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NUTRIENTS AND HEAVY METALS CONCENTRATIONS IN NEEDLES OF *PINUS BRUTIA* TEN. IN THESSALONIKI, GREECE

Maria Aslanidou[1*], Athanasios Papaioannou[2], Elias Pipinis[1], Olga Mavrokordopoulou[1], Matina Katsanidou[1], and Pavlos Smiris[1]

[1]Laboratory of Silviculture, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, P.O. Box 262, 54124 Thessaloniki, Greece. [*]E-mail: maslan@for.auth.gr
[2]Laboratory of Forest Soil, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, P.O. Box 271,54124 Thessaloniki, Greece.

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Abstract

The present study is based on the differential concentrations of nutrients and heavy metals observed in *Pinus brutia* Ten. needles resulting from the influence of various factors, such as traffic and topographical conditions in four sites in urban and periurban areas of Thessaloniki, Greece. The results showed that there were significant differences in nutrient and heavy metal concentrations in the needles collected in the four sites. In particular, the samples from the periurban forest exhibited significantly higher concentrations of Mg and Ca than the samples collected in the sites near the centre of the city. *The s*ignificantly higher concentrations of K observed in the samples collected from two sites in the city, are possibly attributed to the fertilization of the parks. The high concentrations of Na found in the samples collected in sites of the periurban forest, are due to topographical conditions and aerial deposition. However, the highest level of Mn and Zn observed in the samples collected in the site of the periurban forest located far away from the busy ring road, is mainly attributed to soil type. High levels of Cu, Pb, Ni and Cr concentrations were found in the needles collected from the sites close to the busy traffic roads of Thessaloniki. No significant differences in the levels of Fe and Cd among the samples of the four sites were found. However, the concentrations of elements measured in *P. brutia* needles collected in the urban and periurban areas of Thessaloniki are below the normal level and as a result they do not constitute an alarming pollution agent in the city.

Key words: periurban forest, pollution, trace elements, traffic, urban environment.

Introduction

Nutrients and heavy metals released from anthropogenic sources have entered the environment and have followed normal biogeochemical cycles. Although they are natural components and some are essential in plant nutrient (micronutrients), they are accumulated in the soil and air in increasing amounts, as a result of the increased urbanization and expansion of industrial activities, causing a serious risk to human health (Kabata-Pendias 1992, Voutsa et al. 1996, Baslar et al. 2005).

In this context, Thessaloniki, which is the second biggest city in Greece, was chosen for the investigation particularly with regard to airborne particles daily released from vehicular traffic (Ward et al. 2004, Oliva and Espinosa 2007, Vlacho-

Fig. 1. Sites 1 and 2 (located near the busy traffic centre of the city), site 3 (located in the periurban forest besides the ring road) and site 4 (located in the periurban forest far away from the busy ring road).

kostas et al. 2012). An important feature of large urban centers, such as the city of Thessaloniki, is that the intense emission of pollutants and heat are trapped within the city because of the high-density housing, resulting in changes in the balance of temperatures and increased dispersion of pollution (Namdeo et al. 1999).

Analyses of plant sample trace elements have been a fundamental feature of ecological research for a long time. It is now well accepted that trees in urban and suburban areas have been used to detect the deposition, accumulation and distribution of trace metals in the ecosystems, because they first accept the negative effects of large concentrations of air pollutants and then provide the cheapest and simplest method by analyzing their tissues, leaves, twigs or their shanks, to assess the effects of the increased concentration of pollutant substances (Baslar et al. 2009, Kula et al. 2010). In the extant literature, there are many studies focusing on using bark and needles of pine species. Thus, Yilmaz and Zengin (2004) used the needles of Scots pine, Pyatt (1999) – of Corsican pines and Baslar et al. (2003) and Dogan et al. (2010) – of Turkish red pine to monitor heavy metal pollution.

The objective of the present study was to indicate the differential concentrations of nutrients and heavy metals in *Pinus brutia* Ten. needles, in terms of the influence of traffic and topographical conditions in the area of Thessaloniki.

Materials and methods

Two sites (1 and 2) located near the busy traffic centre of the city and two other sites located in the periurban forest, one besides the ring road (site 3) and the other one far away from the busy ring road (site 4) were selected for the investigation (Fig. 1). In all sites, the age of *P. brutia* trees ranged from 50 to 60 years as they were planted during a reforestation program which basically started in the 1950s.

In each site, needles were collected from 20 randomly selected *P. brutia* trees.

