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NAPHTHENIC ACIDS – ALTERNATIVE ROOTING STIMULATORS IN BLACK LOCUST MICROSHOOTS

ABSTRACT: The study describes the rooting effect of naphthenates and their fractions on *in vitro* grown *Robinia pseudoacacia* L. shoots. Natural naphthenic acids have been isolated by alkaline extraction from middle fraction of crude oil type "Velebit" from Vojvodina, characterized and fractionated. Black locust shoot bases were immersed in ACM medium [Ahuja, 1984] without agar supplemented with either 10, 50 or 100 μM of basic naphthenate preparation, naphthenate fractions obtained by extraction at different pHs (pH 2, pH 4, pH 7 and pH 9), or indole-3-butyric acid (IBA). Treated shoots have been then grown on hormone-free medium for four weeks. Significant differences among test treatments were recorded during the third and the fourth week of *in vitro* cultivation. Final evaluation was performed on the basis of rooting percentage after four weeks of cultivation. The highest rooting percentage (>70%) was achieved after the treatment with solution containing 50 μM of IBA. However, treatment with 10 μM of naphthenate preparation achieved also positive effect on rooting (>60%). Average rooting percentage in the control treatment was just 45%. Our results with black locust confirm previous results gained with some other agricultural and forest tree species that naphthenates have the potential to stimulate rooting in shoots and cuttings.

KEYWORDS: naphthenates, micropropagation, rooting stimulators, *Robinia pseudoacacia*

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INTRODUCTION

Naphthenic acids represent a complex mixture of cycloalkyl and alkylcarboxylic acids that are found in raw oil, and could contain more than 3000 compounds [Qian and Robbins, 2001; Clemente and Fedorak, 2005]. These compounds exhibited a certain biological activity with respect to uptake of various ions [Kevrešan et al., 2005a], as well as an activity similar to auxin [Ćirin Novta et al., 2002]. Naphthenic acids from this oil fraction stimulate rooting of sunflower cuttings [Kevrešan et al., 2003a], poplar hardwood cuttings [Kevrešan et al., 2003b] and softwood cuttings of *Thuja occidentalis* L. [Kevrešan et al., 2006]. Naphthenate treatment influenced the rooting of black locust genotype shoots *in vitro* [Kevrešan et al., 2005b] and caused biochemical changes in softwood cuttings of *Robinia pseudoacacia* [Kevrešan et al., 2007].

The aim of this work was to determine if naphthenates could be used as alternative rooting stimulators for black locust shoots.

MATERIAL AND METHODS

Total preparations of naphthenic acids were isolated by alkaline extraction from the middle gas fraction of crude oil type "Velebit" (Autonomous Province of Vojvodina, Republic of Serbia) and characterized by physico-chemical methods, as described earlier [Ćirin Novta et al., 2002]. Preparations of total naphthenic acids were fractionated according to acid ionization constants. The naphthenic acids were dissolved in a 5% solution of NaOH at pH 11, the pH was subsequently decreased by H_2SO_4, and at different pHs (pH 2, pH 4, pH 7 and pH 9); undissolved naphthenic acids were obtained by extraction with petroleum ether. In all experiments the sodium salt of naphthenic acids (sodium naphthenate) was used.

The genotype of *Robinia pseudoacacia* with fastigiata tree form was used in the experiment. The basis of 1.5–2.0 cm long shoot was immersed for one minute in solution prepared as ACM – medium without agar, supplemented with either 10, 50 or 100 μM of basic naphthenate preparation; naphthenate fractions obtained by extraction at different pHs (pH 2, pH 4, pH 7 and pH 9) or indole-3-butyric acid (IBA). Treated shoots were then grown in hormone-free ACM medium (Ahuja, 1984) for four weeks. Treatments are presented in Table 1. In control treatment shoots were immersed in hormone-free ACM medium. Five shoots were placed per jar and five jars were set per treatment. The rooting was analyzed on the basis of average number of roots per shoot (RN) and percentage of rooted shoots (RP [%]) one, two, three and four weeks after the treatment. Statistical analysis included ANOVA and LSD-test. The percentage of rooted shoots (RP) was transformed by arcsine transformation. Statistical program package STATISTICA 12 was used [StatSoft Inc., 2012].

Table 1. Test treatments applied to shoots of *Robinia pseudoacacia in vitro*

Treatment solution	Total naphthenates	Fraction of total napthenates obtained at	Indol-butiric acid	Concentration of tested active substance (μM)
na-tot-10	+			10
na-tot-50	+			50
na-tot-100	+			100
na-pH2-10		pH2		10
na-pH2-50		pH2		50
na-pH2-100		pH2		100
na-pH4-10		pH4		10
na-pH4-50		pH4		50
na-pH4-100		pH4		100
na-pH7-10		pH7		10
na-pH7-50		pH7		50
na-pH7-100		pH7		100
na-pH9-10		pH9		10
na-pH9-50		pH9		50
na-pH9-100		pH9		100
IBA-10			+	10
IBA-50			+	50
IBA-100			+	100
IBA-1g			+	4921.26
Control				

RESULTS AND DISCUSSION

Characterization of total preparation of naphthenic acids showed the presence of five classes of carboxylic acids with different content in total acid mixture (% mass): aliphatic $C_nH_{2n}O_2$ (2%), monocyclic $C_nH_{2n-2}O_2$ (21%), bicyclic $C_nH_{2n-4}O_2$ (42%), tricyclic $C_nH_{2n-6}O_2$ (28%) and tetracyclic $C_nH_{2n-8}O_2$ (6%). The average molecular mass of naphthenic acids was determined to be 262, and this value was used to prepare solutions for rooting experiments.

In all test fractions, at least one of the concentrations had stimulative effect on rooting of black locust shoots (Figure 1). The results of analysis of variance indicated significant differences among test treatments: for number of roots per explant in the third and for the percentage of rooted shoots in the third and the fourth week (Table 2). The best results were obtained in the treatment with 50 μM IBA (IBA-50). Only after the treatment na-pH9-100 (100 μM of fraction extracted on pH 9) the percentage of rooting was not significantly different from RP on the best treatment with IBA (58% and 64%, respectively). However, almost every fraction, except fraction obtained at pH 2, achieved stimulative

effect on rooting percentage comparing to control treatment in at least one test concentration. Stimulative effect of naphthenates total preparation on rooting is in agreement with the results of Kevrešan et al. [2003a] and Kevrešan et al. [2005b]. Rooting activity of test fractions of total preparation of naphtenates, with its different values depending on concentrations, suggests the presence of numerous active substances in the total preparation of naphtenates and their presence in all test fractions. It is especially obvious for the fraction obtained at pH 9 that at concentration of 10 μM it achieved inhibited and at concentration of 100 μM stimulated rooting of black locust shoots.

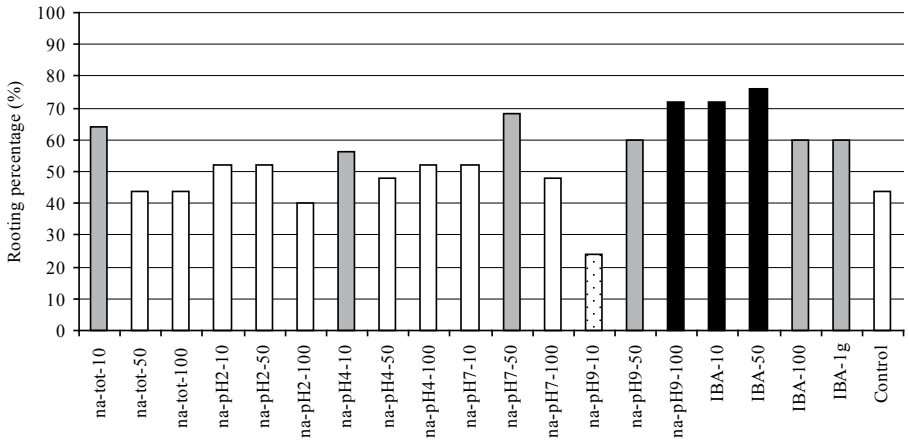

Figure 1. Rooting percentage of black locust (*Robinia pseudoacacia*) four weeks after the treatment with test solutions

Labels of treatments: explained in Tab 1.
Colors of columns: Dotted columns – treatments whose effect was significantly worse than the effect of control treatment; White columns – treatments whose effect was not significantly different from the effect of control treatment, but also significantly worse than the effect of the best IBA-treatment (IBA-50); Grey columns – treatments whose effect was significantly better than the effect of control treatment; Black columns – treatments whose effect was not significantly different from the effect of the best IBA-treatment (IBA-50)

The basis of stimulatory effect of naphthenic acids on rooting is not completely understood [Wort, 1976; Clemente and Fedorak, 2005]. Severson [1972] concluded that potassium-naphthenates stimulated the glucose uptake by root tips of bean plants, while Kevrešan et al. [2005a] showed that low concentrations of Na-naphthenates influence the uptake of some metal ions by soybean plants. Loh and Severson [1975] found that one-day treatment with potassium naphthenates had stimulative effect on the activity the indolacetic acid oxidase, one of the key enzymes in the process of initiation and activation

of root primordia. Ćirin-Novta et al. [2002] found auxinic effect of naphthenic acids, while Kevrešan et al. [2007] found their stimulative rooting potential in *Robinia pseudoacacia* softwood cuttings, on the bases of biochemical indicators of root initiation (activity of IAA-oxidases, peroxidases and amylases and content of glucose). However, the effect of naphthenats on some other important processes that influence rooting, like phenol-peroxidases activity and indolacetic acid conjugation, as well as their influence on ethylene synthesis is still poorly examined.

Treatments with 100 μM of total preparation of napthenates and test fractions (except fraction obtained at pH 9) usually failed to achieve stimulative effect on rooting of tested black locust genotype *in vitro*. Kevrešan et al. [2003a] observed inhibitory effect of high concentrations of Na-naphthenates on rooting of sunflower green cuttings. Also, the inhibitory and toxic effect of higher concentrations of naphthenic acids is a well-known ecological problem [Clemente and Fedorak, 2005].

Table 2. Analysis of variance for test treatments applied to shoots of *Robinia pseudoacacia*

	Number of roots				Percentage of rooted shoots			
	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
Degree of freedom								
Treatment	19	19	19	19	19	19	19	19
Error	80	80	80	80	80	80	80	80
Total	99	99	99	99	99	99	99	99
Sum of squares								
Treatment	0.572	0.591	0.677	0.454	9451.69	8008.60	10574.69	6047.62
Error	1.827	1.801	1.626	1.495	29711.85	21647.01	15932.69	9191.38
Total	2.399	2.392	2.303	1.948	39163.55	29655.61	26507.39	15239
Mean square								
Treatment	0.030	0.031	0.036	0.024	497.46	421.51	556.56	318.30
Error	0.023	0.023	0.020	0.019	371.40	270.59	199.16	114.89
F-test	1.318	1.382	1.754*	1.277	1.339	1.558	2.795**	2.770**
p-value	0.196	0.160	0.044	0.222	0.183	0.089	0.0007	0.0008

CONCLUSION

Our results confirm the possibility of rooting stimulation by naphthenic acids in black locust. The best results we obtained in *Robinia pseudoacacia* with treatment na-pH9-100. The rooting was significantly better than in the control treatment and at the level of the effect of the best IBA treatment (IBA-50). This suggests high potential for implementation of napthenates in rooting of *Robinia pseudoacacia*, which should be tested in the future.

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НАФТЕНСКЕ КИСЕЛИНЕ – АЛТЕРНАТИВНИ СТИМУЛАТОРИ ОЖИЉАВАЊА КОД МИКРОИЗБОЈАКА БАГРЕМА

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РЕЗИМЕ: Рад описује ефекат нафтената на ожиљавање микроизбојака багрема *in vitro*. Нафтенске киселине су изоловане базном екстракцијом из средње фракције сирове нафте типа „Велебит“, која је описана у ранијим радовима. Доњи део микроизбојка је урођен један минут у течни АСМ медијум [Ahuja, 1984] у који је додато 10, 50 или 100 μM основне мешавине натријум-нафтената или њених појединих фракција добијених екстракцијом на различитим pH (pH 2, pH 4, pH 7 или pH 9), односно 10, 50, 100 μM или 1g/l индол-3-бутерне киселине (IBA). Контролни третман је чинио АСМ медијум без испитиваних активних материја. Третирани микроизбојци су затим гајени на чврстој АСМ подлози без хормона. Значајне разлике међу испитиваним третманима су забележене током треће и четврте недеље узгоја у *in vitro* условима. Коначна оцена је изведена на основу процента ожиљавања након четири недеље узгоја. Највиши проценат ожиљавања је постигнут раствором са 10 μM натријум нафтената, након чега је остварен значајан позитиван ефекат на проценат ожиљавања (>60%) у односу на контролни третман (око 45%). Резултати до којих смо дошли код багрема потврђују раније резултате који су добијени код пољопривредних и шумских дрвенастих врста о могућности стимулације ожиљавања микроизбојака и резница солима нафтенских киселина.

