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SYNTHESIS AND CHARACTERISATION OF SOME N-SUBSTITUTED AMIDES OF SALICYLIC ACID

ABSTRACT: The synthesis of some N-substituted aromatic amides in the salicylic acid series was achieved, by direct reaction between primary amines and salicylic acid in inert organic solvent, in the presence of PCl_3. The compounds that were obtained, partially not described in literature, were characterized by chemical-physical methods.

KEY WORDS: synthesis, characterization, salicylic acid, N-substituted aromatic amides

INTRODUCTION

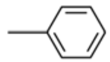
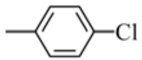
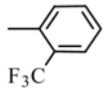
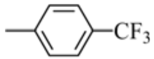
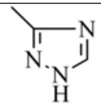
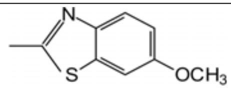
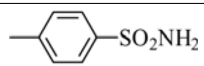
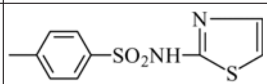
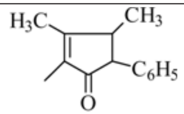
Salicylic acid — their derived in general — and N-substitutes amides in particular are used for their disinfecting, antifungic, antimicotic and antibacterial properties, in human and animal medicine, in different industrial sectors and in agriculture (Neubauer et al., 1984; Lupea et al., 1995; Lupea et al., 2001).

Synthesis of N-substitutes amides of salicylic acid is achieved either via its acid chloride or by direct reaction between salicylic acid and amines in a heterogeneous medium in inert organic solvent at reflux (Bozga et al., 1987; Neubauer et al., 1984; Lupea et al., 2001). The efficiencies are determinated by the nature of the amine component.

MATERIAL AND METHODS

In a 250 ml three-neck round-bottom flask equipped with a stirrer, condenser and dropping funnel, 100 ml of chlorobenzene, 16 g (0,12 moles) of salicylic acid and 0,12 moles of primary amine were introduced. Afterwards, under strong stirring, through the dropping funnel 3,5 ml (0,04 moles) of PCl_3 was added. The dropping rate was adjusted so that the mixture temperature do

Table 1 — N-substituted amides of salicylic acid; characteristics

Comp no.	R-substituent	Molec. form. Mol. mass	Elem. analysisteor./found C%	H%	N%	S%	m.p. (°C)	%	Solubility*
1		C13H11NO2 M = 213	73,23 72,219	5,164 5,245	6,57 6,495		133	89,5	1c 2b 3b 4e 5f 6b 7c 8f 9f 10c
2		C13H10ClNO2 M = 247,5	63,03 62,52	4,04 4,12	5,65 5,47		187—189,5	61,4	1d 2c 3b 4e 5f 6b 7b 8f 9e 10c
3		C14H10F3NO2 M = 281	59,78 59,325	3,56 3,172	4,98 4,384		224—226	56,1	1d 2e 3d 4e 5e 6c 7e 8f 9f 10d
4		C14H10F3NO2 M = 281	59,78 59,258	3,56 3,232	4,98 4,275		204—206	55,6	1c 2d 3d 4d 5e 6b 7d 8f 9f 10c
5		C9H8N4O2 M = 204	52,94 52,895	3,92 3,898	27,45 27,551		275—276	52,6	1f 2f 3b 4f 5f 6d 7f 8f 9f 10f
6		C15H12N2O3S M = 300	60,00 59,863	4,00 3,972	9,33 9,421	10,67 10,585	260—262	62,8	1f 2f 3d 4f 5f 6c 7f 8f 9f 10f
7		C13H12N2O4S M = 292	53,42 53,32	4,138 4,254	9,583 9,5989	10,97 11,126	280—281	66,45	1f 2c 3c 4d 5f 6b 7c 8f 9f 10d
8		C16H13N3O4S2 M = 375	51,19 50,864	3,49 3,671	11,193 11,063	17,082 17,078	285—286	64,1	1d 2d 3b 4e 5e 6b 7e 8f 9f 10f
9		C18H17N3O3 M = 323	66,87 66,751	5,26 5,312	13,00 13,098		204—208	71,5	1d 2d 3c 4d 5f 6b 7d 8f 9f 10d

* The using solvents are: 1 — chloroform; 2 — ethanol; 3 — dimethyl sulfoxid; 4 — dioxane; 5 — toluene; 6 — dimethylformamide; 7 — acetone; 8 — hexane; 9 — carbon tetrachloride; 10 — ethyl-methyl-ketone. Solubility: a — miscible; b — very soluble; c — middling soluble; d — slightly soluble; e — less soluble; f — insoluble

Table 2 — IR, UV, and mass spectra for the substituted amides synthesized

Comp. no.	$\nu_{Ar\text{-}H}$	$\delta_{CH\ arom}$	$\gamma_{CH\ arom}$	$\nu_{OH\ fenolic}$	$\nu_{C\text{-}O\ fenolic}$	$\delta_{OH}+\nu_{CH_2}$	B.am.I	B.am.II	B.am.III	ν_{Ham} γ_{NH}	Other bands	UV spectra (ε)	Mass spectra
1	3070(s)	1020(m) 1060(m)	750(m) 810(m) 880(m) 910(m)	3360(m)	1220(i)	1260(i)	1640(i)	1530(i)	1300(i)	3250(s) 680(m)		224i 267m 299u 340u	93-fenoli, aniline 121-deriv. salicilici 65-comp. arom. $C_5H_5^+$ 77-$C_6H_5^+$ M = 213
2	3060(s)	1020(m) 1050(m)	750(i) 810(m) 890(m)	3630(i)	1230(i)	1230(i)	1600(i)	1530(i)	1300(m)	3050— 3200(s) 750(m)	triazole nucleus 1450(i) 1480(i) 1320(s)	224i 242u 267m 299u 240s	65-arom. comp $C_5H_5^+$ 93-phenols, aniline 121-deriv. salicyl 127-p-chlor aniline rest M = 247,5
3	3100(s)	1020(i) 1070(m)	750(i) 810(m) 860(m) 900(m)	3300(l)	1220(i)	1260(i)	1650(i)	1560(i)	130 0(i)	3100— 3300(l) 670(i)	$\nu_{CF}^{s,as}$ 1170(i) δ_{CF} 1270(i)	224i 240u 266u 340u	93-phenols, aniline 121-deriv. salicyl. 65-arom. comp. $C_5H_5^+$ 77-$C_6H_5^+$ 161-amine rest M = 281
4	3070(s)	1100(s) 1070(i)	710(m) 760(i) 840(i) 900(m)	3300(l)	1220(i)	1290(m)	1630(i)	1560(i)	1320(i)	3000— 3100(l) 690(m)	$\nu_{CF}^{s,\ as}$ 1170(i) δ_{CF} 1400(i)	224i 240u 266u 340u	65-arom. comp. $C_5H_5^+$ 93-phenols, aniline 121-deriv. salicyl. 161-amine rest M = 281
5	3090(s)	1020(m) 1050(m)	730(m) 900(m)	3400(i)	1250(i)	1370(i)	1640(i)	1530(i)	1270(i)	3120(s) 700(i)	triazole nucleus 1510(m) 1460(m) 1420(i) 1290(i)	228i 309m	93-phenols, aniline 121-deriv. salicyl. 65-arom. comp. $C_5H_5^+$ 77-$C_6H_5^+$ M = 204

Comp. no.	$\nu_{Ar\text{-}H}$	$\delta_{CH\ arom}$	$\gamma_{CH\ arom}$	$\nu_{OH\ fenolic}$	$\nu_{C\text{-}O\ fenolic}$	δ_{OH}+ ν_{CH_2}	B.am.I	B.am.II	B.am.III	ν_{Ham} γ_{NH}	Other bands	UV spectra (ε)	Mass spectra
6	3070(s)	1020(m) 1050(i)	740(m) 800(i) 890(m) 920(m)	3370(m)	1220(i)	1260(i)	1650(i)	1530(i)	1300(i)	3050(s) 680(m)	benztiazole nucleus 1600(i) 1450(i) 1120(m) $\nu_{CH3}^{as,\ s}$ 2930	226i 269m 305s	93-phenols, aniline 121-deriv. salicyl. 65-arom. comp. $C_5H_5^+$ 77-$C_6H_5^+$ 165—166 benztiazol. 180-aminobenztiazol. M = 300
7	3100(l)	1050(I) 1070(m)	780(I) 830(m) 890(I)	3400(i)	1180(i)	1320(m)	1650(i)	1530(i)	1300(i)	3300— 3400(l) 690(m)	ν^{as}_{SO2} 1310(i) ν^{s}_{SO2} 1160(i) $\nu_{S\text{-}N}$ 910(s)	224i 244u 278u 284i 300u 342m (8487,56)	93-phenols, aniline 121-deriv. salicyl. 65-arom. comp. $C_5H_5^+$ 64-sulphonamide 172-sulphanilamide fragm. M = 292
8	3060(m)	1100(m)	760(m) 820(m) 930(m)	3350(l)	1220(m)	1220(m)	1660(i)	1560(i)	1300(i)	300— 3100(l) 550(i)	ν^{as}_{SO2} 1310(i) ν^{s}_{SO2} 1100(i) thiazole nucleu 1600(i) 1450(m) 1400(m)	224i 243s 278i 287u 345m (12197,2)	93-phenols, aniline 121-deriv. salicyl. 65-arom. comp. $C_5H_5^+$ 151-sulphamide rest 255-sulphathiazole rest M = 375
9	3070(m)	1180(m) 1090(i)	830(m) 860(m)	3340(m)	1310(m)	1310(m)	1650(i)	1520(i)	1300(i)	3300— 3400(l) 700(m)	$\nu_{Calif\text{-}N}$ 1010(m) $\nu_{Carom\text{-}N}$ 1300(i) $\nu_{CH3}^{s,\ as}$ 2930(m)	223i 244u 269u 333s (6373,44)	93-phenols, aniline 121-deriv. salicyl. 65-arom. comp. $C_5H_5^+$ 202, 203, 204- aminoantipyrine fragm. M = 323

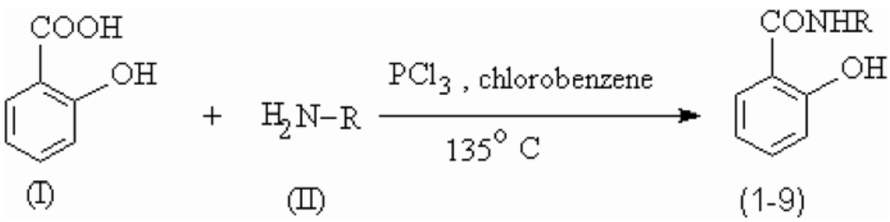
not exceed 20—25°C. After adding of PCl_3, the mixture was heated to reflux, slowly at the beginning to avoid possible foaming and emitting of HCl. The mixture was maintained at the solvent reflux for averaging 6—7 hours. After cooling, the crude product was filtered off on a Büchner funnel and was washed three times successively with 50 ml of hot distilled water, in order to eliminate the unreacted amine chlorohydrates. After that, three successive washings with 50 ml of 10% Na_2CO_3 solution eliminated the unreacted salicylic acid. The obtained precipitates were dried at 80 5°C, and then purified by dissolving in warm DMF and treatement with active carbon. A warm filtering process followed by precipitation with adding of distillated water and eventually few drops of diluted HCl afforded compounds 1—9 (Table 1).

The using reagents were of high purity (Merck produce, 98%), with the exception of sulfonamides (95%).

Elementary analyses were performed using a Vario EL apparatus. UV spectra were recorded on a SPECOL 75 apparatus, in NAOH 0,5 N. Melting points were determinate with a Böetius Carl-Zeiss Jena apparatus. IR spectra were recorded on a Jaskow FT/IR — 430" apparatus, and mass spectra on a GS/MS MAT 212 apparatus.

RESULTS AND DISCUSSIONS

In experimental works for obtaining the N-substitutes amides of the salicylic acid, there was achieved by direct reaction between components in chlorobenzene medium in the presence of phosphorus trichloride at reflux:



Scheme 1

The molar ratio of salicylic acid: amine: PCl_3 was 1: 1: 0,3. Contrarily the literature data (Cojocariu et al., 1980; Bozga et al., 1987; Celianu—Bibian et al., 1983) according that — for aromatic amides from the benzoic and p-hydrobenzoic acid series, increasing of the PCl_3 quantity leads to favorable effects on the efficiency, in the accomplished synthesis the effect of PCl_3 was not favorable. The extension of the concluding time of the reaction at the reflux of the solvent influenced favorable the efficiency. Successive washings from the crude product that was obtained removed the unreacted components. Final purification was achieved by dissolving in warm DMF, treating with active carbon, followed by precipitation with water adding, respectively by acidifying at pH = 3—5 with HCl aq. The obtained amides were characterized by melting point, solubility in various solvents, UV and IR specters, mass spectrometry and elementary analyses. The characterization of the synthesis compounds is presented in Tables 1 and 2.

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СИНТЕЗА И КАРАКТЕРИЗАЦИЈА НЕКИХ N-СУПСТИТУИСАНИХ АМИДА У САЛИЦИЛНОЈ КИСЕЛИНИ

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Резиме

Салицилна киселина, односно њени супституисани ароматични амиди употребљавају се као дезинфекциона средства, антифунгициди, антимикотици и као антибактеријске супстанце у хуманој и ветеринарској медицини, у различитим индустријским секторима и у пољопривреди.

У раду су дате синтезе неких N-супституисаних ароматичних амида салицилне киселине, реакцијом између примарних амина и салицилне киселине у инертним органским растварачима у присуству PCl_3.

Добијени деривати окарактерисани су физичко-хемијским методама при чему су дати њихова молекулска форма, маса и елементарни састав.

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THE AVAILABILITY OF BASE ELEMENTS (CA, MG, NA, K) IN SOME IMPORTANT SOIL TYPES IN SERBIA

ABSTRACT: In this paper results are presented of agrochemical and mineralogical analyses of 14 types of important soils in Serbia (Vojvodina and Central Serbia) and total content and availability of base alkali elements (Ca, Mg, Na, K) are determined. Total element content was as follows: Ca 2.22%; K 1.77%; Na 0.85% and Mg 0.61%. Total content of alkali metals in the soils investigated and their variations within and between the soil types, is in very good/close correlation with contents of primary and secondary minerals as well as their rates of weathering. Taking in account the average availabilities the most abundant is calcium with 947 mg/100 g, whilst the averages of the other elements (Mg, K and Na) are quite similar and are about of 40 mg/100 g of soil. The results obtained have shown that the soils investigated are well to moderate provided with K, Ca and Mg and that their deficit could not be expected in plant nutrition, apart for some plants/cultures in the case of magnesium due to occasionally higher Ca/Mg and K/Mg ratios.

KEY WORDS: soil, alkali metals, total and available contents

INTRODUCTION

According to Haby et al. (1990) from all alkali metals found in soils: K, Ca, Mg and Na, the most important in plant nutrition is potassium and it is occasionally found in plants in higher percentages than nitrogen. Most of agricultural plants, under optimal yield condition, would take up between 100 and 300 kg of potassium per hectare from soils. Calcium is following one in the significance amongst the alkali metals, which is usually in soils in ample supply for plant nutrition. Its contents in plants are about less then half of that of potassium. Third in significance is magnesium, which is present in plants about less then half of calcium, but in soils it is frequently found in deficit for plant nutrition. Sodium is common constituent in plants, but it is not an essential element, although for some plants (e.g. sugar beet) is an important element to achieve high yields.

Total content of potassium in soils varies within wide limits from 0.01% up to 4%, with most common values of about 1% and an average content between 1—2% (Wild, 1988). In soils and many rocks, potassium occurs as major constituent of many rock-forming primary minerals, particularly in the alkali feldspars group: orthoclase and microcline, also as mica group: muscovite and biotite as well as secondary minerals, particularly illite. Principal source of available potassium in soils is its concentration in soils solution, which could be refreshed by cation exchange with adsorbed potassium ions, and which is also affected by content of fixed potassium (strongly bonded and non-exchangeable).

As with potassium, other three alkali metals (Ca, Mg and Na) are also present in silicate rock-forming minerals and later are transferred into mineral fraction of soils. Their contents depend on the rate of mineral weathering and the rate of leaching of weathered products. Principal source of Ca and Na are plagioclase group minerals: anortite and albite, whilst Mg is present in a few silicate minerals ouch as: biotite, pyroxenes and amphiboles. All mentioned minerals are weathered faster than potassium minerals. Beside that magnesium is also present in many secondary minerals (hydrobiotite, vermiculite and montmorillonite). Available forms of Ca, Mg and Na, as with potassium, are their exchangeable cations and various salts in soil solution.

With respect to content and state of potassium in our soils, during the last twenty years (decades) large number of papers was published, but the most of them were dealing with potassium availability and only a few were related to its state and chemical speciation in soils. With regards to the problems of the other three alkali elements there are much less available data and it is occasionally said they seam as ignored elements in soil chemistry investigations. All available data are presented as general chemical soil properties as well as in CEC analyses or in soil solution analyses (Bogdanovic et al. 1973; Jakovljevic and Blagojevic, 1997). However, up to now there was no data dealing with spatial distribution of alkali metals in our soils, apart from a comprehensive/extensive paper by Kostic et al. (2001), about content, concentrations, spatial distribution and chemical speciation as well as mineralogy of magnesium in our soils.

MATERIAL AND METHODS

During a large scale sample collecting for the project, financed by the Ministry of Science and technology and Fund for Land Use, Protection and Organisation of the Republic of Serbia, an orthogonal regular grid was used to avoid bias in site location. For financial reasons sample collection was restricted to the layer 0—20 cm depth. Sampling was based on a 5 x 5 km cell and samples were collected. From this set of samples a selection of samples from Vojvodina, Šumadija and Northern Pomoravlje was taken to represent the most important (14) soil types and the number of 100 samples was obtained.

Total and available contents of the elements (Ca, Mg, K and Na) were determined. Total contents were analyzed after grinding of sample and digesting it in a mixture of aqua regia and HF in Pt crucible. Ca and Mg contents, after taken into solution and addition of lanthanum, were determined by AAS, whilst the K and Na contents were determined by a flame-photometric method. The available contents of alkali metals were determined by the same methods, but after the 1M NH_4OAc (pH 7.0) extraction.

Histograms of the frequency distribution and summary consisting of the mean, quartiles and range in a normal distribution of the concentrations of each element were made using STATISTICA for Windows 4.3b program. Experience in geochemical research has shown that analysing and mapping data by using the box-plot provide the best realization. The classes used to represent the data on the map were chosen from the box and wiskers analysis, as proposed by Kuerzl (1986). So, the map has the 5 classes. Raster map showing the distribution of the elements was drawn by computer, by using UNIRAS subroutine. Each square on the map represents the result from one 10 km grid scale.

RESULTS AND DISCUSSION

The results obtained during this investigation are given in the Table 1., where they are presented as total and available contents of analyzed alkali elements as well as average values and range of minimum and maximum arranged according to various soil types and as summary for all soils. In the soils investigated calcium is the most abundant alkali element with the average content of 2.25%, which is much higher value than the average (1.37%) for the soils in the world. The highest total calcium content is found in chernozem and semigley (3.4%), and the lowest in soil types: pseudogley, luvisol, dystric cambisol and vertisol (about 0.5%). This result have shown that soil types with higher calcium content have a very good correlation with soil mineral composition of primary and secondary minerals bearing calcium, such as: calcite, dolomite, plagioclas, smectite and mixed-layer-silicates (MSS 10—14). The soil types with low calcium content have mineral composition with dominant quartz, and lower content of calcium bearing minerals, particularly calcite and dolomite, as presented in the Table 2.

The average content of available calcium in our soil types is 947 mg/100 g, but it is present in very wide range limits between 93 and 2000 mg/100 g. However, only a limited number of samples of very acid pseudogley soil types have available calcium below 100 mg/100 g, when they could show signs of calcium deficit in plant nutrition, whilst the majority of soil types investigated have shown high contents of available calcium of about 1000 mg/100 g. In acid soil types content of available calcium is lower, but very close to the average contents of available calcium in soils investigated with about 300 mg/100 g. With regards to the afore mentioned, it imply that our soils contain ample/adequate supply of both total and available calcium for plant nutrition, which is evenly distributed over the area investigated, as could be seen from the map presented in Figure 1.

The average content of magnesium in our soils shows entirely adequate values for its average content in pedosphere. Its total content averages show

Table 1 — Total (%) and available (mg/100 gr) contents of alkali elements in the investigated soils

	Magnesium		Calcium		Potassium		Sodium	
Soil type	Total	available	Total	available	Total	available	Total	available
Chernozem	0.67* (0.25–1.00)	42 (27–65)	3.41 (0.87–7.90)	1475 (478–2000)	1.68 (0.84–2.20)	49 (17–90)	0.87 (0.71–1.04)	40 (30–51)
Semigley	0.74 (0.26–1.25)	43 (20–75)	3.29 (0.54–10.40)	1132 (333–1925)	1.77 (1.37–2.14)	45 (17–113)	0.83 (0.43–1.19)	40 (26–54)
Humogley and eugley	0.61 (0.20–1.00)	58 (22–93)	2.40 (0.25–6.00)	984 (225–1950)	1.84 (1.02–2.26)	56 (21–115)	0.94 (0.40–1.58)	43 (32–57)
Halomorphic Soils	0.57 (0.35–0.85)	37 (25–53)	2.05 (0.96–3.30)	1060 (390–1775)	1.85 (1.44–2.22)	83 (36–122)	0.97 (0.34–1.29)	33 (28–42)
Fluvisol	0.75 (0.44–1.00)	51 (32–80)	2.14 (0.61–7.50)	832 (308–1900)	1.81 (0.86–2.30)	26 (14–46)	0.92 (0.60–1.47)	51 (34–130)
Pseudogley	0.42 (0.25–0.57)	32 (10–45)	0.55 (0.17–1.17)	334 (93–703)	1.80 (1.58–2.00)	26 (23–32)	0.69 (0.42–0.81)	32 (18–39)
Eutric Cambisol	0.48 (0.26–1.25)	43 (20–75)	1.07 (0.42–3.51)	592 (275–1908)	1.74 (1.14–2.09)	32 (21–56)	0.76 (0.36–1.26)	37 (23–45)
Vertisol	0.35 (0.20–0.52)	27 (15–38)	0.68 (0.30–0.91)	340 (175–570)	1.94 (1.60–2.85)	31 (22–40)	0.91 (0.34–1.29)	33 (28–47)
Luvisol and Distr. Cambisol	0.36 (0.32–0.43)	30 (22–40)	0.51 (0.40–0.65)	274 (180–378)	1.69 (1.51–1.96)	28 (26–30)	0.75 (0.50–0.91)	36 (33–39)
Ranker and regosol	0.65 (0.39–1.26)	23 (12–38)	1.86 (0.20–4.05)	963 (160–1685)	1.72 (0.95–2.60)	23 (18–26)	0.80 (0.44–1.07)	36 (22–44)
All soils	0.60 (0.20–1.26)	41 (10–93)	2.25 (0.17–10.40)	947 (93–2000)	1.76 (0.84–2.85)	41 (14–122)	0.85 (0.34–1.58)	40 (18–130)

N. B. * Average (min-max)

Table 2 — Average mineral composition (%) of the bulk samples of the soils investigated

	Quartz	Plagio clase	Ortho clase	Chlorite	Mica + Illite	Smectite + Verm.	Mixed Layers	Kaoli-nite	Calcite	Dolo-mite	Goetite
Chernozem	53.8	8.7	0.7	6.0	19.0	0.9	0.3	1.0	4.5	4.9	0.2
Halomorphic soils	57.3	10.4	0.7	4.7	20.6	0.5	0.4	1.1	1.2	2.3	0.2
Semigley	52.5	8.5	0.6	5.4	19.8	1.8	0.2	1.5	4.3	5.1	0.2
Humo- + Eugley	52.9	11.2	0.7	5.3	21.7	2.6	0.3	1.7	1.7	1.5	0.3
Fluvisol	51.2	10.4	0.6	5.7	22.4	3.3	0.2	2.2	2.3	1.7	0.2
Pseudogley	67.6	6.4	0.5	4.6	16.5	1.3	1.3	1.1	0.1	0.1	0.1
Eutric Cambisol	63.5	8.9	0.8	3.7	16.5	2.3	0.2	2.3	0.8	0.2	0.3
Vertisol	70.3	8.8	1.4	2.5	10.8	4.0	0.3	1.1	0.2	0.0	0.3
Distric Cambisol + Luvisol	69.1	7.4	0.8	4.7	13.5	2.3	0.3	1.3	0.3	0.0	0.4
Regosol + Ranker	58.9	6.9	0.8	4.7	18.9	2.5	0.4	1.9	2.5	2.1	0.4
All soils	57.6	8.8	0.7	5.0	18.7	2.0	0.3	1.6	2.5	2.6	0.2

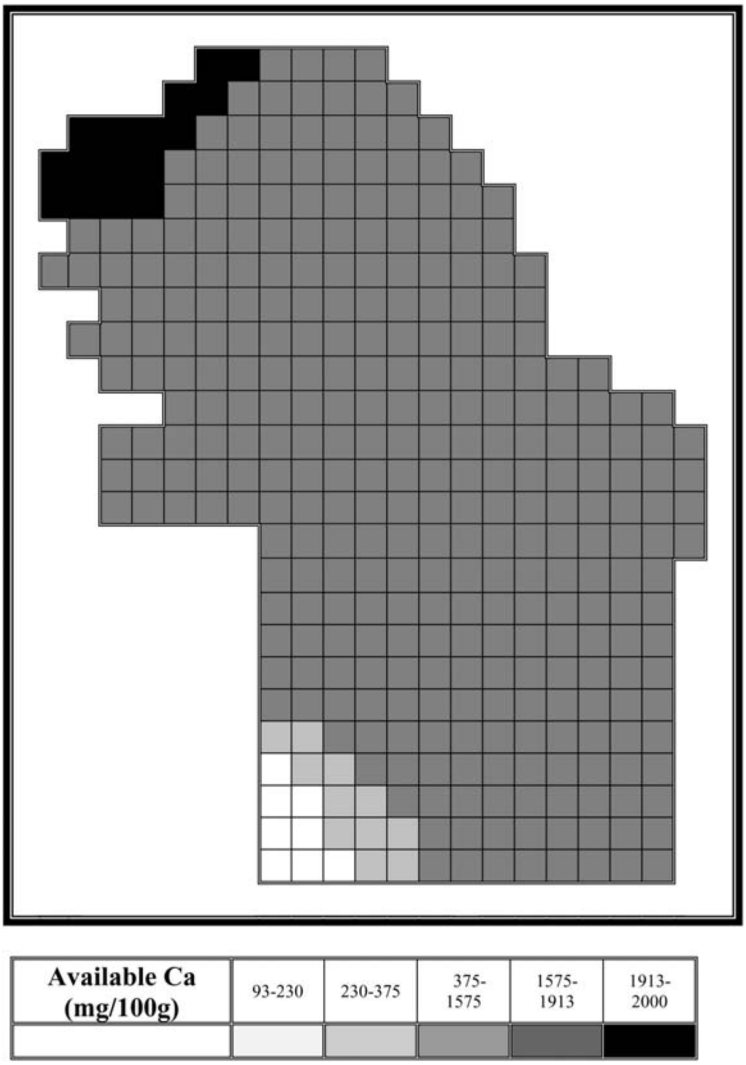
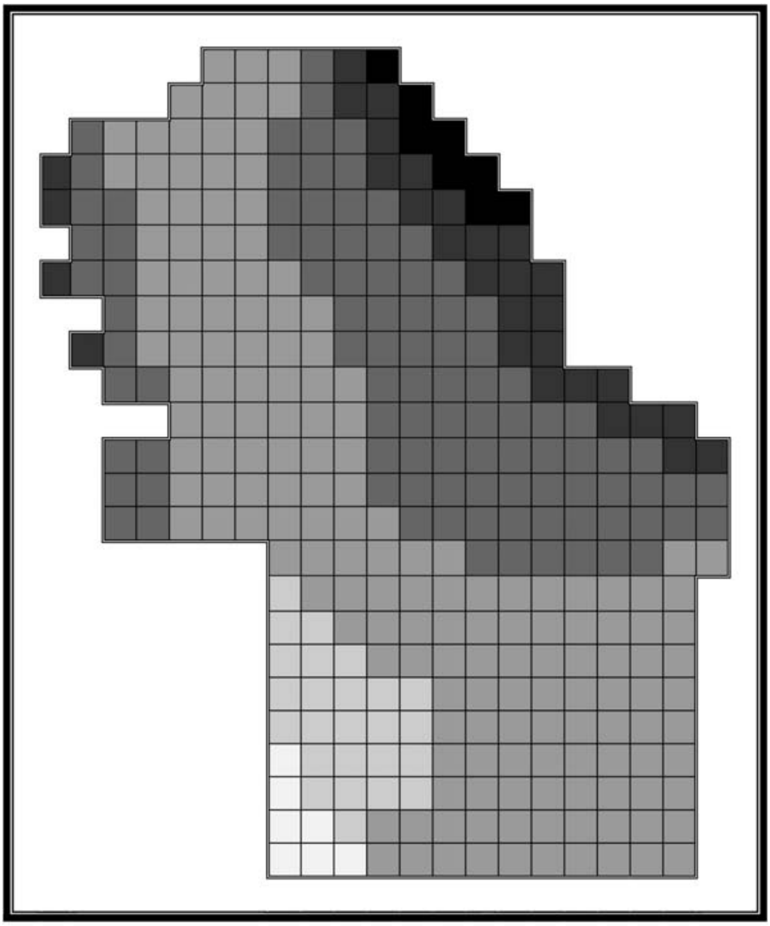


Figure 1. – Content and spatial distribution of available calcium in the investigated soils from Serbia

smaller variations between than within the soil types. In general, magnesium total content varies in wide range limits between 0.20 to 1.26%. Its total content averages are quite low (0.35—0.48%) in the following soil types: vertisol, luvisol, dystric cambisol, pseudogley and eutric cambisol. These are more weathered and more acid soils, with progressive transformation of mica and illite to clay minerals with subsequent leaching of magnesium as well as other base elements.

