

A Study of Air Pollution with Heavy Metals in Thessaloniki City (Greece) Using Trees as Biological Indicators

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Abstract. The air pollution of the city of Thessaloniki was studied, using park trees as biomonitors. The species analyzed were *Ligustrum japonicum*, *Nerium oleander*, *Olea europea*, *Pinus brutia*, *Platanus orientalis*, *Populus alba*, *Populus nigra*, and *Robinia pseudoacacia*. Acid digestion of leaf tissues and subsequent use of atomic absorption spectrometry was the analytical methodology used for the determination of heavy metals (Cu, Zn, and Pb). The concentration of heavy metals in the tree leaves depended on the metal species as well as on the position of the tree in the city. The mean concentrations of Cu ranged between 5 and 10 mg kg⁻¹ dry weight, of Zn between 19 and 85 mg kg⁻¹ DW and of Pb between <1.5 and 4.5 mg kg⁻¹ DW. In some cases, the effect of road junctions proved more significant than the traffic load for the contamination of leaves. Some differentiation was also observed between tree species. Old leaves collected during the fall were more contaminated than young leaves collected during the spring. The roughness of the outer surface leaves contributed to the trapping and retention of air particles. The results were compared with corresponding results from lichens and mosses which were also collected for a similar study.

The industrial activities of man and the uncontrolled development of large cities, especially during the recent past, have resulted in the contamination of soil, water, and air. Burning or combustion of fuels contribute a considerable amount of pollutants to air. According to Mudd and Koslowski (1975), the engines of motor vehicles and the central heating of buildings are the most important sources of air pollutants near the city centers. This fact is also true for the city of Thessaloniki, where there are already more than 400,000 motor vehicles, while most of the buildings use oil for central heating.

Trees are not the best indicators for air pollution monitoring. In recent years the most popular plants for such monitoring studies have been lichens and mosses because of their ability to receive and accumulate chemical substances predominantly from the surrounding atmosphere. Lichens and mosses are rare in urban areas with a high degree of pollution, and trees are the major plant type found in these areas. Therefore, we turn our attention towards using trees for pollution monitoring studies.

Increased heavy metal concentrations in the environment, such as Cu, Cd, Pb, etc., causes damage in vegetation and sometimes visible injuries to trees (Wang and Schaap 1988). Metals may inhibit several biological processes such as hydrolysis of starch and sucrose as well as transportation of sucrose followed by secondary effects of a disturbed nitrogen metabolism. Usually the concentrations of sugars, starch, and certain amino acids are elevated with increased metal concentration while the protein content is lowered (Malhotra and Sarkar 1979; Koziol and Cowling 1980; Hollworth 1981; Grunhage and Hager 1982). It has been reported that treatment of beans with zinc (Agarwala *et al.* 1977) or barley with zinc and copper (Balsberg 1989) increased their carbohydrate content.

There are considerable data regarding the retention and bioaccumulation of heavy metals from tree leaves and the biological affects caused from the toxicity of these metals (Hasemann *et al.* 1990). Until now, metal uptake by different plant species, especially trees, has been unclear (Smith and Brennan 1984; Kabata-Pendias 1992), and also no correlation with high significance has been observed between heavy metal doses and corresponding effects on tree leaves.

Among the wide range of methods used to determine heavy metal concentrations in leaves, flame and electrothermal atomic absorption spectrometry have become some of the most popular and reliable techniques (Ure and Mitchell 1976; Puchades *et al.* 1989). This method requires that the organic part of the sample be destroyed by either a wet or a dry ashing procedure.

In this paper, the chemical analysis of copper, zinc, and lead in tree leaves, collected from the city of Thessaloniki, is described, using atomic absorption spectrometry after a wet ash-

Table 1. List of sampling sites in Thessaloniki city, and tree species selected for the collection of leaves

Sampling sites	Tree species
1. Diavata	1. <i>Ligustrum japonicum</i> Thumb.
2. Kalohori	2. <i>Nerium oleander</i> L.
3. Kordelio	3. <i>Olea europea</i> L.
4. Railway Station	4. <i>Pinus brutia</i> Ten.
5. Ilioupoli	5. <i>Platanus orientalis</i> L.
6. Aristotle Square	6. <i>Populus alba</i> L.
7. Neapoli	7. <i>Populus nigra</i> L.
8. Macedonia Palace	8. <i>Robinia pseudoacacia</i> L.
9. A. Toumba	
10. Depot	
11. Kalmaria	
12. Filyro	