КЉУЧНЕ РЕЧИ: нафтенати, микроизбојци, стимулатори оживљавања, *Robinia pseudoacacia*

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THE EFFECT OF Ni ON CONCENTRATION OF THE MOST ABUNDANT ESSENTIAL CATIONS IN SEVERAL *BRASSICA* SPECIES

ABSTRACT: Some plants from the genus *Brassica* have the ability to tolerate excessive concentrations of heavy metals, including Ni. Considering the fact that Ni is a very toxic element for living beings we wanted to examine its influence on some species from genus *Brassicaceae*. The aim of this study was to investigate the effect of Ni on distribution and accumulation of essential macronutrients from the standpoint of food quality and phytoremediation potential. Experiments were performed using winter (W) and spring (S) varieties of rapeseed (*Brassica napus*, L.), white mustard (*Brassica alba*, L.), black mustard (*Brassica nigra*, L.) and turnip (*Brassica rapa*, L.). The seeds were exposed to 10 μM Ni from the beginning of germination. Plants were grown in water cultures, in semi-controlled conditions of a greenhouse, on ½ strength Hoagland solution to which was added Ni in the same concentration as during germination. Concentrations and distribution of Ca, Mg, K in leaf and stem were altered in the presence of increased concentration of Ni. Significant differences were found between the control and Ni-treated plants as well as among the genotypes.

KEYWORDS: excess nickel (Ni), *Brassicaceae,* concentration of magnesium (Mg), calcium (Ca), potassium (K)

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INTRODUCTION

Nickel (Ni) is a heavy metal that can be present in excessive amounts in the soil and it is one of the most toxic elements for plants, if present in excessive concentration [Rabie *et al.*, 1992]. The main sources of agricultural soil contamination with Ni are contaminated compost and sewage sludge. Inadequate disposal of waste from households, municipalities and industries may increase the concentration of Ni in the soil even more [Alloway, 1995]. This metal is very toxic for humans and animals and it can enter food chain mainly through the food of plant origin [Nellessen and Fletcher, 1993; Guo and Marschner, 1995]. It is important to prevent the accumulation of Ni in edible plant parts. Some plant species are tolerant to Ni toxicity, and the accumulation of a large amount of Ni in their shoots produces no adverse effects. That is the reason why the plant species/genotypes suitable for phytoremediation of Ni-contaminated sites should be found. This is the most appropriate way to reduce Ni concentration in mildly contaminated soils [Salt *et al.*, 1995]. The ability of plants to tolerate excessive concentrations of heavy metals, including Ni, depends, among the other features, on their ability to synthesize sulfur containing compounds. Plants from the genus *Brassica* are among them. Moreover, they are important agricultural crops. Black and white mustard are grown for seeds that are used as spices. Oil of black mustard has strong antibacterial activity and white mustard is also used as feed and green manure. Rapeseed and turnip are important oil crops and are often used as the first spring and the last autumn green feed [Erić *et al*., 2006]. They are also very important for the production of honey [Blažyté-Čereškiené *et al.*, 2010]. Contamination of fields with heavy metals is generally present during the entire vegetation. With respect to this, we studied the effect of continious presence of Ni on chemical composition of winter and spring varieties of rapeseed (*Brassica napus* L.), white mustard *(Brassica alba* L.), black mustard (*Brassica nigra* L.) and turnip (*Brassica rapa* L.).

MATERIAL AND METHODS

To test the Ni accumulation capacity and Ni impact on different species belonging to the family *Brassicaceae*, the experiments were performed under semi-controlled conditions using plants grown in water cultures in the glasshouse. Winter (W) and spring (S) varieties of rapeseed (*Brassica napus*, L.), white mustard (*Brassica alba*, L.), black mustard (*Brassica nigra*, L.) and turnip (*Brassica rapa*, L.) were used in the experiment.

Plant growth

Before sowing, seeds were kept for 24 h in deionized water (control) and in 10 μM Ni (as $NiSO_4$) dissolved in deionized water. Seeds were germinated

in the quartz sand, in an incubator, at 26 °C. Seedlings were planted in pots containing ½ strength Hoagland nutrient solution [Hoagland and Arnon, 1950] (control) to which was added Ni to final concentration of 10 μM. Each treatment was set in 5 replications with 8 plants per replication. Nutrient solution was changed every other day and aerated regularly. Plants were grown for 30 days under semi-controlled conditions.

Plant analyses

Concentrations of K, Ca, Mg, and Ni were determined by atomic absorption spectrophotometry (AAS SHIMADZU AA-6300), after ashing plant material at t = 500 °C and dissolving it in deionized hot water in the presence of 0.25 M HCl.

Statistical analysis

Statistical analysis was performed using STATISTICA 12.0 [StatSoft, University Licence, University of Novi Sad, 2012] and Excell (Microsoft Inc.) software packages. Means of replicates and evaluation of significance of differences between means were determinated with descriptive statistics and ANOVA analysis, followed by LSD *post hoc* test (α=0.05).

RESULTS AND DISCUSSION

All analysed genotypes accumulate Ni in the above-ground parts, esspecialy in the leaves (Figure 1). The biggest concentration of Ni, both in leaf and in stem, was in *B. napus* S, with 67.75 times increase in concentration compared with the control group, and 92.5 times in stem in comparision with the respective control group.

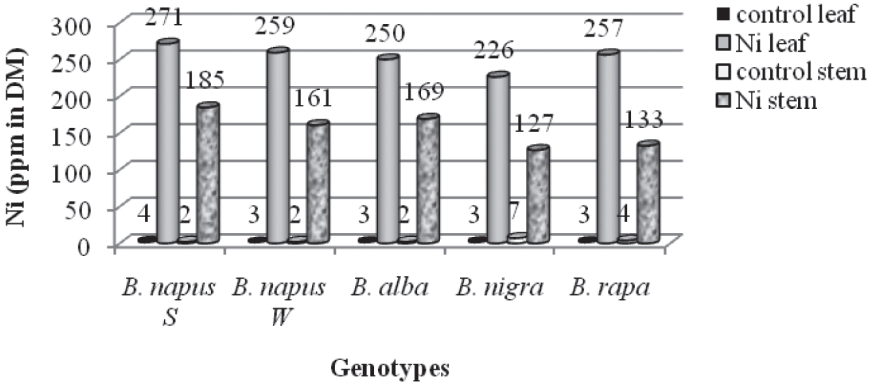

Figure 1. Concentration of Ni in leaves and stems of 5 genotypes belonging to the *fam. Brassicaceae* grown in the presence of 10 μM Ni.

Genetically, metal accumulation is independent of metal tolerance (Assun *et al.* 2006). Therefore, it is not possible to conclude that a plant with an increased metal concentration in leaves is also tolerant to that metal. Metal concentration in leaves *per se* can only be taken as an indication of a plant's potential tolerance to that metal, but not as evidence of tolerance itself [Ernst, 2006].

A certain number of plants belonging to the family *Brassicaceae* have hyperaccumulative properties [Lagercrantz et al., 1998; Paterson et al., 2001]. For this reason, a large number of studies focused on the possibility of using plants from this family for phytoremediation [Zeremski, 2011]. Angelova *et al*. [2008] experimentally found potential of *Brassica napus* for phytoremediation of soils contaminated by Pb and Cd. The ability of *Brassica juncea* to accumulate a high concentration of Cd in shoot was often the research subject in the last ten years [Banuelos, et al., 2005; Ghosh and Singh, 2005]. Because of that, the analysis of the effects of excess Ni on chemical composition of *Brassica napus*, L., *Brassica alba*, L., *Brassica nigra*, L. and *Brassica rapa*, L contributes to an advanced knowledge of species belonging to the family *Brassicaceae*.

Statistically significant difference in the concentration of Ca was found in all analyzed genotypes compared with the controls. Concentration of Ca in leaves decreased in comparison with the control plants, while concentration of Ca in stem increased in comparison with the control plants (Figure 2). Compared with the controls, *B. alba* had the highest (147%) concentration in leaves and the smallest in stem (87%). A similar result was obtained for the Mg (Figure 4), except that the concentration of Mg in both leaf and stem increased as a result of treatment. *B. alba* had the highest concentration of Mg also in leaf (177%). Like other metals, Ni can displace Mg from chlorophyll and enzymes such as RuBisCO that contain Mg ion as cofactor [Furini, 2012]. The results of our previous experiments indicated that concentration of chloroplast pigments as well as Chl. a/b and Chl. a+b/Car declined significantly in the presence of Ni [Maksimović et al., 2011].

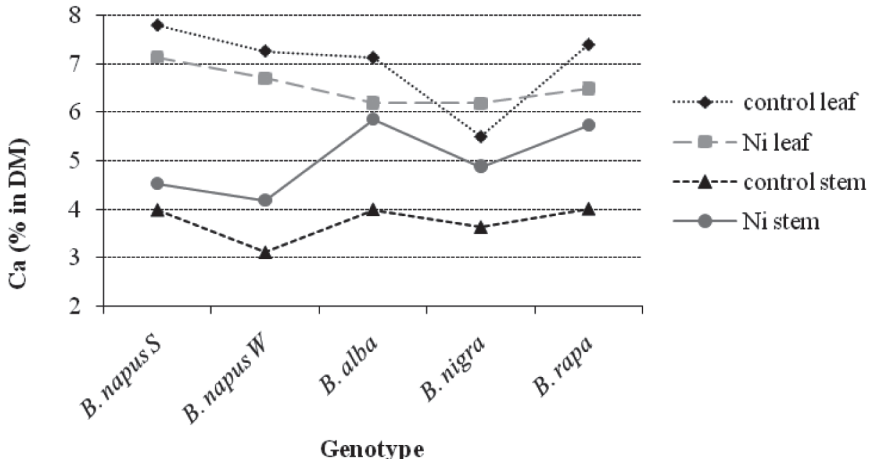

Figure 2. Concentration (%) of Ca in leaves and stems of 5 genotypes belonging to the *fam. Brassicaceae* grown in the presence of Ni

Concentration of K varied depending on genotype. It increased in leaf in *B. napus* S and *B. alba* when compared to controls, and in stem it increased just in *B. napus* W (Figure 3). The greatest differences were observed in concentration of K in genotype *B. napus* W in leaf (125%) and in stem (85%), compared to controls.

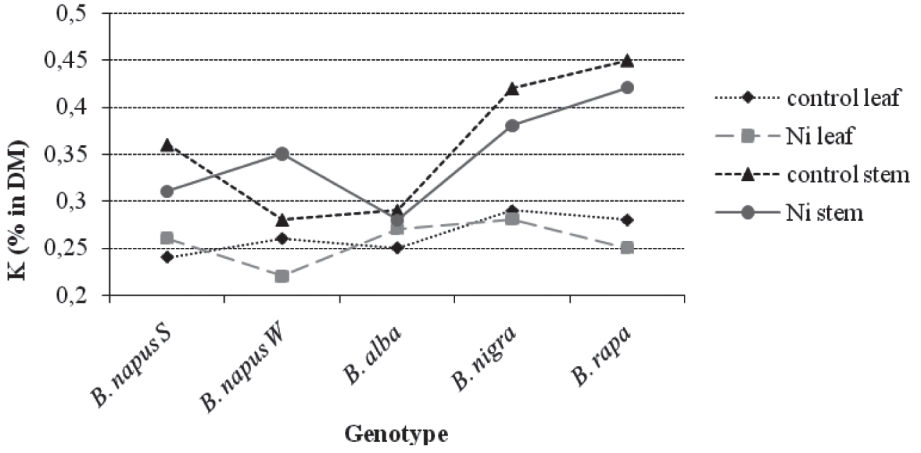

Figure 3. Concentration (%) of K in leaves and stems of 5 genotypes belonging to the *fam. Brassicaceae* grown in the presence of Ni

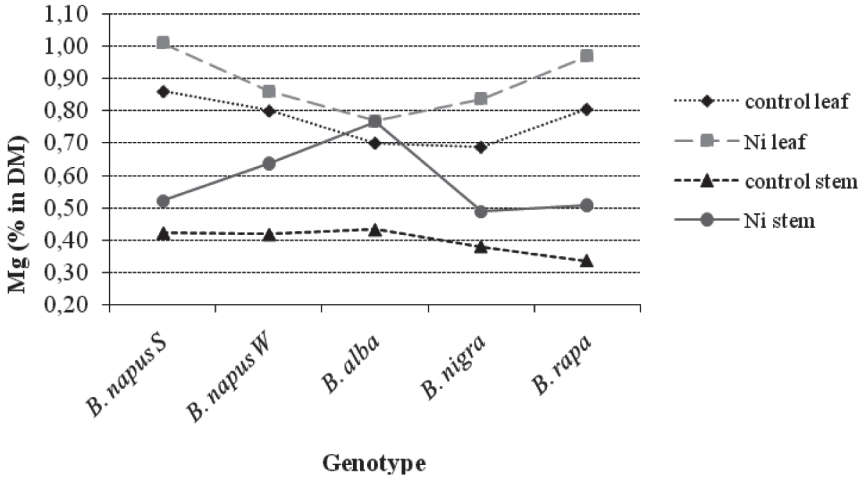

Figure 4. Concentration (%) of Mg in leaves and stems of 5 genotypes belonging to the *fam. Brassicaceae* grown in the presence of Ni

Increase in concentrations of essential elements (not added as a tretament) may also be explained by severe reduction in fresh and dry weight in the plants treated with Ni. The reduced growth is typical response of plants to excess Ni and Cd [Sanita di Topi and Gabbrielli 1999; Sandalio et al. 2001; Moya et al. 1993; Maksimović et al. 2007].