The average content of available magnesium in soils investigated is 41 mg/100 g, with interval from 10 to 93 mg/100 g, as could be seen from the



Available Mg (mg/100g)	< 20	20 - 40	40 - 65	65 - 80	> 80

Figure 2. – Content and spatial distribution of available magnesium in the investigated soils from Serbia

map presented in Figure 2. The average contents of available magnesium show good correlation with its total content as could be seen for vertisol, pseudogley and luvisol from the Table 1. In general, all investigated soils are on average quite well supplied with available magnesium for plant nutrition, with only two soil types (pseudogley and ranker) approaching the upper limits of magnesium deficit (10 mg/100 g) in soils.

Since the Ca/Mg and K/Mg ratios are limiting factor for available magnesium in plant nutrition, its averages, standard deviation and ranges are presented in the Table 3. With respect to plant nutrition Ca/Mg ratio has beneficial

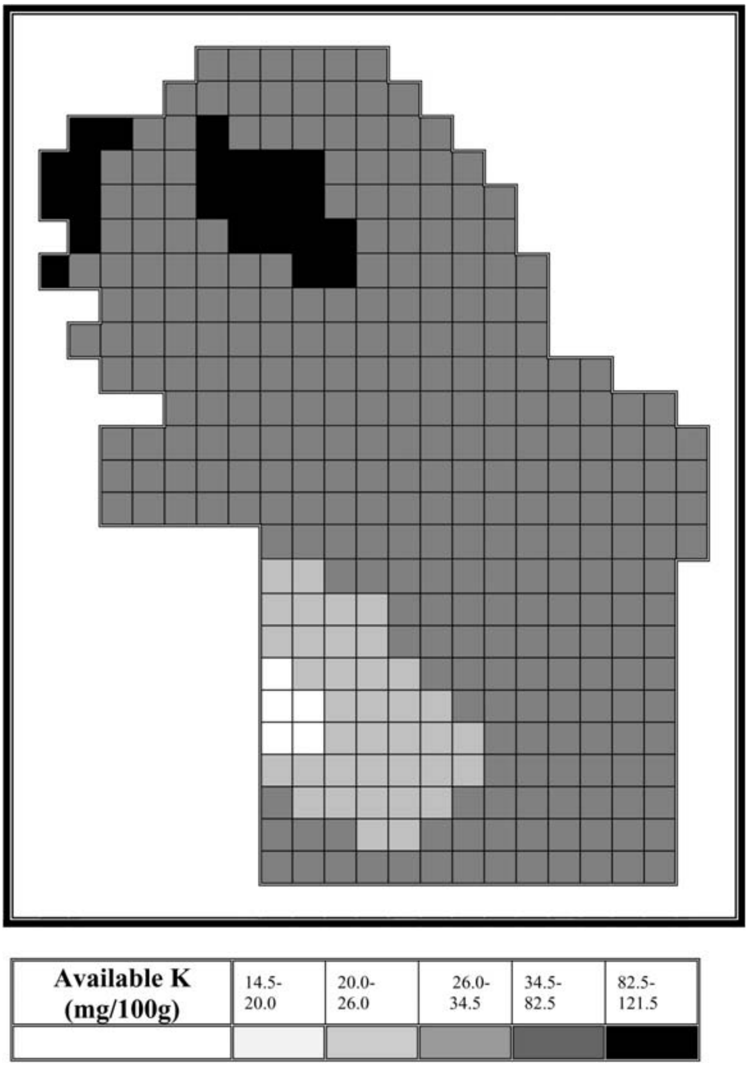


Figure 3. – Content and spatial distribution of available potassium in the investigated soils from Serbia

effects in the range from 1:1 to 5:1. Ratios lower than 5:1 are detected in about 10% of soils investigated, whilst the bulk of soils have shown the ratio between 5:1 and 20:1. The average value for all soils is 15.4:1. Lower Ca/Mg ratio values are limited to some areas in Sumadija and Pomoravlje, whilst the higher values could be associated to far northern areas of Backa, where total content of magnesium is connected to high contents of magnesium bearing minerals (dolomite and chlorite) in soils. On average, even our acid soils have shown Ca/Mg ratio higher then 5:1, whilst chernozem has shown 22:1 and ranker, regosol and rendzina have even 29.9:1. Maximum values were detected

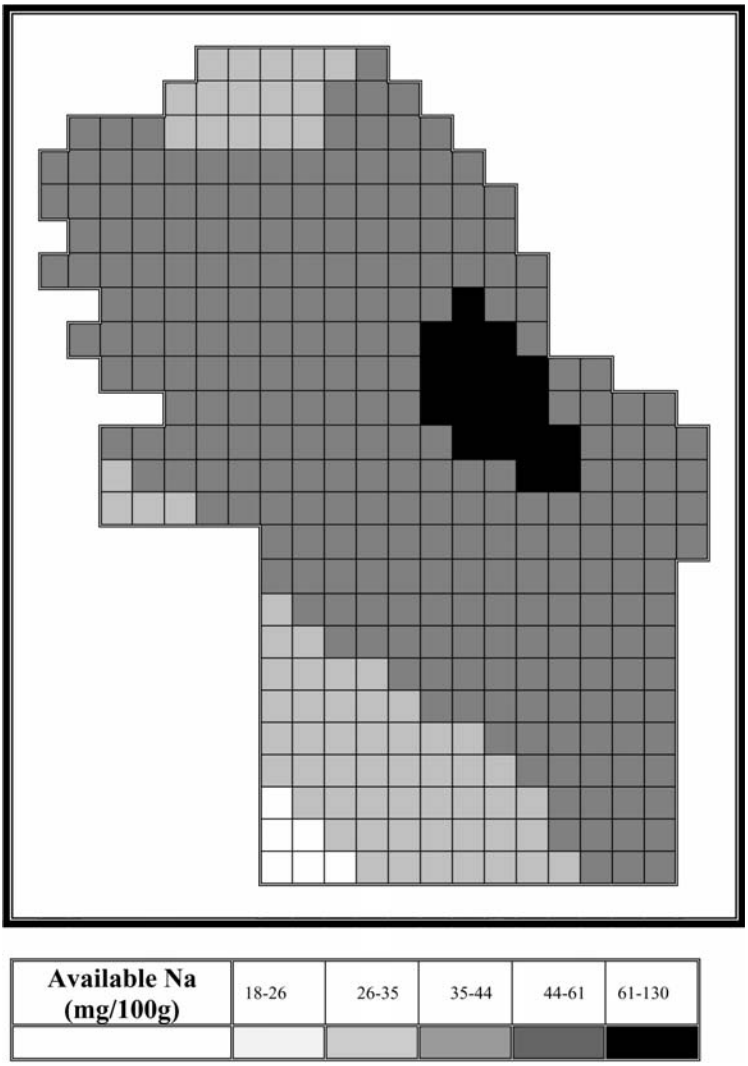


Figure 4. – Content and spatial distribution of available sodium in the investigated soils from Serbia

mostly about 30:1, with some extreme values of 50.4:1 in eutric cambisol and 78.8:1 in rendzina developed on marly limestone. With regards to the afore mentioned, it imply that our soils have slightly higher Ca/Mg ratio, which could affect some sensitive plants to magnesium deficits in their nutrition. Because of this it would be necessary to continue with such, but detailed investigation in future.

Another important factor in magnesium plant nutrition is the K/Mg ratio. The obtained results presented in the Table 3., have shown that the rations for the bulk of samples and soil types are below 1:1, which is adequate/agreeable for the most arable and vegetable crops. Only in more acid soils the K/Mg ratios are over 2:1, which could lead to reduced uptake of magnesium in some plants.

Table 3 — Ca/Mg and K/Mg ratios in the soils investigated (mean, standard deviation and interval)

Soil type and number of samples	Ca/Mg	K/Mg
	m.e./100 g	
Fluvisol (n = 8)	12.3±9.2 3.9—28.9	0.3±0.1 0.2—0.5
Eutric cambisol (n = 14)	11.1±12.0 3.3—50.4	0.6±0.2 0.3—1.0
Pseudogley (n = 5)	6.0±2.0 4.8—9.5	0.6±0.4 0.4—1.4
Semigley (n = 20)	15.8±7.1 5.6—30.8	0.6±0.2 0.3—1.2
Humogley and eugley (n = 12)	11.2±7.8 2.5—31.5	0.7±0.5 1.2—1.9
Regosol, Ranker, Rendzina (n = 6)	29.9±28.7 5.3—78.8	0.7±0.2 0.4—0.9
Chernozem (n = 20)	22.1±8.7 5.6—36.6	0.7±0.3 0.3—1.5
Vertisol, Luvisol, Distric cambisol (n = 10)	6.8±2.9 4.0—17.3	0.8±0.3 0.4—1.2
Halomorphic soils (n = 5)	17.1±9.2 7.2—30.0	1.2±0.3 0.9—2.5
All soils (n = 100)	15.4±12.4 2.5—78.8	1.5±0.8 0.1—2.5

The average total content of potassium in soils investigated is slightly higher (1.76%) then the average (1.36%) for the world soils. The results presented in the Table 1., have shown only slight variation (1.68—1.98%) in the average values of total potassium content for soils investigated. This could be only explained with an even and uniform distribution of potassium bearing minerals (orthoclase, micas and illite) in the soils. However, these variations between investigated soil types could be quite significant, within wider limits (0.84—2.85%) or even within the same soil type e.g. fluvisol (0.86—2.30%).

The average content of available potassium is 41 mg/100 g, and according to the map in the Figure 3. the most of soils comprise of over 20 mg/100 g of available potassium, which could purport that our soils are quite well supplied with this important plant nutrient. It is necessary to emphasize that the range limits for available potassium are quite wide, between 14 and 122 mg/100 g. The highest values and the averages are observed in halomorphic soils, eugleys, humogleys and smigleys. Only a limited number of samples have shown values below 20 mg/100 g, which could be considered as moderate contents in supply of this important plant nutrient.

Our soils have shown slightly higher averages (0.85%) of total sodium than the average world soils (0.6%). Its behavior is similar to that of plagioclase and has shown similar average values of soils investigated, but the variations within the soil types are quite significant and range between 0.34 to 1.58%.

The average content of available sodium is 40mg/100 g, with uniform and slight variations between the soil types, which are between 32 and 51 mg/100 g. However, these variations within the samples are in much wider interval between 18 and 130 mg/100 g. Maximum content of available sodium is determined in fluvisols, which could be an effect of high concentration of soluble salts. Content of available sodium in soils investigated is almost the same as for potassium and magnesium, so it could be concluded that our soils are well supplied with available sodium, but its limits are not determined, since there is no published data for this element, which is considered as an no-essential in plant nutrition.

CONCLUSIONS

The comprehensive investigation of soils from Vojvodina, Sumadija and Northern Pomoravlje, of our main arable regions, has showed a wide range of base alkali metals concentrations. Based on the results the following conclusions could be presented:

Total contents of all investigated alkali metals in our soils and their variations between and within various soil types are in close correlation with concentrations of primary and secondary minerals and their rate of weathering in soils.

The averages of total content of Ca, Mg and K in our soils are slightly higher than their averages in world soils, whilst sodium content is similar to the world average.

Taking in account average values of available K, Ca Mg and Na, our soils could be considered as well supplied for the plant nutrition. Only a limited number of samples has shown lower values and are considered as deficient in base alkali metals.

However, in the case of deficit of available magnesium in soils with high ratios of Ca/Mg and K/Mg, it could be a limiting factor in available magnesium uptake and utilisation by plants, particularly to the sensitive plants with higher demand for magnesium as well as favourable ratios of available cations such as: Ca, K, Na, and H.

This paper has shown a need for the wider investigation of chemistry and mineralogy of soils in Serbia, which are considered in general as well supplied with base alkali metals.

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СНАБДЕВЕНОСТ ВАЖНИЈИХ ТИПОВА ЗЕМЉИШТА СРБИЈЕ ОСНОВНИМ АЛКАЛНИМ ЕЛЕМЕНТИМА (Ca, Mg, K и Na)

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Резиме

У овом раду је испитивано 100 репрезентативних узорака 14 типова земљишта Србије (Војводина, Шумадија и Северно Поморавље) у погледу укупног и приступачног садржаја основних алкалних метала (Ca, Mg, K и Na). Такође је приказан и просечан минералошки састав испитиваних земљишта. Нађени су следећи средњи укупни садржаји испитиваних елемената: Ca — 2,25%; K — 1,77%; Na — 0,85% и Mg — 0,61%. Укупни садржаји испитиваних алкалних метала у нашим земљиштима и њихова варирања између и унутар земљишних типова у уској су вези са заступљеношћу одређених примарних и секундарних минерала и њиховом отпорношћу на распадање. Према средњим вредностима за приступачне садржаје највише има калцијума (947 mg/100 g), а средњи садржаји за остале базе (Mg, K и Na) су врло слични и крећу се око 40 mg/100 g. Добијени резултати показују да су испитивана земљишта добро и средње обезбеђена K, Ca и Mg и да се не могу очекивати њихови дефицити за исхрану биљака, осим за неке културе у случају магнезијума, због понекад сувише повишених односа Ca/Mg и K/Mg.

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SELENIUM IN SOIL

ABSTRACT: Selenium (Se) is an essential microelement, necessary for normal functioning of human and animal organisms. Its deficiency in food and feed causes a number of diseases. In high concentrations, selenium is toxic for humans, animals and plants. Soil provision with selenium affects its level in food and feed via nutrition chain. However, selenium reactivity and bioavailability depends not only on its total content in soil but also on its chemical forms. Distribution of the different forms of selenium depends on soil properties such as reaction, aeration, contents of clay and organic matter and microbiological activity.

KEY WORDS: selenium, total Se, water-soluble Se, soil

INTRODUCTION

The biological importance of selenium is reflected in the fact that it is essential for humans and animals on one side (Rayman, 2000), and toxic when in high concentration on the other. This controversial element was discovered by Berzelius, a Swedish scientist, in 1817, although it was Marco Polo during his travel to Asia, as early as the 13th century, who observed toxic symptoms in animals feeding on plants that contained a high concentration of selenium. In the first half of the 20th century, selenium was considered exclusively from the standpoint of its toxicity, until it was identified as a component of a preparation which prevented the necrosis of liver in rats (Scwarz and Foltz, 1957). Soon afterwards, selenium was found to play a key role in glutathione peroxidase, an enzyme that catalyzes the decomposition of hydrogen peroxide thus protecting cells from oxidative damage. Selenium also plays an important anti-carcinogenic role and it neutralizes heavy metals toxicity.

Soil provision with selenium affects its level in food and feed via nutrition chain (Finley et al., 2000). However, selenium reactivity and bioavailability depends not only on its total content in soil but also on its chemical form. Depending on oxidation state, selenium is present in soil as selenide (Se^{2-}), elemental selenium (Se^0), selenite (SeO_3^{2-}), selenate (SeO_4^{2-}) and organic selenium. The water-soluble selenium fraction is considered to be most available to plants. Its content in normal tilled soil does not exceed 50 ppb

(Workman and Soltanpour, 1980). Although selenium is not essential for plants, they take up selenium and incorporate it in their amino acids and proteins (Shrift, 1973). The level of selenium accumulation in plants depends on the amount of available Se, pH value and redox potential, contents of sesquioxide, clay and organic matter and the microbiological activity of soil (Jump and Sabey, 1989).

GEOGRAPHIC DISTRIBUTION OF SELENIUM

Selenium is abundant in different parts of the world but its level in soil varies with native substrate, climatic conditions and vegetation cover (McNeal and Balistrieri, 1989). In central US, for example, there are regions in which plants contain Se levels 10 times higher than the toxic level, while Se levels in plants in eastern and western US are low (Kubota et al., 1967). In eastern Canada, Se concentrations in plant dry matter are much below 0.1 ppm (Winter and Gupta, 1979), while they are 10 times higher in western Canada. Selenium deficiency was observed in some parts of South America, selenium toxicity in others (Jaffe, 1973). Selenium deficiency was noted in western and southern parts of Australia and in New Zealand (Welsh et al., 1981).

Severe selenium deficiency registered in southeastern China has been associated with Keshan disease, a disease that occurs almost exclusively in children. In India, toxicity was observed in the region of Harayana and deficiency in the other parts of the country (Dhillon and Dhillon, 1991). Total Se content is slightly increased in Japanese soils as compared with the contents established in other countries (Kang, 1990). However, because these soils have an acid reaction and because of the humid climate of Japan, the concentration of water-soluble Se is low, making only 2.3—3.85% of the total Se content (Kang, 1990).

In Europe, toxic level was registered in several locations in Wales and Ireland (Fleming, 1962). Symptoms of Se deficiency were observed in cattle in England and Scotland (Rimmer et al., 1990). Suboptimal Se contents were reported for northern Europe. In Finland, before Se fertilization became a regular practice, wheat contained only 10—15 ppb Se (Koivistoinen and Varo, 1981). Low Se levels were registered in Poland (mean value 0.27 $\mu g\ g^{-1}$) (Borowska, 1998) and Hungary (Gondi et al., 1992; Horvath et al., 1996).

In our country, preliminary studies of soil, cereal and animal feed samples (Krajinović, 1983) indicated the presence of low Se levels. Later studies of Se content in geological materials (Jović et al., 1995; Dangić et al., 1989), soil (Jakovljević et al., 1995; Maksimović et al., 1992; Čuvardić et al., 1997) and biological materials (Maksimović et al., 1992) confirmed a pronounced Se deficiency on this territory. Perilously low Se contents were found in blood serum and hair of two groups of male examinees, miners in the Majdanpek coal strip mine and inhabitants of a region in the vicinity of Belgrade (serum — 31 ppb and 25 ppb, respectively; hair — 76 ppb and 73 ppb, respectively). According to these results, the analyzed populations were comparable to the inhabitants of the countries suffering a severe Se deficiency, such as China, New Zealand and Finland (Maksimović et al., 1992).

SELENIUM IN LITOSPHERE

The primary sources of Se in nature are volcanic rocks and metal sulfides formed by volcanic activity (NAS, 1974). Although some 50 minerals contain Se, it occurs most frequently in sulfides of heavy metals (Ag, Cu, Pb, Hg, Ni, etc.), either in the form of selenide or substituting the sulfur ion (S) in the minerals' crystal grid (Adriano, 1986). Average Se content in the Earth's crust is considered to vary from 0.05 to 0.09 $\mu g\ g^{-1}$ (Lakin, 1972). Total Se content varies from 0.1 to 2 $\mu g\ g^{-1}$ in most soils (Swaine, 1955). Increased amounts of Se are found in the soils formed by the decomposition of shale, mostly in arid and semiarid regions (Rosenfeld and Beath, 1964).

Selenium content in magmatic rocks seldom exceeds 0.05 $\mu g\ g^{-1}$ (Kabata-Pendias, 1993). An extremely low Se content was found in magmatic rocks from Serbia (mean value 46 ppb Se) (Maksimović et al., 1986). In these rocks, Se is part of sulfides in which it substitutes sulphur. So, Se content depends on the size of sulfide phase. In magmatic rocks, Se concentration decreases from basic and ultra basic to acid and neutral rocks (Dangić et al., 1989).

In sedimentary rocks, Se is bound to the organic and clay fractions. Lowest Se concentrations are recorded in sandstones and limestones (Table 1). Selenium is most frequently found in phosphates, uranium ore, fossil coal and oil and in shale with a high content of organic matter. Soils formed on such substrates typically have high or toxic Se concentrations, unlike the soils formed on magmatic rocks which have a low Se content (Fleming, 1980).

The metamorphic rocks of Serbia have a lower Se content than the magmatic and sedimentary rocks. An analysis of 89 samples of metamorphic rocks from Serbia showed a low Se content of 28 ppb (Jović et al., 1995), leading to a conclusion that the high temperature and pressure present during metamorphic processes tend to extrude Se from these rocks.

Tab. 1 — Selenium in main rock types — μg Se g^{-1} (Kabata-Pendias, 1993)

Igneous rocks	μg Se g^{-1}
Ultra basic (dunit, peridotite, pyroxenite)	0.02—0.05
Basic (basalt, gabbro)	0.01—0.05
Intermediate (syenite)	0.02—0.05
Intermediate to acidic (rhyolite, trachyte, dacite)	0.02—0.05
Acidic (granite, gneiss)	0.01—0.05
Sedimentary rocks	
Shale	0.40—0.60
Sandstone	0.05—0.08
Limestone, dolomite	0.03—0.10

SELENIUM IN WATER AND AIR

Besides litosphere, atmospheric precipitation is another source of selenium in soil. Selenium is released into the atmosphere from natural deposits or due to anthropogenic influences (burning of coal and other fuels, releases for mines, oil refineries and other industries). Soils, plants, microorganisms, animals and volcanoes release up to 0.04 ng m^{-3} Se (EHC, 1987). The total annual anthropogenic emission of selenium in the USA was estimated at 4670 t (Herring, 1991). The atmospheric precipitation in Norway and Sweden brings annually 0.5—1.0 g Se ha^{-1}. Under natural conditions, Se contents in air and water are quite low — less than 10 ng m^{-3} in air and a few μg l^{-1} of water (EHC, 1987). Se contents in rain and snow samples gathered in different parts of the world ranged from 0.03 to 0.3 μg l^{-1} (Cutter, 1989). Se concentration in atmospheric precipitation in Sweden was below 0.1 μg l^{-1} (Johnsson, 1991).

SELENIUM IN SOIL

Selenium content in soil is highly variable. It varies from 0.1 to 2 μg Se g^{-1} in most soils, but it is most frequently between 0.2 and 0.4 μg g^{-1} (McNeal and Balistrieri, 1989). Some soils are low in selenium, from 0.03 to 0.08 μg g^{-1} (NAS, 1976). Toxic concentrations occur in arid and semiarid parts of China, in Hawaii, Mexico, Columbia and western parts of the USA and Canada (McNeal and Balistrieri, 1989). Soils containing less than 0.5 μg g^{-1} of total Se are considered as deficient in this element (Mayland et al., 1989).

Selenium concentration in soil is determined by selenium content in soil native substrate, climate, relief and age (Mayland et al., 1989). These factors may contribute either to selenium accumulation during soil forming or its removal during or after soil forming. The soils formed on sedimentary rocks that contain high amounts of organic matter typically have high to toxic selenium concentrations. The soils formed on magmatic rocks, which are poor in Se, usually have a low Se content (Fleming, 1980).

Besides native substrate, amount of rainfall plays an important role in determining selenium content in soil (Fleming, 1980). In regions with less than 500 mm of rain, the soil formed from rocks with a high Se content contains potentially toxic Se concentrations. The same kind of soil formed in humid regions typically contains high amounts of Se, but it is bound to iron in a poorly soluble complex. When the substrate is low in selenium, the soil forming on it will have a low Se concentration regardless of climate.

Since the total Se content in soil is predominantly determined by its content in the native substrate, it is understandable why the soils in certain regions of our country have very low Se concentrations. For example, the soils in northern Pomoravlje have the Se contents from 0.12 to 0.44 ppm, with the mean value of 0.24 ppm (Jakovljević et al., 1995), in western Serbia 119.5±70.5 ppb Se (Maksimović et al., 1992), in the valley of the Zeta

River from 0.2 to 0.41 ppm Se, with the mean value of 0.28 ppm Se (Jakovljević et al., 1995)

Low Se concentrations have also been registered in the Vojvodina Province. In 46 soil samples from the regions of Srem and Banat, taken from plots used for vegetable production, the contents of Se ranged from 0.11 to 0.45 ppm, with the mean value of 0.25 ppm Se (Čuvardić et al., 1997). In samples of chernozem, distric cambisol, humogley, humofluvisol, arenosol, solonetz and solonchak taken in the Vojvodina Province, Se contents in the surface soil layer ranged from 0.024 to 0.194 μg Se g^{-1} (Čuvardić, 2000).

SELENIUM DISTRIBUTION ALONG SOIL PROFILE

Selenium content in soil typically decreases with depth because it binds with proteins, fulvic acids and other organic compounds that content nitrogen. As organic matter decreases along soil profile, so does total selenium (Abrams et al., 1990; Alemi et al., 1991; Gustafsson and Johnsson, 1992). Selenium fixation may also be due to microbiological incorporation into amino acids and other Se-containing organic compounds (Mao, 1999). In addition to humus distribution, Se distribution along soil profile resembles those of Fe, Al and clay (Levesque, 1974; Gondi et al., 1992; Čuvardić, 2000). Selenium binds with clay and arranges itself along the profile. Total Se increases with the increase of small fractions in the soil (Čuvardić, 2000). The most important characteristics of the fine soil fraction are the large total area of its particles and specific mineralogical composition which distinguishes it from the coarse fractions. Secondary minerals predominate in the finest fraction, clay, while the coarsest fraction is composed mainly of quartz (Jakovljević and Pantović, 1991). For that reason, the fractions of coarse and fine sand have a low adsorption capacity. Ions of Al octahedral layer, whose sides are bare, may be considered as positively charged sites on the surface of clay minerals. Ions of the Al octahedral layer of caolinite, which, in contrast to the minerals from the montmorillonite group, are not covered with the tetrahedral sheet, may be positively charged not only at the sites of crystal fractures but also their planes may be charged, attracting $HSeO_3^-$ as a substitute for OH^- (Bar-Yosef and Meek, 1987).