Table 2. Graphite furnace atomizing conditions of temperature and time, for the analysis of copper, zinc, and lead in acid-digested leave solutions

Element	Drying step		Charring step		Atomization step	
	°C	s	°C	s	°C	s
Cu	110	40	1,000	20	2,700	6
Zn	110	40	300	35	2,100	5
Pb	110	40	500	50	2,700	6

Table 3. The mean copper concentrations in leaves from sampling sites 1–12 (mean values in mg.kg⁻¹ DW, spring and fall 1992, Thessaloniki, Greece). Sampling sites described in Table 1

Tree species	Sampling site (mg kg ⁻¹ DW)											
	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>Ligustrum japonicum</i>												
Flesh	4.2	5.2	6.2	8.2	7.0	6.7	5.2	7.0	5.0	5.0	4.2	4.2
Leaf	7.4	7.8	8.5	10.2	8.3	6.7	10.3	8.3	6.8	4.8	7.5	9.8
Sperm	8.8	7.5	12.5	6.7	15.0	10.4	5.2	10.8	7.5	11.2	10.0	9.3
2. <i>Nerium oleander</i>	10.8	8.2	11.6	17.8	8.0	11.5	12.6	15.5	7.2	7.8	9.7	3.9
3. <i>Olea europea</i>	6.2	4.9	8.4	12.9	6.7	5.4	5.8	7.0	4.3	11.9	8.9	4.2
4. <i>Pinus brutia</i>	4.0	2.2	4.9	8.1	6.6	3.7	4.2	7.6	5.1	2.7	5.0	3.4
5. <i>Platanus orientalis</i>	6.0	7.4	12.7	14.5	4.9	7.7	8.8	13.9	7.2	11.4	8.5	5.3
6. <i>Populus alba</i>	5.4	3.3	6.2	13.4	5.5	6.0	8.5	9.8	6.1	4.8	6.1	6.6
7. <i>Populus nigra</i>	4.4	2.7	9.1	9.4	5.8	5.8	6.3	6.2	5.9	6.7	7.0	4.1
8. <i>Robinia pseudoacacia</i>	5.3	6.2	8.3	9.1	6.1	7.4	6.9	5.6	5.7	9.5	4.6	7.4

Table 4. The mean zinc concentrations in leaves from sampling sites 1–12 (mean values in mg.kg⁻¹ DW, spring and fall 1992, Thessaloniki, Greece). Sampling sites described in Table 1

Tree species	Sampling site (mg kg ⁻¹ DW)											
	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>Ligustrum japonicum</i>												
Flesh	16.8	18.5	27.0	20.0	12.3	11.1	6.2	24.6	20.9	12.3	9.8	8.6
Leaf	27.1	26.6	42.2	34.3	28.1	15.5	25.6	37.7	27.3	19.1	29.6	27.6
Sperm	33.2	18.0	48.0	27.1	35.7	33.3	12.3	49.2	36.9	34.5	33.5	38.1
2. <i>Nerium oleander</i>	33.9	29.2	26.8	42.2	27.1	35.0	30.2	40.5	34.3	26.8	30.8	18.7
3. <i>Olea europea</i>	23.3	22.0	31.1	50.8	22.2	13.0	18.9	21.6	19.0	21.0	32.2	17.2
4. <i>Pinus brutia</i>	20.9	15.5	21.3	21.8	25.1	15.1	16.5	29.7	13.1	14.9	18.3	16.6
5. <i>Platanus orientalis</i>	17.7	14.6	31.6	37.6	23.1	24.3	64.9	30.1	26.8	27.9	21.8	11.9
6. <i>Populus alba</i>	52.1	45.6	73.9	74.4	71.8	30.2	88.5	12.2	58.6	61.4	54.2	30.9
7. <i>Populus nigra</i>	41.1	64.1	86.5	123	103	53.7	106	193	68.2	56.2	79.8	49.7
8. <i>Robinia pseudoacacia</i>	40.6	24.9	28.5	37.8	23.0	24.4	19.9	21.1	21.7	28.8	21.4	18.1

ing procedure. Eight species of trees were selected to investigate changes in the heavy metal content of their leaves and in one case, of their flesh and sperm. The aim of the collection was to cover a wide spectrum of tree species (gymnosperms–angiosperms, deciduous–evergreen) or leaf types (blade–needle, simple–composed, smooth–rough).

