Arch. Biol. Sci., Belgrade, 66 (4), 1345-1355, 2014 DOI:10.2298/ABS1404345B

POPULATION VARIABILITY AND COMPARATIVE ANALYSIS OF MACROELEMENT CONCENTRATIONS IN PEDUNCULATE OAK (*QUERCUS ROBUR* L.) LEAVES AND SURROUNDING SOILS

BRANISLAVA BATOS[1,*], SAŠA ORLOVIĆ[2], ZORAN MILETIĆ[1], LJUBINKO RAKONJAC[1] and DANIJELA MILJKOVIĆ[3]

[1]*Institute of Forestry,* 11000 Belgrade, Serbia
[2]*Institute of Lowland Forestry and Environment, University of Novi Sad,* Novi Sad, *Serbia*
[3]*Institute for Biological Research "Siniša Stanković", University of Belgrade,* 11000 Belgrade, *Serbia*

*Corresponding author: danijela.miljkovic@ibiss.bg.ac.rs

Abstract - The concentration of macroelements in plant biomass is determined by nutrient uptake from the soil and depends on the spatially variable environmental conditions and nutrition status in the soil. The aim of this study was to examine the macroelement concentrations in pedunculate oak (*Quercus robur* L.) leaves and to compare them with soil heterogeneity. *ANOVA, MANOVA and* discriminant canonical analysis (CDA) were used to estimate population variability in the macroelement concentrations in pedunculate oak leaves and surrounding soil. The results of ANOVA revealed a statistically significant difference between populations – in ash content, N, Ca, Mg, P, and all nitrogen scaled variables (all P <0.05) in *Q. robur* leaves. MANOVA revealed a statistically significantly difference between populations – in soil pH, P_2O_5 and K_2O concentrations (all P <0.05). The P in *Q. robur* leaves had the highest total canonical standardized coefficients on the first canonical variable (CDA1) and was responsible for discrimination between populations. Foliar analysis of pedunculate oak leaves and soil analysis showed statistically significant spatial variability in macroelement concentrations between populations in Serbia, which were determined by the bioavailability of essential minerals in the soil.

Keywords: Population variability; macroelement concentrations; *Quercus robur*; discriminant canonical analysis (CDA).

INTRODUCTION

The macroelement concentration in plant biomass is determined by nutrient uptake, plant growth and nutrient translocation from soil to plant organs (Hagen-Thorn et al., 2004). The species, plant genomes, soil fertility, nutrient elements and their role in various physiological processes, root architecture and other environmental conditions (Marschner, 1995; Ayres et al., 2009) are influenced by foliar nutrient concentrations (Augusto et al., 2000). Plant growth and development both depend on nutrient uptake from the soil as a growth media. The bioavailability of essential minerals depends on different factors (solubility of minerals, soil structure and pH) (Ghandilyan et al., 2009) that control the complex mechanism of the mineral pathway from roots to shoots (Tremolieres et al., 1999; Clemens, 2001; Covelo et al., 2008; Ghandilyan et al., 2009). As the results of Ghandilyan et al. (2009) revealed, variation in mineral concentrations exists between different organs and populations, and depends on growth media properties. Biomass and nutrient concentrations in different tree compartments are commonly used for the evaluation of plant nutrient status, soil nutrient availability and as indicators of forest health, which is of fundamental importance for understanding nutrient circulation in forest ecosystems (Hagen-Thorn et al., 2004).

Leaves as the primary photosynthetic organs have an important role in the survival and growth of a plant (Xu et al., 2008). The morphological, structural and physiological changes of leaves indicate that leaves are very sensitive to environmental conditions, the significance of which has been emphasized by some studies (Charles and Garten, 1976; Gallardo and Covelo, 2005; Nikolić et al., 2006). The heterogeneity of environmental conditions surrounding plants in natural habitats (Gallardo and Covelo, 2005) induce phenotypic variation between genotypes within populations and between them (**Primack and Kuang, 1989)**. On the one hand, the elements available in soils can be absorbed through the root and *translocated* to the *leaves* through the *xylem*, and on the other, some elements from the air surrounding leaf surfaces are directly absorbed in leaves by the stomata (De Nicola et al., 2003).

The spatially variable environmental conditions and nutrition status in soil can be studied through the analyses of element concentrations in leaves and soils (**Primack and Kuang, 1989)**. Variations in mineral concentrations of plant (in foliage, for example) depend on the soil characteristics, as a growth media (Gallardo and Covelo, 2005). Predicting forest-stand productivity from soil properties seems difficult (Jandl and Herzberger 2001). The interactions between macroelements in the soil and plants, as well as the circulation of individual elements from soil to plant parts, creates a reciprocal control of their element compositions and determines plant growth (Ladanai et al., 2010).

The ecosystems' components, organisms and their environment, are closely related, and the chemical stability in plants and other living organisms are directly dependent on the chemical composition of the external environment (Ågren and Bosatta, 1996). The maximum growth rate of a plant depends on the disposable element concentrations in the soil that are related to the control of macroelement absorption. If variability of element concentrations between different populations and within species is low, this indicates that the element is under biological control. If the variability in element concentrations differs between populations, it may point to locality-specific properties rather than ecosystem characteristics, and perhaps adaptation to local properties (Knecht and Goransson, 2004; Csikász-Krizsics and Diófasi, 2008; Ladanai et al., 2010).