FACTORS AFFECTING SELENIUM SOLUBILITY AND AVAILABILITY TO PLANTS

Organic and mineral forms of selenium are present in the soil (Cary and Allaway, 1969). The organic forms of Se result from partial decomposition of selenium-loving vegetation. Mineral Se occurs as a selenide of metals (Se^{2-}), elementary Se (Se^0), selenite ($HSeO_3^-$) and selenate (SeO_4^{2-}). Se partitioning is affected by soil pH and redox potential, content of sesquioxides, clay, organic matter and microbiological activity (Elrashidi et al., 1987; Jayaweera and Biggar, 1996).

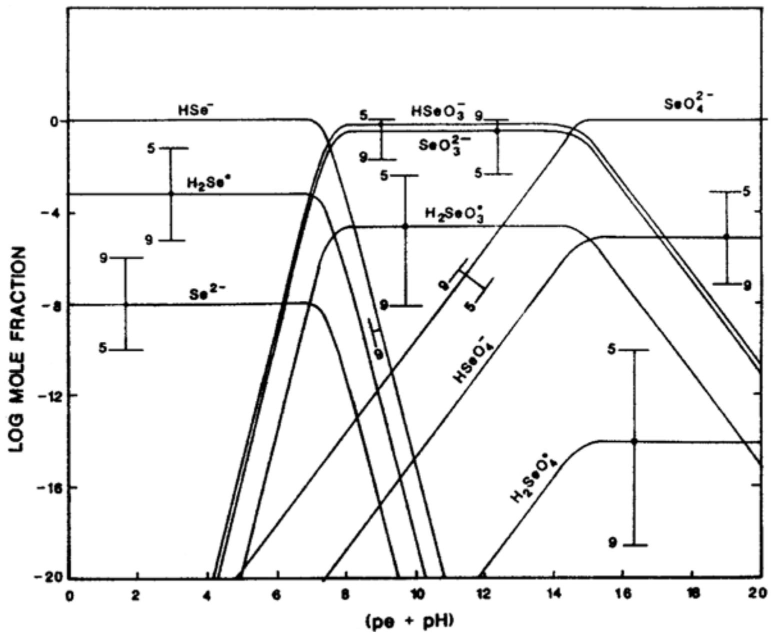

Fig. 1 — The effect of redox on Se species in solution (E l r a s h i d i et al., 1987)

Se solubility and availability to plants is predominantly affected by pH--value and redox potential (G e e r i n g et al., 1968). According to pe+pH diagram (Figure 1) (E l r a s h i d i et al., 1987), selenide and elementary Se are favored in a reducing environment (pe+pH < 7.5), selenite in a slightly oxidizing environment (pe+pH 7.5—15.0), and selenate in an oxidizing environment (pe+pH > 15.0). In humid regions and acid soils, the prevailing form is selenite, which is firmly adsorbed on sesquioxides and clay minerals and thus not readily available to plants. High annual precipitation and low temperature tend to reduce Se content in plants. This is due to reducing conditions in the soil and reduction of selenate (SeO_4^{2-}) into less available forms: selenite (SeO_3^{2-}); elementary selenium or selenids (Se^{2-}) (G e e r i n g et al., 1968). Increased leaching of available Se forms also leads to reduction in uptake (J o h n s s o n, 1991). Under well-aerated conditions in the alkaline soils of semiarid regions, selenium is present in the form of selenate which is not adsorbed, does not form insoluble salts and is readily available to plants (C a r y et al., 1967).

Although soil reaction plays a major role in determining Se solubility and availability, its influence lessens as the contents of clay and organic matter in the soil increase (J o h n s s o n, 1991). Because of Se affinity for clay minerals, the soils having increased clay content are typically better provided with Se than sandy soils (G i s s e l - N i e l s e n, 1975). As described in the previous chapter, Se binds with the positively charged ions of AL-octahedral sheets, which occur on the edges of clay minerals, primarily caolinite (B a r - Y o s e f, 1987). G i s s e l - N i e l s e n (1971) studied the effect of pH value and texture on Se uptake from loamy and sandy soils. In the first two cuttings of *Lo-*

lium multiflorum grown on a slightly acid soil, Se uptake dropped with increase in clay content. In subsequent cuttings, however, the effect of soil acidity evidently weakened because Se became more and more available. When the soil pH exceeded 7, the effect of clay disappeared altogether. Se adsorption occurs also on iron (Fe), aluminium (Al) and manganese (Mn) oxides and hydroxides in acid environments, when these particles become positively charged (B a l i s t r i e r i and C h a o, 1990). Namely, a specific characteristic of these oxides is that they change their electric charge with a change of ambient reaction. The charge is negative under alkaline conditions and positive under acid conditions.

Selenium also binds well with organic matter in acid soils (C h r i s t e n s e n et al., 1989; G u s t a f s s o n and J o h n s s o n, 1992). Because of a high content of organic matter in topsoil, higher selenium content may be expected there than in deeper soil layers (L e v e s q u e, 1974). When in the organic fraction, selenium either forms complexes with organic compounds or is built in amino acids and proteins by microorganisms and plants (C a r y et al., 1967; H a m d y and G i s s e l - N i e l s e n, 1976). Se fractionation from three profiles of Swedish podzols showed that considerable amounts of Se were present in the soil organic matter, especially in the Bs horizon which has a high capacity for adsorption of anions (G u s t a f f s o n and J o h n s s o n, 1992).

Microorganisms play an important role in Se transformations in the soil. They are capable of transforming the absorbed selenite into organic compounds or inot selenate (D o r a n, 1982). It was also found that microorganisms take part in the transformation of selenate into less mobile forms, especially in soils rich in carbon (A l e m i et al., 1991). Products of microbiological activity are also volatile Se organic compounds, primarily dimethyl selenide. This is a way for selenium depletion from the soil (F r a n k e n b e r g e r and K a r l s o n, 1989). H a m d y and G i s s e l - N i e l s e n (1976) measured the volatilization of added selenite under laboratory conditions. The volatilization was higher from a sandy soil (about 8% of the added Se) than from a humic soil (about 5% of added Se).

Se availability to plants depends also on competing ions, especially phosphates and sulfates. Interaction between selenium and phosphorus (P) in the soil has been observed by several researchers, but its mechanisms have not been fully revealed. In a greenhouse test, S i n g h (1979) examined the effect of added Se and P on dry matter mass and chemical composition of *Brassica juncea* Cos. In the variant without P and with 10 ppm Se, the selenium content in the plant was 100 times higher than in the control. The increases were 258- and 336-fold with 50 and 100 ppm P. Similar results were obtained by S i n g h and M a l h o t r a (1976) who found that the application of 50 and 100 ppm P positively affected Se uptake by *Trifolium alexandrinum* in all variants of Se level, as well as in the experiment with residual Se. Effect of P on Se availability depends on the auxiliary ion in the phosphorus fertilizer, Se form and the presence of other ions (K h a t t a k et al., 1991). F l e m i n g (1962) studied the effect of P from superphosphate, $Ca(H_2PO_4)_2$, on Se uptake in an experiment in pots. All three doses of superphosphate reduced Se uptake, probably due to the influence of sulfate that was a component of the superphosphate. Unlike superphosphate, dicalcium phosphate and monocalcium phosphate increased Se content in grasses (F l e m i n g, 1965). A possible explanation is that P, if incorporated into the soil together with Se, substitutes Se in some sorption points, thus increasing Se mobility and uptake (C a r t e r et al., 1972). Another possible explanation of this interaction is that P increases Se uptake by stimulation cell division in plant root. High cell division increases the contact between the root and soil, which increases the active root surface and Se uptake (C a r t e r et al., 1972).

Because of a large chemical similarity between sulfur (S) and selenium and because of the fact that most S compounds have Se analogues, it seems reasonable to expect antagonistic interaction during uptake of these two elements. Several authors found that Se concentration in the plant decreases after adding sulfate to the nutritive medium (W a n et al., 1988; M i k k e l s e n and W a n, 1990). In an experiment in pots filled with peaty soil, W i l l i a m s and T h o r n t o n (1972) examined the effects of different S compounds on Se uptake. All S compounds tested (elementary S, sulfate S, sulfite S, sulfide S and methionine) tended to reduce Se uptake. The situation was opposite in the case of alkaline soils, where the application of sulfate increased Se uptake (C a r t e r et al., 1972).

WATER-SOLUBLE SELENIUM IN SOIL

Total Se content in the soil is not always in correlation with its content in plants because Se solubility is affected by several factors: soil reaction, the contents of sesquioxides, clay and organic matter (E l r a s h i d i et al., 1987; J a y a w e e r a and B i g g a r, 1996; P e z z a r o s s a, 1999). Because of that, when assessing soil provision with selenium, concentration of available Se and factors that affect the dynamics of Se in the soil have to be taken into account in addition to the total Se content. The water-soluble Se fraction is considered

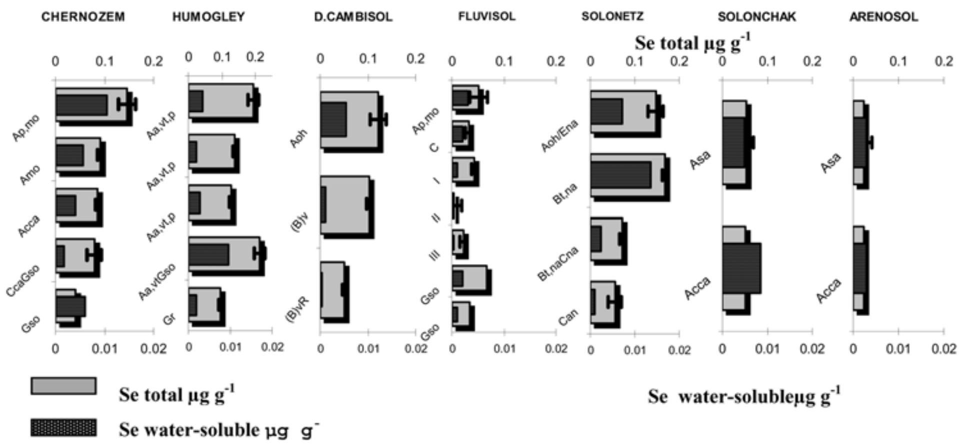

Fig. 2 — Contents of total and water-soluble Se in the profiles of the examined soils in the Vojvodina Province (Č u v a r d i ć, 2000)

to be most available to plants (J u m p and S a b e y, 1989). Non-adsorbed Se and water-soluble organic Se are released from the soil by extraction with boiling water (W a n g and S i p o l a, 1990; S e b y et al., 1997; K a n g et al., 1993).

In normal tilled soils, the concentration of the water-soluble Se fraction typically does not exceed 0.050 μg g^{-1} Se (W o r k m a n and S o l t a n p o u r, 1980). Exceptionally low concentrations of water-soluble Se, ranging from traces to 0.013 μg g^{-1}, were registered in the soils of Srem and Banat used for vegetable production (Č u v a r d i ć et al., 1997). Low concentrations of water-soluble Se were found for seven different soil types in the Vojvodina Province (Č u v a r d i ć, 2000). These concentrations ranged from traces to 0.014 μg Se g^{-1} of soil, or from 0.59% to 16.35% of the total Se (Figure 2). In Hungary, G o n d i et al. (1992) found that the mobile Se fraction did not exceed 10% of the total Se in most of the tested soils, with the exception of several profiles of alkaline soils in which this fraction ranged between 20% and 35% of the total Se.

SELENIUM UPTAKE

Plants take up Se in the form of selenate or selenite, or as organic Se (H a m i l t o n and B e a t h, 1963, 1964). Probably they may also take up atmospheric Se (A s h e r et al., 1967). Capacity for Se uptake varies among plant species (H u r d - K a r r e n, 1935). Still, differences among plant species were smaller regarding Se uptake from low Se soils than regarding Se uptake from high Se soils (E h l i g et al., 1968). Furthermore, the rate of uptake depends on the form of Se prevailing in the soil or a nutritive medium (Z h a n g et al., 1988). Generally, plants exhibit preference for selenate over selenite (B a n u e l o s and M e e k, 1990). This seems to be due to high adsorption of selenite by hydrated oxides, or selenite reduction to elementary Se or selenide (C a r y et al., 1967; G i s s e l - N i e l s e n et al., 1984). In an experiment in vegetation pots, G i s s e l - N i e l s e n (1973) found that the Se uptake by barley was 40 times higher from selenate than from selenite.

CONCLUSION

Selenium is an essential microelement, necessary for normal functioning of human and animal organisms, whose deficiency in food and feed causes a number of diseases. In high concentrations, selenium is toxic for humans, animals and plants. Soil provision with selenium affects its level in food and feed via nutrition chain. Selenium bioavailability depends not only on its total content in soil but also on its chemical forms.

Organic and mineral forms of Se are present in the soil. The organic forms of Se result from partial decomposition of seleniferous vegetation. Mineral Se occurs as a selenide of metals (Se^{2-}), elementary Se (Se^0), selenite ($HSeO_3^-$) and selenate (SeO_4^{2-}). Se partitioning is affected by soil pH and redox potential, content of sesquioxides, clay, organic matter and microbiological activity.

In humid regions and in acid soils, selenite is the predominant form. It is firmly adsorbed on sesquioxides and clay minerals and thus not readily available to plants. In well-aerated alkaline soil in semiarid regions, Se is found in the form of selenate, which is not adsorbed, does not form insoluble salts and is readily available to plants.

Since Se solubility and availability to plants are governed by several factors, concentration of available Se and factors that affect Se dynamics in the soil should be considered in addition to the total Se content in the soil when estimating soil provision with selenium.

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СЕЛЕН У ЗЕМЉИШТУ

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Резиме

Селен је есенцијални микроелеменат, неопходан за нормално функционисање организма људи и животиња, чији недостатак у храни доводи до низа обољења. У исто време, у високим концентрацијама, селен (Se) је токсичан, како за

људе и животиње, тако и за биљке. Обезбеђеност земљишта се преко ланца исхране одражава на ниво селена у храни људи и животиња, али реактивност и биоприступачност селена не зависи само од његовог укупног садржаја, него и од хемијских форми у којима се селен налази у земљишту. На дистрибуцију између појединих облика селена утичу својства земљишта, као што су: реакција земљишта, аерација, садржај глине и органске материје и микробиолошка активност.

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THE EFFECT OF WATER POTENTIAL ON ACCUMULATION OF SOME ESSENTIAL ELEMENTS IN SUGARBEET LEAVES (*Beta vulgaris* ssp. *vulgaris*)

ABSTRACT: An investigation has been conducted on the effect of reduced water potential in nutrient solution on the accumulation of some essential macro- and micronutrients in the aboveground parts of young sugarbeet plants. Plants of 8 different sugarbeet genotypes were exposed for 21 days to a nutrient solution whose water potential of 0.1 MPa was regulated by PEG. Contents of N, P, K, Ca, Mg, Fe, Mn, Cu and Zn declined in all genotypes under water deficiency, but the intensity of reduction varied among the genotypes. The results indicated that some harmful effects of water deficiency could be attributed to disturbances in plant mineral nutrition, especially the lack of N, P, and Mg, as well as to impaired ratios between the contents of particular elements, especially K/Ca.

KEY WORDS: sugarbeet, PEG, drought, N, P, K, Ca, Mg, Fe, Mn, Cu, Zn

INTRODUCTION

Content of mineral elements in plants (mineral composition of plants) depends on numerous internal and external factors which have to be taken into account while optimizing plant nutrition. One of the ecological factors that affect plant provision with essential elements is water deficit. Drought is the most limiting factor of yield worldwide, our country included, and it often reduces crop quality. The impact of drought is complex, as there is no process in plant metabolism that remains unaffected by water deficiency. In addition to various levels of water requirement, plant species and genotypes differ in their ability to adapt their metabolism to water deficiency. The problem in drought investigation is that there is no single indicator of drought tolerance that could serve as a reliable criterion for evaluation of genotypes. It is therefore necessary to monitor different indicators of plant water and osmotic status (e.g.

fresh and dry matter, leaf area, characteristics of stomata), biochemical parameters (e.g. content of osmotically active substances, activity of particular enzymes) as well as eventual changes in the elemental composition of plants. Analysis of such data and their interactions may lead to a conclusion about the degree of tolerance/sensitivity to drought in a particular genotype. Differences in plant mineral composition are the result of ***1)*** genotypic differences in plant mineral composition, and ***2)*** different levels of tolerance towards water deficit exhibited by different genotypes, as manifested through differences in the uptake and accumulation of particular mineral elements.

Sarić and Kovačević (1981) and Petrović and Kastori (1990) reported that genotypic specificity plays an important role in sugarbeet mineral nutrition. Petrović et al. (1991) reported that the mineral composition of sugarbeet plants changed under drought conditions. Balanced mineral nutrition of sugarbeet has also been mentioned as a factor affecting the contents of various elements and the ratios between essential elements in plant tissues: K/Ca, S/N, P/Zn, P/Ca, P/Fe, Fe/Mn, Mg/Mn, etc. (Kastori et al., 1996). It was shown that water deficiency causes disturbances in biochemical pathways such as the contents of free proline, DNA and RNA, activity of RUBISCO, and others (Krstić et al., 1997; Kevrešan et al., 1998).

The aim of this investigation was to monitor the influence of long-lasting mild water deficit on the accumulation of several essential mineral elements in leaves of eight sugarbeet genotypes. The genotypes, taken from the breeding program of the Institute of Field and Vegetable Crops, had shown variability in drought tolerance under field conditions. Experiments were conducted in semi-controlled conditions. Preliminary tests showed that the osmotic value of 0.1 MPa in the nutrient medium provokes symptoms of water deficiency in sugarbeet plants, but at the same time it does not completely inhibit plant growth. The water potential of 0.1 MPa was therefore chosen for testing the effect of water deficit over a period of time during which young sugarbeet plants could exhibit their capacity to adapt to this stress.

MATERIALS AND METHODS

Of the eight sugarbeet genotypes included in the experiment, seven belonged to *Beta vulgaris* ssp. *vulgaris* (genotypes 1 to 8), and one to *Beta vulgaris* ssp. *maritima* (genotype 9): 2nmm "O"1102-3-7 (line 1234, p. 399) (**1**), 2nMM C-39 (line 3484, p.25) shows quantitative resistance to rhizomania (**2**), 2nmm "O"11547 originates from Ukraine (K-2, p. 574) (**3**), 2nMM C-78 (line 3486, p. 27) (**4**), 2nMM MTRB (line 3465, p. 6) shows quantitative resistance to rhizomania (**6**), 2nmm "O"21223 (3412, p. 553) shows resistance to Cercospora (**7**), 2nmm "O" GRRT (line 3416, p. 555) shows qualitative resistance to rhizomania (**8**), and 2nMM C-51 (line 3493, p. 34) shows resistance to rhizomania, 2nMm, *B. mar* (**9**). These genotypes had come from the breeding program of Sugarbeet Department of the Institute of Field and Vegetable Crops Novi Sad and they had shown variability in drought tolerance under field conditions. The experiment was done under semi-controlled conditions. Plants were grown for 30 days in the complete nutrient medium and additional 21 days under conditions of water deficiency. After that, the contents of some essential macro- and micronutrients were determined in sugarbeet leaves.

Four hundred seeds of each genotype were soaked overnight in distilled water and subsequently sown in vermiculite. Trays with vermiculite and seeds were kept in a thermostat, in the dark, at the temperature of 24°C. After 8 to 10 days (depending on genotype) plantlets were replanted in plastic pots for water culture. The plants were grown in the complete nutrient medium after Hoagland ½ (Hoagland and Arnon, 1950). Eight plants of each genotype were planted in 12 replications (96 plants per genotype).

Polyethylene glycol (PEG) was used to induce water deficit. PEG is an inert, non-ionic polymer, which does not interfere with plant metabolism. PEG is often used in studies of plant water regime (Lawlor, 1970; Oertli 1985, 1986).

After 30 days of growth in the full nutrient medium, plants from 7 pots of each genotype were transferred to the full medium to which PEG 6000 was added in the concentration that provided the final osmotic value of the medium of 0.1 MPa (PEG). Plants of the remaining 5 pots of each genotype continued their growth in the full nutrient medium (control). The mass of PEG 6000 to be added to each pot in order to achieve the final osmotic value of 0.1 MPa was calculated by the regression equation $y = 0.0028\ x^2 + 0.0052\ x$, which was experimentally obtained for the concrete PEG, as described by Money (1989), using automatic micro-osmometer Roebling, type 12/12 DR. Nutrient solutions were changed at 3-day intervals and aerated daily.

After 21-day treatment, plants were taken for analyses. Fresh matter was measured first, dry matter after drying the samples at 70°C to constant mass. Leaf area was measured by an automatic photoelectric meter LI-3000 (Automatic Area Meter LI-3000, LICOR, USA).

Total nitrogen was determined by the micro-Kjeldahl method, potassium content flamephotometrically, phosphorus content by the ammonium-vanadate-molybdate method. Contents of Ca, Mg, Fe, Zn, Mn and Cu were determined by standard atomic absorption spectrophotometry, using a Varian model SPECTR AA-10.

The obtained results were statistically processed by calculating means and the least significant differences (LSD) between the means, using the program MstatC (Michigan State University, USA).

RESULTS AND DISCUSSION

Dry matter mass of the aboveground parts of plants grown in the conditions of water deficiency was 50% lower than that in the control (Arsenijević-Maksimović et al., 2002). Experimental results in this paper are expressed as content of mineral elements per plant. Nitrogen and P contents were significantly reduced in all genotypes exposed to water deficit. The reduction of N content was lowest in genotype 3 (13.8%), highest in genotype 7 (71.8%), with the average of about 50% (Figure 1). Generally, N content was

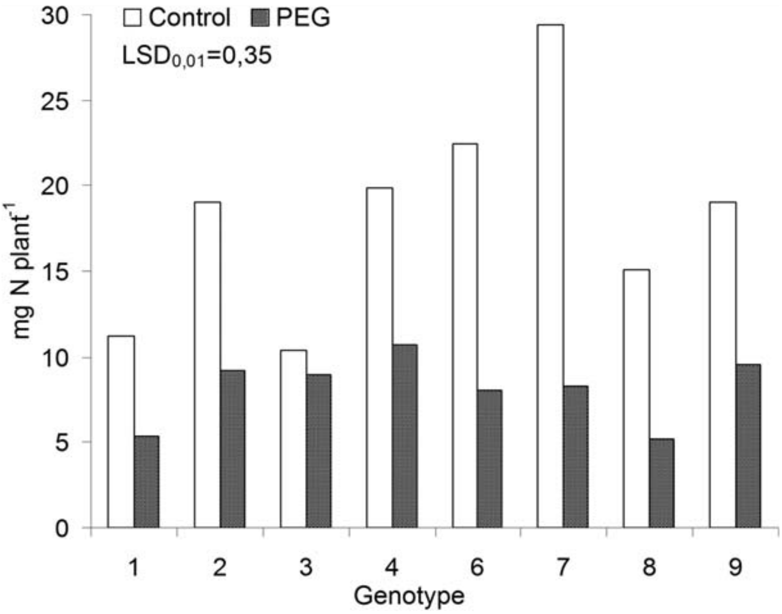

Fig. 1. Nitrogen content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

more variable in the control plants than in those exposed to drought. This can be explained by differences in growth rate during early stages of onthogenesis among the genotypes. Nitrogen content in leaves is considered as a better indicator of water deficit effects than N content in roots (Foster et al., 1991). Reduction of N content under water deficit was reported for many plant species including maize, been, citruses, Festuca (Bruce et al., 2002; Foster et al., 1991; Zekri, 1995; Huang, 2001). Beside its effect on nitrogen content itself, water deficit affects N metabolism as well. This is evident through the inhibition of nitrate reductase activity and increase in nitrate and proline contents in young sugarbeet plants (Petrović et al., 1987; Petrović et al., 1991; Arsenijević-Maksimović et al., 2002). Genotypes tolerant to drought have higher N use efficiency than less tolerant genotypes (Van den Boogaard, 1995; Foster et al., 1991).

Phosphorus content was least reduced in genotype 3 (18.4%), most reduced in genotype 8 (74.8%), while the average reduction was 50.5% (Figure 2). P uptake is sensitive to increased osmotic pressure in nutrient solution (Kastori, 1976). It is well documented that P plays a role in drought tolerance in many plant species including sugarbeet (Petrović, 1987), beans and sorghum (Al-Karaki, 1995) and wheat (Gutierrez-Boem and Thomas, 1998). Under conditions of water deficit, disturbances in P provision as well as in P metabolism occur in sugarbeet, as indicated by a change in mineral and organic P ratio in favor of the former (Petrović, 1987).