The species analyzed were *Ligustrum japonicum* Thumb. (privet), *Nerium oleander* L. (oleander), *Olea europea* L. (olive-tree), *Pinus brutia* Ten. (calabrian pine), *Platanus orientalis* L. (plane tree), *Populus alba* L. (white poplar), *Populus nigra* L. (black poplar), and *Robinia pseudoacacia* L. (false acacia). The tree leaves were examined for their capacity to

Table 5. The mean lead concentrations in leaves from sampling sites 1–12 (mean values in mg.kg⁻¹ DW, spring and fall 1992, Thessaloniki, Greece). Sampling sites described in Table 1

Tree species	Sampling site (mg kg ⁻¹ DW)											
	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>Ligustrum japonicum</i>												
Flesh	<1.5	<1.5	<1.5	6.3	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Leaf	<1.5	<1.5	<1.5	4.4	<1.5	2.2	<1.5	<1.5	2.2	<1.5	2.1	<1.5
Sperm	<1.5	<1.5	<1.5	2.0	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
2. <i>Nerium oleander</i>	<1.5	<1.5	2.4	14.0	<1.5	<1.5	2.9	<1.5	2.2	<1.5	<1.5	<1.5
3. <i>Olea europea</i>	<1.5	<1.5	<1.5	8.0	<1.5	<1.5	<1.5	<1.5	2.2	<1.5	2.7	<1.5
4. <i>Pinus brutia</i>	2.4	2.7	<1.5	7.3	3.3	<1.5	<1.5	5.5	2.7	<1.5	2.1	<1.5
5. <i>Platanus orientalis</i>	<1.5	<1.5	<1.5	11.5	2.2	<1.5	<1.5	6.9	<1.5	2.7	2.4	<1.5
6. <i>Populus alba</i>	<1.5	<1.5	<1.5	14.5	<1.5	<1.5	<1.5	5.6	<1.5	3.0	<1.5	<1.5
7. <i>Populus nigra</i>	<1.5	<1.5	<1.5	4.2	<1.5	<1.5	<1.5	<1.5	<1.5	2.2	2.7	<1.5
8. <i>Robinia pseudoacacia</i>	<1.5	<1.5	5.0	7.3	2.4	<1.5	<1.5	2.1	2.7	2.4	2.5	<1.5

accumulate the above metals in relation to the tree species, the site of origin, and the season.

Materials and Methods

Sampling Procedures

Twelve sites were selected in the city of Thessaloniki and the surrounding suburbs (Table 1). Three factors were taken into account during site selection: industrialization (sites 1, 2, 3), main city roads (sites 3, 4, 6, 8), and meteorological data. Tree leaves from a clear region, 15 km away from Thessaloniki city (site 12) were also collected as reference material.

Leaves were sampled twice in the year 1992 from the eight different tree species (Table 1). From *Ligustrum japonicum*, sperm and flesh were also collected. Young tender leaves were collected in the spring, and in the fall, older leaves before leaf fall were collected. Leaves were collected uniformly from around the trees at a trunk height of about 1.5–2.0 m above the ground.

After drying at 60°C and pulverizing, the leaves were placed in polyethylene containers and stored in the refrigerator. Subsamples of the homogenized material were taken by means of plastic spatulas.

Analytical Methods

The wet digestion of organic matrix samples (plants, fishes) by the use of concentrated HNO₃ is the most common procedure although other acids or mixtures of acids are also reported (H₂SO₄ + HNO₃, HNO₃ + HClO₄). The digestion time was extended so as to achieve maximum recoveries of metals in the solution.

About 1 g DW of each subsample was placed in an open quartz tube. Eight ml of concentrated HNO₃ (Merck, *pro analysi*) were added to each tube and the mixture was left at room temperature over night. It was warmed for 2 h at 50°C and heated at 180°C for 4 h. The solution was filtered through Whatman type 589/2 filters and the filtrate was diluted to 25 ml volume with double deionized water. Each final solution was analyzed for copper (Cu), zinc (Zn), and lead (Pb) concentrations.

