as for other organisms. The aim of this work was to investigate the distribution of these trace metals in the leaf surface deposit, leaf tissue, and soil, with special attention to relationships (1) between leaf and soil metal concentrations; (2) between elemental content in leaf surface deposit and leaf tissue; and (3) between total and available content in the soil. The paper also includes a comparative analysis of trace metal dynamics over a 2-yr period in leaves of *Q. ilex* from a remote area and a highly polluted urban site in order to evidence the influence of leaf age and sampling period on leaf metal content.

MATERIALS AND METHODS

Q. ilex (Holly Oak) is an evergreen mediteranean oak very common in parks and gardens, as well as along major roads in Naples. In January 1994, several branches were sampled around the lower tree foliage from nine sites in the urban area exposed to different loads of trace metals *(17)*. The sampling sites referred to as CI, OB, and CE are in urban parks, the first two in internal positions, the last facing a road. The other sites are in squares (PT, PC) or major roads (CC, GI, VM, ST) supporting intense traffic flows. The site CI, in the interior of the largest park in the urban area of Naples, was used as a reference for comparison between urban sites.

From each site, two comparable samples of 30 leaves of first year were obtained, from two or three trees, taking in succession two adjacent leaves in good condition, one for the first sample and the other for the second sample. One of the samples was washed by shaking in tap water for 30 min in order to remove the leaf surface deposit *(19)*, whereas the other was left unwashed for comparison.

Soil samples were collected from the same sites chosen for leaf sampling, as well as from a wood (RA) out of the city. At each site, three soil samples were collected with a cylinder of PVC and a trowel of plexiglass from the surface horizon (0–10 cm) at the trunk base and stored in polythene bags. In laboratory, the soil samples were passed through a 2-mm mesh sieve.

Samples of soil and of washed and unwashed leaves were oven-dried at 75°C to constant weight and grounded to a fine powder by an agate pocket in a Fritsh pulverisette. Care was taken at all stages of sampling, storage, and preparation for analysis to avoid metallic contamination.

To estimate Pb, Cu, Fe, and Mn total content, leaf and soil samples (250 mg) were digested in a Mileston (mls 1200) Microwave Laboratory Systems with a mixture of nitric and hydrofluoric acid (HF 40% v/v; HNO_3 65% v/v = 2:4).

The available amounts of Pb, Cu, Fe, and Mn in the soil were estimated in extracts obtained by shaking soil samples for 2 h in Lindsay and Norwell reactive (DPTA, $CaCl_2$, and TEA at pH 7.3) in ratio 1:2 *(20)*. The extracts were filtered (Whatman 42) and the resulting solution diluted to volume with distilled water.

The elemental concentration in leaves and soil was measured by atomic absorption spectrophotometry (SpectrAA20 Varian) using standard solutions (STD Analyticals Carlo Erba) diluted in the same acid matrix as for extraction. Leaf analyses have been carried out on two subsamples, soil analyses on three subsamples. Elemental contents in soil and washed and unwashed leaves exhibited a SD < 10%; higher SD values (<20%) referred only to samples from sites with low Pb contamination. With remarkable leaf surface deposit, trace metal content in washed and unwashed leaves differed significantly ($p < 0.05$). The elemental concentration in the leaf surface deposit was evaluated on the basis of differences between unwashed and washed leaves. This procedure is supported by experimental evidence showing that careful rinse in water removes a very large percentage of metal burden from the leaf surface *(19)*. Accordingly, the values of elemental concentration in washed leaves are referred to as leaf tissue values.

Relationships between leaf and soil metal concentrations, as well as between elements, were tested by the Spearman's coefficient of rank correlation (r_s). The significance of differences between sites was analyzed using one-way ANOVA. The seasonal dynamics of Pb, Cu, Fe, and Mn contents in unwashed leaves of *Q. ilex* were studied twice, during the vegetative season in 1990–91 and in 1991–92. The leaves were collected at 3- to 4-mo intervals from a control site in a remote area (Mount Tubenna) and from an urban site with high traffic flow (*via S. Teresa*) in the heart of Naples. For each sampling, metal contents were measured separately in both 1- and 2-yr-old leaves, as described.