From each tree, a significant amount of needles was collected from four sides (west, east, south and north) in the middle of the crown length. Sampling was carried out after a prolonged rainless period in mid September 2012. Each collected sample was stored in clean sealing plastic bags and transported to the laboratory for further processing, after they had been labeled. The total number of samples was 80. In the laboratory, the samples were placed unwashed in an oven and dried at 70 °C for about 48 hrs to remove all moisture. Subsamples of needles were then ground in a Willey mill to pass through a 40 mesh stainless steel sieve before being analyzed. An amount of 0.35 to 0.45 g was taken from each sample and was digested in a mixture of HNO_3, $HClO_4$ and H_2SO_4 (5:1:1) following the method of Allen et al. (1986). The elements Ca, Mg, K, Na, Mn, Fe, Cu, Zn were determined by means of the atomic absorption spectroscopy (Perkin-Elmer AAnalyst 300), whereas the concentrations of the elements Pb, Cd, Cr and Ni were determined by an atomic absorption spectrometer with flame and graphite furnace modes.

Statistical analysis

For each site, the concentration of each element in *P. brutia* needles was calculated as the average of the 20 samples. For each element, one-way analyses of variance (ANOVA) were performed to determine whether there are any significant differences among the four different sites in urban and periurban areas of Thessaloniki. Duncan and Dunnett's T3 tests were used for the comparison of means. For the statistical analysis the statistical software SPSS 20.0 was used.

Results

According to Table 1 in both sites located near the busy traffic centre of the city, the needles of *P. brutia* exhibited the lowest ($p < 0.05$) Mg and Ca concentrations. The highest ($p < 0.05$) Mg concentration was observed in the needles of site 4 in the periurban forest far away from the busy ring-road, whereas no significant difference ($p > 0.05$) in Ca concentration of *P. brutia* needles was observed between the sites in the periurban area. In both sites located in the periurban forest, the needles of *P. brutia* exhibited the lowest K concentration, whereas the highest concentration was observed in the needles of site 1. The collected needles of *P. brutia* in site 3 showed higher ($p < 0.05$) Na concentration than those which were collected in sites 1 and 2. In the needles collected in site 4, the highest ($p < 0.05$) Zn, Mn concentrations were observed and the lowest ($p < 0.05$) Cu, Ni concentrations. No significant differences ($p > 0.05$) in Cu, and Ni concentrations were observed among the other three sites. In both sites located in the urban area, the needles of *P. brutia* exhibited the lowest ($p < 0.05$) Mn concentration, whereas the lowest ($p < 0.05$) concentration of Zn was observed only in site 2. The results of the statistical analysis showed no significant differences ($p > 0.05$) in Fe, and Ni concentrations among the four sites. In *P. brutia* needles collected in site 1 and 3, the highest ($p > 0.05$) Pb concentration was observed. No significant difference ($p > 0.05$) was observed between the other two sites. In the collected needles from site 4 the lowest Ni concentration was observed, whereas no significant differences ($p > 0.05$) were observed among the other three sites. In both sites located near the busy traffic centre of the city, the needles of *P. brutia* exhibited the highest ($p < 0.05$)

Table 1. Nutrients and heavy metal concentrations in the needles of *Pinus brutia*.

Element	Site 1	Site 2	Site 3	Site 4
		Concentrations ($mg \cdot g^{-1} \pm$ S.D.)		
Mg	1.76[1] c[2] ± 0.41	1.92 c ± 0.25	2.37 b ± 0.38	2.72 a ± 0.33
K	7.16 a ± 1.49	5.48 b ± 0.99	3.24 c ± 0.80	2.65 c ± 0.67
Ca	5.37 b ± 1.84	6.03 b ± 1.58	7.33 a ± 1.93	7.89 a ± 1.57
Na	0.91 c ± 0.19	1.13 bc ± 0.88	1.95 a ± 1.03	1.50 ab ± 0.77
		Concentrations ($\mu g \cdot g^{-1} \pm$ S.D.)		
Cu	6.59 a ± 0.75	6.80 a ± 0.77	7.29 a ± 1.10	5.98 b ± 0.66
Fe	120.08 a ± 28.03	110.69 a ± 37.98	120.91 a ± 36.90	115.15 a ± 27.18
Zn	23.66 c ± 3.67	20.60 d ± 3.85	26.53 b ± 4.31	32.94 a ± 4.05
Mn	25.53 c ± 8.57	38.93 c ± 20.05	73.42 b ± 22.17	178.90 a ± 53.27
Pb	0.971 a ± 0.27	0.602 b ± 0.10	0.818 a ± 0.14	0.606 b ± 0.10
Cd	0.086 a ± 0.03	0.074 a ± 0.03	0.081 a ± 0.02	0.096 a ± 0.02
Ni	1.311 a ± 0.45	0.954 a ± 0.66	1.028 a ± 0.21	0.351 b ± 0.12
Cr	0.924 a ± 0.74	0.925 a ± 0.87	0.298 b ± 0.39	0.060 b ± 0.00

Note: [1] – mean concentration of 20 samples; [2] – in a row, the means are statistically different at $p < 0.05$ when they do not share a common letter. The comparisons of K, Na, Cu, Mn, Pb, Ni and Cr concentrations were made using Dunnett T3 test. The comparisons of Mg, Ca and Zn concentrations were made using Duncan test.