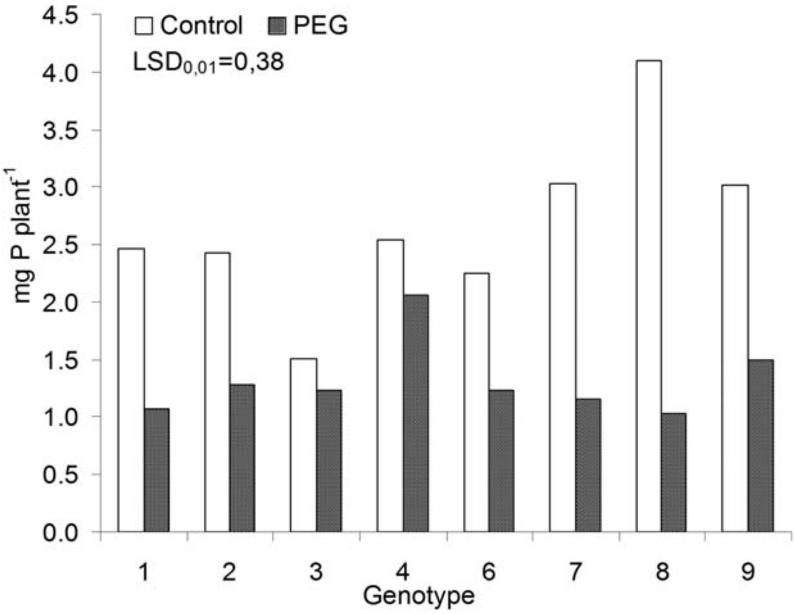

Fig. 2. Phosphorus content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

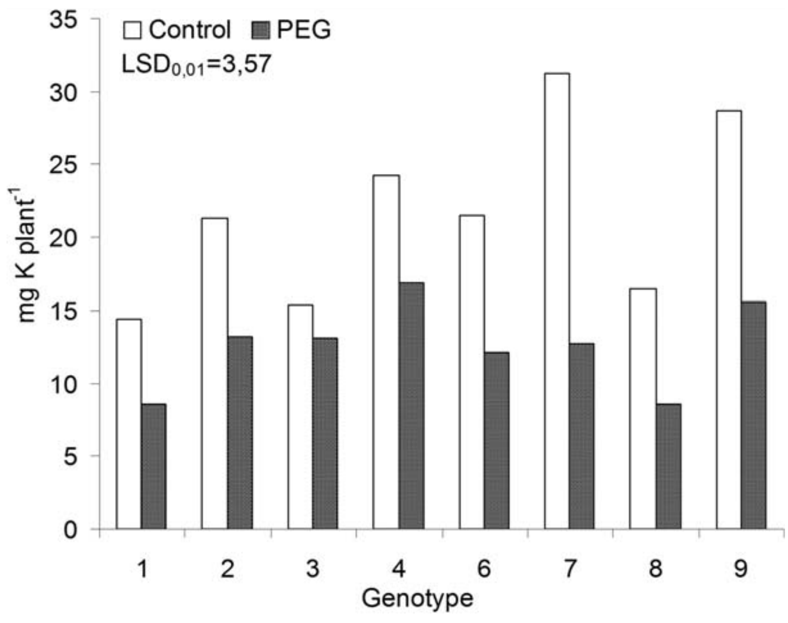

Fig. 3. Potassium content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

Water deficiency provoked statistically significant reductions of K content in leaves in all genotypes except genotype 3 (Figure 3). The average reduction of K content was 41.8%, the highest reduction was registered in genotype 7 (59.27%). Potassium participates in osmoregulation and a change in its content is usually coupled with changes in the contents of sugar and amino acids (J o n e s et al., 1981; F o r d and W i l s o n, 1981) which play a role in plant adaptation to the lack of water. Potassium is most probably important in keeping the balance with negatively charged amino acids (J o n e s et al., 1981). Calcium and magnesium contents declined significantly in all genotypes exposed to water stress, on average by 63% and 54%, respectively (Figures 4 and 5). The reduction of K and Mg contents was found in 7 different citrus genotypes suffering from PEG-induced water deficit (Z e k r i, 1995). The ratio between K and Ca contents in leaves of sugarbeet genotypes was disturbed under the experimental conditions. In the control plants, the average value of this ratio was 13.5 while in the plants exposed to PEG-induced water deficit it was 21.2, meaning that its average increase was 57.22%. Potassium and calcium are not equally mobile in plants: K is very mobile and easily reutilized, while Ca is poorly mobile and it is practically not reutilized at all. Since transpiration flow is the key factor in provisioning the aboveground plant parts with Ca, and since this flow is impaired under the conditions of water deficit due to reduction in water uptake and transpiration (K a s t o r i, 1968), a change occurs in the K/Ca ratio in the shoots of plants exposed to PEG. Changes of this ratio affect the functioning of stomata as well. Increase in Ca con-

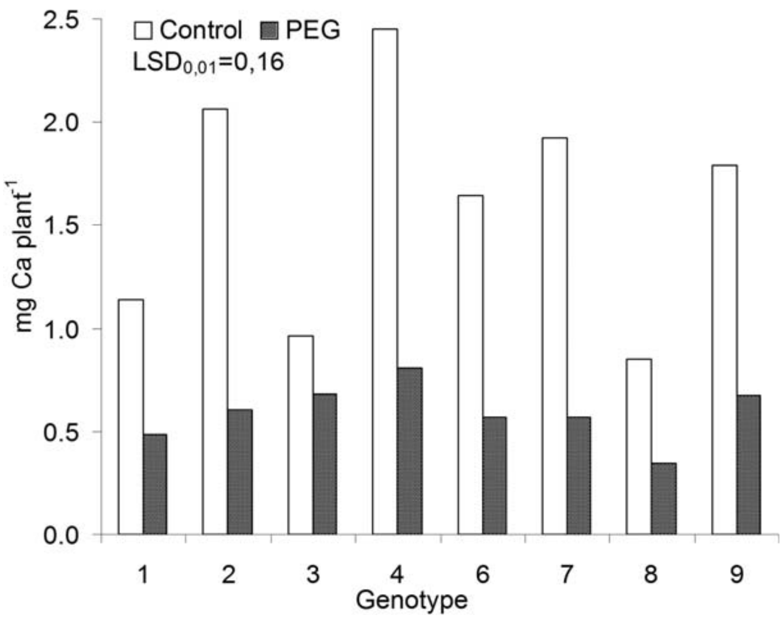

Fig. 4. Calcium content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

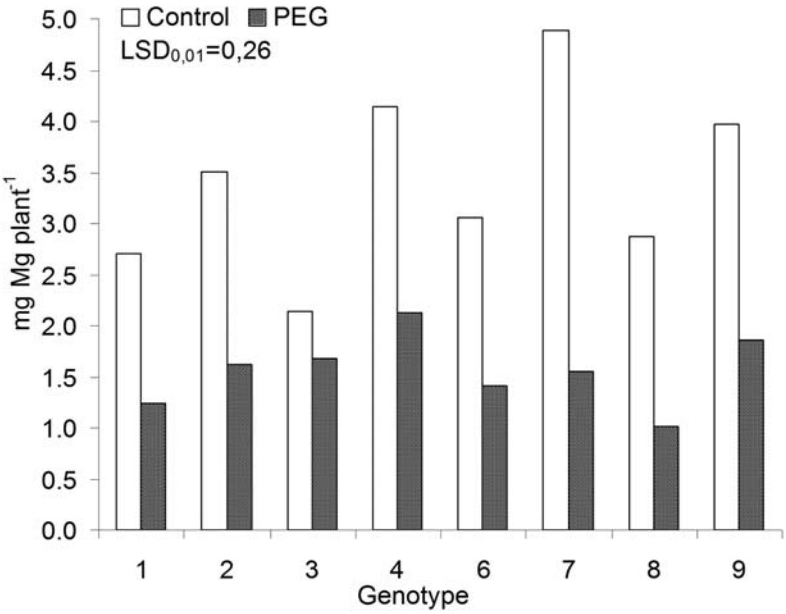

Fig. 5. Magnesium content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

tent in guard cells may be a sufficient signal for stomatal closure (Luan, 1993), the process in which ABA (Schauf et al., 1987) and auxin (Marten et al., 1991) are involved. Therefore, Ca deficiency could cause a disturbance in the stomatal control of transpiration.

The P/Ca ratio increased by 33.7% under the water deficit, suggesting that the Ca content in leaves declined to a higher extent than the P content, which again can be explained by poor mobility of Ca in plants.

Iron content declined significantly in all genotypes under the water stress (51.4 μg on average) (Figure 6). The lowest relative decreases in iron content were found in genotypes 1, 3 and 8, the highest in genotypes 7 and 9. The P/Fe ratio changed only slightly (less than 10%) under the experimental conditions. This reduction in Fe content can therefore be assigned to the generally reduced uptake of iron from the nutrient solution.

The content of Zn was significantly reduced in all genotypes under the water deficiency conditions. On average, this reduction was 54.8% (Figure 7). The decrease in Zn content was relatively lowest in genotypes 1, 3 and 4 and highest in genotypes 7, 8 and 2. Although reduced under the water deficit, Cu content was not significantly changed in all genotypes (Figure 8). Cu content was not significantly reduced in genotypes 1, 3, 4 and 6, it was significantly reduced for ” = 0.05 in genotype 9, while the reduction was significant for ” = 0,01 in genotypes 2, 7 and 8. Similar to Fe and Zn, Mn content was also significantly reduced in all genotypes, with the average reduction of 42.86% (Figure 9). The lowest reduction was recorded again in genotype 3, the highest in

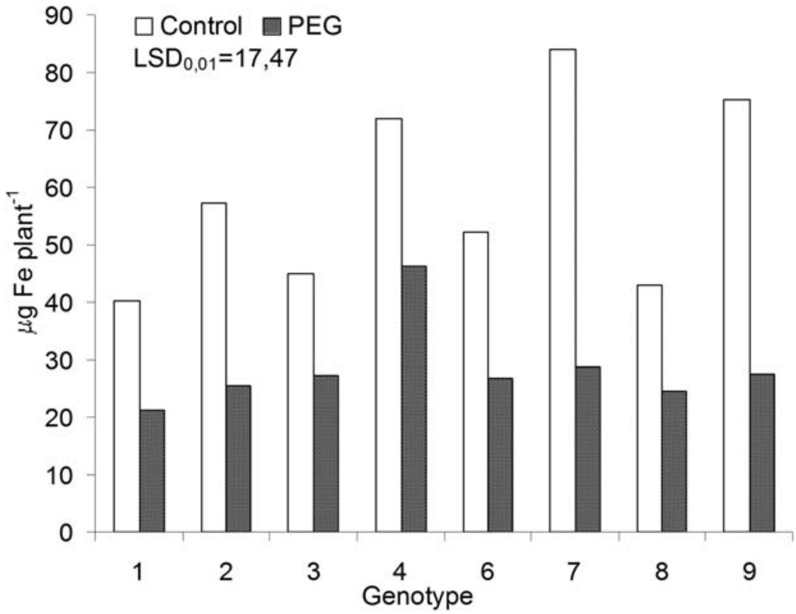

Fig. 6. Iron content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

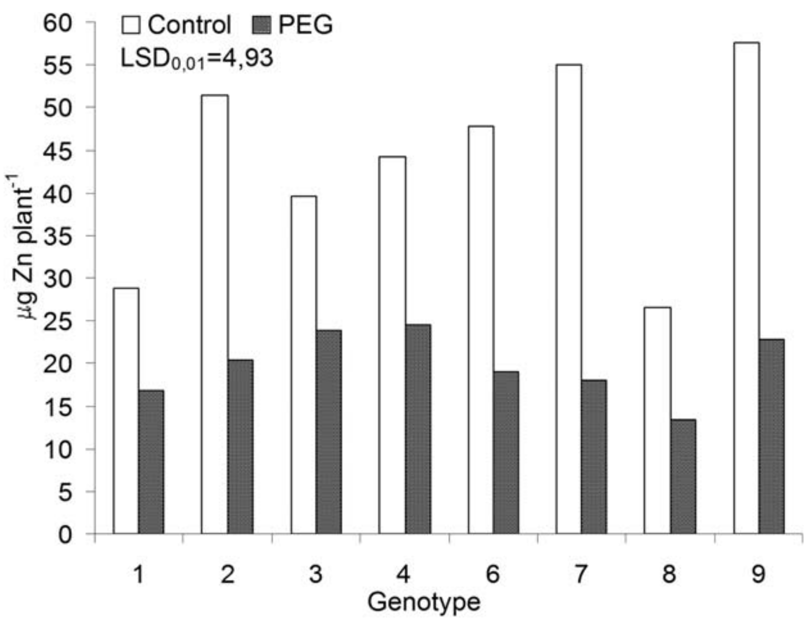

Fig. 7. Zinc content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

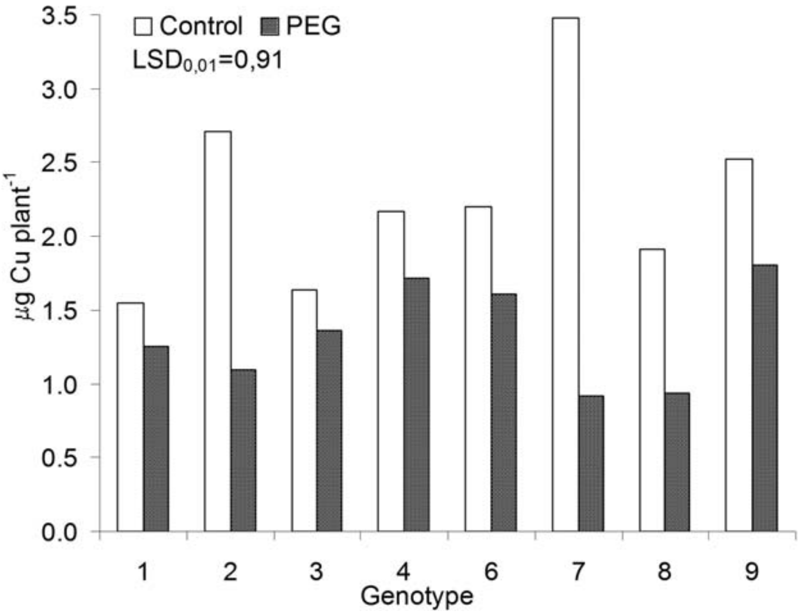

Fig. 8. Copper content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

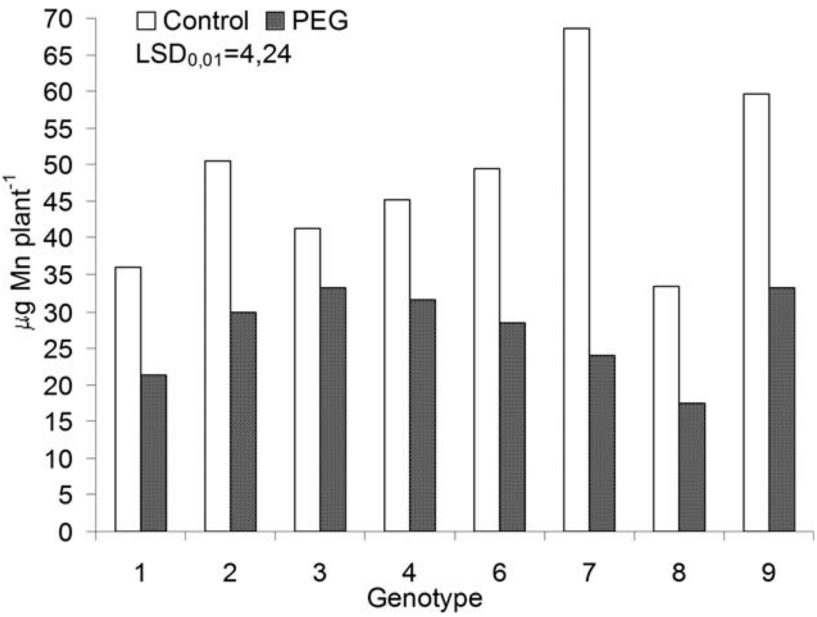

Fig. 9. Manganese content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

genotype 7. Manganese is involved in the process of photosynthesis and activation of several enzyme systems. It was shown that Mn deficiency inhibits cell elongation (Mukhopadhyay and Sharma, 1991). Cu, Zn and Mn are components of superoxide dismutase (SOD), which exists in two forms: Cu/ZnSOD and Mn/SOD. It was found that the expression of genes coding for these enzymes can be induced by the lack of water and/or by rehydration of plants which had experienced drought. Also, an increase in corresponding mRNA content was recorded during the period of plant adaptation to changes in the amount of water available to plant (Perl-Treves and Galun, 1991; Wu et al., 1999).

CONCLUSIONS

The contents of N, P, K, Ca, Mg, Fe, Zn, Mn and Cu in the leaves of young plants of all 8 sugarbeet genotypes declined under water deficiency conditions. However, the intensity of reduction differed among the genotypes. Therefore, it was not possible to establish a general pattern for the observed changes in the contents of the tested elements.

The results suggest that some detrimental effects of water deficiency were due to impaired mineral nutrition, especially the lack of N, P and Mg, and also due to disturbed ratios between the contents of certain elements, especially K/Ca.

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УТИЦАЈ ВОДНОГ ПОТЕНЦИЈАЛА НА НАКУПЉАЊЕ НЕКИХ НЕОПХОДНИХ ЕЛЕМЕНАТА У ЛИСТОВИМА ШЕЋЕРНЕ РЕПЕ (*Beta vulgaris*, ssp. *vulgaris*)

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Резиме

Циљ рада је био да се испита утицај смањеног водног потенцијала хранљивог раствора на накупљање неких неопходних макро- и микроелемената у надземном делу младих биљака шећерне репе. Биљке 8 различитих генотипова шећерне репе су 21 дан гајене на хранљивом раствору чији водни потенцијал је подешен на 0,1 MPa помоћу полиетилен гликола (PEG). Садржај N, P, K, Ca, Mg, Fe, Mn, Cu и Zn је у свим генотиповима опао у условима недостатка воде, али интензитет овог смањења није био једнак код свих генотипова. Резултати указују да се неки од штетних утицаја водног дефицита могу приписати поремећајима у минералној исхрани, посебно недостатку N, P и Mg, као и измењеном односу између садржаја појединих елемената, посебно K/Ca.

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AQUATIC MACROPHYTES AS INDICATORS OF HEAVY METAL POLLUTION OF WATER IN DTD CANAL SYSTEM

ABSTRACT: The aim of this investigation was to establish the presence or absence of chemical contamination of water and the littoral zone (banks) of Danube-Tisza-Danube (DTD) canal system. The investigation covered the canal section from Bezdan to Prigrevica. By analyzing the chemical composition of dominant aquatic species in four locations of the section, we defined the species with the highest capacity to accumulate nutrients and heavy metals. Concentrations of P and K as well as of a beneficial element Na in the tissues of the analyzed macrophytes were both species- and site-dependent. The highest accumulation was registered for *Ceratophyllum demersum* while the species *Elodea canadensis* showed increased P and K accumulation values in the location Sombor. The lowest concentrations of almost all heavy metals were recorded near Sombor, indicating that this section suffered the lowest chemical pollution. Highest concentrations of all of the analyzed heavy metals were recorded in the tissue of *Ceratophyllum demersum* from the location Prigrevica, possibly due to the influx of polluted drainage waters from surrounding agricultural areas as well as industrial wastewaters. The obtained results showed that the enforcement of biomonitoring and analyses of other parameters indicative of ecosystem conditions might be useful for improved protection of areas experiencing a strong human impact.

KEY WORDS: aquatic macrophytes, DTD canal system, nutrient and heavy metals accumulation, chemical contamination, bioindication

INTRODUCTION

Diversity of species and varying distribution of macrophytic vegetation are responsible for the visual aspect of any aquatic ecosystem. Morphological and physiological characteristics of aquatic plants show variations which depend on development stage, nutrient concentration, light conditions, etc. (Janauer, 2001). Therefore, aquatic macrophytes may play a central role in the biological monitoring since changes in the composition of aquatic vegetation are reliable indicators of the quality of water and littoral zone of aquatic ecosystems (P a l l et al., 1996; R a v e r a, 2001). Aquatic macrophytes are good indicators of water quality also because of their remarkable ability to accumulate chemical elements since an elevated accumulation of individual elements in plant tissue might be associated with their increased concentrations in an aquatic environment (Y u r u k o v a and K o c h e v, 1996; M a t a g i et al., 1998). By accumulating such increased amounts of chemical elements, aquatic macrophytes contribute to the nutrient cycling and sediment stabilization, thus significantly affecting the extent of eutrophication (C h a m b e r s and P r e p a s, 1994). Very important is their role in heavy metal accumulation since heavy metal concentrations in macrophytic tissues may be 10^6 times as high as their concentrations in an aquatic environment (K o v á c s et al., 1984; P a j e v i ć et al., 2002). Remediation (bioaccumulation) of heavy metals from water environment depends on plant species, plant organ and numerous abiotic factors, making all of them indispensable for biofiltration and heavy metal cycling in aquatic ecosystems (L e w i s, 1995). A recent survey of aquatic ecosystems examined the possibility of using aquatic vegetation to purify water and littoral zone by removing from them nutrients, heavy metals, and other pollutants (D e B u s k et al., 2001).

The aim of the survey was to assess the level of chemical contamination of canal water and its littoral zone by determining the chemical composition on dominant aquatic species at four sites of Danube-Tisza-Danube (DTD) canal system. Our survey concentrated on the canal section Bezdan—Prigrevica which is subject to a strong human impact as reflected by the presence of waste material coming from surrounding industrial facilities and agricultural areas.

MATERIAL AND METHODS

Four sites were surveyed, Bezdan—Šebeš fok, Bezdan—Siget most, Sombor, and Prigrevica. Chemical analyses included dominant macrophytic species, i.e., *Ceratophyllum demersum* L., *Nymphaea alba* L. and *Phragmites communi*s T r i n. (emergent part and rhizome) from Bezdan—Šebeš fok, *Ceratophyllum demersum* L., *Trapa longicarp*a J a n k., *Typha angustifolia* L. and *Sparganium erecta* L. from Bezdan—Siget most, *Ceratophyllum demersum* L., *Elodea canadensis* L., *Nuphar luteum* L. and *Phragmites communi*s T r i n. (emergent part) from Sombor and *Ceratophyllum demersum* L. and *Potamogeton pectinatus* L. from Prigrevica. Sampling was done in June and July of 2002, at sites characterized by the greatest plant density and coverage.

Plant material was classified, rinsed in distilled water several times, dried at 105°C, and prepared for chemical analyses using standard procedure for water and aquatic plants (APHA, 1995). From stock solution, total P concentration was determined using spectrophotometry, concentrations of total K and Na using flame photometry and the concentration of total N using standard micro Kjeldahl method (N e l s o n and S o m m e r s, 1973).

Concentrations of heavy metals, Fe, Mn, Ni, Cu, Co, Pb, and Cd, were determined directly from stock solution using atomic absorption spectrophotometry.

Data analysis was done using multiple interval test (Duncan). Values with the same letter did not differ significantly at p = 0.05.

RESULTS AND DISCUSSION

Location Bezdan — Šebeš fok. Concentrations of P and K varied among the analyzed plant species. A submersed species *C. demersum* showed the highest accumulation (Figure 1).

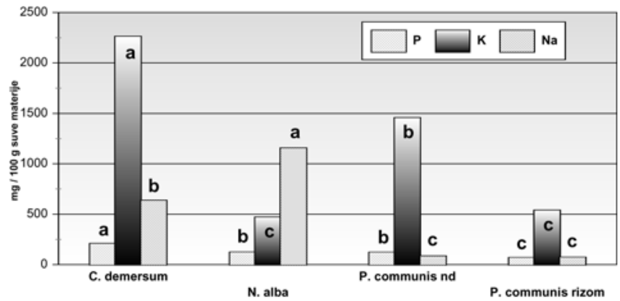

Fig. 1. Concentrations of macronutrients (P and K) and Na in macrophytes from location Bezdan — Šebeš fok

P concentrations were between 211mg% (*C. demersum*) and 73 mg% (rhizome of *P. communis*). Comparing P concentrations in *C. demersum* recorded in DTD canal system near Novi Sad refinery (Pajević et al., 2002) and at Danube—Subić immediately after the canal empties into the Danube (Pajević et al., 2002a) with those recorded at Bezdan—Šebeš fok site, it was seen that P accumulation was several times lower in the latter site. According to Gerloff and Krombholz (1966), critical P content in macrophytic tissue is around 0.13% (130 mg%). Although the obtained results show that the P amounts accumulated in the surveyed macrophytic species were rather small, the role of aquatic plants in taking up excess P from wastewater is important. P amounts taken up by aquatic plants from water depend on plant species and P concentration in water and its substratum. The surface mud layer is frequently the main P source for macrophytes (Kim and Geary, 2001).

The highest K accumulation (2264 mg%), also recorded in *C. demersum*, was five times as high as the concentration in floating *N. alba* leaves (Figure 1). Very low K, P and Na concentrations were registered in *P. communis* rhizomes. Their concentrations were higher in the emergent parts of *P. communis*, obviously due to an intensive element transport. Still, the latter concentrations were low when compared with those in terrestrial plants (Kastori, 1997). Floating leaves of the aquatics accumulated high amounts of Na (Figure 1). Significant Na concentrations in *N. alba* leaves were also reported in previous investigation (P a j e v i ć et al., 2002).

With respect to the accumulation of heavy metals (Fe, Mn, Ni, Cu, Co, Pb), *C. demersum* showed significantly highest values for all elements except Pb (Figures 2 and 3).

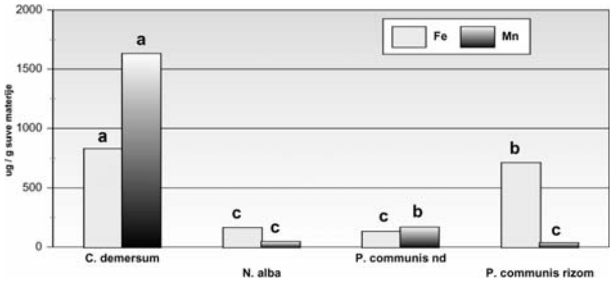

Fig. 2. Concentrations of Fe and Mn in macrophytes from location Bezdan — Šebeš fok

The Fe concentrations ranging from 835 μg · g^{-1} in *C. demersum* tissue to 134 μg · g^{-1} in emergent parts of *P. communis* were significantly lower than those found in the same species at the Danube site (P a j e v i c et al., 2002b). The above data may be due to reduced concentrations of this metal in water and mud, but we should not ignore the fact that the concentrations of total and soluble forms of Fe available to plants as well as the ionic form of Fe are influenced by a large number of factors, particularly by the amounts of soluble O_2 and pH (G o u l e t and P i c k, 2001).

The low Mn concentrations recorded in plant species of the surveyed site further support the conclusion that the canal water of this site was not exceedingly loaded with the analyzed heavy metals.

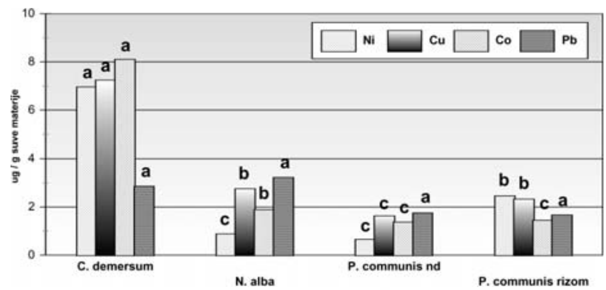

Fig. 3. Concentrations of Ni, Cu, Co and Pb in macrophytes from location Bezdan — Šebeš fok

The concentrations of Ni, Cu and Co below 10 μg 15 g^{-1} again confirmed the above statement that the canal water of this site was not exceedingly loaded with the analyzed heavy metals, in spite of the presence of Pb in all of the analyzed plant species but in concentrations below those indicating pollution.

Location Bezdan — Siget most. Significantly low P concentrations concurrently with significantly high K concentrations were recorded in *C. demersum* tissue. The highest P concentration (257 mg%) was obtained from the emergent parts of *S. erecta*, the lowest (116 mg%) from *C. demersum* (Figure 4).

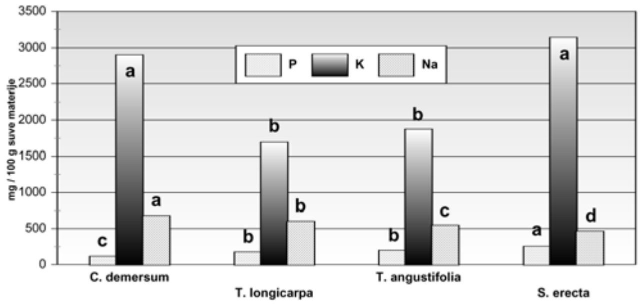

Fig. 4. Concentrations of macronutrients (P and K) and Na in macrophytes from location Bezdan — Siget most

The highest K concentrations were recorded in *S. erecta* (3146 mg%) and *C. demersum* (2903 mg%), the highest Na concentration in *C. demersum* (683 mg%). The obtained Na value (683 mg%) was common for all the surveyed sites where *C. demersum* occurred.

Concentrations of heavy metals, i.e., Fe and Mn micronutrients, were highest in *C. demersum* tissue. Their concentration values were opposite to those reported for Bezdan—Šebeš fok, namely, the Fe concentration (1232 $\mu g \cdot g^{-1}$) was significantly higher than the Mn concentration (858 $\mu g \cdot g^{-1}$) (Figure 5). The obtained values were due to a large number of factors such as the form of the elements and their ratios in the aquatic environment and the substratum (Karpiscak et al., 2001).

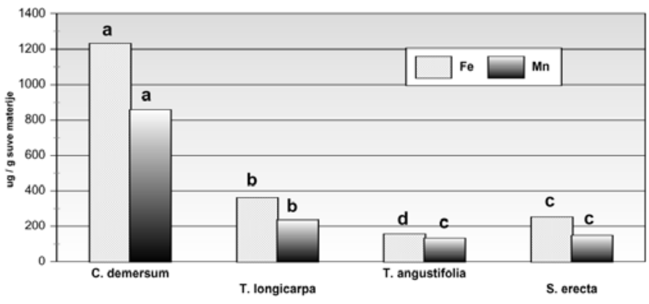

Fig. 5. Concentrations of Fe and Mn in macrophytes from location Bezdan — Siget most

The accumulation rates of the other analyzed elements (Ni, Cu, Co, and Pb) were somewhat lower than those recorded in the location Bezdan—Šebeš fok (Figure 6).

No significant differences in Pb accumulation were found among the macrophytes while the recorded Pb concentrations were similar to those from

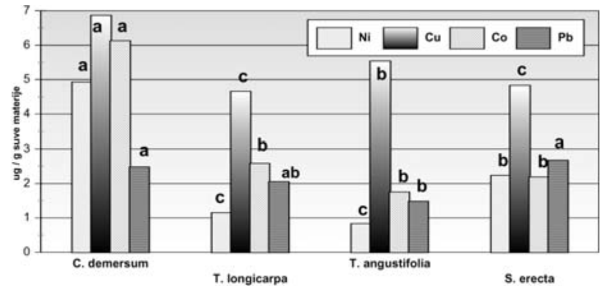

Fig. 6. Concentrations of Ni, Cu, Co and Pb in macrophytes
from location Bezdan — Siget most

the other sites. The lowest Pb concentration (1.48 $\mu g \cdot g^{-1}$) was obtained for the emergent part of *T. angustifolia*.