RESULTS

Soil Analysis

Total Pb (Fig. 1A) and Cu (Fig. 2A) contents in the soil were respectively in the range 80–757 and 25–192 μg/g dry wt, whereas the available contents were in the range 0.5–50 μg/g dry wt for Pb (Fig. 1B) and 2–80 μg/g dry wt for Cu (Fig. 2B). Total and available Pb and Cu contents in the soil from a remote area (RA) were significantly lower ($p < 0.001$) than in urban sites. Accumulation factors of 10 and 8 for total Pb and Cu and 37 and 13 for available Pb and Cu were estimated on the basis of the ratio between the highest value in urban sites and the value at the remote site (RA). Soil Pb and Cu contents in urban sites (parks, squares, and roads) were significantly different from the lowest value in the site CI in the interior of a large park (Fig. 3).

Total soil contents of Fe (Fig. 4A) and Mn (Fig. 5A) were in the range 12.5–24.4 mg/g dry wt and 463–755 μg/g dry wt, respectively, with smaller differences among sites compared to Pb and Cu (Fig. 3). Total Fe and Mn values did not reflect the degree of pollution of the different sites. The available Fe (Fig. 4B) and Mn (Fig. 5B) contents ranged respectively from 0.073–0.486 mg/g and from 27–180 μg/g dry wt; differences among sites were significant (Fig. 3) but, unlike Pb and Cu, Fe and Mn available contents were higher in soil samples from the remote site and urban parks than from roadside sites (Figs. 3, 4B, and 5B).

Total and available soil contents of Pb, Cu, and Mn were positively correlated ($r_S = 0.717$, $p < 0.05$; $r_S = 0.933$, $p < 0.001$; and $r_S = 0.833$, $p < 0.01$, respectively). By contrast, total and available Fe contents were not correlated. The available Pb was negatively correlated to the available Fe soil content ($r_S = -1$, $p < 0.001$). A negative correlation was also found between the available Pb and available K and Mg content (unpublished data).

Leaf Analysis

Metal contents in leaf surface deposit and leaf tissue were respectively in the range 1.2–23.5 and 1.5–21.4 μg/g dry wt for Pb (Fig. 1C,D); 0.3–80.5 and 7.2–30.5 μg/g dry wt for Cu (Fig. 2C,D); 1–1459 and 197–914 μg/g dry wt for Fe (Fig. 4C,D); and 0–19 and 70–248 μg/g dry wt for Mn (Fig. 5C,D). The levels of Pb, Cu, and Fe in the surface deposit and tissue were significantly higher in leaves from roadside sites than in leaves from squares; the latter showed significantly higher contents compared to leaves from urban parks (Fig. 3). In contrast, Mn content in the surface deposit and leaf tissue did not reflect a gradient of pollution (Fig. 5C,D).

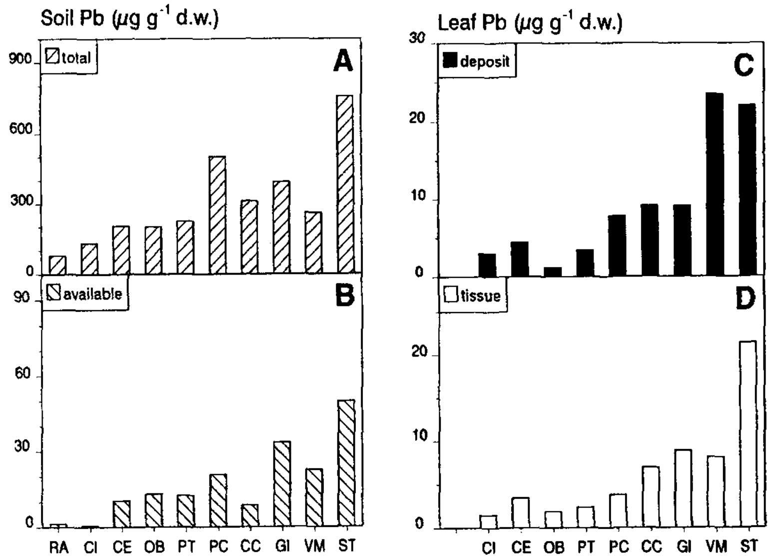

Fig. 1. Total **(A)** and available **(B)** Pb content in the soil (0–10 cm depth) of several sites in the urban area of Naples; Pb concentration in leaf surface deposit **(C)** and leaf tissue **(D)** of *Q. ilex* leaves from the same sites.