Cr concentrations. No significant difference ($p < 0.05$) in Cr concentration was observed between the sites both in the researched periurban and urban areas of the city.

Discussion

It is now well accepted that plants can be effectively used for monitoring environmental pollution in urban areas. There are many studies focusing on using the bark and leaves of tree species to provide valuable information about the quantity and quality of pollutants in the atmosphere (Ceburnis and Steinnes 2000, Migaszewski et al. 2002, Kaya and Yaman 2008, Sawidis et al. 2011). The present study was focused on the differential concentrations of nutrients and heavy metals in *P. brutia* needles in four sites in the urban and periurban area of the city in order to determine if they resulted from the influence of various factors, such as traffic and topographical conditions, and might be related to heavy metal pollution in urban environments. Mg and Ca concentrations were higher in the needles collected from the sites located in the periurban forest (sites 3 and 4) than those collected from the sites located near the busy traffic centre of the city, which may be due to the region bedrock. According to Papaioannou (1993), Ca and Mg have common sources, which could be attributed to the region bedrock and the territories full of bases.

The highest concentration of K observed in the needles of site 1, was possibly originated from the fertilizers used in the herbaceous and shrubby plants of the park. Large amounts of Na ions can cause toxicity and other problems if they overcome the permitted concentration limit which is 0.5 mmol·l^{-1} (Venter 2010). Its high concentration observed in the needles collected in the periurban area is due to the combination of site location and upward movements of the winds coming from the sea (Papaioannou 2015).

Cu is essential for plant nutrition and it mainly originates from the metal industry, mining, coal-fired plants, traffic, copper-containing fungicides and fertilizers used in agriculture (Ruhling and Steinnes 1988, Otvos et al. 2003). Its penetration into the leaves depends upon the plant species (Siedlecks et al. 2001). In addition, Greger (1999) states that Cu can be directly taken up by leaves from the air due to direct atmospheric pollution. Its concentration in the needles of sites 1, 2 and 3, affected by traffic, was higher than that of site 4, which is located in the periurban forest far away from the busy ring road. According to Alifragis and Papamichos (1994), Cu concentrations up to 20 ppm will become toxic whereas values less than 4 ppm will lead to deficiency in plants. The results of the present study showed that Cu concentrations are close to the minimum limit proposed by Alifragis and Papamichos (1994). This proved that there is no Cu pollution in the investigated area. Similar results were also given by Sawidis et al. (1995*)* in their study of air pollution with heavy metals in Thessaloniki using trees as biological indicators. Dogan et al. (2010) found the concentrations of Cu in the needles of *P. brutia* collected from suburban and roadside areas equal to 3.10 and 5.30 μg·g^{-1}, respectively.

The wear of tyres and of brake pads, and the combustion of fuel and oil/lubricants are recognized as major sources of Zn and Cd alongside roadways within the Thessaloniki area (Ewen et al. 2009). Bowen (1979) mentioned that the concentration of Zn in plants ranges from 20 to 400 μg·g^{-1}, dry weight. Thus, the concentrations of Zn (which are close to minimum limit proposed by Bowen (1979) in *P. brutia* needles in all researched area) do not constitute an alarming pollution agent in the city of Thessaloniki. This is due, firstly, to the small industrial activity in the urban and periurban area and, secondly, to the winds prevailing in this area which blow northerly and, as a result, clean the atmosphere.

Fe is one of the principal elements in the Earth's crust (Baslar et al. 2005) and is considered to be geogenic but can also be emitted by industries. The main emission sources of iron can be the steel industry, coal burning and intensive traffic (Ruhling and Steinnes 1988). For plants, the concentration of Fe required for growth is considered to be 100 mg·kg^{-1} (Galatis et al. 2003). The results showed no significant differences in its concentrations among the four sites. The concentrations in the needles collected from the sites located near the busy traffic centre as well as in site 3 located in the periurban forest, besides the ring road, were as high as the concentration in the needles collected from site 4 located in the periurban forest, far away from the busy ring road. In contrast, Dogan et al. (2010) found that Fe concentration in the needles of *P. brutia* collected from industrial and roadside areas (595.50 and 500.90 $\mu g \cdot g^{-1}$, respectively) was much higher than in the needles collected from the control area. Thus, the concentrations of Fe in *P. brutia* needles (in the investigated area) do not constitute an alarming pollution agent in the city of Thessaloniki.