Thus, following this hypothesis, the aim of the present study was to determine the macroelement concentrations in pedunculate oak leaves and surrounding soils in five populations in northern Serbia, and to define differences in the macroelement contents and correlations within and between soil and plant as parts of the ecosystems. The objectives of this study were: (i) to investigate foliar nutrient concentrations within and between populations; (ii) to detect differences in the available macroelements in surrounding soil, and (iii) to estimate the differences between populations in foliage macroelement content, and if differences exist, to detect which of the analyzed macroelements play the major role in causing them.

MATERIALS AND METHODS

Site description

The population Ada Ciganlija (AC) is situated on the river Sava island near Belgrade on the boundary of the Pannonian and the Moesian Provinces. The dominant forests are Ass. *Fraxino angustifoliae-Quercetum roboris* Jov. et Tom., and Ass. *Populeto-salicetum* Raj. The soil type is fluvisol calcaric. The pedogenetic processes in the surface part of the solum have acquired the terrestrial character.

The forest population Bojčinska Šuma (BS) belongs to the Municipality Zemun, in the vicinity of Belgrade; the soil type in the studied populations is dystric planosol. This is a belt of alluvial-hygrophilous forests *Carpino betuli-Quercetum roboris* Rauš.

The analyzed pedunculate oak population Sombor (SO) belongs to Ass. *Carpino betuli-Quercetum roboris* Anić, as a part of the Gornje Podunavlje (Upper Danube Basin) forest area, in gleysol calcaric soil type.

The population Subotica (SU), from the coenoecological aspect is Ass. *Quercetum roboris* Jov. et Tom, on arenosol calcaric soil type, located in the southern part of the Subotica-Horgoš sandy heath.

In eastern Serbia, along the border with Romania, in the riparian area of the river Karaš, on a gleysol mollic soil type, is found the population Vršac (VR). The typology of this forest of pedunculate oak, hornbeam, ash and field maple is Ass. *Carpino-Fraxino-Quercetum roboris* Miš. et Broz, Subass. *inundatum* (Table 1).

Study species

Pedunculate oak (*Quercus robur* L.) is a common European tree species, adapted to both continental-forest and forest-steppe climates, and to submediterranean and Mediterranean climates throughout Europe. Regarding the adaptability to soil conditions, pedunculate oak does not thrive on shallow dry soil and requires deep and fertile soils affected by groundwater and occasional flooded. This study was conducted in Serbia, on three populations in Vojvodina (Sombor, Subotica, Vršac) and two in the Belgrade area (Bojčinska šuma and Ada Ciganlija).

Foliar and soil analysis

In Serbia, a more in-depth study of pedunculate oak from this aspect has not been performed to date. This study includes the analysis of six macroelements (N, P, K, Ca, Mg and Na) in the leaves. The leaves were sampled from 150 pedunculate oak trees of five populations in Serbia (Ada Ciganlija, Bojčinska Šuma forest, Sombor, Subotica and Vršac). The studied trees, aged 80 years, were selected from populations of both natural and artificial origin. The leaves were sampled from all four sides of the tree crown, at a height of 3-5 m, in the second half of August and the first half of September. The leaves were air-dried, ground and the powder was used for foliar analysis (Davis et al., 1995; Sabate et al., 1995; Bonneau, 1996; Oliveira et al., 1996). The content of nutrients in pedunculate oak foliage was examined by leaf ash analysis, at 40°C during 24 h. Nitrogen content was determined by the distillation of ammonium from the samples prepared by Kjeldahl method. Ash analysis included the determination of phosphorus content by colorimetric method, potassium and sodium contents by flame photometry, and calcium and magnesium contents by titrimetric method with complexon III as the titration agent.

The soil profiles at fixed depths were taken for laboratory analyses. Soil sample analyses included particle size distribution by sedimentation, texture class by the soil texture triangle, active acidity of soil solution electrometrically, the organic C content by the Tyurin method, total N content by the Kjeldahl method, and available forms of P_2O_5 and K_2O by the AL method.

Statistical analyses

Concentrations of macroelements were presented as a percentage of total leaf dry mass. For all statistical analyses the *SAS* version 9.1.3 (*SAS* Institute, 2003) was used. Assumptions of normality were checked with the Shapiro-Wilk test, using PROC UNIVARIATE NORMAL. Normality for these traits was obtained after arcsine transformation of raw data. For the soil macroelement content, we used a logarithmic and square root transformation in order to fit the ANOVA assumption of normality (Sokal and Rohlf, 1995).

For all parameters of descriptive statistics, we used the PROC MEANS statement. The differences in means of macroelement concentrations among the populations were performed using Scheffe's multiple-comparison test as the option in the PROC MEANS statement in the SAS GLM procedure. To estimate the variation in leaf and soil macroelement content between and within populations, we used the coefficient of variance (CV% = standard deviation /mean) (Sokal and Rohlf, 1995).