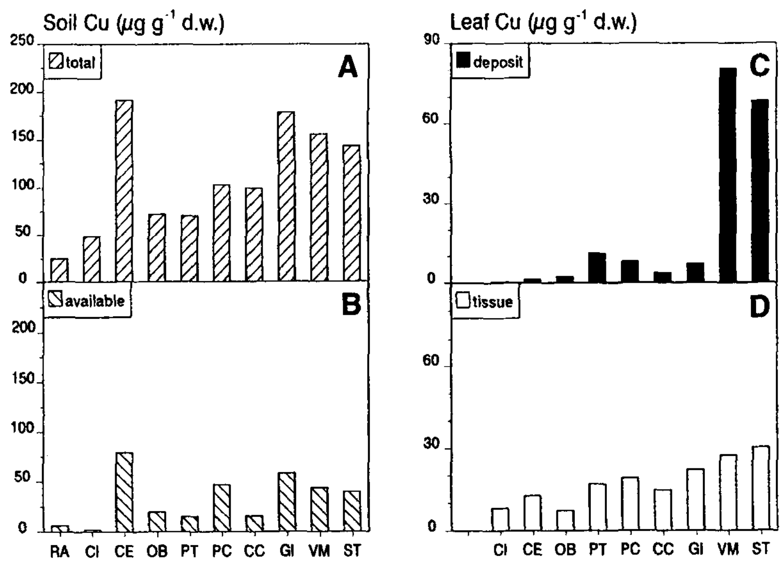

Fig. 2. Total **(A)** and available **(B)** Cu content in the soil (0–10 cm depth) of several sites in the urban area of Naples; Cu concentration in leaf surface deposit **(C)** and leaf tissue **(D)** of *Q. ilex* leaves from the same sites.

Pb levels in leaf deposit and leaf tissue were about an order of magnitude higher in samples from the sites with heavier traffic flow (VM and ST) than in leaves from urban parks (CI and OB, Fig. 1C,D). It has to be emphasized that, in all the sites considered in the present study, the Pb

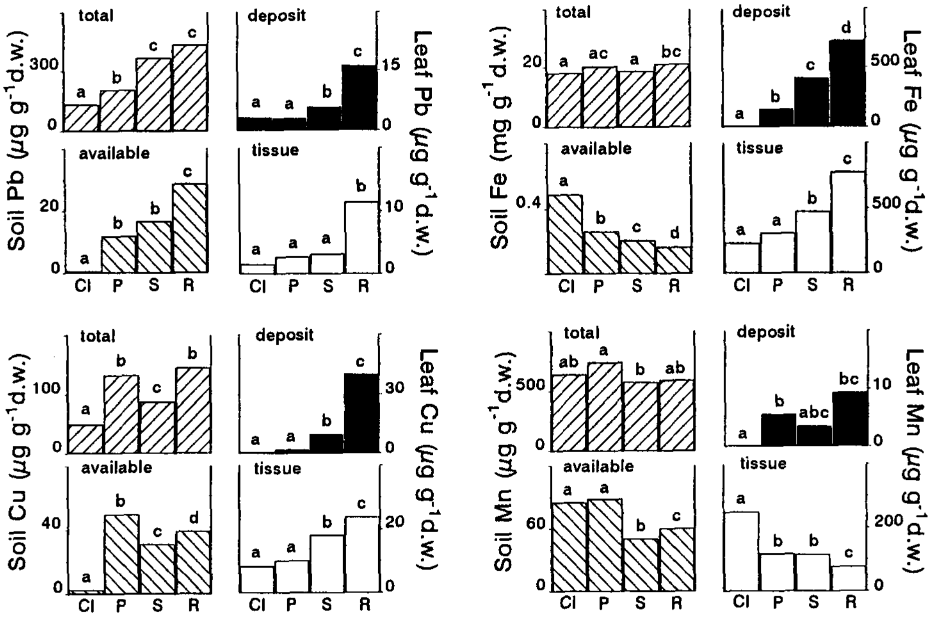

Fig. 3. Significance of differences in trace metal contents of soil and leaves of *Q. ilex* from sites with different typology (CI, in the interior of a large park; P, parks; S, squares; R, roadside). Bars with different letters represent significantly different values ($p < 0.05$ as determined by ANOVA test).