The presence of Ni in *Brassica napus*, L., *Brassica alba*, L., *Brassica nigra*, L. and *Brassica rapa*, L. had a great influence on concentration of essential macronutrients. The largest changes in concentrations of Ca were in the presence of Ni when compared to control plants (Figure 2). Minor changes among the genotypes were also observed for K. Only *B. nigra* had a statistically significant difference in the concentration of K in leaf. Statistically significant difference in stem had *B. napus* (S) when compared to controls (Figure 3). There are variations in the response of the genotype to the presence of nickel.

Chen et al. [2009] concluded that the uptake of nutrients was also affected by Ni excess, and its chemical similarity to Ca, Mg, Fe, Cu, Mn and Zn indicated that Ni can compete with these minerals in absorption and subsequent utilization. Toxic levels of Ni may inhibit the absorption of these elements, decrease their concentration and even lead to their deficiency in plants [Brune and Dietz, 1995]. In the presence of Ni, differences between genotypes were obvious for Ca and Mg, and especially for K (Figure 2, 3, 4).

Generally, *Brassica rapa* had the highest concentration of analyzed elements, and *Brassica alba* exhibited differences of concentration in comparison with controls, particularly of Ca and Mg (Figure 2 and 4).

Accumulation of Ni in the above-ground parts of of these 5 genotypes is very important because of their use for agricultural purposes, as well as because of the potential use for the purpose of phytoremediation.

CONCLUSION

Continious presence of 10 μM Ni significantly altered concentration of Ca, K and Mg. Differences were found between the genotypes. Concentration of Ca in leaf decreased in plants exposed to treatment. The results for the concentration of K varied among genotypes, but generally there was a reduction in concentration due to effects of Ni. The content of Mg increased in all genotypes tested under excessive Ni.

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ЕФЕКАТ Ni НА КОНЦЕНТРАЦИЈУ НАЈЗАСТУПЉЕНИЈИХ ЕСЕНЦИЈАЛНИХ КАТЈОНА У НЕКИМ ВРСТАМА ИЗ РОДА *BRASSICA*

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РЕЗИМЕ: Неке биљке из рода *Brassica* имају способност толеранције прекомерне концентрације тешких метала, укључујући и никал (Ni). Испитивање ефикасности апсорпције и акумулације тешких метала интересантно је са становишта: 1) безбедности хране, и 2) потенцијала за фиторемедијацију. Циљ овог рада је да се испита ефекат никла на дистрибуцију и акумулацију неких есенцијалних катјона као што су калцијум (Ca), магнезијум (Mg) и калијум (K). Експерименти су изведени над озимом и јаром уљаном репицом (*Brassica napus* L.), белом слачицом (*Brassica alba*, L.), црном слачицом (*Brassica nigra* L.) и купусном уљаном репицом (*Brassica rapa* L.). Семе је било изложено утицају 10 μM никла (Ni) од почетка клијања. Биљке су гајене у воденим културама, у полуконтролисаним условима у стаклeнику, на ½ Хогланд-овом хранљивом раствору, односно потпуном хранљивом раствору у који је додат никал (Ni) у истој концентрацији као и током клијања. Садржај калцијума (Ca), магнезијума (Mg) и калијума (K) у листу и стаблу измењен је у присуству повећане концентрације никла (Ni). Значајне разлике установљене су како између контроле и третмана, тако и између генотипова. Сви тестирани генотипови испољили су значајну способност акумулације никла (Ni), с тим што је *Brassica napus* јара форма имала највеће разлике у концентрацији у односу на контролу (у листу 67,75 пута, а у стаблу 92,5).

КЉУЧНЕ РЕЧИ: сувишак никла (Ni), *Brassicaceae,* концентрација магнезијума (Mg), калцијума (Ca), калијума (K)

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EFFECTS OF TEMPERATURE AND TIME ON DEOXYNIVALENOL (DON) AND ZEARALENONE (ZON) CONTENT IN CORN

ABSTRACT**:** Fumonisins are *Fusarium* mycotoxins that occur in corn and corn-based foods and they have been implicated in several animal and human diseases. Their effect on human health is unclear, however, fumonisins are considered to be risk factors for cancer. Baking, frying, and extrusion cooking of corn at high temperatures (190 °C) reduce fumonisin concentrations in foods, with the amount of reduction achieved depending on cooking time, temperature, recipe, and other factors. The aim of this work was to evaluate the effectiveness of temperature (200 and 220 °C) and time (15 and 20 min) on the detoxification of corn flour deliberately contaminated with DON and ZON. After processing at 200°C for 15 min, an average of 12% and after 20 min an average of 15% of DON was lost. At 200°C ZON content was reduced by 22% (after 15 min) and by 27% (after 20 min). Higher temperature (220 °C) did not significantly affect further reduction of DON or ZON content. The process was only partially effective in both cases.

KEYWORDS**:** deoxynivalenol, zearalenone, temperature, incubation time.

INTRODUCTION

Fusarium species are widespread in nature, occurring both as facultative saprophytes and parasites of a variety of plants. They are capable of elaborating toxins with varying chemical compositions [Bilgrami and Choudhary 1998]. Various *Fusarium* species can infect cereal crops under different climatic conditions, the most widely distributed being *F. graminearum* and *F. culmorum.*

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Deoxynivalenol (DON) belongs to the trichothecene group of mycotoxins and is formed by fungi of the genus *Fusarium*. DON often occurs in plant products and particularly in cereals. Of the trichothecene mycotoxins, deoxynivalenol, 3-acetyl- and 15-acetyl-deoxynivalenol are the toxins most frequently occurring in Japan, Korea, China, South East Asia, New Zealand and Europe [Ishii 1983; Yoshizawa 1983; International Programme on Chemical Safety 1990; International Agency for Research on Cancer 1993; Yamashita et al. 1995; Lauren et al. 2001]. The toxin concentrations found in wheat, corn or rice are often in the ppm range. Due to their high cytotoxic and immunosuppressive properties these toxins pose a risk to human and animal health.

Zearalenone (ZEA) is a non-steroidal oestrogenic mycotoxin that can occur concomitantly with DON since the two compounds are produced by the same *Fusarium* species. ZEA is a phytohormone which displays, apart from its anabolic properties, mainly estrogenic effects. Because of its estrogenic properties, zearalenone may induce fertility disorders in animals with clinical signs of hyperestrogenism – an aspect of disease mainly reported in hogs but also described in other species such as cows, horses and sheep [JECFA 2002; Zöllner et al. 2002].

With the common occurrence of DON and ZEA, it is important to understand not only the factors that lead to their occurrence, but also the management strategies capable of minimizing their impact and occurrence in food products [JECFA 2002]. Numerous guidelines, ranging from altering field conditions to adequate storage practices, have been suggested to prevent mould growth and mycotoxin development in crops [Patey and Gilbert, 1989]. Safe limits of DON in foods ranging from 500 to 2000 ppb have been fixed in Austria, Canada, Japan, Russia, Switzerland and the USA; France and Romania regulation limits for ZEA are 200 and 30 ppb respectively [Food and Agriculture Organization, 1997].

In Serbia safe limits of DON and ZON in foods range from 500 to 1750 ppb and 50 to 350 ppb according to Rulebook on maximum allowed quantities of residue of plant protection means in food and animal food and on food and animal feed for which the maximum allowed quantities of plant protection means are determined [*Službeni glasnik RS*, 25/2010; 28/2011].

Despite all these efforts, the problem persists. It became necessary to develop processing methods directed at lowering the co-occurring mycotoxins to safe levels and design probabilistic models which consider that processing of raw agricultural commodities may alter the contamination levels in the final products [JECFA 2002].

Table 1. Global occurence of DON and ZON in 2009, 2010 and 2011. (www.mycotoxins.info)

	DON			ZON		
	2009	2010	2011	2009	2010	2011
Number of samples tested	2432	2947	3509	2342	2633	3061
Positive (%)	50	58	59	35	42	40
Average (ppb)	831	722	625	221	108	96

Milling studies evaluating wheat naturally contaminated with *Fusarium* mycotoxins indicate that the mycotoxins were distributed differentially throughout the kernel, with relatively higher levels in the dockage, outer bran fractions, and shorts and lower levels in the inner flour fractions [Scott et al. 1983; Young et al.1984; Lee et al. 1987]. DON levels were not reduced during the production of Egyptian bread, Westernstyle bread or cookies baked from hard wheat flour [El-Banna et al. 1983; Scott et al. 1983; 1984].

In contrast, the effect of baking on DON in non-yeast products was reported as variable, ranging from no effect to 69% reduction [Young et al. 1984; Abbas et al. 1985]. Additional studies indicated that during bread baking DON was reduced by 49 and 57% [Kamimura et al. 1979]. Chemical treatments have been tested for their effectiveness in reducing DON; in general, better results were obtained at basic pH [Young 1986; Lauren and Smith 2001].

In corn wet milling, ZEA concentrates in gluten (49–56%) and milling solubles (17–26%) but not in starch. All dry-milled corn fractions contained ZEA and only 3–10% were removed by dry cleaning [Scott 1984]. Losses of ZEA added to wheat flour were 34–40% during bread-making, 48–62% when making instant noodles, and 16–27% during processing into biscuits. The decomposition products in bread lacked oestrogenic activity [Matsuura et al. 1981].

The present study was conducted to determine the effects of thermal treatments on DON and ZEA and it wanted to establish the basis for a decontamination model of *Fusarium* mycotoxins in corn.

MATERIAL AND METHODS

Ten naturally contaminated corn samples were purchased from Trilogy Analytical Laboratory, 870 Vossbrink Dr. Washington, MO63090. Samples were stored at 4 °C until used. The presence of ZON and DON was studied by *enzyme-linked immunosorbent assay* (ELISA) method. To obtain a whole corn powder, samples were ground to pass through 0.5 mm mesh; the remaining

fraction was ground consecutively since no more than 4% was bigger than 0.5 mm. All fractions were pooled and thoroughly mixed.

Heating treatment: All samples were heated in an electric convection oven at 200 and 220 °C for 15 and 20 min (a total of 24 treatments). Samples (10 g) were put in aluminium vessels (5.5 cm i.d. x 3.5 cm height), extended to form a 1 cm high layer and placed into the oven preheated to the selected temperature. After the treatment, the samples were immediately cooled and weighed.

Chemicals and mycotoxins standards: All reagents were purchased from R-biopharm. Test kit for DON determination contains:

1 microtiter plate with wells coated with capture antibodies against anti-DON antibodies,
1 DON standard solution, 1.3 ml 0 ppm (zero standard) in water, 1 peroxidase-conjugated DON, 1 anti-DON antibody, 1 substrate/chromogen, 1 stop solution containing 1 N sulfuric acid and 1 Washing buffer (Salt) for preparation of a 10 mM Phosphate Buffer (pH 7.4) containing 0.05% Tween 20.

Test kit for ZON determination contains:
1 microtiter plate with wells coated with capture antibodies against anti-zearalenone antibodies, 1 Zearalenone standard, 1.3 ml 0 ppb (zero standard) in methanol/water, 1 peroxidase-conjugated zearalenone, 1 anti-zearalenone antibody monoclonal, 1 Substrate/chromogen and 1 Stop reagent containing 1 N sulfuric acid.

Sample preparation:
DON analysis: weigh 5 g of ground sample, put it into a suitable container and add 100 ml of distilled water; blend the sample by ultra-turrax (or equivalent) for two minutes or shake vigorously for three minutes (manually or with shaker); filter the extract through Whatman No. 1 filter (or equivalent); use 50 µl of the filtrate per well in the test.
ZON analysis: weigh 5 g of ground sample and add it to a suitable container with 25 ml of methanol (70%); shake vigorously for 3 min (manually or with shaker); filter the extract through Whatman No. 1 filter (or equivalent); dilute 1 ml of the obtained filtrate with 1 ml of distilled or deionized water; use 50 µl of the filtrate per well in the test.