Location Sombor. The highest P and K accumulations were recorded in *C. demersum* and *E. canadensis* (Figure 7). The P concentration recorded in *C. demersum* was similar to that in the location Bezdan—Šebeš fok, whereas the K concentration was higher (2820 mg%).

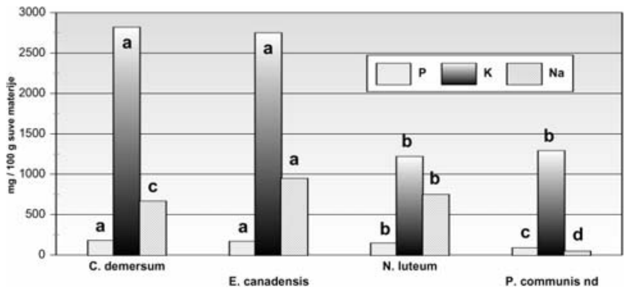

Fig. 7. Concentrations of (P and K) and Na
in macrophytes from location Sombor

Na concentration was species-dependent. The highest accumulation was obtained for *E. canadensi*s (950 mg%). Low Na values were recorded in the emergent parts of *P. communis* (50 mg%).

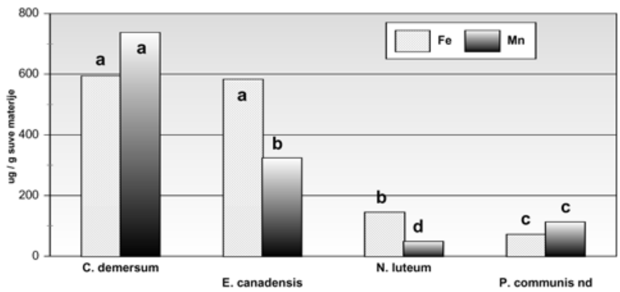

Fig. 8. Concentrations of Fe and Mn in macrophytes from location Sombor

Irrespective of plant species, Fe and Mn concentrations were below 800 μg · g^{-1} (Figure 8). Concentration rates of these two elements showed species-dependent variations. Thus, *C. demersum* and the emergent parts of *P. communis* accumulated more Mn than Fe. Such Fe and Mn distributions were similar to the values obtained for these two species in the location Bezdan—Šebeš fok.

The concentrations of Ni, Cu, Co, and Pb in *C. demersum* tissue resembled those recorded in the locations Bezdan—Šebeš fok and Bezdan—Siget most. *C. demersum* showed significantly highest accumulation rates for the analyzed heavy metals (Figure 9) the concentrations of Ni, Cu, and Co were mutually similar, amounting to 7.56 μg · g^{-1}, 7.01 μg · g^{-1} and 5.29 μg · g^{-1}, respectively. Rather low Pb concentrations, from 3.35 μg · g^{-1} (*C. demersum*) to 0.84 μg · g^{-1} (the emergent part of *P. communis*) were found in all plant samples. The lowest concentrations of all of the analyzed elements were obtained for the emergent part of *P. communis*.

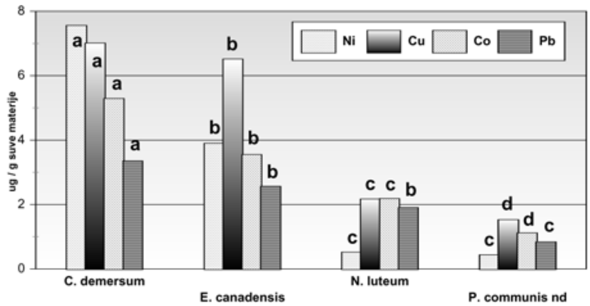

Fig. 9. Concentrations of Ni, Cu, Co and Pb
in macrophytes from location Sombor

Location Prigrevica. The two dominant species, *C. demersum* and *P. pectinatus*, were analyzed. The P concentration in *C. demersum* was 247 μg · g^{-1}. No higher value was obtained with the analyzed aquatics of any other of the surveyed locations (Figure 10). The K concentration in *C. demersum* was twice as high as that in *P. pectinatus* and it amounted to 2375 mg%. The Na concentrations, amounting to app. 600 mg%, were similar in both species.

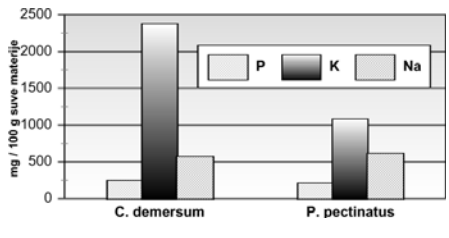

Fig. 10. Concentrations of macronutrients (P and K)
and Na in macrophytes from location Prigrevica

This location was also characterized by the highest Fe and Mn concentrations. The Fe concentration in *C. demersum* was as high as 6487 μg · g^{-1}. It was obviously site-dependent and it was five to ten times higher than the values obtained for the same species elsewhere (Figure 11). The Mn concentration of 2604 $\mu g \cdot g^{-1}$ in *C. demersum* was two to three times higher than the values recorded for the same species in the other surveyed locations. The significant increases in the Fe and Mn concentrations suggest that the canal water of this section contained a high chemical load which was due to wastewater releases. Although the Fe and Mn concentrations in *C. demersum* were rather high, the values obtained for the same species in several Danube sites in the vicinity of Novi Sad were still higher.

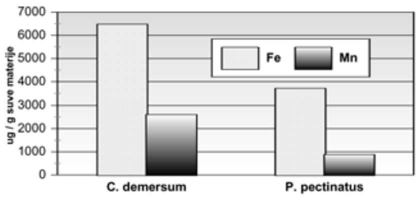

Fig. 11. Concentrations of Fe and Mn in macrophytes from location Prigrevica

Canal water contamination with heavy metals was also confirmed by the concentrations of Ni, Cu, and Co recorded in *C. demersum* (Figure 12). The Pb concentration, however, was not elevated. This was more likely due to antagonistic effects of increased concentrations of other ions than due to low Pb in water and the littoral zone.

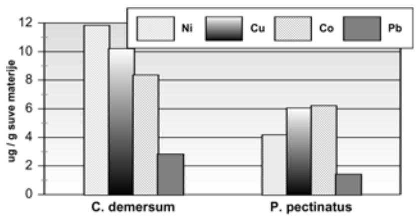

Fig. 12. Concentrations of Ni, Cu, Co and Pb in macrophytes location Prigrevica

CONCLUSION

The concentrations of P and K, as well as that of a beneficial element Na in the tissues of the analyzed macrophytes were both species- and site-dependent. The average P concentrations were rather low, below 300 mg%. The highest P accumulation was obtained for *Ceratophyllum demersum*. Also, the species *Elodea canadensis* showed increased P and K values in the location Sombor. Lowest concentrations of almost all heavy metals were recorded for the DTD canal near Sombor indicating the lowest chemical pollution of this site. Highest concentrations of all of the analyzed heavy metals were recorded in *Ceratophyllum demersum* tissue from the location Prigrevica, possibly due to the influx of polluted drainage waters from the surrounding agricultural areas and industrial wastewaters.

The obtained results show that biomonitoring enforcement and analyses of other parameters indicative of ecosystem conditions might be helpful in protecting areas experiencing a strong human impact.

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АКВАТИЧНЕ МАКРОФИТЕ КАО ИНДИКАТОРИ ЗАГАЂЕЊА ВОДЕ ТЕШКИМ МЕТАЛИМА У СИСТЕМУ ДТД

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Резиме

Концентрације макроелемената P и K, као и корисног елемента Na у ткиву испитиваних макрофита зависиле су од врсте и локалитета. Највећи акумулатор је била врста *Ceratophyllum demersum*, а на локалитету Сомбор по већој акумулацији P и K издвојила се и врста *Elodea canadensis*, што указује на органско оптерећење воде. У поређењу са осталим локалитетима, концентрације готово свих тешких метала у макрофитама локалитета Сомбор биле су најниже, те се на основу тога овај локалитет може сматрати најмање угроженим хемијским полутантима. Највећи акумулатор тешких метала била је субтерзна врста *Ceratophyllum demersum*. На локалитету Пригревица у ткиву врсте *Ceratophyllum demersum* регистроване су највеће концентрације свих испитиваних тешких метала. Високе концентрације тешких метала у макрофитама указују на хемијско загађење каналске воде на овој деоници, што може бити последица уливања загађене дренажне воде са околног пољопривредног подручја, као и индустријских отпадних вода. Добијени резултати јасно указују да би се планским праћењем (мониторингом) хемијског састава биљака узетих са угрожених локалитета, уз анализу и других показатеља стања екосистема, могло брже и свеобухватније доћи до решења проблема заштите од загађења подручја под великим антропогеним утицајем.

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DISTRIBUTION OF THE *Lathyrus* L. 1753 (Fabales, Fabaceae) SPECIES IN THE VOJVODINA PROVINCE

ABSTRACT: Most of the *Lathyrus* species of the Vojvodina Province are cultivated for fodder. They are protein-containing herbs which easily recover after grazing. Some perennial species survive in grassland communities for ten years or more. Certain species are important melliferous plants. In the Vojvodina Province, they inhabit different habitats like forests and grasslands, dry and wet sites, thus showing a wide distribution range. Besides their floristic and vegetation aspects, their role as green and dry fodder crops should be emphasized.

KEY WORDS: floral element, fodder crop, *Lathyrus* species, range, UTM grid, the Vojvodina Province

INTRODUCTION

The genus *Lathyrus* includes about 120 species. They occur mainly in the Northern Hemisphere, from the extreme north to tropic African and South American regions. Annual herbs grow mostly in the Mediterranean and Front Asia (G a m s, 1964). About 55 species have been recorded in the European flora (B a l l, 1968). Of about 30 species recorded in the Serbian flora (K o j i ć, 1972), 16 can be found in the Vojvodina Province. This paper deals with their distribution in the Vojvodina Province, habitat types, essential ecological characteristics, floral elements, and economic importance.

MATERIAL AND METHODS

Literature data, dried plant material (herbarium of the Department of Biology and Ecology, BUNS), and field surveys served as the basis for the development of UTM grid maps showing distribution of the *Lathyrus* species in the Vojvodina Province. The charts were developed by the indirect mapping puncturing method (Walter and Straka, 1970). UTM codes, literature sources, and the specified sites are presented. Literature data cover the period of approximately 140 years. Besides distribution, floral elements and habitat types (Soó, 1966), distinction between annual and perennial taxa and possible economic importance of species (Федченко, 1948) are discussed.

RESULTS AND DISCUSSION

Sect. Aphaca (Adans.) Rchb. 1832 Fl. Germ etc. 533

Lathyrus aphaca L. 1753 Sp. Pl. Ed 1: 729

Distribution in the Vojvodina Province (Figure 1):

Bačka region: the Titelski Hill DR 31 (Stanojev, 1981; Djurčjanski, 1980); Mošorin DR 31 (Stanojev, 1981; Djurčjanski, 1980); Kovilj DR 20 (Prodán, 1916); Futog CR 91 (Prodán, 1916); Rumenka DR 01 (Kupcsok, 1929); Novi Sad—Liman DR 01 (HIB; Budak, 1998); Žabalj DR 22 (Djurčjanski, 1980; Budak, 1978); Bečej DR 25 (Petrović, 1978); Subotica CS 90 (Tóth, 1975; Prodán, 1916); Čantavir DR 08 (Prodán, 1916); Žednik CR 98 (Igić, 1991); Orešković CR 87 (Igić, 1991); Lok DR 30 (Stanojev, 1983); Kanjiža DS 20 (Andrejević, 1976; Budak, 1998); Horgoš DS 11 (Andrejević, 1976); Bačka Palanka CR 71 (Budak, 1998); Sombor CR 57 (Grdinić, 1995); Sonta Velika Bara CR 55 (Grdinić, 1995); Kać DR 11 (Babić and Parabućski, 1971); Vilovo DR 31 (Babić and Parabućski, 1971); Vajska CR 53 (Prodán, 1916); Crvenka CR 85 (Kupcsok, 1929); Doroslovo CR 55 (Purger, 1993); Bečej DR 25 (Kovács, 1915).

Banat region: Sajan DR 47 (Andrejević, 1976); Sečanj DR 82 (Vučković, 1980, 1982); eastern part of the Tamiš River basin DR 82 (Vučković, 1985); Konak DR 91 (Knežević, 1990); Novi Bečej DR 35 (Knežević, 1990); Starčevo DQ 76 (Knežević, 1990); Banatska Dubica DR 81 (Knežević, 1990); Sekuš DQ 99 (Knežević, 1990); Alibunar DQ 99 (Knežević, 1990); Vatin ER 11 (Knežević, 1990); Kuštilj EQ 28 (Seležan 1973); Tomaševac—Botoš DR 71 (Savić, 1993); Deliblato Sands EQ 07 (Diklić, Vasić, 1983); Bočar DR 46 (Knežević, 1990); Novi Bečej DR 35 (Knežević, 1990); Seleuš DQ 99 (Knežević, 1990); Orlovat DR 61 (Drašković, 1996).

Srem region: Petrovaradin DR 11 (Vučković, 1972; Rajačić, 1971; HPM[1]); Mutinci and Gornji Karaš DR 20 (HPM); Belješevo CR 90 (HPM); Obrež DQ 16 (Acević, 1973); Kamenica DR 10 (Zorkóczy, 1896); Venac DQ 09 (HPM); Testera CR 90 (HPM); Čerević CR 90 (Obradović, 1966); Vrdnik DQ 09 (Igić, 1999); by the railroad track Čortanovci—Beška

[1] HPM — Herbarium of Museum of Natural History of the Vojvodina Province, Novi Sad, today Herbarium of Institute of Nature Protection of Serbia, Novi Sad.

DQ 29 (B u t o r a c, 1981); Beška DQ 29 (B u t o r a c, 1981); Stari Slankamen DQ 49 (B u t o r a c, 1981).

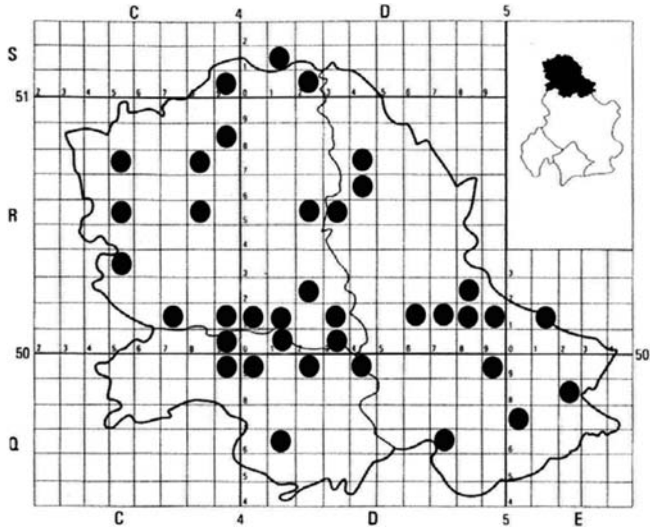

Fig. 1: Distribution *Lathyrus aphaca* in The Vojvodina Province

A sub-Mediterranean, namely, south Eurasian species of east Mediterranean origin. Occurs on warm and rich substrata, humose, neutral, argillaceous and loess soils, cultivated ground, in stubble, in cropped fields, in fallow fields and in weed communities. Rare in wet grasslands. Annual herb cultivated for fodder. Frequent in the Vojvodina Province. Chromosome number n = 7.

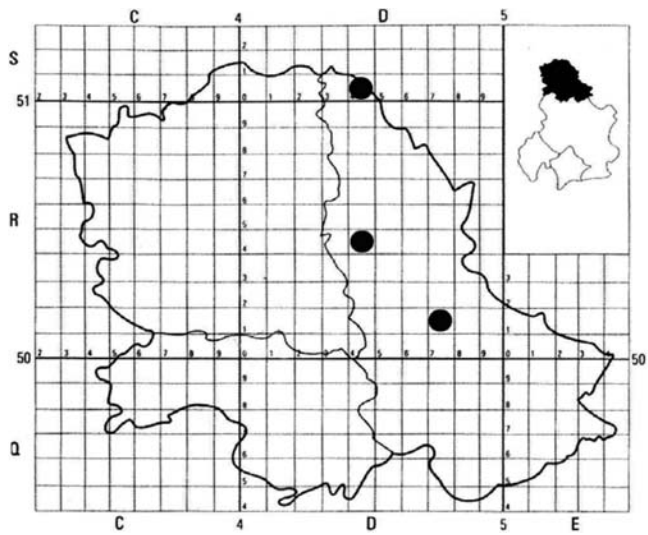

Fig. 2: Distribution *Lathyrus aphaca* var. *aphaca* f. *laetus* in The Vojvodina Province

***L. aphaca* L. var. *aphaca* f. *laetus* Posp. 1898 in Soó Syn. Syst. Gerb. Fl. Veg. Hung. 2: 370**

Distribution in the Vojvodina Province (Figure 2):

Banat region: Mali Siget—Veliki Siget DS 40; Melenci—Rusanda DR 44; Jarkovac DR 7140 (K n e ž e v i ć, 1990).

***Sect.* Nissolia (Adans.) Rchb. 1832 Fl. Germ etc. 533**

***Lathyrus nissolia* L. 1753 Sp. Pl. Ed 1: 729**

Distribution in the Vojvodina Province (Figure 3):

Bačka region: Kovilj DR 20 (Z o r k o c z y, 1896); Šajkaš DR 21 (Z o r k o c z y, 1896); Stari Futog CR 91 (P r o d á n, 1916); Sombor CR 57 (P r o d á n, 1916;); Novi Sad DR 01 (R a j a č i ć, 1971); Deronje CR 63 (P r o d á n, 1916); Apatin—Kurjačica CR 45 (G r d i n i ć, 1996).

Banat region: Kuštilj EQ 28 (S e l e ž a n, 1973); Mokrin DR 58 (K n e ž e v i ć, 1994); Vatin ER 11 (K n e ž e v i ć, 1994); Tomaševac DR 71 (S a v i ć, 1993); Deliblato Sands EQ 07 (D i k l i ć , V a s i ć, 1983); Mesić EQ 39 (V u č k o v i ć, 1991); Markovac EQ 39 (V u č k o v i ć, 1991); Sočica EQ 39 (V u č k o v i ć, 1991); Jablanka EQ 39 (V u č k o v i ć, 1991); ther Vršac Mountain—Karaula EQ 39 (V u č k o v i ć, 1991); Široko bilo EQ 29 (P a n j k o v i ć, 1983; V u č k o v i ć, 1991); Guzajna EQ 39 (P a n j k o v i ć, 1983; V u č k o v i ć, 1991); Vršačka kula (P a n j k o v i ć, 1983).

Srem region: Rivica DQ 09 (S t e v a n o v i ć, 1984); Neštin CR 70 (S t e v a n o v i ć, 1984); Koševac DR 30 (S t e v a n o v i ć, 1984); Kalakač DQ 29 (S t e v a n o v i ć, 1984); Glavica—Širine DR 10 (S t e v a n o v i ć, 1984); Čerević CR 90 (S t e v a n o v i ć, 1984; O b r a d o v i ć, 1966); Banoštor CR 90 (S t e v a n o v i ć, 1984); Grgurevci CQ 99 (S t e v a n o v i ć, 1984); Sremski

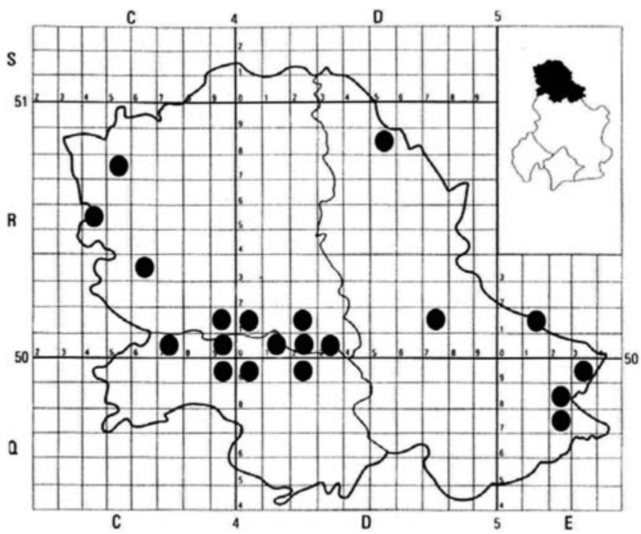

Fig. 3: Distribution *Lathyrus nissolia* in the Vojvodina Province

Karlovci DR 10 (Z o r k ó c z i, 1896); Beleševo CR 90 (HPM); Stražilovo DR 10 (HPM).

An Atlantic-Mediterranean species. Occurs on warm, dry, and rich substrata, humose, neutral, argillaceous and loess soils, in grasslands, in thinned forests, in scrubs, on cultivated ground and saline soils. Rare in marshes and boggy regions. An annual herb without economic importance. Grown scattered, mostly on the Fruška Gora Mountain and the Vršac Mountain while rare elsewhere. Chromosome number n = 7.

Sect. Cicercula (Medik.) Gren. Et Godr. 1848 Fl. Franc. 1: 481

L. cicera L. 1753 Sp. Pl. ed 1: 730

Distribution in the Vojvodina Province (Figure 4):

Srem region: Petrovaradin DR 11 (Z o r k ó c z i, 1896); Sr. Kamenica DR 00 (Z o r k ó c z i, 1896); around the Manđelos Brook CQ 89 (B u t o r a c, 1989).

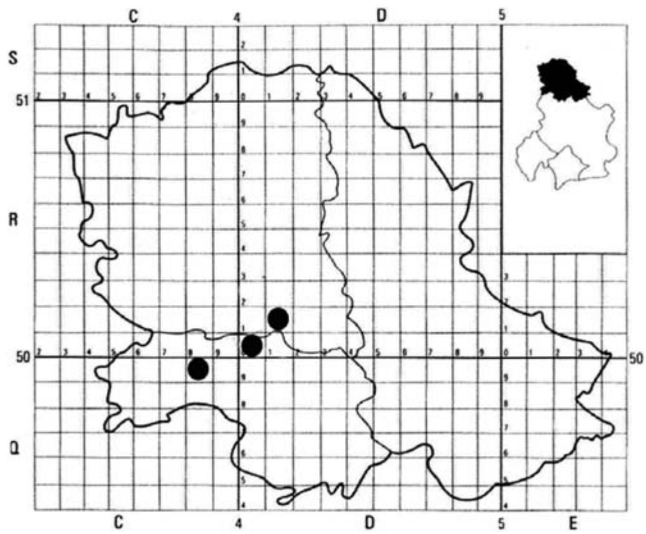

Fig. 4: Distribution *Lathyrus cicera* in the Vojvodina Province

A Mediterranean species, rarely cultivated. No data on its occurrence in natural flora and vegetation. Subspontaneous only on the Fruška Gora Mountain (3 sites). An annual herb cultivated for fodder. Chromosome number n = 7.

L. sativus L. 1753 Sp. Pl. ed 1: 730

Distribution in the Vojvodina Province (Figure 5):

Bačka region: Telečka CR 77 (I g i ć, 1991); Čantavir DR 08 (I g i ć, 1991); Titel DR 40 (S t a n o j e v, 1983); Lok DR 30 (S t a n o j e v, 1983).

Srem region: Stražilovački breg DR 10 (HPM); Susek CR 80 (B u g a r s k i, 1979); Sr. Kamenica DR 00 (Z o r k ó c z i, 1896); Iriški venac DR

00 (HPM); Paragovo DR 10 (Obradović, 1966); Sviloš CR 80 (Obradović, 1966).

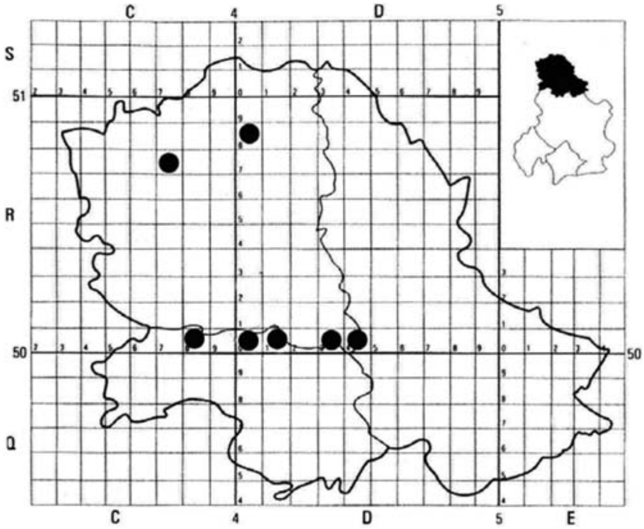

Fig. 5: Distribution *Lathyrus sativus* in the Vojvodina Province

An East Mediterranean neophyte. Rare in natural flora and vegetation, cultivated for fodder, vegetable plant. Subspontaneous on warm, fixed, argillaceous and saline soils, in scrubs and in weed communities. An important annual, melliferous, protein-rich herb cultivated for fodder. Rare in the Vojvodina Province. Chromosome number n = 7.

***L. hirsutus* L. 1753 Sp. Pl. ed. 1: 732**

Distribution in the Vojvodina Province (Figure 6):

Bačka region: Ratno ostrvo DR 01 (Zorkóczi, 1896); Rumenka DR 01 (Kupcsok, 1915); Bečej DR 25 (Kovács, 1929); Veternik—Futog DR 01-CR 90 (BUNS); Žabalj DR 22 (Djurčjanski, 1980; Budak, 1978); Novi Sad DR 01 (Prodán, 1916; Budak, 1998); Kovilj DR 20 (Budak, 1978); Mošorin DR 31 (Stanojev, 1983); Bačka Palanka CR 71 (Budak, 1986, 1998); Bačko Gradište DR 24 (Budak, 1998); Apatin CR 45 (Prodán, 1916); Doroslovo CR 55 (Purger, 1993); Sonta—Velika Bara CR 55 (Grdinić, 1995); the Titel Hill DR 31 (Babić and Parabućski, 1971); Subotica CS 90 (Prodán, 1916).

Banat region: Kuštilj, 31. V 1973. EQ 28 (Seležan, 1975); Novi Kneževac DR 39 (Knežević, 1990); Čoka DR 38 (Knežević, 1990); Bočar DR 46 (Knežević, 1990); Aradac DR 42 (Knežević, 1990); Ečka DR 51 (Knežević, 1990); Vlajkovac EQ 19 (Knežević, 1990); Tomaševac—Botoš DR 71 (Savić, 1993); Jablanka EQ 39 (Vučković, 1991).

Srem region: Karlovčić DQ 26 (Stanojević, 1996); Petrovaradin DR 11 (Zorkóczi, 1896; Rajačić, 1970); Sremska Kamenica DR 10 (Zork ó c z i, 1896); Čortanovci DQ 29 (K u p c s o k, 1914); Stražilovački breg DR 10 (O b r a d o v i ć, 1966); Susek CR 80 (B u g a r s k i, 1975); Koruška CR 80 (B u g a r s k i, 1975); Dolina Kukavica DQ 19 (B u t o r a c, 1989); Sremski Karlovci DR 10 (K u p c s o k, 1914; H i r c, 1919); Ribnjak DR 11 (O b r a d o v i ć, 1966); Sremski Karlovci DR 10 (B u t o r a c, 1981); Banstol DR 10 (B u t o r a c, 1981); Krčedin DQ 39 (B u t o r a c, 1981).