The *analyzed* and compared *variations* for analyzed macroelement contents, and estimated statistical significance of variation between populations, were conducted with analysis of variance ANOVA,. The populations were treated as fixed factors, while the tree was treated as a random factor. The results of this analysis described the statistical significance of phenotypic variation of the following sources: populations – phenotypic variation caused by environmental differences between populations, and tree (nested in the populations) – genetic differences of trees within populations.

The following ANOVA model was used to analyze the contribution of two sources of variation: the populations and trees (nested in populations) and the macroelement content. The value of each trait included:

$$Y_{ijk} = \mu + S_i + T_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the mean value of the trait Y in the leaf *k* of the tree *j* from populations *i*; μ is the mean main effect (known as main effect) and deviations occurred as a result of the following effects: the effect of S_i (populations *i*), and T_{ij} the nested effects of tree *j* within populations *i* of all measured traits; ε_{ijk} represents the error term specified by the effect of leaf *k* of the tree *j* from populations *i*. Multivariate analysis of variance (MANOVA) was used to test for differences in the macroelement content between populations and trees as a source of variation for the P, N, K, Na, Ca and Mg contents.

All variables that displayed a significant population effect in the previous analysis of variance were subjected to discriminate canonical analysis (CDA) in order to assess the most useful characters for discrimination between populations and to determine how well the nutrient component contributed to the separation of individuals into groups.

RESULTS

Our analyses focused on identifying differences between five populations of *Q. robur*. Based on these results, the concentration of major elements had the same pattern for all five of the analyzed sites. The sequence of macroelement concentrations was N > Ca > K > Mg > P > Na. Variations in concentrations of major elements in the leaves of *Q. robur* between individuals, quantified with a coefficient of variation (CV%), were average for all sites and ranged from the largest concentration of Na (mean = 37.02%) to the lowest concentration of N (mean = 13.93%). Macronutrient ratio as compared to N (nitrogen scaled variable) also had the same pattern for all analyzed sites (Ca/N < K/N < Mg/N < P/N < Na/N) (Table 2A).

Observed leaf nitrogen for the scaled variable had the following pattern for the K/N and P/N ratios: Subotica had a minimum value, while Bojčinska Šuma and Vršac had the highest value compared to the other locations. For the Ca/N ratio, Sombor stood out with the highest, and Vršac with the lowest value, compared to the other locations. According to the results of Scheffe's test, the mean concentration of N, Ca, Mg and P in leaves differed between locations (Table 2). The results of the ANOVA revealed a statistically significant difference between locations in the ash content, N, Ca, Mg, P, and all nitrogen-scaled variables (all P <0.05). Statistically significant variations between individual trees within locations (all P >0.05) was not observed (Table 3). The content of macroelements in the leaves of *Q. robur* was statistically significantly different between sites for N, Ca, Mg and P (all P <0.05), and all nitrogen contents, except for the scaled variable Na/N.

The average individual variability of the C/N ratio had the greatest value (CV% = 74.96) compared to other analyzed macroelement ratios in the soil. The highest value of CV% was determined for the Sombor location and the minimum on the location Bojčinska Šuma (131.23 % vs. 34.27 %, respectively) (Table 2B).

Table 1. Soil properties in the analyzed locations.

Site	Above sea altitude (m)	Soil type	Depth (cm)	Texture	ph
Ada Ciganlija (N 44˚48', E 20˚24')					
			0-5	Loam	7.9
			5-10	Clay loam	8
	70-76	Fluvisol Calcaric	10-20	Clay loam	8.3
			20-40	Loam	8.4
			40-80	Clay loam	8.3
Bojcinska suma (N 44˚43', E 20˚10')					
			0-5	Clay loam	4.7
	77-78	Planosol Dystric	5-10	Clay loam	4.9
			10-20	Clay loam	4.7
			20-40	Clay	5
Sombor (N 45˚46', E 18˚56')					
			0-5	Loam	7.3
			5-10	Loam	7.4
			10-20	Clay loam	7.5
	83-86	Gleysol Calcaric	20-40	Loamy sand	7.8
			40-80	Sandy loam	7.8
			80-100	Loamy sand	8
Subotica (N 46˚07', E 17˚18')			0-5	Loamy sand	7.7
			5-10	Loamy sand	7.9
	137	Arenosol Calcaric	10-20	Loamy sand	8.1
			20-40	Loamy sand	8.1
			40-80	Loamy sand	8.1
Vrsac (N 45˚07', E 21˚25')					
			0-5	Clay	7.4
			5-10	Clay	7.2
	76-80	Gleysol mollic	10-20	Clay	7.1
			20-40	Clay	7.2
			40-80	Clay	7.3

Concentrations of macroelements in the soil observed at the sites varied in content of K_2O and P_2O_5, and nitrogen-scaled variables C/N, K_2O/N and P_2O_5/N (Table 2B). N concentration was greatest at the Ada Ciganlija site (0.38 %), while the lowest value was obtained for the Subotica site (0.13 %). A similar pattern was obtained for the concentration of K (K_2O), with the highest value obtained for the sites of Bojčinska Šuma and Ada Ciganlija (15.85 % and 14.78 %, respectively) and lowest value for Subotica (3.38 %). The population of Ada Ciganlija had the highest concentration of P (P_2O_5, 6.80%) and the lowest scores were those of Sombor and Subotica (1.30 % and 1.36 %; respectively). The concentration of C/N was the largest for the site of Bojčinska Šuma and Ada Ciganlija (2.29 % and 2.15 % respectively), while the lowest value was obtained for the Subotica site (0.50 %). The nitrogen-scaled variables for K_2O/N ratio in the soil had the highest value in Subotica (98.70 %) and lowest in Vršac (19.21 %). The maximum values were obtained for Subotica (24.50 %), a minimum was observed on the Sombor site (5.98 %) when we compared the concentrations of nitrogen-scaled variables for the P (P_2O_5/N) ratio between sites (Table 2B).