Test procedure:
DON analysis:
1. Insert a sufficient number of wells into the microwell holder for the standard and samples to be run. Record standard and sample positions.

2. Pipet 50 µl of standard or prepared sample into separate wells; use a new pipette tip for the standard or each sample.
3. Add 50 µl of enzyme conjugate to the bottom of each well.
4. Add 50 µl of anti-DON antibody solution to each well. Mix gently by shaking the plate manually and incubate for 5 min (+/-1) at room temperature (20–25 °C / 68–77 °F).
5. Dump the liquid out of the wells into a sink. Tap the microwell holder upside down onto a clean filter towel (three times in a row) to remove all remaining liquid from the wells. Using a wash bottle or multichannel pipette fill the wells with distilled or deionized water (250 µl per well). Empty the wells again and remove all remaining liquid. Repeat the washing steps two more times.
6. Add 100 µl of substrate/chromogen to each well. Mix gently by shaking the plate manually and incubate for 3 min (+/-0.5) at room temperature (20–25 °C / 68–77 °F) in the dark.
7. Add 100 µl of stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 10 minutes after the addition of stop solution.

ZON analysis:

1. Insert a sufficient number of wells into the microwell holder for the standard and samples to be run. Record standard and sample positions.
2. Pipet 50 µl of standard or prepared sample into separate wells; use a new pipette tip for the standard or each sample.
3. Add 50 µl of enzyme conjugate solution to each well.
4. Add 50 µl of anti-zearalenone antibody solution to each well. Mix gently by shaking the plate manually and incubate for 10 min (+/-1) at room temperature (20–25 °C / 68–77 °F).
5. Dump the liquid out of the wells into a sink. Tap the microwell holder upside down onto a clean filter towel (three times in a row) to remove all remaining liquid from the wells. Using a wash bottle or multichannel pipette fill the wells with distilled or deionized water (250 µl per well). Empty the wells again and remove all remaining liquid. Repeat the washing steps two more times.
6. Add 100 µl of substrate/chromogen to each well. Mix gently by shaking the plate manually and incubate for 5 min (+/-0.5) at room temperature (20–25 °C / 68–77 °F) in the dark.
7. Add 100 µl of stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 10 minutes.

RESULTS AND DISCUSSION

All corn samples examined were contaminated with deoxynivalenol (DON) in concentration of 1.9 ppm and with ZON in concentration of 91.1 ppb.

After heating treatment at 200 ºC for 15 and 20 minutes the results showed the reduction of DON concentration by 11.58% and 15.26% respectively (Figure 1), and the reduction of ZON concentration by 21.79% and 26.65% respectively (Figure 2).

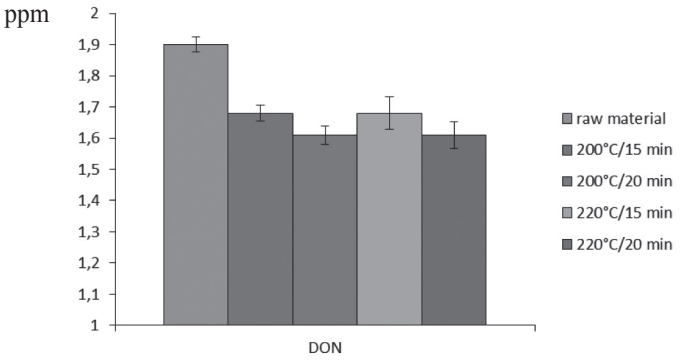

Figure 1. DON concentration in naturaly contaminated corn flower after heating treatments

By increasing the temperature of the heating treatment to 220 ºC the reduction of the DON and ZON concentration after 15 and 20 minutes was not signicantly different from the level of DON concentration from the previous case (Figure 1). However, the level of ZON decreased up to 21.44% and 27.68% respectively (Figure 2).

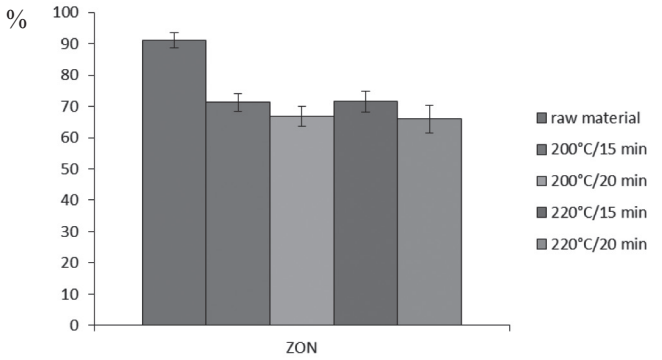

Figure 2. ZON concentration in naturaly contaminated corn flower after heating treatments

Similar examinations conducted on samples of grain powder by Yumbe-Guevara et al. (2003) showed the reduction of DON concentration after heating treatments for 60 minutes at temperatures of 140 ºC, 160 ºC, 180 ºC, 200 ºC and 220 ºC were by 7.5%, 24%, 38%, 48% and 50% respectively. Not significatly different results regarding ZON concentration reduction were obtained.

A large number of other examinations that included heating treatments while baking samples were conducted and showed various results. Young et al. [1984] treated samples of soft white winter wheat, naturaly contaminated with DON (range: 0.28–0.44 ppm), using a baking cookies standard receipe that included heating treatment and the results showed that DON level was decreased up to 35%. On the other hand, Lancova et al. [2008] showed that baking at 210 ºC for 14 minutes had no significant effect on DON levels in examined samples (DON range 0.09–2.99 ppm). Different and sometimes contradictory results were presented by Samarajeewa [1991] in her studies which stated that low penetration of heat, which varies from sample to sample, may be the reason for variuos results.

CONCLUSION

Reduction of DON and ZON concentrations in examined corn powder samples simple by their exposure to high temperature can be achieved to the level of 15% and 27% respectively. This level of reduction for itself cannot be sufficient. However, it can be very significant and taken into consideration if heating treatment is used as a part of food processing in which the concentration of DON and ZON in the processes of cleaning and wet and dry milling are already reduced to some level.

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УТИЦАЈ ТЕМПЕРАТУРЕ И ВРЕМЕНА НА САДРЖАЈ ДЕОКСИНИВАЛЕНОЛА (ДОН) И ЗЕАРАЛЕНОНА (ЗОН) У КУКУРУЗУ

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РЕЗИМЕ: Фумонизини су микотоксини рода *Fusarium*, који се јављају код кукуруза и прехрамбених производа на бази кукуруза и укључени су у неколико животињских и људских обољења. Иако је њихов утицај на људско здравље нејасан сматрају се фактором ризика код појаве рака. Печење, пржење и екструзија кукуруза на вишим температурама (190 °C) такође смањује концентрацију фумонизина у храни, а количина остварeног смањења зависи од времена кувања, температуре, рецептуре и осталих фактора. Циљ овог рада је био да процени ефективност температуре (200 и 220 °C) и времена (15 и 20 мин.) на детоксикацију кукурузног брашна које је било контаминирано са деоксиниваленолом и зеараленоном. Просечно смањење концентрације деоксиниваленола након третмана на 200 °C у трајању од 15 мину-

та износило је 12%, а након трајања од 20 минута 15%. Просечно смањење концентрације зеараленона након третмана на 200 °C у трајању од 15 минута износило је 22%, а након трајања од 20 минута 27%. Више температуре (220 °C) нису значајно утицале на даље смањење концентрација деоксиниваленола и зеараленона. Третман је само парцијално утицао у оба случаја.

КЉУЧНЕ РЕЧИ: деоксиниваленол, зеараленон, температура, период инкубације

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INVASIVE SPECIES IN ASS. *TRIFOLIO-AGROSTIETUM STOLONIFERAE* MARKOVIĆ 1973 IN BAČKA (SERBIA)

ABSTRACT: In the vegetation of meadows and pastures, due to climate changes and an inadequate and intensive use of hydromeliorative measures, invasive species play a significant role in the degradation of biodiversity. Secondary development of ass. *Trifolio-Agrostietum stoloniferae* Marković 1973 stands was observed in Bačka, in periodically flooded pastures. Floristic composition of these stands consists of 117 plant species, of which 94 grow in the Danube riverbank region and 97 around the Tisa river. According to the floristic analysis, *Ambrosia artemisiifolia*, *Bellis perennis, Carduus nutans, Cirsium arvense, Eupatorium cannabinum, Linaria vulgaris, Lotus corniculatus, Lythrum salicaria, Rumex crispus,* and *Trifolium repens* are characterized as invasive plants of the European region. Moreover, *Ambrosia artemisiifolia, Eleusine indica* and *Xanthium spinosum,* included in the *List of invasive species in AP Vojvodina*, are also present. *Lythrum salicaria* is regarded as one of the 100 most dangerous invasive alien species in the world.

KEYWORDS: invasive species, pasture, *Trifolio-Agrostietum stoloniferae,* vegetation

INTRODUCTION

Biodiversity conservation and environmental protection are primary concerns of contemporary society. Biodiversity reduction in ecosystems is a direct consequence of environmental changes. Expansion and retraction of species are natural phenomena that, owing to the intensive and often unfavorable human treatment, are increasingly expedited [Van Kleunen and Richardson, 2007]. By polluting habitats, humans are disrupting living conditions of many species, which consequently retreat or disappear. On the other hand,

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species that have successfully adapted to the changed habitat conditions rapidly proliferate and often become expansive. Anthropogenic factors thus strongly influence the changed relationships in ecosystems, leading to depletion of species and changes in ecosystem structures. Additional ecological pressure on the ecosystem imbalance is created by invasive plant species that further contribute to the biodiversity reduction [Wetzel, 2005]. According to the Convention on Biological Diversity, invasive species are one of the main threats to biological diversity [Šilc et al., 2012].

The main aim of the invasion ecology studies is identifying the determinants of invasiveness, many of which focus on the species' traits. Effective physiological and reproductive capacities, such as high growth rates, vitality, high plasticity and flexibility of the natural resource utilization and short life cycle, are known factors that promote invasiveness [Wetzel, 2005; Bernez et al., 2006; Van Kleunen and Richardson, 2007; Bekavac et al., 2010]. However, as the majority of the comparative studies of this phenomenon involve a small number of species, broader generalization of their findings is not possible [Van Kleunen et al., 2010]. On the other hand, it is necessary to emphasize the importance of interactions between species' traits and habitat invasiveness [Chase and Knight, 2006; Thiébvaut, 2007]. In that respect, high fluctuations in the available ecosystem resource levels have been identified as one of the key causes of invasiveness [Davis et al., 2000].

The necessity of invasive species monitoring has led to the development of international programs and databases, such as the Global Invasive Species Database − GISD (ISSG, GISP, IUCN), Delivering Alien Invasive Species Inventory for Europe − DAISIE, etc. More recent extensive research on the invasions and attempts to solve the problem of alien species includes floristic network mapping, as well as—empirically proven as more reliable—mapping using phytocoenological records [Šilc et al., 2012].

In the vegetation of meadows and pastures, in addition to climate changes, improper use, hydromeliorative measures, and conversion of meadows and pastures into arable land, invasive species have a significant influence on biodiversity degradation and decreased productivity. In the areas surrounding the rivers Danube and Tisa in Bačka, in the periodically flooded pastures in close proximity to human settlements, ass. *Trifolio-Agrostietum stoloniferae* Marković 1973 stands have developed. This association develops due to grazing, trampling and fertilization. Stands of ass. *Trifolio-Agrostietum stoloniferae* typically cover higher parts of the riverbanks, which are only temporarily flooded during high tides. In this area, the analyzed phytocoenosis is represented by stands belonging to two subassociations, the formation of which was caused by the microrelief differences.

Trifolio-Agrostietum stoloniferae subass. *agrostetosum albae* Marković 1973 stands are formed in the parts that remain hydrated throughout the year, whereas *Trifolio-Agrostietum stoloniferae* subass. *cynodontetosum* Marković 1973 stands are found in the higher parts of the riverbanks, characterized by markedly xerophilic habitat. In terms of syntaxonomic classification, ass. *Trifolio-Agrostietum stoloniferae* belongs to *Agropyro-Rumicion crispi* Nordh. 1940 alliance, *Agrostietalia stoloniferae* Oberdorf. 1967 order and *Molinio-Arrhenatheretea* Tx. 1937 class.

The aim of this paper is to demonstrate that the presence of invasive species ass. *Trifolio-Agrostietum stoloniferae* stands in Bačka can be a potential cause of biodiversity depletion and decreased pasture productivity.

MATERIALS AND METHODS

Phytocoenological studies of ass. *Trifolio-Agrostietum stoloniferae* stands in Bačka were conducted in the areas surrounding the Danube River (Sombor, Apatin, Bogojevo and Koviljski rit) and the Tisa River (Titelski breg, Žabalj-Jegrička, Bečej and Senta).