Two standards (National Bureau of Standards, NBS) with numbers 1573 (tomato leaves) and 1575 (pine needles) were also analyzed, using the same procedure and the recoveries were 95.5% for copper,

Table 6. Results of statistical analysis using paired t-test, for the significance of differences (x_d) between fall and spring concentrations of Cu and Zn. From each species, twelve samples collected from Thessaloniki were tested. The experimental (t_{exp}) values obtained were compared to t_{crit} = 2.20 (probability 95%). (+) = significant, (–) = not significant

Tree species	Cu		Result	Zn		Result
	X _d	t _{exp}		X _d	t _{exp}	
1. <i>Ligustrum japonicum</i>	0.083	0.900	–	0.917	0.296	–
2. <i>Nerium oleander</i>	3.125	2.282	+	6.533	2.374	+
3. <i>Olea europea</i>	2.855	2.418	+	0.258	0.102	–
4. <i>Pinus brutia</i>	2.717	2.368	+	6.242	2.579	+
5. <i>Platanus orientalis</i>	0.367	0.166	–	1.142	0.190	–
6. <i>Populus alba</i>	4.242	2.725	+	2.917	0.271	–
7. <i>Populus nigra</i>	1.875	2.534	+	46.5	2.230	+
8. <i>Robinia pseudoacacia</i>	0.825	0.835	–	8.667	3.322	+

97.5% for zinc, and 94.2% for lead. Thus, the above digestion method was effective and used in all tree species collected.

Copper, zinc, and lead concentrations were determined by atomic absorption spectrometry, with flame and graphite furnace modes. A Perkin Elmer 2380 atomic absorption spectrometer was used, coupled with a HGA-400 graphite furnace controller. The analytical wavelengths were set at 283.3 nm for Pb, 324.8 nm for Cu, and 213.8 nm for Zn. In the majority of the samples, the determinations of Cu and Zn were performed with flame atomic absorption mode, while for very low concentrations the graphite furnace mode was necessary. In all graphite furnace determinations, pyrocoated graphite tubes were used and their performance was very good even after 180 injections of the above nitric acid-sample solutions. The conditions programmed in the graphite furnace are given in Table 2.

To ensure the complete ashing of the injected sample, the drying and charring times were expanded. The gas was interrupted (stop flow mode) during the atomization step in all the graphite furnace determinations. After an optimization of the time needed for the quantitative atomization of the metals, it was shown that 6 s for copper and lead and 5 s for zinc were satisfactory.

The above digestion and determination procedures were followed for the determination of the Cu, Zn, and Pb concentrations in plant tissues. The concentrations are given as mg kg⁻¹ dry weight (DW).

The concentrations of Cu and Zn in leaves of all species collected from the twelve sites during spring, were compared to those collected during fall. The resulting differences were tested for their significance