The results of ANOVA revealed a statistically significant difference between sites in soil pH (F = 193.89, P <0.0001), and the concentrations of P_2O_5 and K_2O in the soil (F = 3.94, P <0.05, F = 5.27, P <0.001, respectively) (Table 3). According to MANOVA, we also found significant differences between localities (F = 12.95, P <0.0001).

The results of discriminant canonical analysis (CDA), based on standardized coefficients of canoni-

Table 2. Mean values of macroelements concentrations in pedunculate oak (*Quercus robur* L.) leaves (A) and surroundings soils (B) on five populations in Serbia. The values of different letters are significantly different at the 0.05 probability level according to the results of the Scheffe's test.

A	Ada Ciganlija (n=29)			Bojcinska suma (n=30)			Sombor (n=29)			Subotica (n=31)			Vrsac (n=31)		
	mean value	SE	CV (%)	mean value	SE	CV (%)	mean value	SE	CV (%)	mean value	SE	CV (%)	mean value	SE	CV (%)
Leaf															
macroelements															
N	2.61^{A}	0.06	11.77	2.20^{C}	0.10	24.43	2.27^{BC}	0.05	12.71	2.52^{AB}	0.04	9.46	2.44^{ABC}	0.05	11.26
Ca	1.62^{A}	0.08	27.98	1.40^{BA}	0.07	28.47	1.62^{A}	0.10	34.84	1.19^{B}	0.05	21.26	1.24^{B}	0.04	16.30
K	1.27	0.06	24.59	1.22	0.04	15.74	1.19	0.06	25.00	1.01	0.06	32.16	1.20	0.05	21.70
Mg	0.91^{A}	0.04	22.09	0.71^{B}	0.03	25.37	0.69^{B}	0.04	28.65	0.70^{B}	0.03	25.33	0.49^{C}	0.04	48.70
P	0.15^{C}	0.00	20.30	0.21^{B}	0.00	13.06	0.16^{C}	0.00	12.48	0.16^{C}	0.00	13.11	0.23^{A}	0.00	13.81
Na	0.02	0.00	26.78	0.02	0.00	49.08	0.02	0.00	44.08	0.03	0.00	44.40	0.02	0.00	20.76
nitrogen scaled variables															
Ca/N	0.63^{BA}	0.03	28.11	0.69^{A}	0.05	39.64	0.72^{A}	0.05	37.77	0.47^{B}	0.09	21.79	0.51^{B}	0.01	13.25
K/N	0.49^{BA}	0.03	27.61	0.60^{A}	0.04	37.65	0.53^{BA}	0.03	26.55	0.44^{B}	0.03	32.61	0.50^{BA}	0.02	27.35
Mg/N	0.35^{A}	0.01	19.98	0.35^{A}	0.03	47.39	0.31^{A}	0.02	32.68	0.28^{BA}	0.01	27.02	0.20^{B}	0.02	49.39
P/N	0.06^{B}	0.00	25.36	0.10^{A}	0.00	34.22	0.07^{B}	0.00	12.82	0.06^{B}	0.00	13.72	0.01^{A}	0.00	18.17
Na/N	0.01	0.00	29.80	0.01	0.00	52.15	0.01	0.00	45.46	0.01	0.00	46.81	0.01	0.00	25.08

B	Ada Ciganlija (n=5)			Bojcinska suma (n=4)			Sombor (n=6)			Subotica (n=5)			Vrsac (n=5)		
	mean value	SE	CV (%)	mean value	SE	CV (%)	mean value	SE	CV (%)	mean value	SE	CV (%)	mean value	SE	CV (%)
Soil															
N	0.38	0.08	45.54	0.26	0.03	23.56	0.26	0.07	65.19	0.13	0.05	82.36	0.30	0.06	42.82
K_2O	14.78^{BA}	4.42	66.91	15.85^{A}	1.97	24.90	11.85^{BA}	1.78	36.96	3.38^{B}	0.81	53.68	5.45^{BA}	0.92	37.65
P_2O_5	6.8^{A}	2.57	84.43	1.58^{B}	0.05	6.18	1.30^{A}	0.22	42.13	1.36^{B}	0.27	44.60	2.87^{B}	0.47	36.59
nitrogen scaled variables															
C/N	2.15^{A}	0.58	59.97	2.29^{B}	0.39	34.27	1.93^{BC}	1.03	131.23	0.50^{BA}	0.24	107.42	1.26^{C}	0.24	41.93
K_2O/N	41.04	11.17	60.87	60.53	3.07	10.13	52.96	6.68	30.92	98.70	59.44	134.66	19.21	2.67	31.02
P_2O_5/N	15.44^{BA}	5.10	73.87	6.33^{B}	0.94	29.71	5.98^{B}	0.95	38.92	24.5^{A}	10.43	95.23	11.44^{BA}	3.31	64.60

Table 3. Results of ANOVAs for the macroelements concentrations (Na, Ca, K, Mg, P, Na) in pedunculate oak (Quercus robur L.) leaves and surrounding soil conditions in five populations in Serbia.