Biogeographical classification into floristic elements was performed according to Gajić [1980], and that pertaining to life forms followed Soó [1980].

RESULTS AND DISCUSSION

Floristic composition of ass. *Trifolio-Agrostietum stolonifeae* stands in the Bačka region comprises 117 plant species, of which 94 and 97 are found in the areas surrounding the rivers Danube and Tisa respectively. In the Danube region there are characteristic species of *Trifolium fragiferum* association, characteristic species of *Cynodon dactylon* subassociation, characteristic species of *Agropyro-Rumicion crispi* alliance and *Agrostietalia stoloniferae* order: *Agrostis verticillata, Rumex crispus,* and *Festuca arundinacea*, as well as characteristic species of *Molinio-Arrhenatheretea* class: *Trifolium repens, Lolium perenne, Achillea millefolium, T. pratense, Cichorium intybus, Pastinaca sativa* and *Plantago lanceolata* [Lazić, 1995; Stojanović et al., 1996; Džigurski and Nikolić, 2012]. In the area surrounding the Tisa River there are characteristic species of *Trifolium fragiferum* association, characteristic species of *Agropyro-Rumicion crispi* alliance and *Agrostietalia stoloniferae* order: *Agrostis verticillata, Rumex crispus* and *Inula britannica,* and characteristic species of *Molinio-Arrhenatheretea* class**:** *Lolium perenne* and *Andropogon ischaemum* [Stojanović et al., 2000; Džigurski et al., 2012]. Stand analysis points to the rich flora diversity of the investigated phytocoenosis, which is in line with the findings pertaining to Eastern Slavonia and Baranja, where 122 taxa were found [Šegulja and Topić, 1987]. Similarly,

80 species were identified in Srem [Butorac, 2004] and 43 in the region of northeastern Croatia [Rauš et al., 1985].

Table 1. Floristic composition of the ass. *Trifolio-Agrostietum stoloniferae* stands in the Bačka region, with life forms, floristic elements and invasiveness of the plant species, according to GISD (∗) and the *List of invasive species in AP Vojvodina* (∗∗)

Plant species	Podu-navlje	Poti-sje	Life forms	Floristic elements	Invasive spesies
Achillea millefolium L.	+	+	H.	Evr.	
Agrimonia eupatoria L.	+	+	H.	Evr.	
Agropyrum repens (L.) P. B.	+	+	G.	Evr.	
Agrostis alba L.	+	+	H.	Subevr.	
Agrostis verticillata Vill.	+	+	H.	Subm.	
Alopecurus pratensis L.		+	H.	Evr.	
Althea officinalis L.	+		H.	Subpont.-ca.	
Ambrosia artemisifolia L.		+	Th.	Adv.	*, **
Andropogon ischaemum L.	+	+	H.	Pont.-ca.-subm.	
Atriplex litoralis L.		+	Th.	Evr.	
Bellis perennis L.	+	+	H.	Subse.	*
Bidens tripartitus L.	+	+	Th.	Subse.	
Bromus arvensis L.	+		Th.-TH.	Evr.	
Bromus commutatus Schr.		+	Th.	Subse.	
Bromus tectorum L.		+	Th.	Evr.	
Calamagrostis epigeios (L.)Roth.	+		H.-G.	Evr.	
Calamintha vulgaris (L.)Druce.	+	+	H.	Cirk.	
Calystegia sepium (L.) Br.	+	+	H.	Evr.	
Capsella bursa-pastoris (L.)Med.	+	+	Th.-TH.	Kosm.	
Carduus acanthoides L.	+	+	TH.	Subse.	
Carduus nutans L.	+	+	TH (Th).	Subevr.	*
Carex distans L.	+	+	H.	Evr.	
Carex hirta L.	+	+	G.	Subevr.	
Carex praecox Schreb.	+		G.-H.	Evr.	
Carex vulpina L.	+	+	H.-HH.	Subevr.	
Cerastium caespitosum Gilib.	+		TH.-H(Ch.)	Kosm.	
Cichorium intybus L.	+	+	H.(TH.)	Subevr.	
Cirsium arvense (L.) Scop.,		+	G.	Subevr.	*
Cirsium lanceolatum (L) Scop.	+	+	TH.	Subevr.	
Convolvulus arvensis L.	+	+	H.-G.	Kosm.	
Crataegus monogyna Jacq.	+		M.	Subse.	
Crepis setosa Hall.	+	+	Th.	Subm.	
Cynodon dactylon (L.) Pers.	+	+	G.(H.)	Kosm.	
Dactylis glomerata L.	+	+	H.	Subevr.	

Daucus carota L.	+	+	Th.-TH.-H.	Subevr.	
Dipsacus laciniatus L.	+	+	TH.	Pont.-ca.-subm.	
Dipsacus sylvestris Huds.	+	+	TH.	Subse.	
Eleusine indica (L.) Gaertn.	+	+	Th.	Adv.	**
Epilobium adnatum Gris.		+	H.	Subevr.	
Erigeron canadensis L.	+	+	Th.-TH.	Adv.	
Eryngium campestre L.	+	+	H.	Subpont.-subm.	
Eupatorium cannabinum L.		+	H.	Subse.	*
Euphorbia cyparissias L.	+	+	H.(G.)	Evr.	
Festuca arundinacea Schreb.	+	+	H.	Subevr.	
Festuca pratensis Huds.	+	+	H.	Evr.	
Festuca pseudovina Hack.	+		H.	Evr.	
Galium aparine L.		+	Th.	Evr.	
Galium mollugo L.		+	H.	Subse.	
Galium verum L.	+	+	H.	Evr.	
Geranium pusillum Burm.		+	Th.	Subse.	
Glechoma hederacea L.	+	+	H.(Ch.-G.)	Evr.	
Gratiola officinalis L.	+	+	H.	Cirk.	
Heleocharis palustris (L.)R.Br		+	G.-HH.	Kosm.	
Helminthia echioides Gaertn.		+	TH.-Th.	Subm.	
Hordeum murinum L.		+	Th.	Subm.	
Inula britannica L.	+	+	TH.-H.	Subse.	
Juncus articulatus L.	+	+	H.	Cirk.	
Juncus compressus Jacq.	+		G.	Evr.	
Juncus gerardii Lois.	+		G.	Subcirk.	
Juncus inflexus L	+		H.	Subcirk.	
Kickxia elatine (L.) Dum.		+	Th.	Subatl.-subm.	
Linaria vulgaris Mill.	+	+	H.(TH.)	Subse.	*
Lolium perenne L.	+	+	H.	Subse.	
Lotus corniculatus L.	+	+	H.	Subevr.	*
Lotus tenuis W. et K.	+		H.	Subse.	
Lycopus europaeus L.	+	+	HH.	Subevr.	
Lycopus exaltatus L.	+	+	HH.	Subj.sib.	
Lysimachia nummularia L.	+	+	Ch.	Subse.	
Lythrum salicaria L.		+	H.-HH.	Pont.-ca.-subm.	*
Malva sylvestris L.	+	+	Th.-TH.-H.	Subse.	
Matricaria chamomilla L.	+	+	Th.	Evr.	
Medicago falcata L.		+	H.	Subpont.-ca.	
Medicago lupulina L.	+	+	Th.-TH.-H.	Subevr.	
Mentha aquatica L.		+	H.-HH.	Evr.	
Mentha longifolia (L.) Nath.	+	+	H.(G.)	Subse.	
Mentha pulegium L.	+	+	H.	Subse.	

Odontites rubra Gilib.	+		Th.	Subse.	
Ononis arvensis L.	+	+	H.-Ch.	Subse.	
Pastinaca sativa L.	+	+	H.	Evr.	
Phragmites communis Trin.	+	+	HH.	Kosm.	
Picris hieracioides L.	+		TH.-H.	Subpont.-ca.	
Plantago lanceolata L.	+	+	H.	Evr.	
Plantago major L.	+	+	H.	Evr.	
Plantago media L.	+	+	H.	Evr.	
Poa pratensis L.	+	+	H.	Subcirk.	
Poa trivialis L.	+		H.	Subevr.	
Polygonum aviculare L.	+		Th.	Kosm.	
Potentilla anserina L.	+	+	H.	Subcirk.	
Potentilla argentea L.	+	+	H.	Subpont.-ca.	
Potentilla reptans L.	+	+	H.	Evr.	
Prunella vulgaris L.	+	+	H.	Subevr.	
Ranunculus polyanthemus L.	+	+	H.	Subpont.	
Ranunculus repens L.	+	+	H.	Evr.	
Ranunculus sardous Cr.	+	+	Th. (-H.)	Subse.	
Rhinanthus rumelicus Vel.		+	Th.	Subsrbalk.	
Rorippa austriaca (Cr.) Bess.		+	HH.	Subpont.	
Rorippa sylvestris (L.) Bes.	+	+	H.	Subpan.	
Rumex crispus L.	+	+	H.	Evr.	*
Rumex hydrolapathum Huds	+	+	H.-HH.	Subse.	
Rumex pulcher L.	+		Th.-TH.	Subm.	
Sambucus ebulus L.	+	+	H.	Subpont.-subm.	
Setaria glauca (L.) P. B.		+	Th.	Kosm.	
Sinapis arvensis L.	+		Th.	Subevr.	
Sonchus arvensis L.	+	+	H.	Evr.	
Statice gmelinii Willd	+		H.	Pont.-pan.	
Stenactis annua (L.) Nees.		+	Th.	Adv.	
Taraxacumn officinale Web.	+	+	H.	Evr.	
Torilis arvensis (Huds.) Link.	+		Th.	Evrafr.	
Trifolium campestre Schreb.	+	+	Th.-TH.	Subse.	
Trifolium fragiferum L.	+	+	H.	Subse.	
Trifolium pratense L.	+	+	H.	Subevr.	
Trifolium repens L.	+	+	H.	Subevr.	*
Urtica dioica L.	+	+	H.	Evr.	
Verbena officinalis L.	+	+	Th.-H.	Kosm.	
Xanthium italicum Mor.	+	+	Th.	Adv.	
Xanthium spinosum L.	+	+	Th.	Adv.	**
Xeranthemum annum L.	+		Th.	Pont.-subm.	

Life form spectrum of the analyzed pasture vegetation indicates the dominance of hemicryptophytes (52.99%), including the species of the highest diagnostic significance and the highest values regarding abundance and ground cover: *Trifolium fragiferum*, *Agrostis verticillata, Rumex crispus, Festuca arundinacea, T. repens, Lolium perenne, Achilea millefolium, T. pratense*, etc. Significant presence of therophytes (27.35%) indicates warmer climate effects. Based on the percentage participation, these are followed by hemiterophytes (7.69%) and geophytes (6.84%). The presence of hydrophytes (3.42%) was noted in microdepressions that are moist throughout the larger part of the growing season. Hamephytes and phanerophytes are represented by a single taxon each (0.85%). Like other meadow phytocoenoses, the studied one is of therophytic-hemicryptophytic character. Tomić et al. (2010) indicate that in Karakuša region (Srem), the ass. *Trifolio-Agrostietum stolonifeae* is dominated by hemicryptophytes (64.3%). Much lower participation of therophytes (11.9%) is a result of the specific microclimatic conditions of this hunting region, which is under intense anthropogenic-zoogenic influence. Butorac [2004] also characterized the analyzed association found in Mt. Fruška Gora loess plateau as a therophytic-hemicryptophytic (with 57.50% hemicryptophytes and 23.75% therophytes). According to Jovanović-Dunjić [1969], the percentage increase in therophytes and decrease in hemicryptophytes, starting from parts of Croatia characterized by humid climate, through Serbia, to Macedonia is primarily driven by climatic conditions. Hence, the therophytes/hemicryptophytes ratio, proportional to the reduction in humidity in meadow phytocoenoses, points to the transitional ecological character of the studied region.

Phytogeographical analysis of the ass. *Trifolio-Agrostietum stolonifeae* stands in Bačka region indicates the dominance of plants belonging to the groups characterized by the floristic elements of wide distribution (61.53%). Eurasian group of floristic elements is the most common (42.73%), followed by cosmopolitan (7.69%), circumpolar (5.98%) and adventive (5.13%) groups. Groups of floristic elements of narrower distribution participate with 38.45%, and comprise Central European (19.66%), Pontic-Central Asian (11.96%), sub-Mediterranean (5.98%) and sub-Atlantic (0.85%) groups.