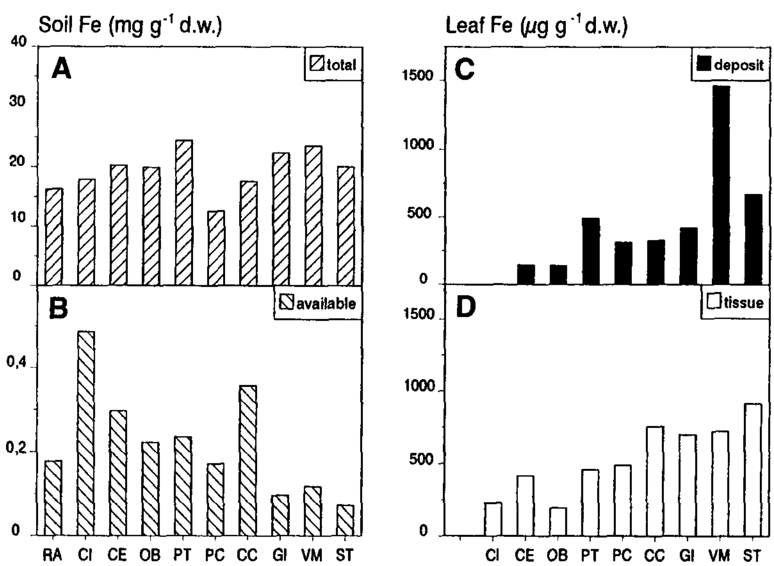

Fig. 4. Total **(A)** and available **(B)** Fe content in the soil (0–10 cm depth) of several sites in the urban area of Naples; Fe concentration in leaf surface deposit **(C)** and leaf tissue **(D)** of *Q. ilex* leaves from the same sites.

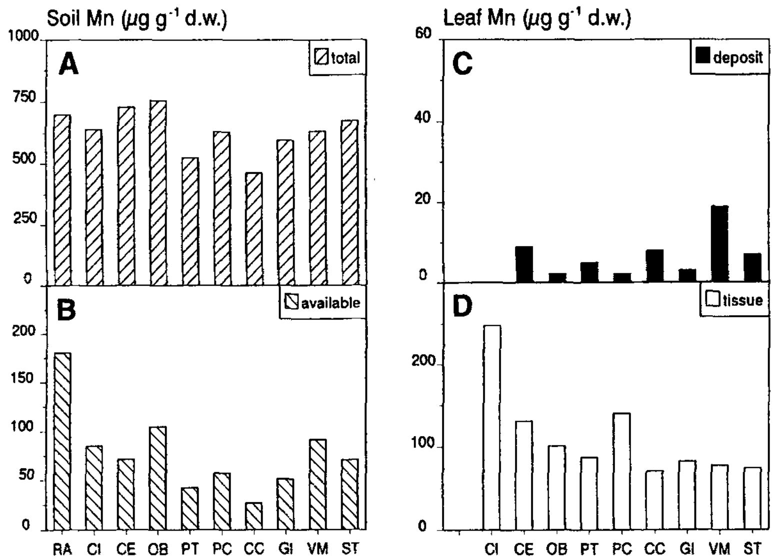

Fig. 5. Total **(A)** and available **(B)** Mn content in the soil (0–10 cm depth) of several sites in the urban area of Naples; Mn concentration in leaf surface deposit **(C)** and leaf tissue **(D)** of *Q. ilex* leaves from the same sites.