	Leaves		Soil	
	Location (df= 4)	Tree (df=4)	Location (df= 4)	
macroelements			ph	193.89***
N	7.26***	1.26		
Ca	8.62***	1.19		
K	1.26	1.00	C/N	1.25
Mg	12.16***	0.50	N	2.02
P	52.25****	0.90	K_2O	5.27**
Na	0.76	0.87	P_2O_5	3.94*
nitrogen scaled variable				
Ca/N	9.93****	1.18	K_2O/N	1.14
K/N	4.03**	1.32	P_2O_5/N	1.99
Mg/N	8.09***	0.98		
P/N	31.96***	1.10		
Na/N	1.11	1.10		
Ca/N	9.93****	1.18		
K/N	4.03**	1.32		
Mg/N	8.09***	0.98		

* P<0.05. ** P<0.01. *** P<0.001. **** P<0.0001

Table 4. Standardized canonical coefficients on two axes from a canonical discriminant analysis (CDA) based on macroelements concentrations in pedunculate oak (*Quercus robur* L.) leaves from five populations in Serbia.

Variable	Root1	Root2	Root3
P	0.95	0.12	-0.40
Ca	-0.09	0.72	0.09
Mg	-0.05	0.25	-0.73
N	-0.30	-0.74	-0.41
Eigenval	2.36	0.30	0.11
Cum.Prop.	0.83	0.94	0.98

cal variables, showed *differences between* populations according to the first axis for the concentration of P in the leaf (Table 4). The first axis represents 83% of the total variability and the second only 10% (Fig. 1). According to the first axis, Vršac differed in comparison to other populations (with the highest concentration of P in the leaf (0.23%) see Table 3.

DISCUSSION

Forest tree nutrition depends on locality conditions (Kunzova et al., 2007). The optimal amount of nutrients and their ratios in plants are the basic elements for successful growth and development, total production potential, and plant resistance to biotic and abiotic agents. A complex mechanism regulates the uptake of nutrients from the soil (Tremolieres et al., 1999; Covelo et al., 2008). The highest concentrations of nutrients are observed in the assimilation organs, i.e. leaves. As fundamental and very sensitive plant organs, leaves reflect the morphological, structural and physiological changes caused by environmental changes. In foliar analysis of pedunculate oak clones in the seed orchard Banov Brod in Vojvodina (Serbia), by Nikolić et al., (2006), and for *Q. ilex* (Canadell and Vila, 1992), a similar sequence of average values of nutrient macroelement percentages (N>Ca>K>Mg>P>Na) was reported. Based on an estimation of the coefficient variation, the highest variability of N and P in pedunculate oak was found by Gallardo and Covelo (2005). The results obtained by Nikolić et al. (2006) were similar to ours; the highest individual variability in pedunculate oak foliage was shown by Na, Ca, K and P and the lowest by N, while Canadell and Vila (1992) reported the lowest variation in N and K in their study of the nutrient