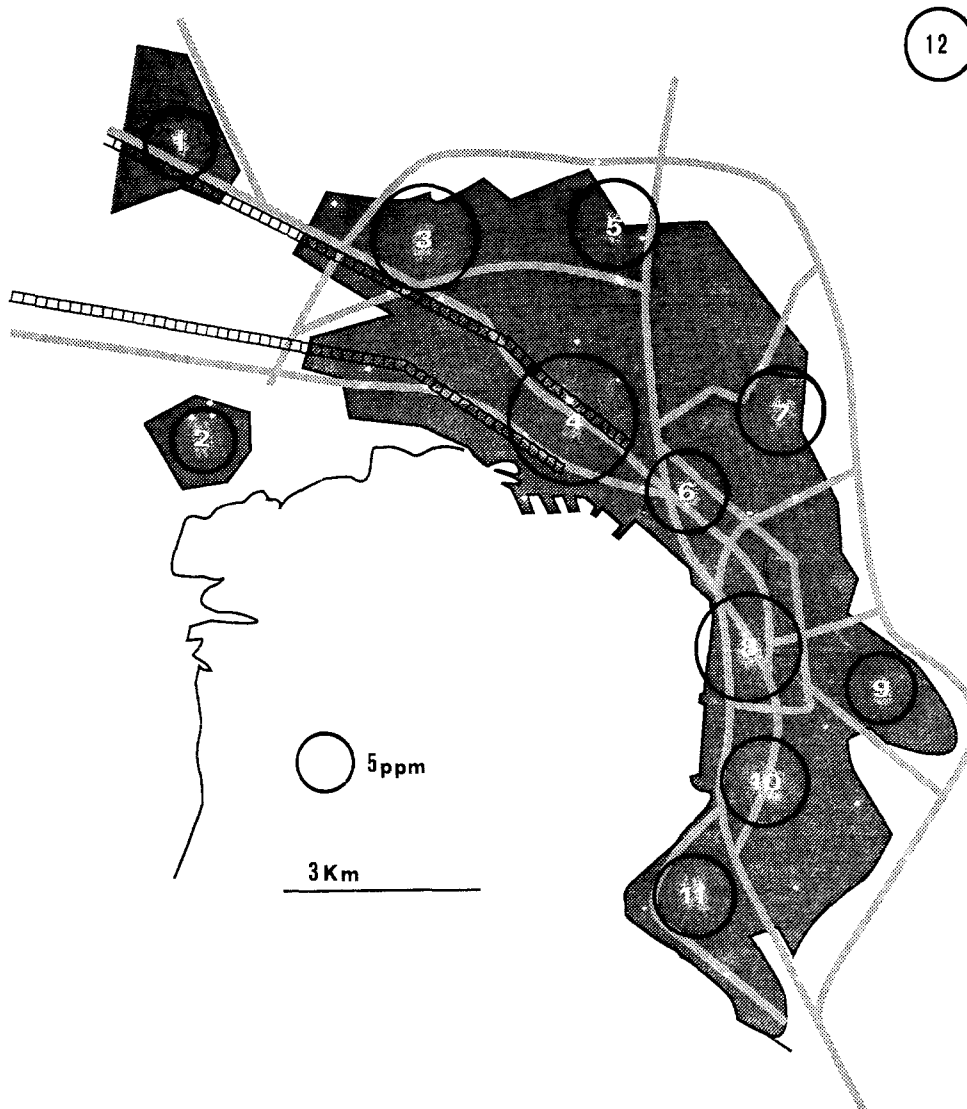


Fig. 1. Schematic representation of mean copper levels in various sampling sites from Thessaloniki city (1992). The size of the circles reflects the concentration level in comparison to 5 mg kg^{-1} of Cu. The numbers refer to the following sampling sites: (1) Diavata, (2) Kalohori, (3) Kordelio, (4) Railway Station, (5) Ilioupoli, (6) Aristotle Square, (7) Neapoli, (8) Macedonia Palace, (9) A. Toumba, (10) Depot, (11) Kalamaria, (12) Filyro

using the paired t-test as an appropriate statistical method, at 95% probability level.

Results

The mean concentrations of copper, zinc and lead of the tree leaves from each site are given in Tables 3, 4, and 5, respectively. For the species *Ligustrum japonicum*, the concentrations were determined both in the leaves and in the fleshy part of the fruit and the seed.

The bioaccumulation of the heavy metals was dependent on the species of trees used as indicators. Only in the case of copper the differences observed between the tree species were small, with the exception of *Nerium oleander* and *Platanus orientalis* (Table 3). However, zinc concentrations were greater in *Populus nigra* and *Populus alba* (Table 4), while lead concentrations were higher in the species *Populus alba*, *Platanus orientalis*, and also in *Nerium oleander* and *Pinus brutia* (Table

5). With reference to the uptake of the metals in the plant organs of *Ligustrum japonicum*, the accumulation of Pb in the seed was lower than in the fleshy parts. The distribution was reversed for Zn and Cu.

The concentrations of the metals are expressed as the mean values obtained from the analysis of the specimens collected in the two different seasons, as described in the Materials and Methods section. The differences between the metal content of the leaves collected in spring and the leaves collected in fall represented the behavior of young and older leaves, respectively. The significance of these differences at the 95% level was tested using the paired-t test. The 12 values of each species in fall samples were compared with the corresponding values of the same species in spring samples. The results of this statistical analysis are listed in Table 6.