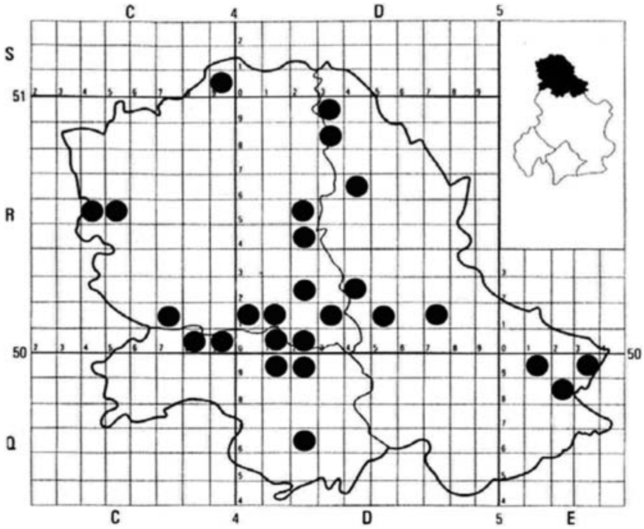

Fig. 6: Distribution *Lathyrus hirsutus* in the Vojvodina Province

A south (-central) Eurasian species of sub-Mediterranean importance. Occurs on dry, porous and rich substrata, on argillaceous, loess and sandy soils, on cultivated ground, in crops, in scrubs, on embankments, along roadways, and in dry saline grasslands. A soil-fixing, long-lived, annual herb cultivated for fodder. Scattered in the Tisza River basin and on the Fruška Gora Mountain while rare elsewhere. Chromosome number n = 7.

Sect. **Eulathyrus Ser. in DC 1825 Prodr. 2: 369**

L. tuberosus **L. 1753 Sp. pl. ed 1: 730**

Distribution in the Vojvodina Province (Figure 7):

Bačka region: Vrbas CR 94 (P r o d á n, 1916); Sombor CR 57 (P r o d á n, 1916); Bajša CR 87 (B o ž a, 1976; I g i ć, 1991); Zobnatica CR 97 (P a r a b u ć s k i, 1982); Bačka Topola CR 97 (I g i ć, 1991; D u d a š, 1996); Mošorin DR 31 (S t a n o j e v, 1981; B u d a k, 1978, 1986); Žabalj DR 22 (D j u r č j a n s k i, 1980; B u d a k, 1978, 1998); Kisač DR 02 (K u p c s o k, 1915; B u d a k, 1978); Novi Sad DR 01 (BUNS); Kać DR 11 (B a b i ć and P a r a b u ć s k i, 1971; HIB); Gospođinci DR 22 (B a b i ć and P a r a b u ć s k i, 1971); Čurug DR 23 (B a b i ć and P a r a b u ć s k i, 1971); Titel DR 40 (BUNS); Kovilj DR 20 (D j u r č j a n s k i, 1980; B u d a k, 1978); Rumenka

DR 01 (D j u r č j a n s k i, 1980; B u d a k, 1978); Futog CR 91 (D j u r č j a n s k i, 1980); Bečej DR 25 (K o v á c s, 1929; P e t r o v i ć, 1979); Subotica CS 90 (C e k u š, 1975; S a b i ć, 1975.); Senta DR 28 (M a r i ć, 1979); Crvenka CR 85 (I g i ć, 1991); Kula CR 85 (B u d a k, 1986; I g i ć, 1991; G r d i n i ć, 1996); Karavukovo CR 53 (G r d i n i ć, 1996); Sombor CR 57 (G r d i n i ć, 1996); Doroslovo CR 55 (G r d i n i ć, 1996); Apatin—Kurjačica CR 45 (G r d i n i ć, 1996); Lalić CR 74 (G r d i n i ć, 1996); Bački Monoštor CR 37 (G r d i n i ć, 1996); Stanišić Cr 58 (G r d i n i ć, 1996); Deronje CR 63 (G r d i n i ć, 1996); Bač CR 62 (G r d i n i ć, 1996); Kruščić CR 75 (G r d i n i ć, 1996); Odžaci CR 64 (G r d i n i ć, 1996); Sonta—Velike bare CR 55 (G r d i n i ć, 1996); Čonoplja CR 67 (G r d i n i ć, 1996); Sivac CR 76 (I g i ć, 1991; G r d i n i ć, 1996); Lipar CR 86 (I g i ć, 1991); Đurđin CR 88 (I g i ć, 1991); Mali Iđoš CR 96 (I g i ć, 1991); Lovćenac CR 95 (I g i ć, 1991); Svetozar Miletić CR 67 (I g i ć, 1991); Riđica CR 59 (B u d a k, 1998); Ruski Krstur CR 74 (B u d a k, 1998; G r d i n i ć, 1996); Srpski Miletić CR 54 (B u d a k, 1998); Bačka Palanka CR 71 (B u d a k, 1998; R a d o n i ć, 1979); Bačko Gradište DR 14 (B u d a k, 1998); Senćanski Trešnjevac DR 29 (B u d a k, 1998).

Banat region: Horgoš DS 11 (A n d r e j e v i ć, 1976); Kanjiža DS 20 (A n d r e j e v i ć, 1976); Sajan DR 47 (A n d r e j e v i ć, 1976); Zrenjanin DR 52 (V u k o v, 1999); Kuštilj EQ 28 (S e l e ž a n, 11. 06. 1973.); Sajan DR 47 (K n e ž e v i ć, 1990); Banatsko Aranđelovo DS 40 (K n e ž e v i ć, 1990); Zrenjanin DR 52 (K n e ž e v i ć, 1990); Mali Siget—Veliki Siget DS 40 (K n e ž e v i ć, 1990); Filić DR 39 (K n e ž e v i ć, 1990); Bočar DR 46; Mokrin DR 58; Torda DR 54; Banatski Dvor DR 64; Melenci—Ostrvo DR 44; Melenci—Rusanda DR 44; Melenci—Aradac DR 44—42; Perlez DR 50; Jazovo DR 38; Boka DR 82; Konak DR91; Alibunar DQ 99; Pavliš EQ 19; Vršac EQ 29; Vatin ER 11 (K n e ž e v i ć, 1990); Tomaševac DR 71 (S a v i ć, 1993); Orlovat DR 61 (S a v i ć, 1993; D r a š k o v i ć, 1996); Jarkovac DR 71 (S a v i ć, 1993); Deliblatska peščara EQ 07 (D i k l i ć , S p a s i ć, 1983; Deliblatski pesak, 1970); Stari Lec DR 91 (K n e ž e v i ć, 1990); the Vršac Mountain — Karaula EQ 39 (V u č k o v i ć, 1991); Mesić EQ 39 (V u č k o v i ć, 1991); Pančevo DQ 76 (B u d a k, 1998); Malo Središte EQ 39 (P a n j k o v i ć, 1983; V u č k o v i ć, 1991); Markovac EQ 39 (P a n j k o v i ć, 1983; V u č k o v i ć, 1991); Guzajna EQ 28 (P a n j k o v i ć, 1983; V u č k o v i ć, 1991); Gudurica ER 30 (P a n j k o v i ć, 1983; V u č k o v i ć, 1991); Široko Bilo EQ 39 (P a n j k o v i ć, 1983).

Srem region: Karlovčić DQ 26 (S t a n o j e v i ć, 1996); Petrovaradin DR 11 (HPM; C r n č e v i ć, 1994); Čortanovci DQ 29 (HPM); Petrovaradin—Tekije DR 11 (B u t o r a c, 1981); Beška DQ 29 (B u t o r a c, 1981); Kalakač DQ 29 (B u t o r a c, 1981); Pećinci DQ 17 (A c e v i ć, 1973); the Fruška Gora Mountain — Kukavica Valley DQ 19 (B u t o r a c, 1989); between Bačinci and Erdevik CQ 69—79 (B u t o r a c, 1989); Čerević CR 90 (O b r a d o v i ć, 1966); Bukovac — Iriški venac DR 00—10 (C r n č e v i ć, 1994); Jančikovac CQ 99 (C r n č e v i ć, 1994); Vetrenjača CQ 99 (C r n č e v i ć, 1994); Divoš CQ 89 (C r n č e v i ć, 1994); along the Rovača Brook DQ 09 (C r n č e v i ć, 1994); along the Mutalj Brook CQ 99 (C r n č e v i ć, 1994); between Divoš estate and the Jaroš Brook CQ 89 (C r n č e v i ć, 1994).

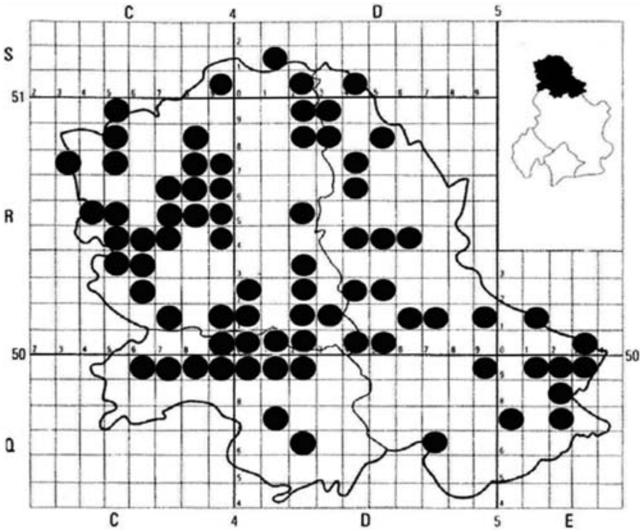

Fig. 7: Distribution *Lathyrus tuberosus* in the Vojvodina Province

A Eurasian (-Mediterranean) species. Occurs on calcareous, warm, dry and rich substrata, on neutral, humose, argillaceous and loess soils, in crops, grassland, weed phytocoenoses, barrens; infrequent in wet places. A perennial herb cultivated for fodder. Frequent in the Vojvodina Province. Chromosome number n = 7.

***L. latifolius* L. 1753 Sp. pl. ed 1: 733**

Distribution in the Vojvodina Province (Figure 8):

Bačka region: Bečej DR 25 (K o v á c s, 1929); Titel DR 40 (S t a n o j e v, 1983; P r o d á n, 1916); Futog CR 91 (P r o d á n, 1916); Lok DR 30 (S t a - n o j e v, 1980); Deronje CR 63 (P r o d á n, 1916); Lok — Vilovo DR 30—31 (S t a n o j e v, 1983).

Banat region: Vršački breg — Babin do EQ 39 (P a n j k o v i ć, 1983); the Vršac Mountain — Karaula EQ 39 (V u č k o v i ć, 1991); Magareći vrh EQ 29 (V u č k o v i ć, 1991).

Srem region: Karlovčić DQ 26 (S t a n o j e v i ć, 1996); Sremski Karlovci DR 10 (H i r c, 1919); Lipje DQ 09 (H i r c, 1919); Stražilovo DR 10 (HPM); Beleševo CR 90 (HPM); Širine DR 10 (HPM); Čortanovci DQ 29 (B u t o r a c, 1981; O b r a d o v i ć, 1966); Čortanovci — Beška DQ 29 (B u t o r a c, 1981); Venac DQ 09 (HPM); Sviloš CR 80 (O b r a d o v i ć, 1966); Banstol DR 10 (H i r c, 1919); Ledinci DR 00 (H i r c, 1919); Stražilovački breg — Lipe DR 10 (S t e v a n o v i ć, 1984); Glavica — Širine DR 00 (S t e v a n o v i ć, 1984); Bukovac DR 10 (S t e v a n o v i ć, 1984); Čerević CR 90 (S t e v a n o v i ć, 1984); Selište — Direk DR 10 (S t e v a n o v i ć, 1984); Banoštor CR 90 (S t e v a n o v i ć, 1984).

A sub-Mediterranean species. Cultivated as ornamental plant in the past. Occurs on calcareous, warm, dry or wet, porous, rich substrata, on neutral,

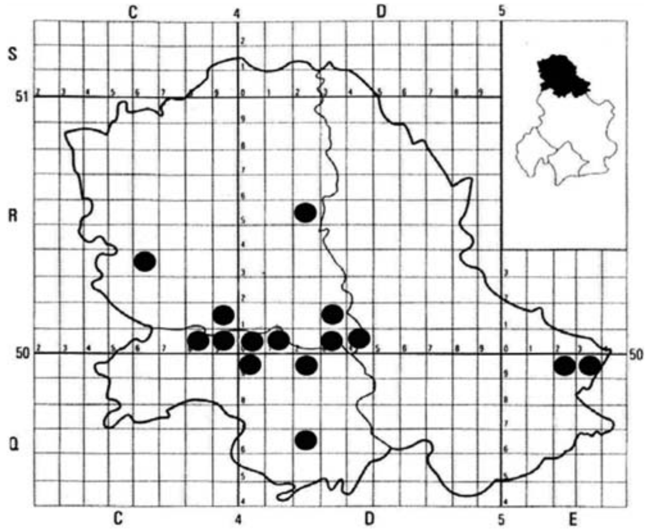

Fig. 8: Distribution *Lathyrus latifolius* in the Vojvodina Province

humose, argillaceous, loess and sandy soils, in barrens, mountain grassland scrubs, thin and dry oak forests, vineyards, rare on cultivated ground and along roadways. A perennial herb of no economic importance. Could be cultivated for fodder and as a melliferous species. Chromosome number n = 7.

***L. silvestris* L. 1753 Sp. pl. ed 1: 733**

Distribution in the Vojvodina Province (Figure 9):

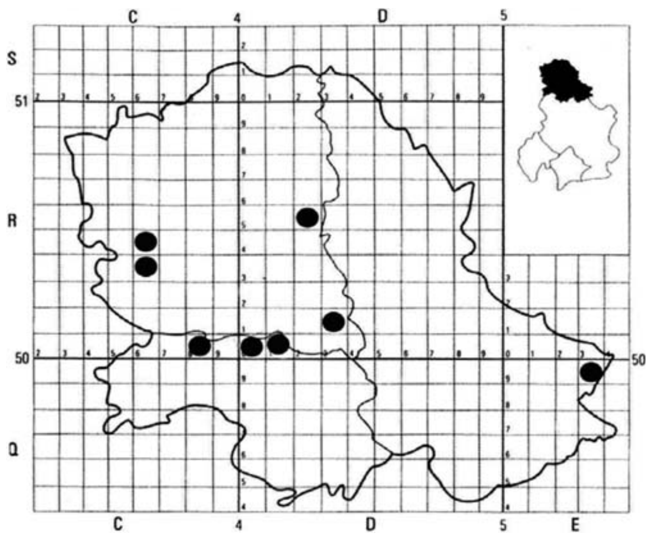

Fig. 9: Distribution *Lathyrus silvestris* in the Vojvodina Province

Bačka region: Bečej DR 25 (K o v á c s, 1929); Deronje CR 63 (P r o d á n, 1916); Mošorin DR 31 (S t a n o j e v, 1981); Bački Gračac CR 64 (G r d i n i ć, 1995).

Banat region: Vršački breg EQ 39 (BUNS).

Srem region: Sremski Karlovci DR 10 (O b r a d o v i ć, 1966); Susek CR 80 (B u g a r s k i, 1979); Venac DR 00 (O b r a d o v i ć, 1966); Karlovčić DQ 26 (S t a n o j e v i ć, 1996).

A European (-Mediterranean) species. Occurs on calcareous, warm, semi-dry or wet substrata and on neutral, humose, argillaceous and sandy soils, on edges of oak forests and hornbeam and European chestnut forests. A soil-fixing species. An important melliferous perennial containing up to 21% proteins, cultivated for fodder. Rare in the Vojvodina Province. Chromosome number n = 7.

Sect. Orobastrum Bois. 1872 Fl. or. 2: 601

L. sphaericus **Retz. 1785 Observ. bot. 3: 39**

Distribution in the Vojvodina Province (Figure 10):

Bačka region: Novi Sad DR 00

Banat region: northeastern slopes of the Titel Hill DR 41 (I g i ć et al., 1999)

Srem region: between Kamenica and Venac DR 00 (Z o r k ó c z i, 1896); Zmajevac DR 00 (BUNS); Širine DR 19 (HPM); Brankovac CR 90 (O b r a - d o v i ć, 1978); Andrevlje CR 90 (O b r a d o v i ć, 1978).

A south (-central) Eurasian species of sub-Mediterranean importance. Occurs on calcareous, warm, dry, porous and argillaceous soils, in barrens, on

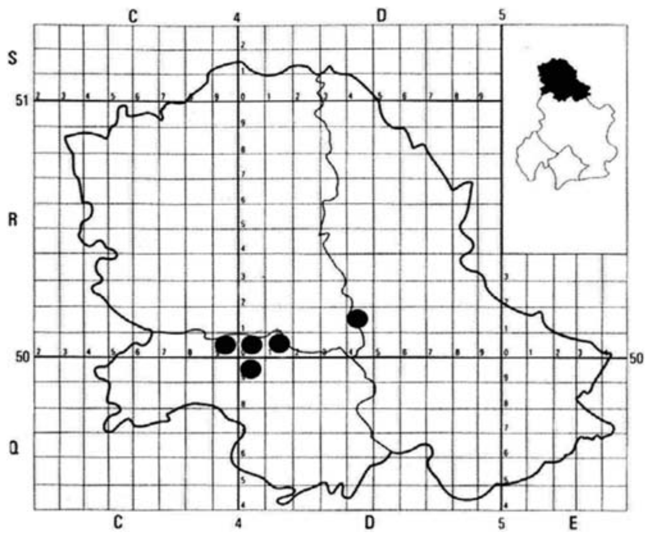

Fig. 10: Distribution *Lathyrus sphaericus* in the Vojvodina Province

rocky slopes. An annual herb of no economic importance. Could be cultivated for fodder.

***L. pratensis* L. 1753 Sp. pl. ed. 1: 743**

Distribution in the Vojvodina Province (Figure 11):

Bačka region: Kovilj DR 20 (Zorkóczi, 1896); Ratno ostrvo DR 01 (Zorkóczi, 1896); Kisač DR 02 (Kupcsok, 1915); Bečej DR 25 (Petrović 978; Kovács, 1925); Subotica CS 90 (Šabić, 1975); Kula CR 85 (Prodán, 1916; Budak, 1998); Lipar CR 86 (Igić, 1991); Srpski Miletić CR 64 (Budak, 1998); Bačka Palanka CR 71 (Budak, 1986); Doroslovo CR 55 (Purger, 1993); Deronje CR 63 (Prodán, 1916).

Banat region: Zrenjanin DR 52 (Vukov, 1999); Sečanj DR 82 (Knežević, 1990); Konak DR 91 (Knežević, 1990); Vršac EQ 29 (Knežević, 1990); Tomaševac — Botoš DR 71 (Savić, 1993); Sočica EQ 39 (Vučković, 1991); Gudurica ER 30 (Vučković, 1991); Jablanka EQ 39 (Vučković, 1991); the Vršac Mountain — Karaula EQ 39 (Vučković, 1991); Magareći vrh EQ 29 (Vučković, 1991); Široko bilo EQ 29 (Vučković, 1991); Mesić EQ 39 (Vučković, 1991); Markovac EQ 39 (Vučković, 1991); Guzajna EQ 28 (Vučković, 1991).

Srem region: Karlovčić DQ 26 (Stanojević, 1996); Čortanovci — Beška DQ 29 (Butorac, 1981); Banoštor CR 90 (Bugarski, 1976); Perljuša CR 80 (Bugarski, 1976); Obrež DQ 15 (Acević, 1973); Iriški venac DQ 09 (Obradović, 1966); the Fruška Gora Mountain — Paragovo DR 10 (Obradović, 1966); Sviloš — Ravno CR 80 (Obradović, 1966); Krušedol DQ 19 (Butorac, 1981); Rivica DQ 09 (Butorac, 1981); Ležimir CQ 89 (Butorac, 1981); the Vranjaš Brook valley CQ 99 (Butorac, 1981); the Mutalj Brook valley CQ 99 (Butorac, 1981); Privina Glava CQ

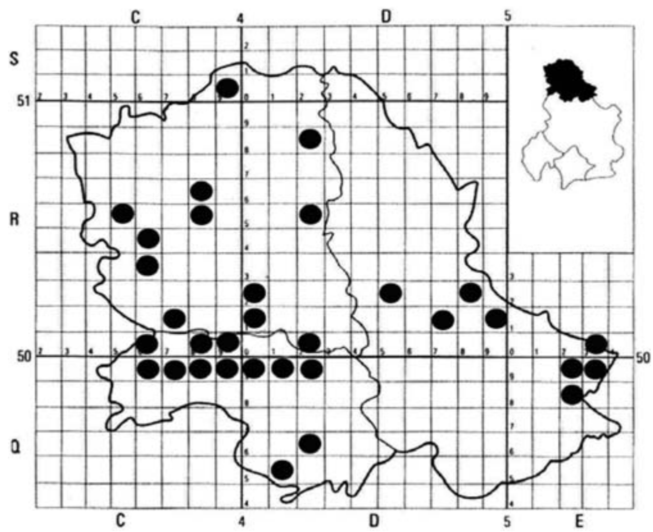

Fig. 11: Distribution *Lathyrus pratensis* in the Vojvodina Province

69 (B u t o r a c, 1981); the Kajnovac Brook valley DQ 19 (B u t o r a c, 1981); along the Luka Brook DQ 19 (B u t o r a c, 1981); between Erdevik and Sot CQ 79-CR 60 (B u t o r a c, 1981).

A Eurasian (-Mediterranean) species. Occurs on nitrogen rich, calcareous, wet, semiacid or neutral, humose, argillaceous, peaty and sandy soils, in grasslands, forests, ruderal, dry and wet habitats. A perennial herb cultivated for fodder which easily recovers after grazing. Frequent in the Vojvodina Province. Chromosome numbers n = 7 and 14.

***L. hallersteinii* Baumg. 1816, Euvm. Strip. Transs. 2: 333**

Distribution in the Vojvodina Province (Figure 12):
Srem region: Čerević, Katanske livade CR 90 (O b r a d o v i ć, 1966)

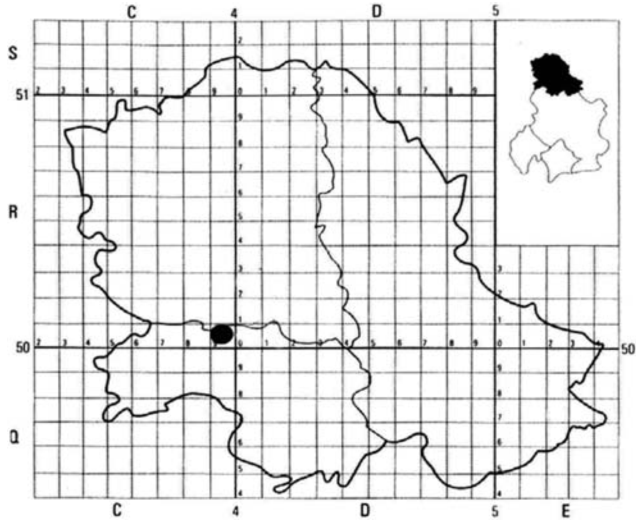

Fig. 12: Distribution *Lathyrus hallersteinii* in the Vojvodina Province

Widespread in Serbia, Greece, Rumania (K o j i ć, 1972), and Bulgaria (K o ž u h a r o v, 1976). Occurs on hill and mountain grasslands and in beech forests and shrubs. A Perennial herb (K o j i ć, 1972). Chromosome number n = 7 (B a l l, 1968). In the Vojvodina Province, found only in the Fruška Gora Mountain. The Fruška Gora population might belong to a form of *L. pratensis* since populations intermediary between *L. pratensis* and *L. hallersteinii* (B a l l, 1968) occur frequently in central parts of the Balkan Peninsula. Also, the species *L. pratensis* is highly variable in the Pannonian Plain (S o ó, 1966). The only distribution data for this species were presented by O b r a d o v i ć (1966).

***L. palustris* Baumg. 1816, Euvm. Strip. Transs. 2: 333**

Distribution in the Vojvodina Province (Figure 13):
Bačka region: Veternik DR 01 (B u d a k, 1978); Žabalj DR 22 (B u d a k, 1978); Bečej DR 25 (K o v á c s, 1929).

Srem region: Stražilovo DR 10 (HPM); Petrovaradin, Tekijski rit DR 11 (BUNS).

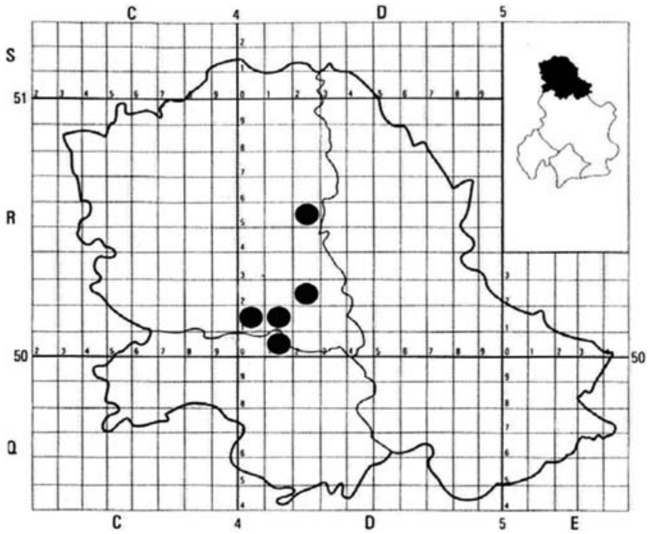

Fig. 13: Distribution *Lathyrus palustris* in the Vojvodina Province

A circumpolar species. Occurs on calcareous, alkaline, semiacid, humose, marshy, argillaceous and peat soils with fluctuating water regimen, on reedy terrains, in flooded grasslands and woods. A perennial herb of wet pastures containing app. 20% proteins, cultivated for fodder. Very rare in the Vojvodina Province. Chromosome number n = 21.

Sect. Orobus L. 1754 Gen. pl. ed. 5: 524

L. pannonicus (Kramer) Garcke 1863 Fl. Deutschl. G. Aufl.

Distribution in the Vojvodina Province (Figure 14):

Banat region: Vršački breg EQ 39 (BUNS); Deliblato Sands EQ 07 (Diklić, Vasić, 1983).

Srem region: Čerević CR 90; Andrevlje CR 90; Vrdnik DQ 09.

A Eurasian (-Mediterranean) continental species. Occurs on calcareous, wet, neutral, humose, argillaceous and peat soils, on reedy tarrains, and barrens. A forest-steppe plant. A perennial herb cultivated for fodder. Very rare in the Vojvodina Province, found only on the Fruška Gora Mountain, Deliblato Sands and Vrsački Breg. Chromosome number n = 7.

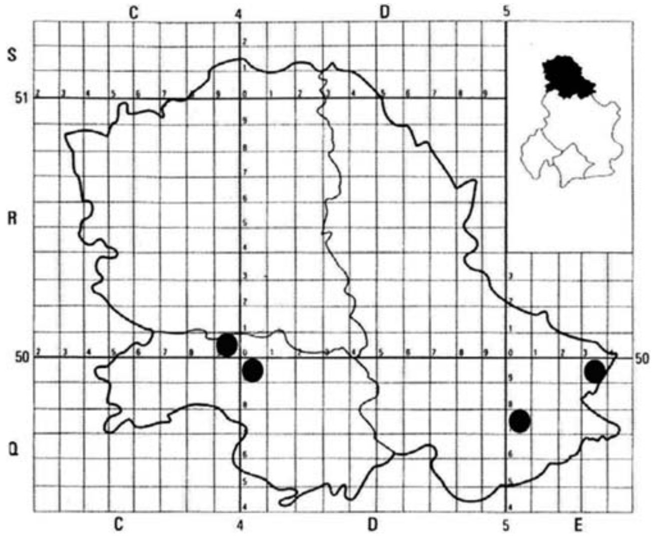

Fig. 14: Distribution *Lathyrus pannonicus* in The Vojvodina Province

L. vernus (L.) Bernh. 1800 Syst. Verz. Erf. 247

Distribution in the Vojvodina Province (Figure 15, C):

Bačka region: Deronje CR 63; Bođani CR 52 (Prodán, 1916).