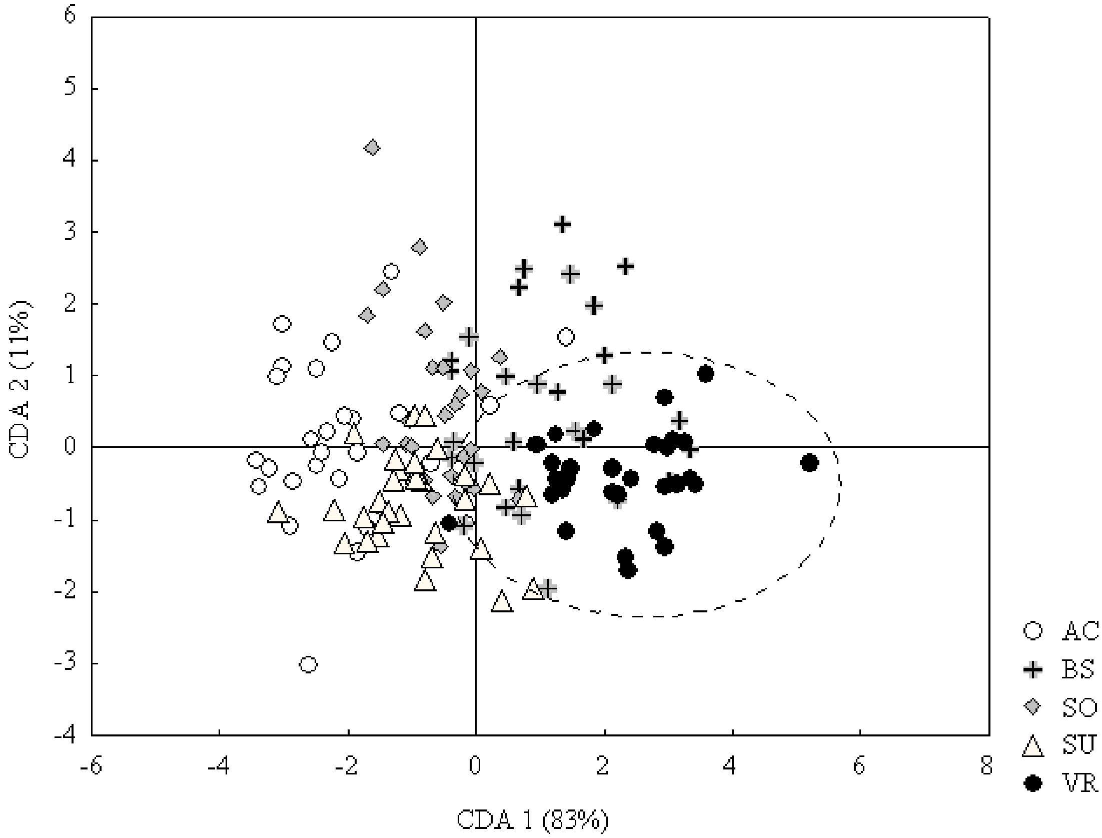

Fig. 1. *Discrimination between populations* of pedunculate oak *Quercus robur* (L.) based on the *canonical discriminant analysis* using four macroelements concentrations (P, Mg, Ca, N) in dry leaf mass. Populations: Ada Ciganlija (AC), Bojčinska šuma (BS), Sombor (SO), Subotica (SU) and Vršac (VR).

content in *Q. ilex* leaves in three types of soil.

The concentration of N in soil is influenced by the function of plants, by biotic and abiotic factors, environmental factors, soil moisture, and temperature changes that affect N transformations (mineralization and nitrification) (Xiaoli et al., 2011; Nunes et al., 2012). Nitrogen mineralization is affected by several environmental factors (Herrman, 2003). Ada Ciganlija was distinguished by high values of soil solution pH, light texture and good aeration of the topsoil layers, i.e. favorable oxidative conditions that enable rapid mineralization of nitrogen organic forms. After the phase of ammonification, nitrogen mineralization passed through the phase of nitrification. This was enabled by the high pH value of the soil solution and by the favorable oxidative conditions of the pedochemical environment in surface parts of the solum. The nitrates of all soil bases were extremely mobile and they readily migrated through the solum to the deeper layers where they were absorbed by pedunculate oak roots. Soil microorganisms are dynamically involved in many basic ecological processes, such as the biogeochemical cycling of elements and the mineralization of carbon, nitrogen, phosphorous and sulfur (Narasimha et al., 2011). On the planosol at Bojčinska Šuma, the soil solution pH is acidic. Nitrogen mineralization is reduced, resulting in ammonification. Some forms of nitrogen, particularly organic nitrogen and ammonium, can also be adsorbed on active sites of the soil, limiting movement through the soil profile (Felton et al., 2008). As the soil texture is heavy, the adsorption capacity is high, and NH_4^+ ions are, for the most part, adsorbed to the adsorption complex (Savić and Jekić, 1975). This only allows for the nutrition of plants with shallow roots. Low amounts of nitrates formed in the acidic medium at this locality are subject to denitrification because of the anaerobic and anoxidative conditions on planosols, thanks to the prolonged water stagnation in the soil profile. In such cases, organic matter decomposition is performed by anaerobic and facultative anaerobic microorganisms that use chemically bound oxygen from NO_3 and transform it into the molecular form. There is no migration of plant-available nitrogen outside the impermeable stagnic g-horizon at this locality. This explains the poor nitrogen nutrition of pedunculate oak at this locality.

Statistically highly significant differences among the populations were observed for the concentration of P (P <0.05). Vršac exhibited the highest values (0.23%) and Ada Ciganlija had the lowest values (0.15%). Although the total amount of P in the soil may be high, it is often present in unavailable forms or in forms that are only available outside of the rhizosphere (Schachtman et al., 1998). The higher content of P at Vršac, on gleysol mollic, was the consequence of higher extractable amounts of P forms in the deeper layers of the solum. In these layers of soil, the pedunculate oak root system absorbs water and nutrients. For other study populations, soil P forms were observed only in surface layers, to the depth of 20 cm, which did not have much effect on pedunculate oak nutrition, because this element is poorly soluble and its migration through the solum is very difficult.

Highly significant differences were observed for the contents of Ca and Mg. The highest values of earth alkaline elements in pedunculate oak foliage Ca and Mg were measured in Ada Ciganlija (1.62% *vs.* 0.91%, respectively), the lowest values of Ca (1.19%) in Subotica, while the Vršac population exhibited the lowest Mg values (0.51%). The River Sava's sediment is rich in carbonates of earth alkaline elements (Miljković, 1972) and this high concentration of earth alkaline elements in the oak leaves in Ada Ciganlija, and the high pH soil solution, were the consequence of the higher content of these elements in the soil. According to the characteristics of the aeolian sands of Subotica-Horgoš sandy heath, which is rich in carbonates of earth alkaline elements (Letić et al., 2001), the high quantities of earth alkaline elements in pedunculate oak leaves could also be expected, but the results showed the lowest calcium percentage in the leaves. However, this locality was characterized by the most unfavorable conditions for pedunculate oak. The existing pedunculate oak stand had no natural regeneration because the topsoil was extremely dry. Even the attempts made to artificially regenerate the pedunculate oak using different afforestation methods and technologies have had no positive results.