Based on the floristic analysis, *Ambrosia artemisiifolia*, *Bellis perennis, Carduus nutans, Cirsium arvense, Eupatorium cannabinum, Linaria vulgaris, Lotus corniculatus, Lythrum salicaria, Rumex crispus* and *Trifolium repens* were identified, according to Global Invasive Species Database (ISSG, GISP, IUCN), as invasive plants for the European region. Within the aforementioned species, *Lythrum salicaria* is regarded as one of the 100 most dangerous invasive alien species in the world. Among the stands examined in this study, in addition to the already mentioned *Ambrosia artemisiifolia, Eleusine indica* and *Xanthium*

spinosum are also included in the *List of invasive species in AP Vojvodina* (IASV 2011) [Table 1]. Life form analysis of the identified invasive species (58.33% hemicryptophytes, 25% therophytes, and 8.33% hemiterophytes and geophytes each) is proportional to the life form spectrum of the total ass. *Trifolio-Agrostietum stolonifeae* flora. From the geo-floristic perspective, the invasive species are dominated by the widely distributed floral elements (66.67%). This agreement in the biological spectrum analysis and that pertaining to the areal invasive species types on the one hand, and the total stand flora on the other, confirms excellent adaptability of invasive species, in particular to the specific vegetation relationships within this grassland phytocoenosis, and to the climatic conditions as well. Thompson and Davis [2011] also argue that the invasive species characteristics vary only slightly from the indigenous species. Therefore, in order to explain the process of a successful invasion, in addition to understanding the biological characteristics of the invasive species, further research must include interactions between invasive species and autochthonous flora [Keller et al. 2011].

Although the 12 invasive species of stands found in the analyzed pasture vegetation are present in smaller numbers and cover smaller area (with the exception of *Trifolium repens, Ambrosia artemisifolia* and *Cirsium arvense*), monitoring their expansion is necessary in order to prevent structure distortion and floristic composition impoverishment [Džigurski and Nikolić, 2012]. In addition, these species play a significant role in the reduction of pasture productivity because they interfere with the growth of desirable forage species. More specifically, apart from *Trifolium repens* and *Lotus corniculatus*, which are useful forage plants, the remaining species are, according to Mrfat-Vukelić et al. [1996], worthless (*Ambrosia artemisiifolia*, *Bellis perennis, Eupatorium cannabinum, Lythrum salicaria* and *Xanthium spinosum*) or mildly poisonous, i.e. harmful species (*Carduus nutans, Cirsium arvense, Rumex crispus*).

CONCLUSION

Floristic composition of ass. *Trifolio-Agrostietum stolonifeae* stands in the Bačka region comprises 117 plant species. Based on the floristic analysis, *Ambrosia artemisiifolia*, *Bellis perennis, Carduus nutans, Cirsium arvense, Eupatorium cannabinum, Linaria vulgaris, Lotus corniculatus, Lythrum salicaria, Rumex crispus, Trifolium repens, Eleusine indica* and *Xanthium spinosum* were identified as invasive plants. The presence of invasive species in the stands of the analyzed vegetation, along with climate changes and intensive and inappropriate use of these areas, will inevitably lead to the degradation of biodiversity in the future. Therefore, continuous monitoring

of the invasive species proliferation in the ass. *Trifolio-Agrostietum stoloniferae* vegetation is necessary in order to preserve biodiversity and enhance pasture productivity.

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ИНВАЗИВНЕ ВРСТЕ У ФИТОЦЕНОЗИ *TRIFOLIO-AGROSTIETUM STOLONIFERAE* MARKOVIĆ 1973 У БАЧКОЈ

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РЕЗИМЕ: Инвазивне врсте су у вегетацији ливада и пашњака, поред климатских промена, неадекватног и интензивног коришћења, хидромелиоративних захвата, превођења ливада и пашњака у ораничне површине, значајан узрок деградације биодиверзитета. На подручју Бачке, на периодично плављеним пашњацима, се-

кундарно су развијене састојине ass. *Trifolio-Agrostietum stoloniferae* Марковић 1973. Флористички састав ових састојина чини 117 биљних врста, 94 на подручју Подунавља и 97 на подручју Потисја. Анализом флоре утврђено је да су *Ambrosia artemisiifolia*, *Bellis perennis, Carduus nutans, Cirsium arvense, Eupatorium cannabinum, Linaria vulgaris, Lotus corniculatus, Lythrum salicaria, Rumex crispus* и *Trifolium repens*, окарактерисане као инвазивне биљке за подручје Европе. Са Листе инвазивних врста за подручје Војводине присутне су *Ambrosia artemisiifolia, Eleusine indica* и *Xanthium spinosum*. *Lythrum salicaria* је окарактерисана као једна од 100 најопаснијих инвазивних врста на свету.

КЉУЧНЕ РЕЧИ: инвазивне врсте, пашњак, *Trifolio-Agrostietum stoloniferae*, вегетација.

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BACILLUS SPP. IN THE CITY OF NOVI SAD GROUNDWATER SUPPLIES

ABSTRACT: The paper investigates the presence of spore-forming bacteria of the genus *Bacillus* among aerobic mesophilic bacteria found in water samples collected from the City of Novi Sad groundwater supplies. Microbiological examination included the samples from three groundwater supplies: *Štrand* (19 samples), *Petrovaradinska ada* (10 samples) and *Ratno ostrvo* (12 samples). Apart from the analysis of groundwater, the research also included microbiological examination of water from the City of Novi Sad water supply system collected from three localities - Liman 1, Slana bara and Stari grad. All samples were inoculated onto Plate Count Agar and low nutrient medium R2A.

The presence of *Bacillus* spp. was detected in all three groundwater supplies. Comparing the samples obtained from the three sites, a significantly higher number or percentage of the genus *Bacillus,* in comparison with aerobic mesophilic bacteria, was detected in the groundwater supply *Štrand* using R2A medium. No correlation was detected between the total count of aerobic mesophilic bacteria and *Bacillus* spp.

KEYWORDS: aerobic mesophilic bacteria, *Bacillus spp*., groundwater, City of Novi Sad

INTRODUCTION

According to the World Health Organization, drinking water quality is one of the main indicators of the wealth and health conditions of a country's population [World Health Organization and United Nations Children's Fund, 2004]. Apart from its physiological, hygienic and toxicological importance,

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water has a considerable epidemiological significance. Humans can become infected either directly, by consuming polluted drinking water and using it for food preparation, or indirectly, by applying polluted water for recreational purposes. The knowledge concerning the most common genera of bacterial contaminants is of a great significance, not only for water itself, but for groceries of various origin. In this respect, the presence of spore-forming bacteria can be of a great importance [Petrović, 1996].

Spores are primarily formed by gram positive rods, mainly the species belonging to the genera *Clostridium* and *Bacillus,* as well as by certain cocci and gram negative rods [Holt et al., 1994]. The genus *Bacillus* is a frequent contaminant of water, milk and dairy products, wheat, bread and pasta. Some of the common contaminants of food, including drinking water as well, are aerobic mesophilic bacterium *Bacillus subtilis*, aerobic thermophilic spore-forming bacterium *Bacillus stearothermophilus* and aerobic mesophilic spore-forming bacterium *Bacillus cereus* [Kramer and Gilbert, 1989].

The data gathered by the Institute of Public Health of Vojvodina point to a frequent cultivation of *Bacillus* spp. in purified and chlorinated water from the City of Novi Sad water supply system. Accordingly, *Bacillus spp.* appeared as the most commonly cultivated microorganisms in the tested samples of purified and chlorinated drinking water in 2011 [Ekobilten, 2008; Ekobilten, 2009; Ekobilten, 2011].

The City of Novi Sad water supply system uses the groundwater supply sites located in the vicinity of the Danube River. Three groundwater supply sites are in use: *Štrand* and *Ratno ostrvo* on the left bank of the river and *Petrovaradinska ada* on the right bank. A great cause for concern is the fact that, in spite of technological processing of groundwater, the presence of spore-forming bacteria of the genus *Bacillus* has been continually detected in a considerable number of the tested samples of drinking water from the City of Novi Sad water supply system [Ekobilten, 2008; Ekobilten, 2009; Ekobilten, 2011].

The aim of this study was to determine whether and to what extent bacterial spores of the genus *Bacillus* are detected in samples of the City of Novi Sad groundwater supplies. Also, the aim of this study was to determine if this phenomenon can be associated with a sampling site and the presence of large numbers of aerobic mesophiles. Furthermore, the study examined the possible presence of *Bacillus spp.* in purified and chlorinated drinking water distributed by the City of Novi Sad water supply system.

MATERIAL AND METHODS

The samples were collected in January, February and March 2011. The groundwater samples were collected from Štrand, Petrovaradinska ada and

Ratno ostrvo supply sites. The analysis included 19 samples collected from Štrand groundwater supply: Š1, Š3, Š5, Š7, Š8, Š9, Š10, Š16, Š17, Š18, Š19, Š20, Š21, BHD-1-2-20, P1, P2, P5, P8, and P10. From Petrovaradinska ada, 10 samples were collected: PA1, PA6, PA7, PA9, PA11, PA11a, PA12, PA13, PA14, and PAzbirni, while 12 samples were taken from Ratno ostrvo: BHZ1, BHZ3, BHZ5, BHZ7, BHZ9, BHZ11, Mp6, Md6, PJC1, PJC9, PPJC6, and PPJC10.

Apart from the groundwater samples, the study also included the analysis of three drinking water samples collected from different localities of the City of Novi Sad water supply system. The samples were designated as SG, SB and L1.

The analysis focused on the total number of *Bacillus* spp. and the total count of aerobic mesophilic bacteria in all of the collected samples. The total count of aerobic mesophilic bacteria was determined using Plate Count Agar (PCA) (Torlak) and low nutrient medium R2A (HiMedia), while the inoculation was performed using standard spread plate technique directly from the sample and from appropriate dilution in two repetitions, the inoculum quantity being 0.5 ml. With the samples inoculated onto PCA the incubation temperature was 37 °C, while in the case of R2A medium the temperature was 22 °C. The incubation period in both cases was 48 hours. After the incubation period, the counting of all grown colonies was performed. In order to determine the number of spore-forming bacteria of the genus *Bacillus*, the water samples were treated in the water bath at the temperature of 85 °C during 15 minutes prior to the inoculation. As for the inoculation process, the used media and the incubation temperature, the procedure was identical to the one applied to aerobic mesophilic bacteria. The incubation period lasted for 5 days. After the incubation period, the grown colonies were counted and the detection of *Bacillus* spp. was conducted with microscopic examination of preparations stained by Gramm and Schaeffer-Fulton technique.

Differences in number and percentage of *Bacillus* spp. found in groundwater samples were processed by means of Krusakl-Wallis and Mann-Whitney test. The tests, along with the figures and correlation analysis were processed by STATISTICA v. 10.0 [StatSoft, Inc. 1984 – 2011].

RESULTS AND DISSCUSION

Out of 19 samples collected from the Štrand spring, *Bacillus* spp. were present in 18. Only the sample P5 showed no colonies of these bacteria in any of the applied media. The greatest number of *Bacillus* spp. colonies on PCA were detected in sample Š7, with the recorded value of 250 CFU/ml. A slightly lower number was detected in samples BHD-1-2-20 (199 CFU/ml) and Š9 (176 CFU/ml), while the complete absence of spore-forming *Bacillus* genus bacteria was in samples Š21 and P5 (Figure 1). As for R2A medium, the number

of *Bacillus spp.* was the largest in sample Š9 (289 CFU/ml), while sample P5 showed total absence of the bacteria (Figure 1). In relation to the number of aerobic mesophilic acteria, the percentage of *Bacillus* spp. colonies on PCA ranged from 0 to 95.4%, while on R2A medium it spanned from 0 to 29.9 % (Figure 1). In samples Š3, Š9, P1 and P8 on R2A medium, as well as in samples P1 and BHD-1-2-20 on PCA, the percentage of *Bacillus* colonies could not be determined due to the abudance of aerobic mesophilic bacteria.