Banat region: Vršački breg — Lisičja glava EQ 39 (Panjković, 1983).

Srem region: Klenak CQ 95 (Slavnić, 1954); Kupinovo DQ 25 (Slavnić, 1954); Stražilovo DR 10 (Hirc, 1914; Janković and Mišić, 1980); Grgeteg DQ 19 (Janković and Mišić, 1980); Venac DQ 09 (Obradović, 1966); Kamenički park DR 10 (Boža, 1981; Butorac, 1981); Zmajevac DR 00 (Janković and Mišić, 1980); Crveni čot DR 00 (Janković and Mišić, 1980); Venac DR 00 (Janković and Mišić, 1980); Testera CR 90 (Janković and Mišić, 1980); Dobre Vode DR 00 (Janković and Mišić, 1980); Crveni čot DR 00 (Janković and Mišić, 1980); Ravno CR 90 (Janković and Mišić, 1980).

A Eurasian (Eusiberian) continental species. Occurs on calcareous, porous, rich substrata, on alkaline, humose, argillaceous and flooded soils in deciduous forests, in forest of hornbeam and juniper. Rare in the Vojvodina Province. A parennial herb important for forest grazing. Chromosome number n = 7.

L. vernus (Mill.) Rouy et Foucard var. _banaticus_ (Heuff.) A. et G. 1910 Syn. 6, 2:1050

Distribution in the Vojvodina Province (Figure 15, đ):

Bačka region: Deronje CR 63 (Prodán, 1916); Bođani CR 52 (Prodán, 1916).

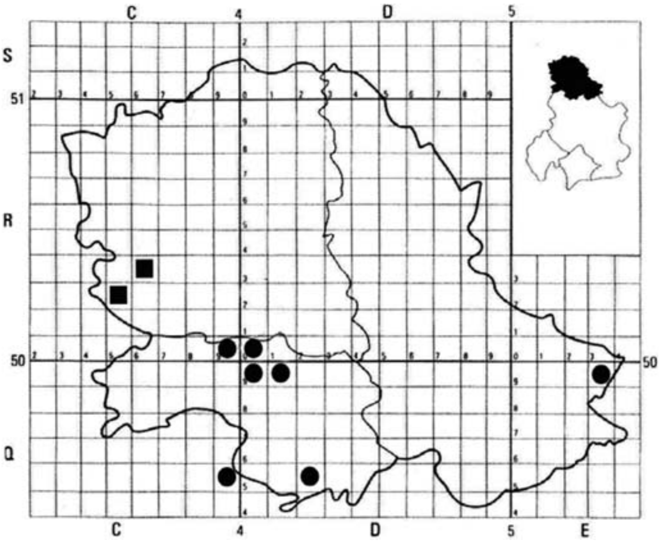

Fig. 15: Distribution *Lathyrus vernus* ● i *Lathyrus vernus* var. *banaticus* ■ in the Vojvodina Province

L. venetus (Mill.) Rouy et Foucard 1899 Fl. France 5: 254

Distribution in the Vojvodina Province (Figure 16):

Banat region: Široko bilo EQ 29 (P e k a n o v i ć, 1991); Guduričkí vrh ER 30 (P e k a n o v i ć, 1991); Vršački vrh ER 30 (P e k a n o v i ć, 1991), Korkana EQ 39 (P e k a n o v i ć, 1991), Kula EQ 39 (P e k a n o v i ć, 1991), Donji Všicor EQ 39 (P e k a n o v i ć, 1991), Mesić EQ 39 (P e k a n o v i ć, 1991),

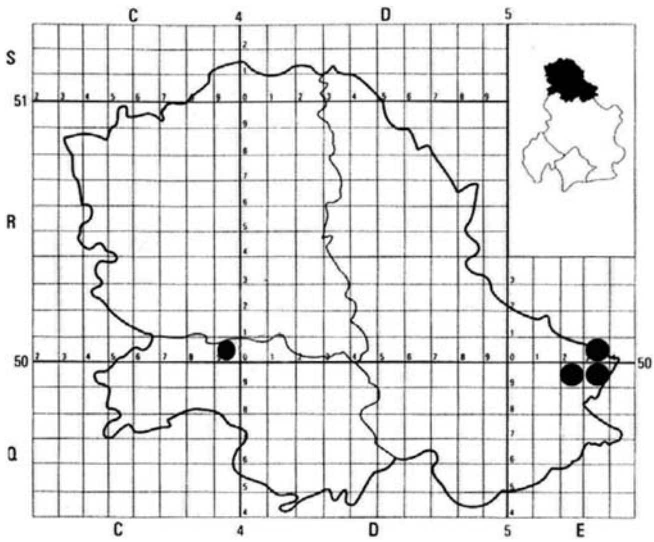

Fig. 16: Distribution *Lathyrus venetus* in the Vojvodina Province

Sočica EQ 39 (P e k a n o v i ć, 1991), surroundings of Markovci, around Karaula ER 30 (P e k a n o v i ć, 1991).

Srem region: the Fruška Gora Mountain CR 90 (Č o l o v i ć, 1956).

A Pontic-Mediterranean, Illyrian species. Occurs on calcareous, porous, rich substrata, on alkaline, humose, forest soils in hornbeam and beech forests. A perennial herb of no economic importance. Very rare in the Vojvodina Province. Chromosome number n = 7.

L. niger **(L.) Bernh. 1800 Syst. Verz. Erf. 248**

Distribution in the Vojvodina Province (Figure 17):

Bačka region: Bezdan CR 37 (P r o d á n, 1916); Apatin CR 45 (P r o d á n, 1916); Vajska CR 53 (P r o d á n, 1916).

Banat region: the Vršac Mountain—Karaula EQ 39 (V u č k o v i ć, 1991); Široko bilo EQ 29 (V u č k o v i ć, 1991; P a n j k o v i ć, 1983); Mesić EQ 39 (V u č k o v i ć, 1991); Sočica EQ 39 (V u č k o v i ć, 1991); Vršački vrh ER 30 (P e k a n o v i ć, 1991); Gudurički vrh EQ 39 (P e k a n o v i ć, 1991); Kula EQ 39 (P e k a n o v i ć, 1991); surroundings of Markovci near Karaula ER 30 (P e k a n o v i ć, 1991).

Srem region: Osovlje DR 00 (J a n k o v i ć et M i š i ć, 1980); Beočinske livade CR 90 (J a n k o v i ć et M i š i ć, 1980).

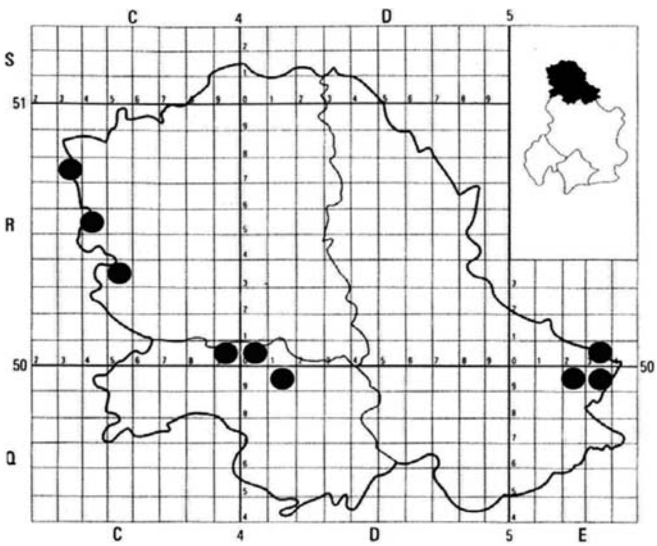

Fig. 17: Distribution *Lathyrus distribution* in the Vojvodina Province

A central European (-Mediterranean) species. Occurs on neutral, limeless, warm, semidry, humose, forest, argillacelous and sandy soils, in deciduous forests, in scrubs and in pine forests plantations. Rare in the Vojvodina Province. A perennial herb important for forest grazing. Chromosome number n = 7.

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РАСПРОСТРАЊЕЊЕ ВРСТА РОДА *Lathyrus* L. 1753 (Fabales, Fabaceae) У ВОЈВОДИНИ

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Резиме

У раду је дато распрострањење врсте рода *Lathyrus* у Војводини, на UTM картама са UTM кодовима и конкретним локалитетима. Подаци потичу из литературе која обухвата временски период од око 140 година, Хербаријума Департмана за биологију и екологију (BUNS) и сопствених теренских истраживања. Даје се њихов флорни елемент, односно ареал распрострањења, типови земљишта и станишта на којима расту, њихов привредни значај и број хромозома.

С обзиром да су дивљи грашкови значајне крмне биљке, могле би бити укључене у гајене или коришћене у селекцији и оплемењивању. У флори Војводине расте 16 врста рода *Lathyrus* од којих 11 већ има привредни значај, а још две би могле бити коришћене као крма. У току је њихово анатомско испитивање, што ће указати на ниво еколошке адаптације и употребљивост у исхрани стоке. Такође је у току физиолошка анализа врста, анализа макроелемената, концентрација пигмената и интензитет дисања и фотосинтезе, од чега зависи продукција органске биљне масе.

ЗАХВАЛНОСТ

Овај рад је део истраживања на пројекту број 1760 „Дивљи сродници гајених биљака: *Lathyrus* spp., *Trifolium* spp. и *Allium* spp.”, финансираног од стране Министарства за науку, технологију и развој Републике Србије.

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Iris spuria L. (*Iridaceae*) AT TWO NEW LOCALITIES IN THE VOJVODINA PROVINCE

ABSTRACT: *Iris spuria* L. was found at two new locations in the Vojvodina Province in 1997, both of them in the vicinity of Sombor: near the village of Kruševlje (CR59 according to 10x10 km UTM grid) and near the village of Bački Monoštor, in the Kozara Forest (CR47 according to 10x10 km UTM grid). Patches of the plants were sketched and observed until 2001. The populations in both locations had been stable, though under possible influence of humans (picking, burning) and animals: bloom-eater (*Trapinota hirta*), and wild pigs (*Sus scrofa*). Fencing off stand with *Iris spuria* L. population in the Kozara Forest is recommended.

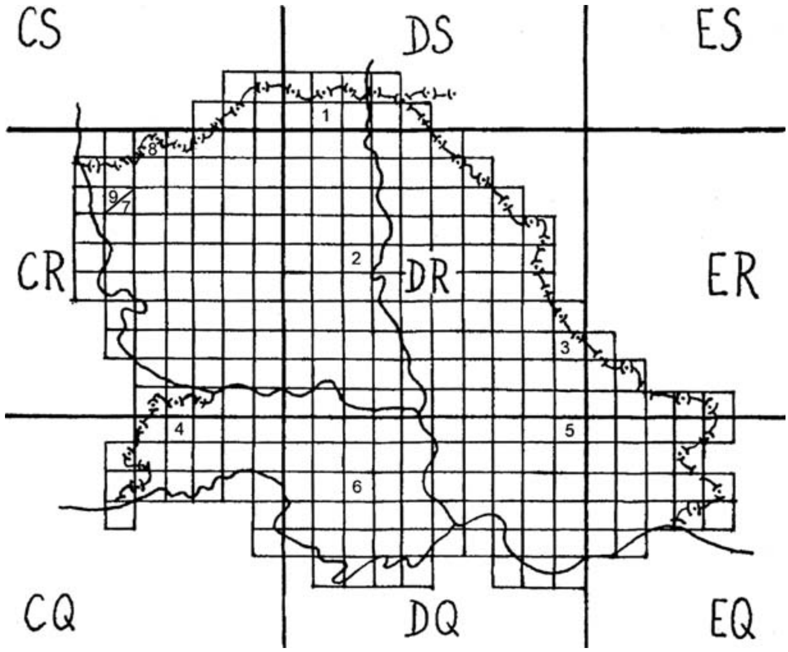
KEY WORDS: conservation, habitat, *Iris spuria*, population, Sombor, the Vojvodina Province

INTRODUCTION

Soó (1973) states that *Iris spuria* L. (*Iridaceae*) is a native in Western Mediterranean and the Pannonian Province with limited occurrence in Central Europe. According to *Flora Europea* (Tutin at al., 1980) the species commonly occurs from eastern Sweden to central Spain, north-eastern Greece and the southern Ukraine.

Iris spuria L. is a rare plant in the floras of the Republic of Serbia and the Vojvodina Province. It has been found in six locations in Serbia, five of these on the territory of the Vojvodina Province (Map 1). Fritsch (taken from Stjepanović-Veseličić, 1976) found it near Gornji Milanovac. Lengyel's (1915) finding of this species northwest of Horgoš was confirmed by Sturc (1973). Kovács (1929) reported (taken from Obradović et al., 1984), that it had disappeared from moist, saline, marshy black soil in the surroundings of Stari Bečej. It was found by Stojanović et al. (1985) near Konak, on the bank of a shallow canal next to a larger patch of shrubbery. Obradović and Boža (1987) found *Iris spuria* in eastern Srem, near the highway Belgrade-Zagreb, without providing a precisely described location.

K n e ž e v i ć (1994) reported a finding for Alibunar, S t o j š i ć and P a n j - k o v i ć (1998) for most places near Šimanovci and for the lowest part of Štrbac — Crna bara.



Map 1. Locations of *Iris spuria* in the Vojvodina Province
1 Horgoš; 2 Stari Bečej; 3 Konak; 4 Eastern Srem (approximation); 5 Alibunar; 6 Šimanovci; 7 Štrbac — Crna Bara; 8 Kruševlje 9 the Kozara Forest near Bački Monoštor

We supposed that *Iris spuria* is native in the vicinity of Sombor. In this paper we describe habitats where it was found in 1997, with estimates on the population status until 2001.

CLIMATE OF SOMBOR VICINITY

The climate is continental with certain specific local features. The annual mean temperature is 10.8°C. The coldest month is January with the average temperature of –1.8°C, the warmest is July with the maximum average of 21.3°C. The absolute minimum is –27.2°C, the absolute maximum 39.6°C (the amplitude is 66.8°C). Early autumn frost and late spring frost are usual, occurring approximately on 24th October and on 10th April, respectively. The earliest autumn frost occurred on 30th September and the latest spring frost on 3rd May. Due to temperature extremes, the local climate is distinctly continental.

Precipitation is largely influenced by cyclone activity. The total annual mean precipitation is 585.5 mm, with variations between 404 mm and 912 mm. The highest average (69.9 mm) occurs in June, the lowest (31.5 mm) in

March. Summer showers (short but heavy) occur frequently. Such distribution of precipitation belongs to the Central European or Danube basin precipitation regime.

According to Lang's rain factor which depends on temperature and precipitation, Sombor vicinity is a moderately humid climate zone, on the border between the steppe and forest types of climate.

The mean annual air humidity is 77.3%, with the highest average in January (87.8%) and the lowest in July (70.0%). The mean annual overcast is 58%, the mean annual insolation is 2171 hours, i.e., 49.4% of the possible insolation with respect to latitude. Northwesterly and northerly are the most frequent winds; koshava, a strong northeasterly wind, also reaches the region. Data on the local climate (for periods 1925—1940 and 1949—1968) were obtained from Dukanović (1970) and supplemented with data (for the period 1948—2000) from the Faculty of Agriculture, University of Novi Sad.

METHOD

The vicinity of Sombor was inspected during the flowering periods of *Iris spuria* in May and June, 1997—2001. The patches covered by fertile and sterile plants were measured and sketched. We counted all individuals and entered them on a millimeter paper (Figures 1 and 2). Status of the population was evaluated 10—15 times each year. Nomenclature of the registered plant species is given according to Soó (1964—1973).

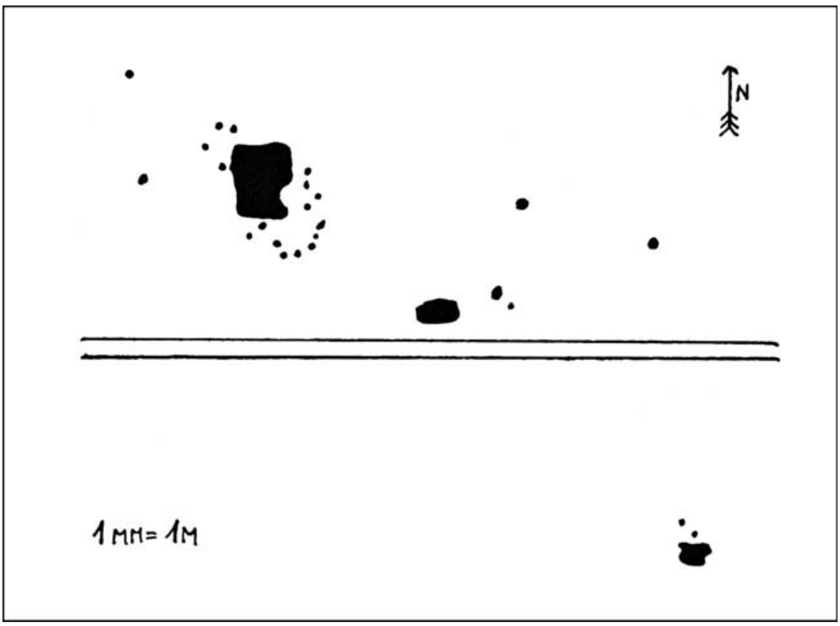


Fig. 1. Habitat of *Iris spuria* near Kruševlje.
Plants of *Iris spuria* are marked in black color.

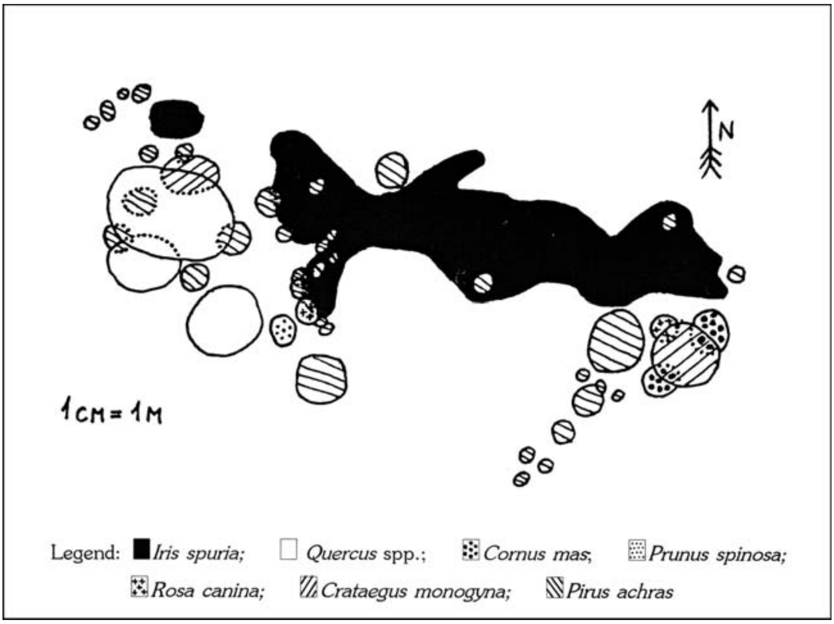


Fig. 2. Habitat of *Iris spuria* in the Kozara Forest near Bački Monoštor

RESULTS

Northeast of the Kruševlje village (CR59 according to 10x10 km UTM grid) approximately 800 fertile individuals of *Iris spuria* were found (Map 1, location 8). The average altitude of the site is 90 m. A drainage canal is 1.5 m deep (Figure 1). About 40 m far from the site, the terrain is about 50 cm lower and, because of a high salt accumulation, only *Lepidium crassifolium* W. et K. grows there. The drainage canal was covered with a typical marshy vegetation predomenated by *Phragmites communi*s T r i n., *Typha latifolia* L. etc. The surrounding flat area was uniformly overgrown by saline vegetation with small patches of meadow vegetation. The most abundant plants were *Euphorbia lucida* W. et K., *Tetragonolobus maritimus* (L.) R o t h., *Plantago schwarzenbergian*a S c h u r., *Plantago lanceolata* L., *Rhinanthus angustifolius* G m e l., *Achillea asplenifoli*a V e n t., *Serratula tinctoria* L., *Centaurea pannonica* (H e u f f .) S i m k., *Briza media* L. and *Ononis* sp. In this location, individuals of *Iris spuria* were fully insolated, both in terms of intensity and duration. A small group of *Iris spuria* individuals grew on the other side of the canal. It may be explained in two ways. The excavation of the drainage canal might have cut through the population of *Iris spuria*, destroying its central part, or it may be that the population expanded to the opposite bank. Since during our four-year observations there were no notable signs of change in the size of population, we concluded that the first option is more probable.

The neighboring meadows are used for haymaking, but the stands of *Iris spuria* are not mowed due to the abundance of *Ononis* sp. In 1998, the site was exposed to fire which spread from the neighboring stubble field. The greater part of seeds inside pods remained undamaged because the firefront swept rapidly across the site. A real danger to the population was the occurrence of a bloom-eater *Trapinota hirta*, the beetle which hatched in large numbers (2—3 insect per inflorescence) in 1999 and almost completely destroyed the flowers. That year, seedpods were very rare.

North of the village of Bački Monoštor (CR47 according to 10x10 km UTM grid), in the Kozara Forest, approximately 80 fertile individuals were found (Map 1, locality 9). The altitude was 91m and the patch itself was in a 10—20-cm microdepression (Figure 2). The vegetation consisted of the herbaceous, shrub and tree layers. With regard to the position and height of the surrounding shrubs and trees (*Quercus* sp., *Cornus mas* L., *Prunus spinosa* L., *Rosa canina* L., *Crataegus monogyn*a J a c q., *Pirus achra*s G a r t n.; Figure 2), the *Iris spuria* patches receive 40% of the annual insolation. The following plant species were found around the iris patches: *Tritolium campestr*e S c h r e b., *Trifolium incarnatum* L., *Chamaecytisus ausfriacus* L i n k., *Genista tinctoria* L., *Cynanchum vincetoxicum* L., *Dorycnium germanicum* (G r e m l i) R i n k l i, *Euphorbia cyparissias* L., *Centaurium vulgar*e R a f. subsp. *uliginosum* (W. et K.) S o ó, *Filipendula vulgari*s M ö n c h., *Anthoxanthum odoratum* L., *Aster punctatus* W. et K. subsp. *canu*s S o ó, *Lysimachia nummularia* L. and *Prunella laciniata* (L.) N a t h.

The population of *Iris spuria* near Bački Monoštor is endangered. It is situated near a forest path and inflorescences are frequently picked by passers-by. The main problem are wild pigs. They dig out rhizomes and either eat them or leave them to dry in the sun. Although the Kozara Forest belongs to the Gornje Podunavlje Protected Area, it seems that additional protection is needed. The stands should be fenced to keep out wild pigs and signs should be posted warming people to save this rare species.

CONCLUSION

Iris spuria was found in two locations in the vicinity of Sombor, near the villages of Kruševlje and Bački Monoštor (the Kozara Forest). The two sites and the conditions in them are different. Near Kruševlje, about 800 individuals grow in a meadow while in the Kozara Forest about 80 flowering plants grow in a brush.

The factors which greatly influence the survival of the *Iris spuria* populations are: human activity (mowing, burning, collecting), bloom-eater (*Trapinota hirta*) and wild pigs. The population in the Kozara Forest should be fenced for improved protection.

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Iris spuria L. (*Iridaceae*) НА ДВА НОВА ЛОКАЛИТЕТА У ВОЈВОДИНИ

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Резиме

Током 1997. године у околини Сомбора је пронађена *Iris spuria* на два локалитета. Североисточно од села Крушевље (на УТМ мрежи 10x10 km у квадрату ЦР59) (Карта 1. локалитет 8) расте око 800 биљака *Iris spuria* које цветају. Надморска висина терена је 90 m, прилично је уједначена, а неравнине су мање од 30 cm. Једина већа депресија је канал за одводњавање дубине око 1,5 m. Околну вегетацију чине нискорастуће, једно- и вишегодишње зељасте биљке, па је *Iris*

spuria изложена пуном осунчавању, како у погледу јачине, тако и у погледу трајања. Популација *Iris spuria* је посматрана до 2001. године. Установљено је да је она стабилна у погледу броја цветајућих биљака. Станиште је 1998. године захватила ватра. Број оштећених семенки у чаурама је био мали јер је ватра трајала кратко. Током 1999. године тврдокрилац цветојед (*Trapinota hirta*) био је многобројан све време цветања. Те године чауре са семенкама су биле права реткост. Други локалитет се налази у шуми Козара, северно од села Бачки Моноштор (На УТМ мрежи у квадрату ЦР47) (Карта 1. локалитет 9). На овом станишту расте око 80 биљака *Iris spuria* које цветају (Скица 2). Надморска висина терена је 91 m, а сама оаза *Iris spuria* је у микродепресији дубокој 10—20 cm у односу на околни терен. Околна вегетација се састоји из три спрата: зељастих, жбунастих и спрата дрвенастих биљака! С обзиром на положај и висину дрвећа и жбуња, биљке *Iris spuria*, добијају око 40% осунчавања на годишњем нивоу. Оаза *Iris spuria* се налази у близини шумског пута, па људи беру ове биљке у време цветања. Ипак највећу штету причињавају дивље свиње које ријући земљу и тражећи храну ископавају ризоме *Iris spuria* из земље. Ископане ризоме дивље свиње поједу или се они осуше и пропадну. Мада је шума Козара у оквиру заштићеног подручја Горње Подунавље, сматрамо да би било потребно додатно заштитити популацију *Iris spuria*, ограђивањем станишта спречити прилаз дивљих свиња, а за људе би требало ставити упозорење да не беру цвасти и не уништавају примерке ове ретке врсте.

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POPULATION DYNAMICS OF *Azotobacter chroococcum* IN SUGARBEET RHIZOSPHERE DEPENDING ON MINERAL NUTRITION

ABSTRACT: Population dynamics of *Azotobacter chroococcum* has been studied in the rhizosphere of a sugarbeet hybrid inoculated with *Azotobacter* strains 5, 8 and 14. Simultaneously we examined the effects of four levels of nitrogen fertilization (non-fertilized control, 50, 100, 150 and 200 kg N/ha) and the applications of manure and harvest residues. Samples were taken three times, in May, July and October. The experiment included inoculated and non-inoculated variants at all four levels of fertilization, in five replicates.

KEY WORDS: *Azotobacter chroococcum*, inoculation, mineral fertilizers, N-fixing bacteria, rhizosphere

INTRODUCTION

Numerous experiments on plant inoculation with free N-fixing bacteria such as *Azotobacter* have shown that it is difficult to foresee plant response to inoculation. Obviously, plant — bacterium interactions are complex and unstable and they vary in dependence of plant genotype and bacterial strain (Sarić et al., 1987; Vassyuk, 1989; Mrkovački, Milić, 2001). The importance of plant genotype was exhibited in experiments with different *Azotobacter* strains (Mrkovački et al., 1995, 2001; Mezei et al., 1999). On the other hand, it was shown that inoculation efficiency is increased when bacterial strains are isolated from a plant species that is to be inoculated (homologous strains) (Arte et Shende, 1981; Wani et al., 1985). Furthermore, only some strains are capable of forming successful associations with several plant species.

To be able to establish a population status, i.e., the number of bacteria in plant rhizosphere, it is essential to have insight into the success of survival of bacterial strains introduced by inoculation. However, in most experiments, especially those on *Azospirillum*, no correlation could be found between the number of applied bacteria and their effect on plant development (Kesava Rao et al., 1990; Bashan and Levanony, 1990; O'Hara et al., 1981). Some degree of association was established between grain yield increase in four barley varieties and the rate of survival of the applied *A. brasilense* (Tilak and Murthy, 1983). This finding opens the question whether an active colonization of rhizosphere (root) with applied bacteria is sufficient to ensure their positive effect on plant development (Belimov et al., 1995).