A profile of environmental contamination at the sampling sites can be described from a botanical perspective as given in Figures 1, 2, and 3 for Cu, Zn, and Pb, respectively. These figures present schematically the mean concentration of each metal in all tree species collected in a site, simultaneously.

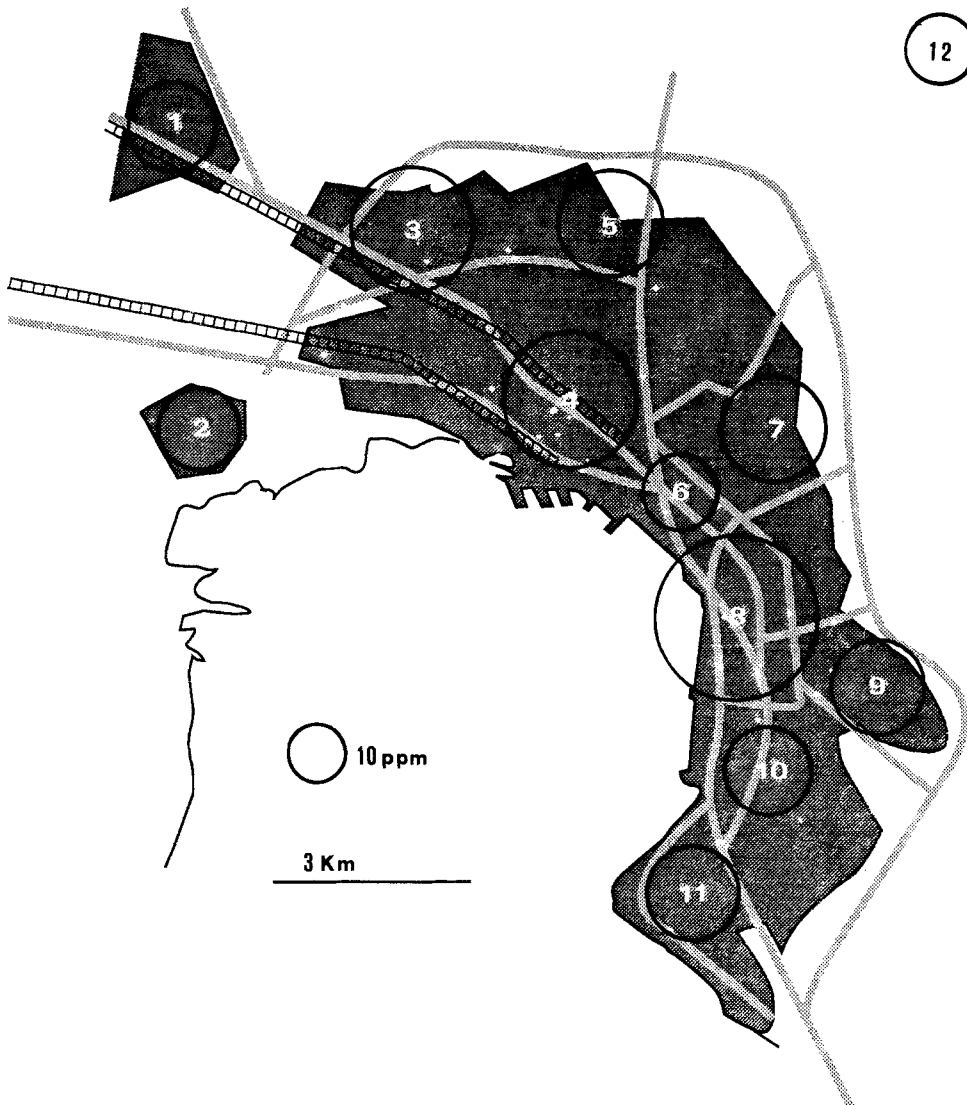


Fig. 2. Schematic representation of mean zinc levels in various sampling sites from Thessaloniki city. The size of the circles reflects the concentration level in comparison to 10 mg kg^{-1} of Zn. Numbers of sampling sites as described in Figure 1

Discussion

The statistical analysis showed that the mean concentrations in all species were higher in the fall than in the spring (Table 6) and in the case of *Nerium oleander*, *Pinus brutia*, and *Populus nigra* the differences were significant for both metals. The higher metal content of the older leaves should be attributed to the longer exposure period in the polluted air environment, although the young leaves are known to have increased ability for nutrient uptake from the environment. Senescing leaves show normal weathering and cracks in the cuticle or complete loss of cuticular cover over necrotic spots and lesions. Reduction of the cuticle integrity with the age allows substantial heavy metal accumulation (Martin and Coughtrey 1982). In the evergreen tree *Ligustrum japonicum* where the senescence of leaves is a long time procedure (over two years), no significant seasonal differences were observed. Also it should be taken into account that the binding of the heavy metals in the tissues is strong and the excretion rate is negligible.