Mn is found in nature and its concentration in the soil is determined by the nature of the parent rock (Aubert and Pinta 1977). As it comes from human activities, such as industry or fossil fuel combustions, Mn concentrations are emitted in the atmosphere and they enrich the ground or surface water and groundwater (Metentzoglou 2009*).* Its concentration, which is considered to be essential for plants, is 50 $mg \cdot kg^{-1}$ (Galatis et al. 2003). The highest concentration in site 4 is attributed to the type of the parent rock. The low concentrations measured in *P. brutia* needles in sites near the busy traffic centre do not constitute an alarming pollution agent in the city.

Pb tends to accumulate heavily in the ground due to its low solubility and desegregation by soil microorganisms and is distinguished by its long residence time in the environment (Mitsios 2004). Leaded fuel is a main source of Pb pollution as well as waste incineration, metal production and mining activities (Dogan et al. 2010). The highest concentrations were found in the needles collected from sites 1 and 3. Possibly, the traffic on the roads close to these sites is denser than the roads close to site 2. Ewen et al. (2009) report that the traffic flow patterns have an impact on Pb concentrations, rather than traffic density and they found the highest concentrations in sites which are road junctions in Thessaloniki. The normal concentration in plants ranges from 0.1 to 10 $mg \cdot kg^{-1}$ (Bhanu Prakash et al. 2007), whereas, according to Malik et al (2010), the normal concentration is considered 5 $mg \cdot kg^{-1}$ dry weight. Therefore, the concentrations of Pb measured in *P. brutia* needles in the investigated area were below the normal level.

Ni is mainly considered to be an essential micronutrient and originates mainly from oil and coal burning, the steel industry and smelters (Otvos et al. 2003). Higher concentrations were observed in the needles collected from both sites located near the busy traffic centre of the city as well as from the site besides the ring road than in the needles collected from the site far away from the busy ring road. The normal Ni concentration in plants is reported to be between 1 and 10 ppm (Rebafka et al. 1990) and toxic symptoms to plants occur at concentrations above 50 $mg \cdot kg^{-1}$ (Bhanu Prakash et al. 2007, Chen et al. 2009). Therefore, there is no critical Ni pollution in the researched areas as the concentrations in *P. brutia* needles were found to be at normal levels.

Among heavy metals, Cd is one of the elements concentrating the greatest environmental concern (Dogan et al. 2007). Pb and Cd do not have any known physiological function in plants and can be toxic. The industrial uses of Cd are the largest sources of environmentally hazardous amounts of cadmium as well as coal burning and the incineration of wastes containing it. According to the results of the present study, the concentrations in the needles collected from the sites located near the busy traffic centre of the city as well as in the site besides the ring road were as high as the concentration in the needles collected from the site lacated far away from the busy ring road. Furthermore, Kabata-Pendias and Pendias (1984) reported that the concentration of Cd in contaminated plants ranges between 5 and 30 mg·kg^{-1}. Therefore, the concentration in *P. brutia* needles collected in the investigated area was within normal levels.

Cr is mainly emitted by the iron and steel processing industry and from coal combustion (Cucu–Man et al. 2004), whereas heavy traffic, and especially transportation near the intercity road, is an additional emission source (Ruhling and Steinnes 1988). According to Bowen (1979) and Cary (1982) the normal Cr concentration in plants that does not cause toxic reactions is 0.23 mg·kg^{-1}, and generally below 1 mg·kg^{-1}. In the present study, Cr concentrations were higher in the needles collected in the urban area of Thessaloniki than in the needles collected in the periurban forest. However, its concentrations were not toxic to plants as they were found to be lower than 1 mg·kg^{-1}.

Conclusions

The results of the present study demonstrate that the concentrations of heavy metals Cu, Pb, Ni and Cr in *P. brutia* needles collected from the sites located near the busy traffic centre of Thessaloniki city or besides the ring road are higher than those of the needles collected from the site located far away from traffic roads. In contrast, the traffic on the roads has no impact on Fe and Cd concentrations in *P. brutia* needles. However, the highest Mn concentration in the site located in the periurban forest far away from the busy ring road is attributed to the type of the parent rock. As far as the concentrations of nutrients are concerned, the concentrations of Mg and Ca observed in the needles collected from the sites located in the periurban forest, which are higher than the concentrations in the needles collected from the sites located in urban areas of the city, may be due to the region bedrock. The high Na concentration in the needles collected in the periurban area is attributed to the combination of the site location and upward movements of the winds which come from the sea. The highest concentration of K observed in sites of the urban area is possibly originated from the fertilizers used in the herbaceous and shrubby plants of the park. However, the concentrations of elements measured in *P. brutia* needles collected in the urban and periurban areas of Thessaloniki are below the normal level and as a result they do not constitute an alarming pollution agent in the city.

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