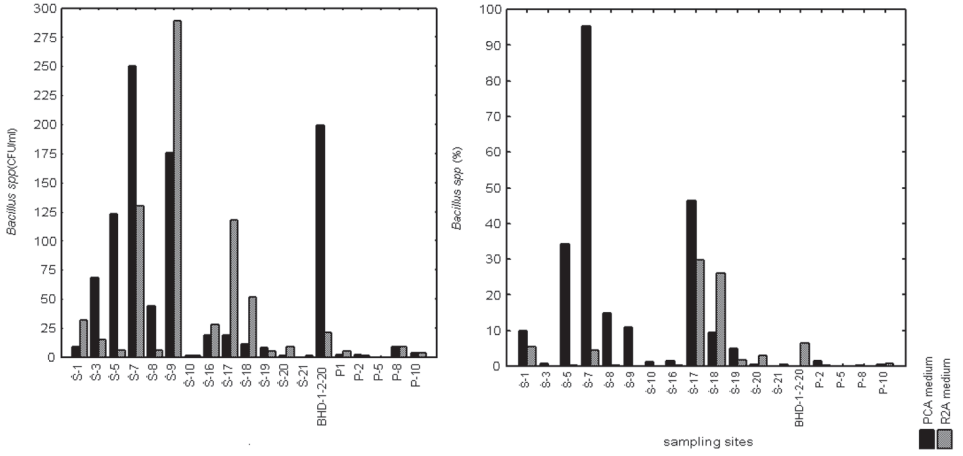

Figure 1. The total number of *Bacillus* spp. in samples from Štrand groundwater supply site isolated on PCA and R2A (left) and the percentage of *Bacillus spp.* among aerobic mesophiles on PCA and R2A (right)

In the case of Petrovaradinska ada supply site, the presence of *Bacillus* spp. was recorded in all of 10 samples included in the analysis. The highest number of *Bacillus* spp. colonies on PCA was detected in sample PA-14, 22 CFU/ml, while the samples PA-1, PA-9 and PA-11 showed the smallest number of the bacteria (Figure 2). As for R2A medium, the largest number of *Bacillus spp.* appeared also in PA-14 sample, the recorded value 6 of CFU/ml, while the samples PA1, PA6, PA9, PA11 and PA12 showed total absence of the bacteria (Figure 2). The percentage of *Bacillus spp.* colonies in relation to the number of aerobic mesophiles grown on PCA and R2A ranged 0.01% – 3.33% and 0% – 2.22%, respectively (Figure 2). In the case of sample PA14 on R2A, due to the abundance of aerobic mesophilic bacteria, the percentage of *Bacillus spp.* colonies could not be determined.

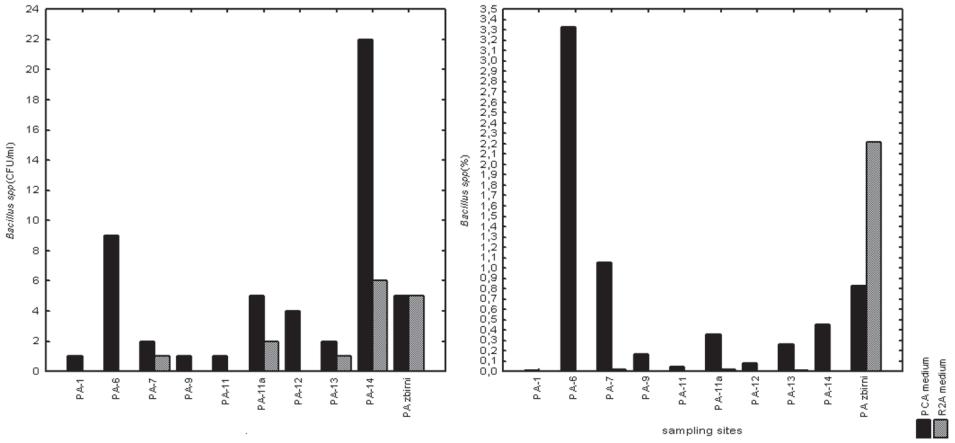

Figure 2. The total number of *Bacillus* spp. in samples from Petrovaradinska ada groundwater supply site isolated on PCA and R2A (left) and the percentage of *Bacillus spp* among aerobic mesophiles on PCA and R2A (right)

In 12 samples collected from Ratno ostrvo, the presence of spore-forming bacteria of the genus *Bacillus* was detected in 9 samples. The sample BHZ5 showed the highest number of *Bacillus* spp. colonies on PCA - 20 CFU/ml, while in samples BHZ3, BHZ11, PJC9 and PPJC6 none of the colonies was detected. As for R2A medium, the greatest number of the colonies was redorded in sample Md6 (14 CFU/ml), while in samples BHZ3, Mp6, PJC9 and PPJC6, *Bacillus* spp. colonies were absent (Figure 3). The percentage of *Bacillus* spp. in relation to the number of aerobic mesophiles on PCA ranged from 0% to 3.5%, while in the case of R2A, the recorded value was rather low ranging from 0% to 0.43% (Figure 3).

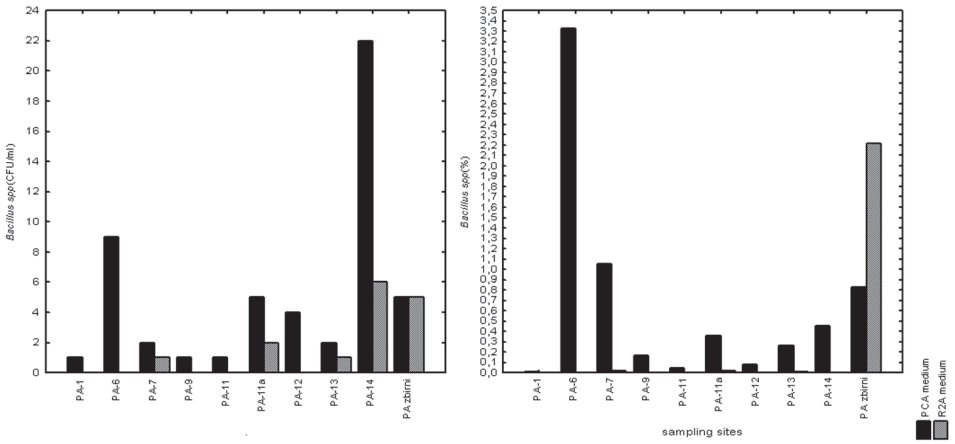

Figure 3. The total number of *Bacillus* spp. in samples from Ratno ostrvo groundwater supply sites isolated on PCA and R2A (left) and the percentage of *Bacillus spp.* among aerobic mesophiles on PCA and R2A (right)

In case of PCA application, no statistical differences were observed regarding the number of *Bacillus spp.* members detected in three groundwater supply sites (Kruskal-Wallis, $X^2 = 6.536579$, df = 2, $p>0.001$). However, with the use of low-nutrient R2A medium, statistical analysis pointed to a significant difference between the bacterial counts obtained for the three supply sites (Kruskal-Wallis, $X^2 = 15.16343$, df = 2, $p<0.001$). A considerably higher number of *Bacillus* spp. on R2A was registered in the samples taken from Štrand supply site, while the values obtained for other two sites showed no statistical differences (Mann-Whitney U Test, $p>0.01$). Comparing all three supply sites, it can be concluded that the sample with the largest count of *Bacillus* spp. detected on PCA was the one that originates from Štrand supply site. It was the sample Š7 with a total of 250 *Bacillus* spp. colonies detected. When applying on R2A medium, the sample Š9, collected from the same site, showed 289 colonies of spore-forming bacteria, which was also the highest number of *Bacillus spp.* detected in all three supply sites. With reference to all three supply sites, no statistical differences were observed in thepercentage of *Bacillus* spp. present among aerobic mesophiles detected on PCA (Kruskal-Wallis, $X^2 = 5.793854$, df = 2, $p>0.01$). However, with the application of R2A,

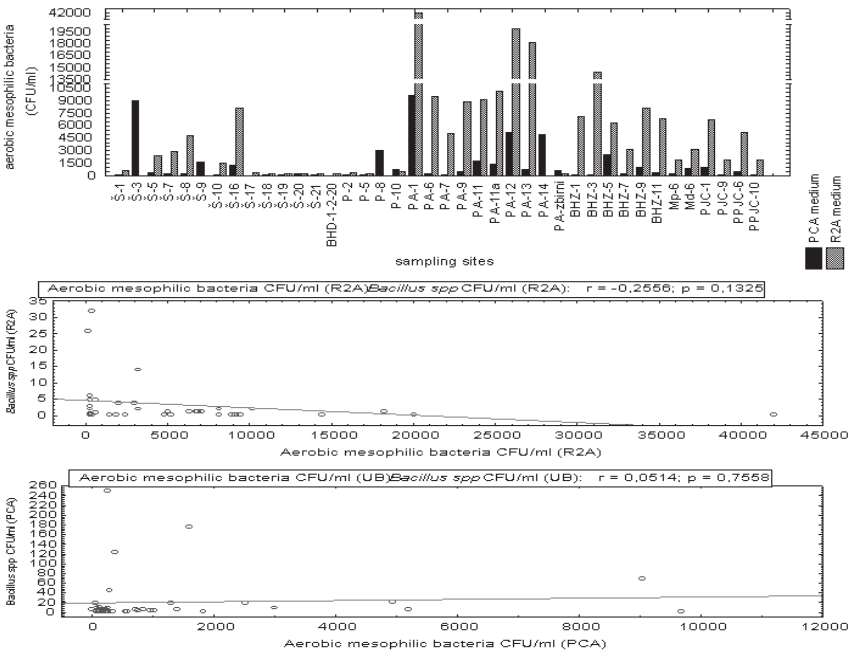

Figure 4. The total number of aerobic mesophilic bacteria for all three supply sites (above) and the results of correlation between the number of aerobic mesophilic bacteria and *Bacillus* spp. number obtained on PCA and R2A (below)

the analysis pointed to a significant difference in percentage of the bacteria detected among aerobic mesophiles (Kruskal-Wallis, $X^2 = 16.11641$, df = 2, $p<0.01$). A higher percentage of *Bacillus* spp. detected on R2A medium was recorded in the case of Štrand supply site, while the other two sites showed no significant differences in percentage of the detected bacteria (Mann-Whitney U Test, $p>0.01$). The results referring to the number of aerobic mesophilic bacteria recorded in the samples from all three supply sites and grown on PCA and R2A can be seen in Figure 4.

No correlation was detected between the number of aerobic mesophilic bacteria and *Bacillus* spp. number recorded for the groundwater supply sites (Figure 4).

Table 1. shows the total count of *Bacillus* spp. and aerobic mesophilic bacteria detected in samples collected from various localities of the city water supply system.

Table 1. The total count of *Bacillus spp.* and aerobic mesophilic bacteria detected in the samples from the water supply system

Sample	Aerobic mesophilic bacteria CFU/ml (PCA)	*Bacillus spp* CFU/ml (PCA)	Aerobic mesophilic bacteria CFU/ml (R2A)	*Bacillus spp* CFU/ml (R2A)
SG	5	0	784	0
SB	6	0	40	0
L1	3	0	19	0

The results presented in Table 1. clearly demonstrate that *Bacillus* spp. were not detected in any of the samples originating from the city water supply system.

The present study points to a high frequency of *Bacillus* spp. in the City of Novi Sad groundwater supplies. These results can be explained by the ubiquity of the bacteria in the environment and by a high resistance of the bacterial spores as well [Hosni et al., 2011]. *Bacillus* spp. members have been isolated from both shallow groundwater supplies [Chapelle et al., 1988] and from samples collected from a considerable depth [Boone et al., 1995]. Their viability in the form of spores in the process of drinking water treatment has been reported as a permanent problem [Bloomfield, 1999; Setlow, 2000; Dalmacija et al., 1996; Zhang et al., 2006; Morrow et al., 2008]. Some studies on the presence of bacterial spores [Sagripanto and Bonificino, 1996] compare the efficacy of glutaraldehydes, formaldehydes, hydrogen peroxide, peracetic acid, cupric ascorbate and natrium hypochlorite in destroying *Bacillus subtilis* spores under various conditions. Each of the above agents were used

considering pH, temperature and period of time. Only three of the chemicals, natrium hypochlorite, peracetic acid and cupric ascorbate, managed to destroy 99.9% of the spores, 30 minutes after incubation at the teperature of 20 °C. Glutaraldehydes inactivated 90% of the spores, while hydrogen peroxide and phenol destroyed a small proportion of spores in the suspension [Sagripanto and Bonificino, 1996].

Referring to *Ekobilten*, a publication comprising data gathered by the Institute of Public Health of Vojvodina, the presence of *Bacillus spp.* has been reported in a large number of samples from the City of Novi Sad water supply system [Ekobilten, 2011]. In the present study, the samples of water from the city water supply system showed no presence of *Bacillus* bacteria. However, as the results of the Institute of Public Health of Vojvodina point to a frequent cultivation of these bacteria in purified and chlorinated water from the city water supply system, this may cast doubt on the efficacy of the applied water treatment technology.

CONCLUSION

The presence of *Bacillus* spp. was detected in groundwater samples from all three supply sites. The percentage of *Bacillus* spp. detected among aerobic mesophilic bacteria widely varied, from 0 to 95%. Comparing all three water supply sites, a significantly higher total count and percentage of the genus *Bacillus,* in relation to the number of aerobic mesophilic bacteria, was detected in the samples from Štrand groundwater supply site using R2A medium. No correlation was detected between the number of aerobic mesophilic bacteria and *Bacillus* spp. number. *Bacillus* spp. were absent from the samples collected from the City of Novi Sad water supply system.

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BACILLUS SPP. У ПОДЗЕМНИМ ВОДАМА НОВОСАДСКИХ ИЗВОРИШТА ВОДЕ ЗА ПИЋЕ

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РЕЗИМЕ: У овом раду праћена је заступљеност спорогених бактерија рода *Bacillus* у оквиру аеробних мезофилних бактерија у узорцима вода новосадских изворишта. Микробилошка испитивања вршена су на узорцима пореклом са изворишта Штранд (19 узорака), Петроварадинска ада (10 узорака) и Ратно острво (12 узорака). Поред микробиолошке анализе вода изворишта вршена је микробиолошка анализа воде из дистрибуционог система водовода града Новог Сада са локалитета Лиман 1, Слана бара и Стари град. Узорци су инокулисани на подлоге *PCA* и *R2A*.