In areas of aerial contamination, such as the city of Thessaloniki, the soil becomes enriched with metals and uptake from

the soil via roots, and subsequent translocation to the leaves, may complicate the interpretation of the results. Attempts to distinguish between the heavy metal burden derived from soil uptake and by aerial deposition have been made by Ter Haar (1970) and McLeod *et al.* (1980).

If we overlook the root uptake and insist on foliar deposition, it is obvious that the most contaminated leaves show variation in surface roughness. In *Populus nigra* and *Platanus orientalis*, the dense piled leaves and the prominent reticulate form of veins trap and retain a considerable number of particles effectively. For this reason, these tree species may be avoided from industrially contaminated areas, because their appearance is not good. In contrast, the waxy cuticle of *Ligustrum japonicum*, which forms a smooth sheet over the epidermal cells, enables the fast removal of the particles by the rain, and so this species is selected from contaminated areas such as city roads. Rabinowitz (1972) reported that rigorous washing of plant samples may remove a large fraction of the aerial deposits.

In *Nerium oleander*, the presence of numerous stomata, grouped in sunken pockets with tangled hairs, is probably the reason for particle trapping and metal uptake. The sunken sto-

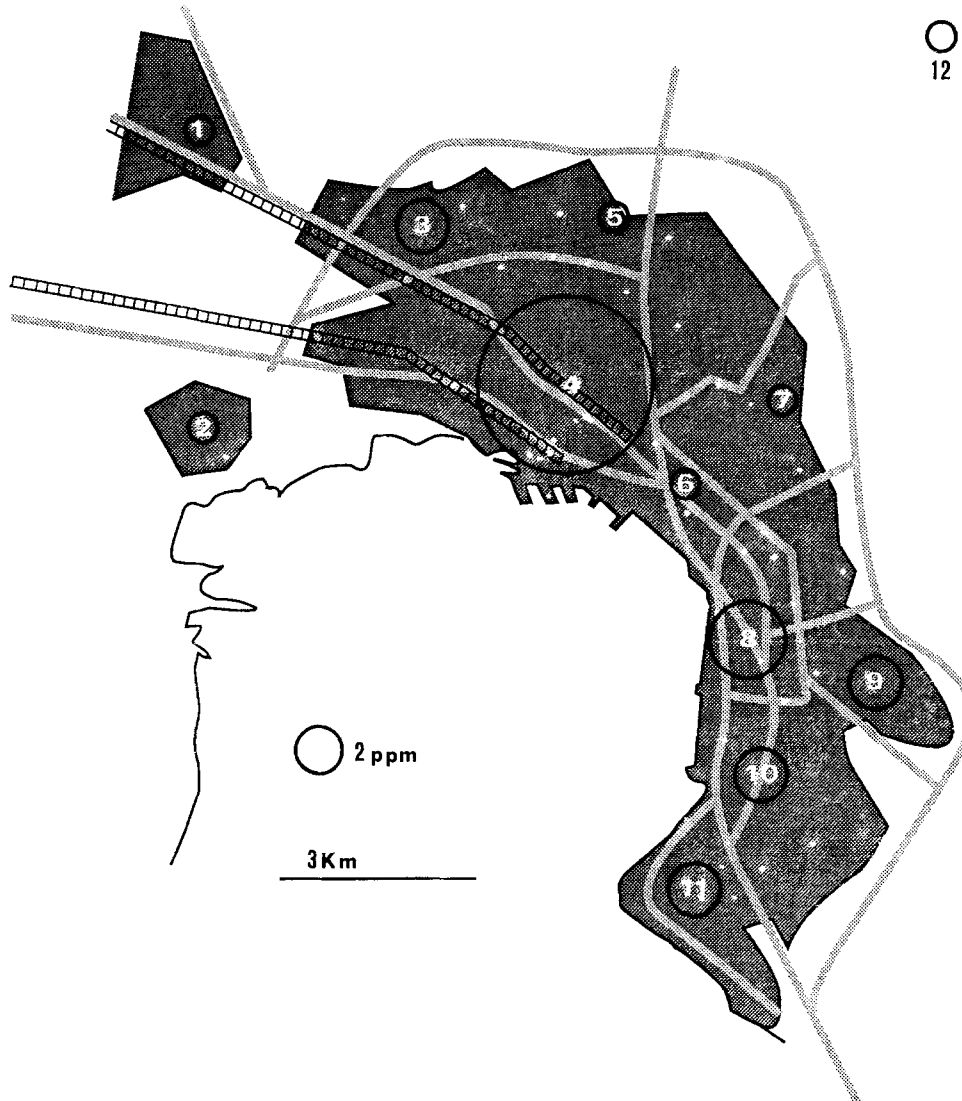


Fig. 3. Schematic representation of mean lead levels in various sampling sites from Thessaloniki city. The size of the circles reflects the concentration level in comparison to 2 mg kg^{-1} of Pb. Numbers of sampling sites as described in Figure 1

mata of *Pinus brutia* borne in longitudinal bands and the rough surface are also the reason of high lead concentration in its leaves. In the case of pine, the presence of resins or honey-dew deposits from aphids, which make the leaf surface sticky, may increase the trapping of air particles. Godzik *et al.* (1979) used chloroform and ultrasonication to remove particulates from the leaves and surface waxes of pine. They found evidence of strong fixing of particles to waxy structures on epidermis.