Based on the results of the analysis at five populations, it can be concluded that the macroelement content in the analyzed pedunculate oak leaves was the highest at Ada Ciganlija and the lowest at Subotica, with the exception of phosphorus. The content of this element in the leaves of the oak was highest in the Vršac population, which was a consequence of the large quantities of available forms of this element in soil. The nutrients are absorbed from the deep layers and they reach the soil surface in the form of leaf litter. The favorable textural composition of the soil, good aeration of the surface soil layers, favorable pH of the soil solution, and the conditions of organic matter mineralization and nutrient migration through the soil profile made the Ada Ciganlija site the most favorable for absorption of the nutrients. In Subotica, the delay in organic matter mineralization and release of plant nutrients on aerosol were caused by the fast drying of surface layers.

Our results are very similar to those reported by other authors, and confirm a spatial dependence in the nutrient concentrations in leaf and soil (Gallardo and Covelo, 2005). Generally, it can be concluded that the macroelement percentage in leaves is determined by the interaction of environmental conditions. The variability in macroelement concentrations in leaves differed between populations as the result of local adaptation to specific-locality soil properties. The changes of groundwater regime (caused by the construction of drainage systems and embankments along the riverbanks, as well as by global climate change), and excessive use of high quality wood in industry, are some of the reasons for the considerable reduction in pedunculate oak forest areas in Serbia during the 20th century. As a species with deep roots, adult trees of pedunculate oak can reach the groundwater level, which enables them to survive more easily, even in areas with a deeper groundwater level. Permanent and rapid changes of environmental conditions in pedunculate oak forests often prevent their natural regeneration. A complete biological study and knowledge of the pathways of macroelement assimilation from the soil can contribute to the preservation of the populations of this very old and industrially valuable plant species in the future.

Acknowledgements – This work was funded as a subproject: "Forestry and mitigation of climate changes", of the project: "Studying climate change and its influence on the environment: impacts, adaptation and mitigation" (No. III 43007) by the Ministry of Education, Science and Technological Development of Serbia.

REFERENCES

Ågren, G. I. and *E. Bosatta* (1996). Theoretical Ecosystem Ecology – Understanding Element Cycles. Cambridge University Press, Cambridge, U.K.

Augusto, L., Ranger, J., Ponette, Q. and *M. Rapp* (2000). Relationships between forest tree species, stand production and stand nutrient amount. *Ann. For. Sci*. **57**, 313-324.

Ayres, E., Steltzer, H., Berg S., Wallenstein, M. D., Simmons, B. L. and *D. H. Wall* (2009). Tree species traits influence soil physical, chemical and biological properties in high elevation forests. *Plos One* **4** (6), 1-11.

Bonneau, M. (1996). Sessile oak seedling fertilization and leaf mineral composition in western France. *Ann. Sci. For.* **53**, 605-613.

Canadell, J. and *M. Vila* (1992). Variation in tissue element concentrations in *Quercus ilex* L. over a range of different soils. *Vegetatio* **99-100**, 273-282.

Charles, T. and *J. R. Garten* (1976). Correlations between concentrations of elements in plants. *Nature* **261**, 686-688.

Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. *Planta* **212**, 475-486.

Covelo, F., Duran, J. and *A. Gallardo* (2008). Leaf resorption efficiency and proficiency in a *Quercus robur* population following forest harvest. *For Ecol. Manage* **255** (7), 2264-2271.

Csikász-Krizsics. A. and *L. Diófasi* (2008). Effects of rootstock-scion combinations on macroelements availability of the vines. *J. Cent. Eur. Agr.* **9** (3), 495-504.

Davis, D. D., Skelly, J. M. and *B. L. Nash* (1995). Elemental concentrations in foliage of red maple, red oak and white oak in relation to atmospheric deposition in Pennsylvania, Individual Papers from Proceedings 10th Central Hardwood Forest Conference March 5-8., Morgantown, WV.: Gen. The. Rep. NE-197. Randnor, PA: US. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station, pp 188-195.

De Nicola, F., Maisto, G. and *A. Alfani* (2003). Assessment of nutritional status and trace element contamination of holm oak woodlands through analyses of leaves and surrounding soils. The Science of the Total Environment 311: 191-203

Felton, G. K., Maimone, D. and *J. S. Kays* (2008). Nitrogen migration from deep-row biosolids incorporation on a hybrid poplar tree farm. Written for presentation at the NABEC Annual International Meeting Clarion Hotel Aberdeen, MD July, 28-July 30, 2008. paper 08-051 http://www.naturalresources.umd.edu/Publications/BiosolidResearch/NABEC08- 051final.pdf

Gallardo, A. and *F. Covelo* (2005). Spatial pattern and scale of leaf N and P concentration in a *Quercus robur* populations. *Plant and Soil* **273**, 269-277.