The objective of this study was to establish the population dynamics of *Azotobacter* in inoculated and non-inoculated plants, i.e., to assess the effect of inoculation on *Azotobacter* population size in sugarbeet rhizosphere in dependence of fertilization intensity.

MATERIAL AND METHODS

Field experiments were conducted on a chernozem soil, in the location of Rimski Šančevi, in 2002. Sara, a sugarbeet hybrid variety developed at the Institute of Field and Vegetable Crops in Novi Sad, was used in the study. Sugarbeet seeds were inoculated by incorporating a culture of *Azotobacter* strains, with the density of 109/ml, into the soil directly before planting. The strains had been isolated from the rhizospheres of the varieties Dana and Hy-11.

The experiments were set up in a block system in five replicates. Seeds in the control were not inoculated. Four variants of nitrogen fertilization were tested (50 kg N/ha, 100 kg N/ha, 150 kg N/ha, 200 kg N/ha) in addition to a variant without nitrogen. Beside the effects of different NPK intensities, we also analyzed the effect of the application of manure and harvest residues.

Samples for microbiological analyses of rhizospheric soil were taken three times, in May, July and October. The number of Azotobacters was determined by the drop method on the Fiodorov medium and expressed per 1 g of absolutely dry soil.

RESULTS AND DISCUSSION

The highest counts of *Azotobacter*, in both inoculated and non-inoculated variants, were obtained at the beginning of the growing season (1st date). These results were in agreement with those of Sarić (1972, 1978). In the chernozem soil and at the beginning of the season, the *Azotobacter* population was more numerous under sugarbeet than under wheat, corn or sunflower (Sarić, 1978).

The *Azotobacter* population increased in all inoculated variants with NPK, the non-fertilized variant and on all sampling dates. On average for all dates, the highest increase was obtained in variants with 150 and 100 kg N/ha. The lowest increase was registered in the variants with 50 and 200 kg N/ha (Table 1). The largest *Azotobacter* population among the inoculated variants was obtained in the variant which received no nitrogen. Lowest *Azotobacter* populations were obtained with 200 kg N/ha in both inoculated and non-inoculated variants. This was an indication of the inhibitory effect of mineral fertilizers, especially when applied in high doses, on the population size of *Azotobacter*.

Tab. 1. Dynamics of *Azotobacter* population in the rhizosphere of the variety Sara depending on fertilization intensity (NPK)

Date	Inoculation	kg N/ha					
		ø	50	100	150	200	Average
I	–A	108.32	126.29	70.91	40.91	33.62	76.01
	+A	258.44	187.09	174.42	115.59	92.74	165.65
II	–A	82.19	87.02	63.07	19.30	54.1 2	61.14
	+A	144.44	113.71	104.95	62.28	68.84	98.84
III	–A	95.22	71.08	57.88	64.88	23.58	62.52
	+A	107.63	156.96	182.09	34.54	30.55	102.35
Average	–A	95.24	91.79	63.95	41.69	37.11	
	+A	170.17	152.58	153.88	104.14	64.04	

All variants of nitrogen application in combination with manure as well as the non-fertilized variant had an increased number of *Azotobacters* in relation to the non-inoculated control variant (Table 2). The highest increase was achieved in the variant with 100 N/ha, the lowest by the variant with 50 kg N/ha. On average for all dates, the lowest numbers of *Azotobacter* were registered with 200 kg N/ha in both inoculated and non-inoculated variants. The highest *Azotobacter* population was found in the variant with inoculation and no nitrogen.

Tab. 2. Dynamics of *Azotobacter* population in the rhizosphere of the variety Sara depending on fertilization (NPK) + manure

Date	Inoculation	kg N/ha					
		ø	50	100	150	200	Average
I	–A	101.49	124.81	50.61	102.66	58.69	87.65
	+A	259.48	151.80	158.86	191.89	130.94	178.59
II	–A	51.73	91.76	32.56	45.01	30.87	50.38
	+A	84.75	125.91	74.42	70.66	99.05	90.96
III	–A	49.57	59.91	47.36	28.99	38.75	44.92
	+A	75.46	81.73	65.23	46.46	39.95	61.77
Average	–A	67.59	92.16	43.51	58.88	42.77	
	+A	139.89	119.81	99.50	103.00	89.98	

Largest increases in *Azotobacter* population were obtained when fertilization was combined with harvest residues on the 2nd date, different from Table 1. and Table 2. The highest increase was registered in the variant with 200 and 100 kg N/ha, the lowest in the variant with 150 kg N/ha (Table 3). The number of Azotobacters was somewhat diminished on the 2nd date while the 3rd date remained at the level of the 2nd. This was similar to the variants that received only different nitrogen doses (Table 4).

Tab. 3. Dynamics of *Azotobacter* population in the rhizosphere of the variety Sara depending on fertilization (NPK) + harvest residues

Date	Inoculation	kg N/ha					
		ø	50	100	150	200	Average
I	–A	121.11	215.15	130.23	101.41	49.96	123.57
	+A	196.75	217.67	166.21	145.63	102.42	165.74
II	–A	193.80	128.84	69.72	61.88	19.44	94.74
	+A	205.79	226.90	160.29	68.56	61.96	144.70
III	–A	85.18	167.02	153.62	85.98	86.53	115.66
	+A	135.79	203.24	183.13	89.71	95.66	141.51
Average	–A	133.36	170.34	117.86	83.09	51.98	
	+A	179.44	215.94	169.88	101.30	86.68	

Tab. 4. Dynamics of *Azotobacter* population in the rhizosphere of the variety Sara depending on fertilization (NPK) + manure + harvest residues

Date	Inoculation	kg N/ha					
		ø	50	100	150	200	Average
I	–A	119.77	185.28	61.47	120.40	85.33	114.45
	+A	160.10	194.90	166.82	150.48	149.36	164.33
II	–A	97.34	117.05	52.33	79.01	24.17	73.98
	+A	220.81	190.66	228.82	85.46	73.26	159.80
III	–A	105.54	67.88	94.21	70.87	81.43	83.98
	+A	127.46	51.60	38.99	27.02	35.50	56.11
Average	–A	107.55	123.40	69.34	90.09	63.64	
	+A	169.46	145.72	144.88	87.65	86.04	

The combining of nitrogen fertilizer, manure and harvest residues had a different effect on the *Azotobacter* population size than when these three types of fertilizers were applied alone. On average, the largest population was found in the non-fertilized variant, which is in agreement with the previous results. On the 3rd date of sampling (October), however, *Azotobacter* populations in the inoculated variants, regardless of nitrogen dose (50, 100, 150 and 200 kg N/ha), were decreased in relation to their non-inoculated counterparts. On the average for the three dates of sampling, the dose of 150 N/ha reduced the *Azotobacter* populations in the inoculated variants as compared with the non-inoculated ones.

Our 1998 study which included different fertilization intensities (0, 40, 80 and 120 kg/ha) showed that the yields of sugarbeet roots and crystal sugar increased with all fertilization intensities. The highest increase was achieved with 80 kg N/ha. The inoculation without fertilization, i.e., without nitrogen application, brought the highest increase in sugar content (%). It is well known that high doses of nitrogen fertilizers inhibit the effect of nitrogen-fixing bacteria. Results of a 5-year study on sugarbeet (S a r i ć, 1978) indicated that nitrogen application and the lowest dose of NPK tended to stimulate *Azotobacter* activity. It is well known that high doses of nitrogen fertilizer inhibit the activity of nitrogen-fixing bacteria.

This study showed that inoculation had the highest effect on the *Azotobacter* population in the rhizosphere of the variety Sara in the variant with 100 kg N/ha. The lowest effect was registered in the variants with 200 and 150 kg N/ha.

The highest *Azotobacter* population in the rhizosphere of the variety Sara was achieved with the combined application of harvest residues and 50 kg N/ha.

The *Azotobacter* population density peaked at the beginning of the season (in May) and then it decreased towards the end of the season.

CONCLUSIONS

— *Azotobacter* populations were largest, in both non-inoculated and inoculated variants, at the beginning of the season (1st date) and they decreased subsequently.

— The inoculation of the rhizosphere of the variety Sara increased the number of *Azotobacters* in relation to the non-inoculated variant at all three dates. The highest increases were registered in the variants with NPK and NPK + manure (1st date) and in the variant with harvest residues and harvest resiued + manure (2nd date).

— Inoculation increased the number of *Azotobacters* in sugarbeet rhizosphere at all four intensities of nitrogen fertilization. The increase was highest in the variant with 100 kg N/ha. The increase was lowest in the variant with 150 kg N/ha.

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ДИНАМИКА БРОЈНОСТИ *Azotobacter chroococcum* У РИЗОСФЕРИ ШЕЋЕРНЕ РЕПЕ У ЗАВИСНОСТИ ОД МИНЕРАЛНЕ ИСХРАНЕ

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Резиме

Резултати многих експеримената инокулације биљака са слободним азотофиксаторима као што је *Azotobacter* показали су да је веома тешко предвидети одговор биљке на инокулацију. Ово указује да су интеракције између биљака и бактерија веома сложене, нестабилне и варирају у зависности од генотипа, како биљака тако и бактерија (С а р и ћ et al., 1987; V a s s y u k, 1989; М р к о в а ч к и, М и л и ћ, 2001). Циљ овог рада је био да се утврди динамика *Azotobactera* у ризосфери инокулисаних и неинокулисаних биљака, т.ј. ефекат инокулације на број *Azotobactera* код шећерне репе у зависности од ђубрења. Пољски огледи изведени су на Римским Шанчевима у 2002. години, на земљишту типа чернозем са хибридном сортом шећерне репе Сара, селекционисаном у Научном институ-

ту за ратарство и повртарство у Новом Саду. Инокулација семена шећерне репе је извршена тако што је култура сојева *Azotobactera* густине 10^9/ml (5, 8, 14) инкорпорирана у земљиште непосредно пре сетве. Сојеви су изоловани из ризосфере Dane и Hy-11. Узорци за микробиолошке анализе ризосферног земљишта узимани су у три рока: у мају I рок, јулу II рок и у октобру III рок. Број *Azotobactera* одређиван је методом капи на Фјодоровој подлози. Бројност је одређивана методом разређења и изражена на 1 грам сувог земљишта. На неинокулисаним и инокулисаним варијантама највећи број *Azotobactera* добијен је почетком вегетације (I рок), а затим опада. Инокулацијом је повећан број *Azotobactera* у односу на неинокулисану варијанту у ризосфери Саре у сва три рока. Инокулацијом је добијено повећање броја *Azotobactera* у ризосфери, са сва четири нивоа ђубрења азотом, највеће са додатком са 100 kg N/ha, а најмање са 150 kg N/ha.

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FISTULAS SECONDARY TO GYNECOLOGICAL AND OBSTETRICAL OPERATIONS

ABSTRACT: The authors present urogenital and rectogenital fistulas treated at the Department of Obstetrics and Gynecology in Novi Sad in the period from 1976 to 1999. The study comprised 28 cases of fistula out of which 17 were vesicovaginal, 3 ureterovaginal, 1 vesicorecto vaginal and 7 recto vaginal. During the investigated period there were 182 Wertheim operations, 3864 total abdominal hysterectomies, 1160 vaginal hysterectomies and 7111 cesarean sections. The vesicovaginal fistulas were most frequent with the incidence of 0.33%, whereas the tocogenic fistulas did not occur. Urogenital fistulas secondary to radical hysterectomy are extremely rare thanks to the administered measures of prevention during the surgical procedure.
KEY WORDS: fistulas, gynecological surgery, urology

INTRODUCTION

The fistulas occuring after either gynecological or obstetrical operations can be divided into two basic groups:

— genitourinary,
— other locations.

In some undeveloped countries, the condition of permanent urinary incontinence has been a frequent consequence of nonprofessional conduct of labor (tocogenic fistulas), whereas in developed countries, fistulas most often occur secondary to gynecological operations resulting from injuries to the ureter, urinary bladder and urethra. The treatment of genitourinary fistulas can be complicated and success depends on medical treatment, surgical technique and the experience of the operator.

MATERIAL AND METHODS

Fistulas secondary to gynecological operations and their prevention

Most surgical procedures performed in the region of the uterine cervix bear risk of injury due to close anatomic-topographic relations with the ureter and urinary bladder. Trauma injuries during the operation may occur in abdominal radical hysterectomy, abdominal hysterectomy, myomectomy of low-located myomas in the anterior uterine wall, endometriosis, chronic inflammatory tumors, sepsis, altered anatomic relations in disturbed statics of genital organs.

In classic hysterectomy it is of importance to have good knowledge or the vesico-cervical region. Upon the incision of the vesicouterine plica, it is important to enter the proper "cleavage" between the bladder and the anterior vaginal wall. The vagina, cervix and bladder are covered by a layer of endopelvic connective tissue in difference from the firm whitish cervicovaginal fascia. The knowledge on these anatomic relations is of utmost importance in prevention of vesicovaginal fistulas. It is most appropriate to use sharp sciccors to separate the bladder from the middle part, in the direction to the vesico-cervico-vaginal ligament (anterior parametria). In case the bladder is separated "bluntly" by a gauze pledget, no matter if entering the proper area, unnecessary injuries and tears may occur with the consequential bleeding. When an injury to the urinary bladder is not recognized and treated in time, an involuntary urine leakage will occur soon following the operation. Under different circumstances, the unrecognized bladder injury may cause the development of a hematoma, which is a convenient soil for the development of infection. In case that the proper drainage accross the vaginal vault has not been established, the bladder wall suffers the pressure, necrotizes and the urinary fistula develops between the urinary bladder and the vaginal vault.

Vaginal operations may also be accompanied by possible urinary tract injuries. Most urinary bladder injuries occur during the incision of the vesicouterine plica. The urethrovesical, layer has always been disposed to injury risk in anterior colporrhaphy, both for direct trauma during operation and possible late necrosis caused by improper placement of the sutures (P. Draca et al., 1979).

Vaginal hysterectomy and Manchester operation bear great risk of injury to the ureter because of their close location to the adjacent uterine cervix. Therefore during the vaginal hysterectomy, after the incision of the vesicouterine plica, the urinary catheter is inserted, elevating the urinary bladder and separating the ureters from the uterus during the surgical procedure (Draca P. et al., 1981, 1984, 1986).

The prevention of urethrovaginal fistulas in radical abdominal hysterectomies is done by preparation of the "Novak's roof" and ureteral mesentery as well as by extraperitoneal abdominal drainage of the parametrial fossae (Draca P., 1973, 1974, 1979). In preoperative intracavitary irradiation for uterine carcinoma, the most optimal time for operation is the period following the three post-irradiation weeks (Draca P. et al., 1980). This is the period when the hyperemia disappears and the postoperative fibrosis has not yet developed. Although the preoperative irradiation improves the five-year survival rate, the very procedure contributes to increased incidence of fistula development.

The following procedures are of significance in the prevention of ureteral injuries during gynecologic operations:

— Complete separation of the urinary bladder and anterior wall of the uterine cervix.

— Clamping, dissection and ligation of the vesicocervical ligament divide the ureter form the operative field, separating it from lateral walls, decreasing the risk of ligation of the terminal part of the ureter and its angulation.

— Correct and complete mobilization of the ureters enables their separation from the lateral walls of the uterus, where clamps and ligatures to a uterine are placed, often resulting in injuries of the ureter.

In intraligamentous myomas operations, the ureter is usually placed either downwards or downwards and out and it is usually adherent to the pseudocapsule of the myoma. The safest procedure is an incision of the peritoneum in the posterior wall of the broad ligament — at the level of a. uterina, and its ligation and dissection with the subsequent enucleation of the myoma. The ureter is left free with its normal adjunction to the peritoneum. The enucelation should be done within the capsule as to avoid injuries to the ureter or its adventitia.

In atypical operations of intraligamentous cysts, tubo-ovarian inflammatory tumors attached to the posterior sheat of the broad ligament or endometriosis spreading to the lateral parametria, it is most important to identify the ureter at the level of its meeting the a. iliaca and follow its descending path. This is a delicate part of the operation with the expecting bleeding episodes and possible injuries to the ureteral adventitia — therefore, a separation of the ureters form the peritoneum has been recommended.

Particular attention is needed in peritonization of the edges of the broad ligament. The ureter can adhere to the posterior sheath of the broad ligament immediately beyond the ligature of the infundibulopelvic ligament. The approachment of peritoneal edges enables the ligature of the ureter.

In vaginal approach to the operation of vesicovaginal fistulas, it is necessary to identify the relation of the fistula and the ureteral orifice.

Cystoscopy and urography have to be performed in order to identify the existing anatomic condition. If the orifices are very close, it is necessary to introduce the urinary catheter.

Tocogenic fistulas and injuries to the urinary tract durig labor

Tocogenic fistulas have been practically missing in pathological conditions in our environment because of an active attitude of the obstetrician in conducting the labor. Due to the increased incidence of operative completion of delivery — cesarean section and repeated cesarean section — possible injuries to the ureter during the low segment transverse cesarean section have to be taken into account, particularly in case of a tear of the uterus spreading to the lateral parametria, sometimes involving the ureters which are firmly attached to the lateral uterine wall. Recognition of these injuries is aggravated by bleeding from the adjacent torn venous vessels. Therefore in cesarean section, the tears in the uterus spreading to the lateral parametria always need revision, because unrecognized injuries to the ureter inwariably lead to the development of fistulas and to other severe complications.

Table 1 — Urogenital fistulas registered at the Department of Obstetrics and Gynecology in Novi Sad in the period 1976—1999

	HTA	HTA Radical	C. S.	C. S. HTA	Colporrhaphy	Episiotomy	Sling	HV	Unknown	Total
Vesicovaginal	10	4	1	1					3	17
Ureterovaginal		2		1						3
Vesicorectovaginal		1								1
Rectovaginal					2	1		1	1	3

Table 2 — Other sites of urogenital fistulas

	HTA	HTA Radical	C. S.	C. S. HTA	Colporrhaphy	Episiotomy	Sling	HV	Unknown	Total
Abdomina wall							1	3		4
Ileoparietoabdomen							1		1	
Vaginoparietoabdomen							1			1
Perineovaginal					2					2
Cervicovaginal									1	1
Perineal					4					4

During the investigated period, there were 138 Werthwim operations, 3864 total abdominal hysterectomies (HTA), 28 subtotal abdominal hysterectomies, 1160 total vaginal hysterectomies (HTV) and 7111 cesarean sections (CS).

In the investigated sample, vesicovaginal fistulas occured with the highest incidence — 17 or 0.33% od the total number of abdominal hysterectomies which were the most frequently performed operations. Conversely, there was not a single case of genitourinary fistula in vaginal hysterectomies.

Abdominal hysterectomy is an operation which any young gynecologist must be able to perform, as far as it needs a minimum of surgical knowledge of any gynecologist and obstetrician, but the lack of experience and the failure to recognize an injury while an operation is still in progress often cause the development of fistulas. Inflammatory processes in the small pelvis, endometriotic lesions and disturbed anatomical position in case of large uterine myomas may also be a cause for development of fistulas.

The fistulas developing secondary to radical hysterectomy — ureterovaginal fistulas — occured in 1.64% (3 patients) localized at the distal part of the ureter. All three patients underwent preoperative cavital irradiation. One patient developed a rectovesicovaginal fistula.

The fistulas occuring secondary to cesarean section always developed following the local infection or unrecognized injury during operation. Two patients with the repeated cesarean section suffered a tear in the uterus spreading towards the lateral parametria, involving the ureters which were firmly attached by connective tissue to the lateral uterine wall. In both patients the unjuries to the ureter were recognized when the operation was still in progress, so that the end-to-end-anastomosis was performed.

The procedures applied in the case of vesicovaginal fistula

Following the recognition of a fistula, a Foley's catheter is inserted in the urinary bladder which often serves as a therapy — in some cases of small-size fistulas, a spontaneous closure occurs following the insertion. If there is no spontaneous closure, the catheter remains inserted till the operation. Immediately before the operation, the location and size of the fistula as well as its relation to ureteral orifices are determined by cystoscopy. Ultrasonography or intravenous pyelography are administered as to determine the condition of the urinary tract. The presence of hydronephrosis and hydroureter point to the development of a scar at the level of ureteral orifice caused by the fistula. Preoperative testing such as urineculture and antibiogram are necessary. The operation timing is scheduled individually, the optimal timing being 3—6 months after the previous operation. The choice of the procedure applied to vesicovaginal fistulas is significant and it is influenced by the specialty of the operator. Gynecologists most often employ the transvaginal approach under condition that the vaginal vault is mobile. If the fistula is placed high in the vagina and fixed, being associated with a mass of surrounding connective tissue, the transperitoneal approach must be employed.

In the vaginal approach to the operation of the vesicovaginal fistula, it is necessary to enable the relaxation of the pelvic floor, which leads to a descent of the vagina. Above and beneath the fistulous opening, a longitudinal incision is made 2 to 3 cm long; the vaginal mucosa is prepared and separated from the urethral wall. It is essential to establish the mobility of the urinary bladder at the level of the fistulous opening as to provide a successful wound healing. The edges of the fistulous canal are incised and the urethral wall sutured by interrupted stitches in two layers using absorbable material and taking care that the edges of the urinary bladder do not turn inside. The hemostatsis must be exact as to avoid the developement of hematoma and infection. The vaginal mucosa is sutured using nylon material and mattress sutures with no empty space between them. Nylon sutures are removed three weeks later. The urinary bladder is drained for the next 14 days with the adequate antibiotic therapy and uroantiseptics. This procedure was administered in 21 cases of vesicovaginal fistula with only one case of recurrence, which was solved by a reoperation using the same procedure.

RARITIES AND ADVICES

For the end, we shall present 4 cases from our long practice, 2 patients from the years 1958 and 1960. The first patient (1958) was admitted to the Department for adnexal tumor. She was prepared for operation by the protocol used at that time, without preoperation chromocystoscopy or pyelography. Explorative laparotomy revealed a kidney that descended into the small pelvis. In another patient (1960), a ligature of the ureter happened during the peritonization following the adnexectomy. The ligature was recognized on the second day, but the patient died of uremia. During the preoperative preparation, no necessary urologic examinations were administered. It is our experience that preoperative urologic examinations such as cystoscopy and IPV necessary.

Out of rare fistula locations, we would like to mention the two postoperative ileocutanous fistulas occuring secondary to the operation of ectopic pregnancy (tubal rupture) associated with a massive abdominal hemorrhage. Both patients with a fistulous opening in the abdomen discharged a green foamy excretion of liquid consistence and unpleasant smell. The postoperative external intestinal fistula was diagnosed and it was managed by resectio intestini ilei cum fistula et anastomosis termino-terminum (Ulic, D. et al., 1981). Subsequent analyses lead to a diagnosis of a fistula developed for ligation of a part of the ileum (meteoristic intestine) during the peritonization of the parietal peritoneum.

CONCLUSIONS

— Vesicovaginal fistulas are most often associated with gynecological operations (0.33%).

— The administered measures of prevention and sound surgical knowledge of the operator result in a low incidence of developing fistulas.

— Similarly, low incidence of tocogenic fistulas points to a good care and active conduct of the labor.

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ФИСТУЛЕ КОД ГИНЕКОЛОШКИХ И АКУШЕРСКИХ ОПЕРАЦИЈА

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Резиме

Аутори приказују урогениталне и ректогениталне фистуле на Клиници за гинекологију и акушерство у Новом Саду у периоду 1976—1990. година. Анализа обухвата 23 фистуле: 17 везиковагиналних, 3 уретеровагиналне, 1 весикоректовагиналну и 7 ректовагиналних. У том периоду урађено је 182 Wertheim-операције, 3864 тоталне абдоминалхистеректомије, 1160 вагиналних хистеректомија и 7111 царских резова. Најчешће су забележене весиковагиналне фистуле 0,33%, док токогених фистула није било. Код радикалних хистеректомија урогениталне фистуле се изузетно ретко срећу као последица превентивних мера током хируршког поступка.

IN MEMORIAM

Prof. Dr. Ivan Mihaljev (1936—2003)

The untimely death of Prof. Dr. Ivan Mihaljev (August 8, 2003), a hard worker, an excellent organizer, a confirmed researcher and an esteemed university professor, leaves Matica srpska short of a prominent permanent member and a very active associate.

Ivan Mihaljev was born on May 5, 1936 in Novi Kneževac. He finished elementary school in Kikinda in 1951 and agricultural highschool in Šabac in 1955. He earned his B. S., M. S. and Ph. D. degrees in agronomy in 1960, 1967, and 1970, respectively, all from Faculty of Agriculture in Novi Sad. He has received a one-year post doctoral training in wheat genetics and breeding at Nebraska University, Lincoln, USA.

After obtaining his B. Sc. degree, he worked as an agronomist in Stara Moravica. In 1962, he was appointed assistant in the course "Plant Breeding" at Faculty of Agriculture in Novi Sad. He retired from the same faculty as full professor of plant breeding.

In addition to teaching the course on plant breeding to graduate students from different departments of Faculty of Agriculture in Novi Sad, Prof. Mihaljev also taught genetics at Faculty of Agriculture in Sarajevo and at the branch of that faculty in Mostar. He taught the course "Theory of Plant Breeding" to postgraduate students taking M. Sc. degree in plant genetics and breeding. He invested much effort in upgrading and modernizing his teaching methods. He has been invited to present lectures at several universities abroad and he has supervised a large number of B. Sc., M. Sc. and Ph. D. theses.

Prof. Mihaljev was not only an excellent and conscientious teacher but also a successful researcher. He was author or co-author of 160 research and technical papers, seven chapters in monographs and co-author of two textbooks. He presented 63 reports at scientific meetings in the country and abroad. His work was focused on hybrid wheat and the occurrence of heterosis in wheat. He developed six varieties of winter wheat.

Prof. Mihaljev also worked as an extension officer, providing advice on wheat production to several agricultural farms in the Vojvodina Province and other parts of the country. In that capacity, he held numerous lectures at seminars and technical meetings.

Prof. Mihaljev was very active in professional affiliations and editorial boards of scientific journals as well as on organization of scientific and technical meetings. He performed numerous functions: vice-dean and dean of Faculty of Agriculture in Novi Sad, head of Department of Plant Genetics and Breeding of the same faculty, president of Association of Faculties of Agriculture, acting director of Institute of Field and Vegetable Crops in Novi Sad, etc. Also, he was leader of a number of research projects and he actively participated in the work of many public, sport and political organizations.

Prof. Mihaljev's activities in Matica srpska were notable. His first paper to appear in "Matica srpska Proceedings for Natural Sciences", titled "Genetic mechanism of fertility restoration in cytoplasmically male sterile varieties of wheat", was published in Vol. 54/73. In 1972, he was admitted to the editorial board and appointed secretary of the board of "Procedings". In recognition for hard work for Matica srpska, Prof. Mihaljev was appointed a member of Management and Executive Boards of Matica srpska and Secretary of Department of Natural Sciences of Matica srpska. In those capacities he participated in the organization of scientific meetings and in the realization of research projects of Matica srpska. In 1977, he was awarded Matica srpska Medal with Diploma for long-term cooperation and accomplishments.

For successful educational, research and public work, Prof. Mihaljev received numerous awards: Medal of Work with Golden Wreath, Medal of Merit to the People and to Development of the Country, Plaque of the City of Novi Sad, Plaque of University of Novi Sad, etc.

The achievements of Prof. Mihaljev will remain a lasting memory to all those who worked with him, researchers, colleagues, practicing agronomists and generations of students. We are grateful for all he did to improve the work and promote the name of Matica srpska. Prof. Mihaljev's passing is a loss of an efficient, devoted and respected associate.

Prof. dr Rudolf Kastori