The highest values were observed for Zn ($19\text{--}85 \text{ mg kg}^{-1}$), followed by Cu ($5\text{--}10 \text{ mg kg}^{-1}$), and Pb ($<2\text{--}3 \text{ mg kg}^{-1}$). A comparison of these levels with the respective levels in lichens from the city of Thessaloniki (unpublished data) undoubtedly proved that lichens accumulated Cu, Zn, and Pb at a higher rate. The rank in the latter case was different because higher concentrations of Pb (102 mg kg^{-1}) were observed in lichens followed by Zn (82 mg kg^{-1}) and Cu (20 mg kg^{-1}). The results were similar in a comparison of the leaves with mosses (Sawidis *et al.* 1993).

It is convenient to consider the use of vegetation composed of vascular plants separately from the numerous surveys which have used mosses or lichens. These epiphytic plants have the great advantage over trees that there is no possibility of taking

up metals from soil. Also, their high surface area to biomass ratio, the absence of cuticle and the slow growth rate are further advantages that must be taken into account.

Site 4 (near the railway station) was the most contaminated with Pb (Figure 3) and Cu (Figure 1), followed by site 8 (Macedonia Palace). The increased Pb content in the leaves from these sites was caused obviously by the heavy traffic (Egnatia Str. more than 22,000 cars/24 h and M. Alexandrou Str. more than 54,000 cars/24 h). Lead contamination was not exactly proportional to the number of vehicles per day. Thus, near the railway station, lead concentration (7.94 mg kg^{-1}) was more than twice the concentration in M. Alexandrou Str. (3.2 mg kg^{-1}), although the traffic load was reversed. Taking into account another factor, the number of road junctions where vehicles must wait longer times, the described phenomenon was then sufficiently explained. Also, in the case of copper the same but minor difference was observed; but in the case of zinc (Figure 2), site 8 seemed to be more contaminated without obvious explanation.

In the triangle which was configured by the sites 1, 2, and 3 (Diavata, Kalohori, and Kordelio) the petrochemical industries are situated. The direction of the local winds (Vardaris) is from

northwest to southeast (Livadas and Sahsamanoğlu 1973); hence a constant increased contamination of site 3 was observed against the two others for all three metals, although this site was located further from the above industries. In case of zinc, site 8 followed by site 4 and 3 (Kordelio, near the chemical industries zone) showed the greater contamination, while the concentrations were also high in the other two sampling stations (5 and 7) located at the northwest suburbs.

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