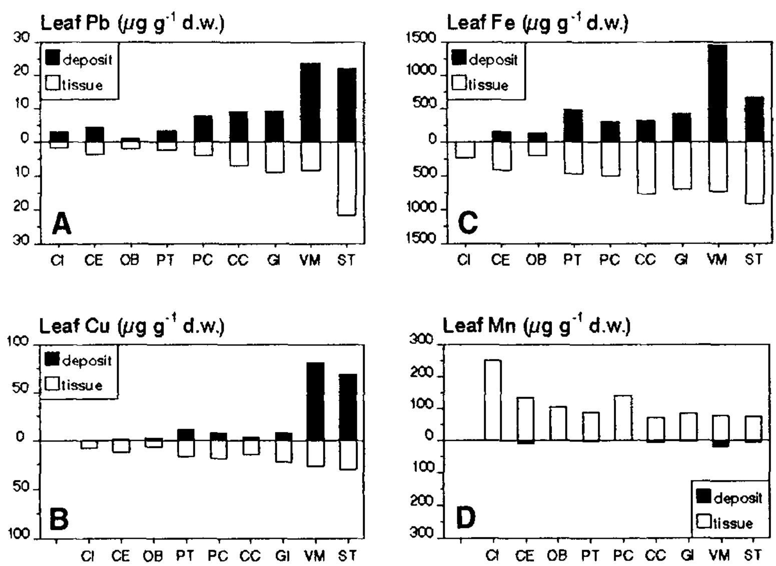

Fig. 6. Comparative analysis of leaf deposit and leaf tissue contents of Pb **(A)**, Cu **(B)**, Fe **(C)**, and Mn **(D)** in *Q. ilex* leaves collected from several sites of the urban area of Naples.

content in leaf deposit was higher than in the leaf tissue (Fig. 6A). The same was observed for Cu and Fe limited to samples from the more contaminated sites (Fig. 6B,C), whereas in samples from squares and parks, the less contaminated sites, these metals were more abundant in the leaf tissue than in the deposit. At all sites, the Mn content in the deposit was much lower than in the tissue (Fig. 6D). In samples from roadside sites VM and ST, Pb, Cu, and Fe contents in the deposit were particularly abundant and much higher than in leaf tissue, with the exception of Fe content at ST site (Fig. 6A–C).

Pb, Cu, and Fe contents in the leaf tissue and in the surface deposit were positively correlated ($r_S = 0.900$, $p < 0.01$; $r_S = 0.867$, $p < 0.01$; and $r_S = 0.783$, $p < 0.05$, respectively), suggesting that deposition of airborne components directly affects leaf elemental composition. By contrast, the Mn content in leaf tissue and surface deposit showed a negative trend ($r_S = -0.617$) but no significant correlation. Pb was positively correlated to Cu and Fe and Cu to Fe both in leaf tissue (Table 1) and in leaf deposit (Table 2).

Relationships Between Soil and Leaf Metal Content

The statistical analysis of data indicates that there was no univocal relationship between leaf and soil contents of the trace metals considered in this study. A significant correlation was observed between total Pb in soil and leaf tissue ($r_S = 0.867$, $p < 0.01$), as well as between total Pb in soil and leaf deposit ($r_S = 0.733$, $p < 0{,}05$); by contrast, no correlation was apparent in the distribution of other elements, with the exception of Fe that showed a significant correlation ($r_S = 0.717$, $p < 0.05$) between soil and leaf deposit.

Leaf Elemental Dynamics

Whereas the absolute concentrations and the seasonal trends of Pb, Cu, and Fe were markedly different in leaves from the urban and control sites (Fig. 7A–C), Mn content and seasonal dynamics were similar at the two sites (Fig. 7D). Very young leaves collected in June from the urban site exhibited (Fig. 7, left of graphs A–C) Pb, Cu, and Fe contents that were respectively 12, 2, and 7 times higher than in control leaves. From June to September no relevant changes of Pb levels were observed, while Cu and Fe showed a decrease in the control as well as in the urban site, probably as a consequence of the dilution effect resulting from biomass production during the growth period. From September to June in the first year, the Pb leaf content in the urban site increased by a factor of three (Fig. 7A), and Cu and Fe contents by a factor of two (Fig. 7B,C). In 2-yr-old leaves from urban site (Fig. 7, right of graphs A–C) the Pb, Cu, and Fe contents lessened markedly from June to September. Subsequently, in 2-yr-old leaves, the concentration of all three elements raised to levels higher than those measured the year before in the same period in 1-yr-old leaves (Fig. 7A–C).

Table 1
Spearman Rank Correlation Coefficients (r_S) Between Trace Metal Concentrations in *Quercus ilex* Leaf Tissue

	Pb	Cu	Fe	Mn
Pb				
Cu	0.883[a]			
Fe	0.900[b]	0.833[a]		
Mn	–0.788[c]	–0.550	–0.817[a]	

[a] $p < 0.01$.
[b] $p < 0.001$.
[c] $p < 0.05$.