Ghandilyan, A., Ilk, N., Hanhart, C., Mbengue, M., Barboza, L., Schat, H., Koornneef, M., El-Lithy, M., Vreugdenhil, D., Reymond, M. and *M. G. M. Aarts* (2009). A strong effect of growth medium and organ type on the identification of QTLs for phytate and mineral concentrations in three *Arabidopsis thaliana* RIL populations. *J. Exp. Bot.* **60** (5), 1409-1425.

Hagen-Thorn, A., Armolaitis, K., Callesen, I. and *I. Stjernquist* (2004). Macronutrients in tree stems and foliage: a comparative study of six temperate forest species planted at the same sites. *Ann. For. Sci.* **61**, 489-498.

Herrman, A. (2003). Predicting nitrogen mineralization from soil organic matter - a Chimera? Doctoral thesis Swedish University of Agricultural Sciences Uppsala, http://pub.epsilon.slu.se/386/

Jandl, R. and *E. Herzberger* (2001). Is soil chemistry an indicator of tree nutrition and stand productivity? *Die Bodenkultur* **52**, 155-163.

Knecht, M. F. and *A. Goransson* (2004). Terrestrial plants require nutrients in similar proportions. *Tree Physiol.* **24** (4), 447-60.

Kunzova, E., Pechova, M. and *M. Vocetkova* (2007). The influence of climatic conditions on nitrogen uptake during the vegetative phases for sunflower. In: Strelcova, K., Skvarenina, J., Blazenec, M. (ed) Bioclimatology and natural hazards, International Scientific Conference, Poľana and Detvou, Slovakia, pp 17-20.

Ladanai, S., Ågren, I.G. and *A. B. Olsson* (2010). Relationships between tree and soil properties in *Picea abies* and *Pinus sylvestris* forests in Sweden. *Ecosystems* **11**, 302-316.

Letić, Lj., Savić, R. and *M. Bozinović* (2001). Turbulent sand. Monograph, (Eds), Tracks, Book 5, Public Enterprise "Palic-Ludas", Subotica, pp 160.

Marschner, H. (1995). Mineral nutrition of higher plants. In: 2nd ed. Academic press, London, pp 889.

Miljković, N. (1972). Alluvial soils. In: Živković B et al. (ed), Soils of Vojvodina, Institute of Agricultural Research, Novi Sad, pp 294-317.

Narasimha, G., Sridevi, A., Venkata Subba Reddy. G. and *B. Rajasekhar Reddy* (2011). Effects of cotton ginning mill effluents on soil enzymatic activities and nitrogen mineralization in soil. *J. Chem. Pharm. Res.* **3**(1), 128-137.

Nikolić, N. P., Krstić, B. D. J., Pajević, S. P. and *S. S. Orlović* (2006). Variability of Leaf Characteristics in Different Pedunculate Oak Genotypes (*Quercus robur* L.). *Proc. Nat. Sci. Matica Srpska*, Novi Sad, **110**, 95-105.

Nunes, J., Madeira, M., Gazarini, L., Neves, J. and *H. Vicente* (2012). A data mining approach to improve multiple regression models of soil nitrate concentration predictions in *Quercus rotundifolia* montados (Portugal) *Agroforest Syst*. **84**, 89-100.

Oliveira, G., Martins-Loucao, M. A., Correia, O. and *F. Catarino* (1996). Nutrient dynamics in crown tissues of cork-oak (*Quercus suber* L.). *Trees* **10**, 247-254.

Primack, R. B. and *H. Kuang* **(1989).** Measuring fitness and natural selection in wild plant populations. *Ann. Rev. Ecol. and Syst.* **20**, 367-396.

Sabate, S., Sala, A. and *C. A. Gracia* (1995). Nutrient content in *Quercus ilex* canopies: Seasonal and spatial variation within a catchment. *Plant and Soil* **168-169**, 297-304.

SAS Institute Inc. (2003). SAS/STAT User's Guide, version 9.1.3, Cary, N.C., SAS Institute Inc.

Savić, B. and *M. Jekić* (1975). Agrochemistry for Students of Agronomy. Svjetlost, Sarajevo

Schachtman, P. D., Reid, J. R. and *S. M. Ayling* (1998). Phosphorus uptake by plants: from soil to cell. *Plant Physiol.* **116**, 447-453.

Sokal, R. R. and *F. J. Rohlf* (1995). Biometry: The principles and practice of statistics in biological research. In: Freeman WH (3rd ed), New York pp 887.

Tremolieres, M., Schnitzler, A., Sanches-Perez, J. M. and *D. Schmitt* (1999). Changes in foliar nutrient content and resorption in *Fraxinus excelsior* L., *Ulmus minor* Mill. and *Clematis vitalba* L. after prevention of floods. *Ann. For. Sci.* **56**, 641-650.

Xiaoli, C., Yiqi, L., Bo, S., Wan, S., Dafeng, H. and *Z. Quanfa* (2011). Plant carbon substrate supply regulated soil nitrogen dynamics in a tallgrass prairie in the Great Plains, USA: results of a clipping and shading experiment. J. Plant Ecol. **4** (1-4), 228-235.

Xu, F., Guo, W., Xu, W. and *R. Wang* (2008). Habitat effects on leaf morphological plasticity in *Quercus acutissima*. *Acta Biol. Crac. S. Bot.* **50** (2), 19-26.