Table 2
Spearman Rank Correlation Coefficients (r_S) Between Trace Metal Concentrations in Surface Deposit of *Quercus ilex* Leaves

	Pb	Cu	Fe	Mn
Pb				
Cu	0.700[a]			
Fe	0.800[b]	0.933[c]		
Mn	0.717[a]	0.467	0.650	

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$.

DISCUSSION

The amounts of trace metals detected in the soil and leaves of *Q. ilex* from the urban area of Naples indicate a marked contamination by Pb, Cu, and Fe.

Particularly conspicuous was soil contamination by Pb, with a peak of 757 µg/g dry wt at site ST and values in all the sites largely exceeding the range of baseline values, estimated at 2–20 µg/g dry wt *(21)*. Despite the absence of vehicular traffic, even the site RA, in a wood outside the city, showed considerable contamination with a Pb content of 79.5 µg/g dry wt. Total Cu level in roadside soil is also indicative of heavy contamination whereas the remote site RA and the site CI, in the interior of a large urban park, have Cu contents within the baseline range 6–40 µg/g dry wt after Allen *(21)*. Total Fe and Mn content in all soil

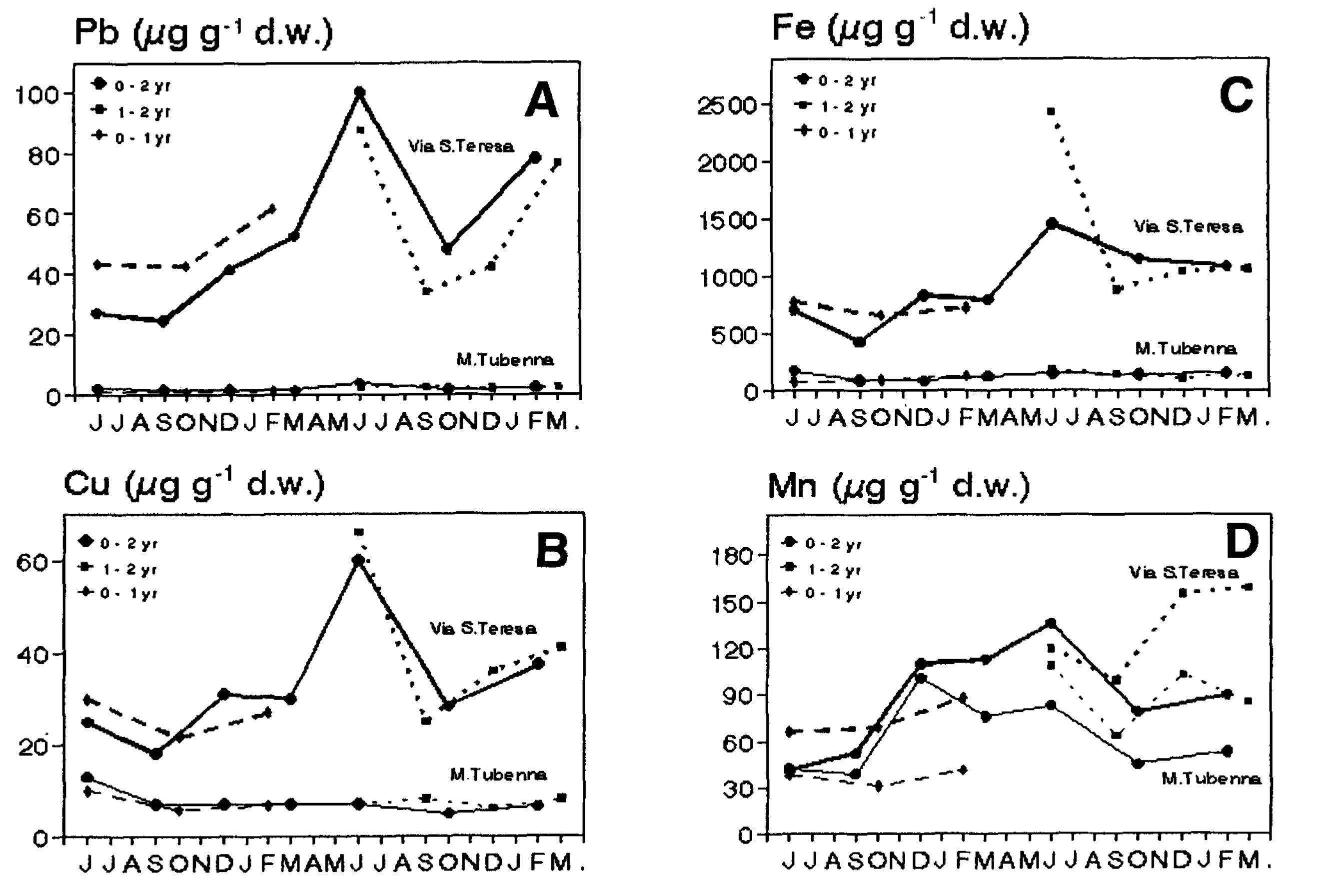

Fig. 7. Seasonal dynamics of Pb (**A**), Cu (**B**), Fe (**C**), and Mn (**D**) in leaves of *Q. ilex* from a remote site (M.te Tubenna) and a polluted urban site (via S. Teresa), studied during two vegetative seasons (1990–91 and 1991–92). On the left is shown the dynamics in first year leaves, on the right the dynamics in second year leaves. The continuous line represents the seasonal dynamics in leaves of the same generation during the first and second year of life. The dotted line represents the seasonal dynamics in leaves of first and second year of two consecutive generations. Ticker lines refer to the polluted site, thin lines to the control site.

samples exceeded the baseline range 0.2–5 mg/g and 50–500 μg/g dry wt, respectively, for Fe and Mn *(21)*, though not so much as Pb and Cu. The contents of available Fe and Mn, though falling in the normal range (0.05–1 mg/g and 5–500 μg/g dry wt, respectively), were higher in the soil from the remote area and urban parks than in more polluted sites.

The values of soil trace metal concentration in the urban area of Naples were similar to those reported by other studies on urban soils *(5,22,23)*. An analysis of 2 cm depth roadside soils in Hong Kong reported levels of Pb ranging from 50 to 2215 μg/g dry wt *(7)*, i.e., higher than those measured in 10 cm depth roadside soils in Naples. However, it must be emphasized that Pb concentration decreases in the soil with depth *(1,4,24)*.

Within the urban area of Naples, both soil and leaves of more exposed sites showed about a 10-fold accumulation of Pb as compared to the less exposed site (CI). The soil tends to accumulate Pb on a relatively long-term basis since this element is not highly mobile *(4)* and becomes more insoluble with time, probably as a result of combination with soil organic complex *(24,25)*. This might account for the relatively high levels of Pb even in soil samples from urban parks. Pb content in leaves reflects more recent accumulation than in soil, since leaf turnover prevents uninterrupted incorporation of this element; as a matter of fact, Pb content of leaves from urban parks is below the upper limit of Allen's baseline range *(21)*.

Whereas Fe levels in leaves from roadside sites in Naples were higher both in the surface deposit and in leaf tissue compared to leaves from urban parks, Fe levels in roadside soils did not significantly exceed those measured in the remote area (RA) and urban parks. However, Fe is a major soil component whose normally high concentration may partially mask the input by deposition.

The relatively low levels of Mn in both soil and leaves indicate that contamination by this element, if present, is low. The same conclusion has been drawn in other studies *(7,26)*.

The positive correlation found between available and total Pb, Cu, and Mn concentrations in the soil indicates that load of metals by external sources directly affects their availability and consequently their potential toxicity to the soil-microorganisms system. Consistent with this conclusion is the previous observation that the soil microbial biomass and activity, notably enzyme activity, and CO_2 production, showed a significant decrease in the presence of high levels of trace metals (*27*, unpublished data).

Pb content in plants normally ranges from 0.05 to 3 μg/g dry wt *(21)*. With the exception of samples from urban parks, the values of Pb measured in the leaves of *Q. ilex* in Naples were higher than baseline level. The values determined in leaves from a road with heavy flow of vehicular traffic (ST) exceed by a factor of about seven the maximum value of the normal range. Pb contents in leaves of *Q. ilex* collected in

Naples are comparable to those measured in *Populus tremuloides* and *Quercus velutina* in East Chicago *(4)*, and in needles of *Taxus baccata* in numerous urban sites *(22)*.

The contents of Cu and Fe in *Q. ilex* leaves exceed the baseline range values, respectively, 2.5–25 and 40–500 μg/g dry wt *(21)*, to a lower extent than Pb; the higher levels found in leaves from roadside sites, compared to leaves from urban parks, are indicative of a conspicuous accumulation of these two elements.

Mn levels in leaf tissue were always in the normal range. At sites VM and ST the leaves with the most abundant surface deposit *(28)* also presented the high Pb, Cu, and Fe contents in surface deposit. This fits well with the results of previous investigations, indicating that the level of vehicular traffic was positively correlated to Pb, Cu, and Fe contents *(17)* as well as to the amount of surface deposit in *Q. ilex* leaves *(28)* in the urban area of Naples.

Our results are consistent with the notion that metal aerosols may enter the leaves directly through stomata and cuticle *(34,35)*. Trace metal accumulation in *Q. ilex* leaves in Naples appeared primarily to come from airborne metal deposition and leaf uptake than from available content in the soil and root absorption. Indeed lead translocation from the roots to the shoots of plants is minimal *(29)*.

The leaves of *Q. ilex*, notably those from roadside sites, presented a conspicuous surface deposit. Most likely the stellate trichomes on their abaxial surface increase the surface area effective in metal interception *(3)*. The deposition of large foliar pollutant burdens may not only increase the absorption of pollutants by the plants but may also result in greater contamination of the soil underneath by throughfall, stemflow, and litterfall *(30,31)*.

The Pb content in leaf deposit was higher than in leaf tissue. Pb was found to be a major component of leaf surface deposit of oak trees (*Quercus palustris*) in sites influenced by vehicular traffic *(32)*. Accordingly, very high levels of Pb have been measured in dust samples from high density traffic roads *(33)*. The superficial deposit removed by washing includes soluble and insoluble fractions that can penetrate the leaf tissue through the guard-cells and cuticle *(34,35)*. Most of the Pb is in the insoluble fraction and this may probably account for its slow incorporation and accumulation in the leaf tissue *(19)*. Our data indicate that much of the total lead burden in *Q. ilex* leaves exists as a superficial deposit, whereas a higher proportion of the Cu and Fe is probably incorporated into the leaf. Infact Cu and Fe contents of surface deposit were lower than tissue content, except for the more contaminated sites where the deposit contents of Cu and Fe were particularly high.

Our study of elemental dynamics calls attention to a progressive bioaccumulation of trace metals in leaves of plants living in urban area. Deciduous leaves of *Aesculus hippocastanum* collected in six parks in the urban area of Christhurch (New Zealand) also showed continuous and conspicuous increment of Pb concentration, during their life-span, whereas Cu content decreased with respect to the initial values *(36)*.

It is not clear whether the consistent decrease of Pb, Cu, and Fe contents observed in June to September in 2-yr-old leaves affects the surface deposit, the leaf tissue, or both components. It seems unlikely, however, that in both years, the surface deposit was removed by rain, since the period considered is the dry season. Sloughing of the cuticle has been reported as a mechanism of removal of the surface deposit *(37)*.

The trend of elemental concentrations, exhibiting great variation during the life of leaves of *Q. ilex*, indicates that the leaf age, and the vegetative season are important prerequisites to choose a leaf sampling period, when elemental analysis of leaves is used for air quality biomonitoring.

CONCLUSIONS

The results of soil and leaf analysis are indicative of a conspicuous contamination by Pb, Cu, and Fe in the urban area of Naples. By contrast, Mn cannot be considered a significant air contaminant in Naples. Our results suggest that both soil and leaves may be utilised for monitoring metal deposition in polluted areas. The soil appears to be a suitable indicator for long-term total deposition, because of its uninterrupted exposure; however it cannot be utilized for highly mobile metals nor for metals that are major soil components, because normaly high levels may cover up accumulation from external sources.

By contrast, leaf analysis in plants such as *Q. ilex* permits a more careful evaluation of short-term accumulation. The positive correlation found between deposit and tissue content of metal contaminants suggests that both the leaf deposit and leaf tissue are suitable materials for the biomonitoring of air quality. Nevertheless, the conspicuous variations in leaf metal contents observed during the year in polluted sites emphasize the necessity of considering leaf age and sampling period in order to obtain comparable data.

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