У подземној води сва три изворишта констатовано је присуство врста рода *Bacillus*. Посматрано за сва три изворишта значајно већа бројност и процентуална заступљеност представника рода *Bacillus* у односу на аеробне мезофилне бактерије регистрована је у води изворишта Штранд коришћењем R2A подлоге. Између бројности аеробних мезофилних бактерија и бројности бактерија рода *Bacillus* у изворштима подземних вода није утврђена корелација.

КЉУЧНЕ РЕЧИ: аеробне мезофилне бактерије, *Bacillus sp*, Нови Сад, подземне воде

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PHOMA MACDONALDI ON SEED AND ITS IMPORTANCE IN ETIOLOGY OF PHOMA BLACK STEM IN SUNFLOWER

SUMMARY: *Phoma macdonaldi* Boerema, teleomorph *Leptosphaeria lindquistii* Frezzi, is a widespread pathogen of sunflower. The aim of this research was to identify the presence of fungus *P. macdonaldi* in seed of different sunflower hybrids, as well as the correlation between seed and field infection. Phoma black stem assessment was performed on three hybrids grown in six localities in Serbia. Untreated and processed seeds of these hybrids were used in the seed health test. Severity of the disease did not differ between localities. Average disease index for hybrids H7, H9 and H19 was 14.01%, 13.25% and 11.83% respectively, and it shows that there are no significant differences in hybrid susceptibility. The index of disease indicates tolerance of these hybrids to Phoma black stem. Seed analysis showed the presence of fungi from the following genera: *Phoma, Alternaria, Botrytis, Sclerotinia, Penicillium* and *Aspergillus*. Seed infection with Phoma (of the untreated seeds) per hybrid ranged from 1.2–3.5%. There is no significant correlation between stem and seed infection.

KEYWORDS**:** *Phoma macdonaldi*, sunflower, seed and field infection

INTRODUCTION

Diseases are one of the limiting factors in sunflower production worldwide [Škorić et al., 2006]. Sunflower is a host plant for approximately 40 pathogens, but only some of them, depending on region, have a potential to reduce yield [Gulya et al., 1997]. In the favorable conditions, sunflower could be attacked by pathogens in all stages of development.

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Disease severity and yield losses depend on genotype resistance, aggressiveness of pathogen and climatic conditions. Intensity of the most important sunflower diseases such as downy mildew (*Plasmopara halstedii*), sunflower stem canker (*Phomopsis helianthi*), Phoma black stem (*Phoma macdonaldi*) and white rot (*Sclerotinia sclerotiorum*) is related to higher amount of precipitation [Maširević and Forgić, 2000]. *Phoma macdonaldi* Boerema, teleomorph *Leptosphaeria lindquistii* Frezzi, is a widespread and moderately damaging pathogen of sunflower [Maširević and Jasnić, 2006]. Phoma black stem has been spreading rapidly on the global scale in the last 10–15 years [Škorić et al., 2012].

P. macdonaldi overwinters on infected plant debris in form of mycelium and pycnidia and after the third year it could form perthecia as teleomorph *Leptosphaeria lindquistii* [Frezzi, 1968; Marić et al., 1981]. Penetration of fungus into plant tissue is done mechanically through wounds or through natural plant openings such as stomata [Al Fadil et al., 2011]. Symptoms of Phoma black stem could be seen on the all above ground plant organs [Maširević and Jasnić, 2006]. The most typical symptoms appear on the stem as circle shaped, oval or irregular large black spots (5–10 cm in diameter). Number of the spots increases during vegetation, and in some cases could encircle the stem. In conditions favorable for disease development, a stem could be completely covered with black spots [Marić et al., 1988]. Lesions are limited to the surface layer of the stem [Debaeke and Pérès, 2003]. However, in highly susceptible genotypes during later stages of plant development the lesions could sometimes penetrate into the central part of the stem and could cause its breaking. A severe attack of the pathogen causes the diseased plants to wilt and die prematurely [Marić et al., 1988]. Their heads become smaller; seeds are empty or shriveled; seed and oil yields decrease [Darvishzadeh et al., 2008]. The role of seed in the process of fungus transmission is not clear.

The aim of this research was to identify the presence of fungus *Phoma* in seeds of different sunflower hybrids in different localities in Serbia, as well as the correlation between stem and seed infection. The aim was also to show the possibility and importance of seed infection for spreading of the pathogen.

MATERIAL AND METHOD

Disease assessment

Disease assessment (presence of *P. macdonaldi*) was performed on three hybrids grown in six localities. Sunflower hybrids marked H7, H9 and H19 were from the Institute of Field and Vegetable Crops in Novi Sad, and the six localities were as follows: Neštin, Kula, Pančevo, Vrbas, Zaječar and Sombor. The experiment was conducted during 2010.

The evaluation of Phoma black stem natural infections in the field was done according to scale 0-9 (0 - no disease; 1 - 10–20%; 2 - 21–30%; 3 - 31–40%; 4 - 41–50%; 5 - 51–60%; 6 - 61–70%; 7 - 71–80%; 8 - 81–90%; 9 - 91–100%) when sunflower plants were in physiological maturity [Maširević, 1995]. Approximately 150 plants of each hybrid in each locality were assessed. McKinney's disease index was calculated. Fungicide treatments during the vegetation were not applied to the tested sunflower hybrids.

Examination of seed health status

Untreated and processed seeds of sunflower hybrids evaluated in the field were used for testing. Sunflower seeds were sterilized by 1% sodium hypochlorite (NaOCl) for 5 min followed by draining. Sterilized and dried seeds were put on wet filter paper. Seed analysis of every hybrid was done in four replicates with 25 seeds. Sunflower seeds were incubated for 7 days at the temperature between 25 and 26 °C. Presence of the causal agent of Phoma black stem was identified according to morphological characteristics of isolates and forming of pycnidia and pycniospores on the surface of sunflower seeds and in the pure culture isolates [Boerema et al., 2004].

Seed germination data were transformed into Arcsin values and analyzed by factorial ANOVA and Duncan test. Correlation between the level of infection in the field and the level of seed infection in laboratory conditions was calculated. Statistical analysis was performed using Statistica 10 software.

RESULTS AND DISCUSSION

Field evaluation

Severity of Phoma black stem did not vary between five examined localities and it ranged from 12–16%. Significantly lower index of disease, comparing to the other localities, was noticed only in the locality Zaječar (3.3%). Average disease index for hybrids H7, H9 and H19 was 14.01, 13.25% and 11.83% respectively and it shows that there are no significant differences in hybrid susceptibility (Table 1). The index of disease indicates the resistance of hybrids to Phoma black stem. A large majority of sunflower plants had infection intensity 10–20%, although in four out of six localities (Neštin, Kula-Vitovnica, Pančevo and Vrbas) there were individual plants with higher infection level, but not over 40%. Genotypes with disease attack of 20% and less could be classified as resistant [Goian, 1984].

Table 1. McKinney's disease index of Phoma black stem in tested hybrids in different localities

Locality	McKinney's disease index (%)			Average disease index per locality
	H7	H9	H19	
Neštin	23.08	12.5	12.75	**16.11**
Kula-Vitovnica	11.11	20.76	15.30	**15.7**
Pančevo	11.11	17.11	12.30	**13.5**
Vrbas	22.83	12.73	14.77	**16.7**
Zaječar	4.01	5.30	1.03	**3.3**
Sombor	11.93	11.11	14.84	**12.6**
Average	**14.01**	**13.25**	**11.83**	**13.0**

The amount of precipitation during 2010 was highly above the average in five out of six localities [Republic Hydrometeorological Service of Serbia, 2010] (Figure 1). According to Aćimović [1998] infections in natural conditions usually occur in the first half of July. Optimal conditions for disease development are temperatures around 25 °C, relative humidity 100% and presence of water drops. Zaječar was the locality with the lowest amount of rainfalls during vegetation period and the lowest disease incidence. The amount of rainfalls in Zaječar was at the level of multiannual average of rainfalls in Serbia, with significantly lower precipitation in August and September. Our results are in accordance with Marić et al. [1988] who reported that lower humidity in August and September lead to weaker attack of Phoma black stem. Disease intensity in other tested localities was higher than in Zaječar. During their four-year research, Fayzalla and Marić [1981] found that disease severity was greater in years with less favorable distribution of rainfalls and these factors lead to weakness of plant vitality and increased their

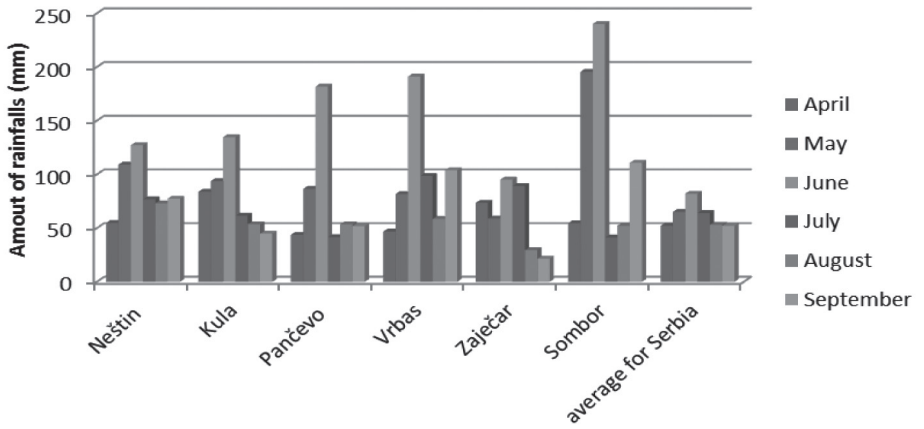

Figure 1. Monthly amount of rainfall per locality

susceptibility to disease. Testing the sunflower inbred lines tolerance to Phoma black stem during 2010 in Rimski Šančevi in the conditions of artificial infection, Dedić [2012] obtained higher disease intensity in the non-irrigated fields.

Seed health testing

Analysis of sunflower showed presence of fungi from the following genera: *Phoma, Alternaria, Botrytis, Sclerotinia, Penicillium*, and *Aspergillus*. The most frequent were fungi from genus *Alternaria*. The appearance of other fungi in sunflower seed were below 5%. The seed infection with *Phoma* was low, but there were two localities where a significantly higher level of seed infection was detected (Pančevo and Neštin) (Table 2).

There is no significant correlation between stem and seed infection. This could be explained by lower field infection and the presence of symptoms only in stems but not in head.

Table 2. Seed infection depending on locality

Locality	Seed infection with *Phoma* (%)
Sombor	0.67[a]
Vrbas	1.00[a]
Kula-Vitovnica	1.33 [a]
Zaječar	1.67 [a]
Nestin	3.67 [b]
Pančevo	6.33 [b]
F= 7.41* p= 0.0001*	

Seed infection with Phoma (identified in untreated seed) per hybrid ranged from 1.2–3.5% (Table 3). Average level of seed infection is different and depends on hybrid in contrary to stem field infections. Hybrid H7 had the lowest percent of seed infection. Darvishzadeh et al. [2007a] reported that the level of seed infection depended on the variability in resistance among the genotypes as well as among the isolates and their interaction.

Table 3. Seed infection depending on hybrid

Hybrid	Seed infection (%)
H7	1.2 [a]
H19	2.7 [ab]
H9	3.5 [b]
F=5.77* p=0.008*	

According to some earlier researches, the presence of *P. macdonaldi* was not noticed in sunflower seed and the conclusion was that seed could not serve as a source of inoculum [Fayzalla, 1978]. However, other authors confirmed that pathogen could develop in seed and could be transmitted by seed [El-Sayed and Marić, 1981; Bhutta et al., 1997; Darvishzadeh, 2007b] and that the infection could also lead to reduction of germination [Saharan et al., 2006]. Lević et al. [2012] in a recent study of seed mycoflora (seed samples originated from different localities in Serbia) did not detect *P. macdonaldi* in sunflower seed. Our results showed the presence of *P. macdonaldi* in seed. Similar percent of seed infection (0.25–3.25%) was also confirmed by other authors [Stajić et al., 2001]. Infected seeds could be eliminated through fungicide treatment [Marić et al., 1988]. It is well known that all commercial seed is treated with some fungicides and that is one of the most important reasons why this pathogen could not be widespread from region to region by contaminated seed. These results indicate that this pathogen is seed borne.

BLACK STEM IN SUNFLOWER

CONCLUSION

The tested sunflower hybrids had stem infection intensity from 10–20% and seed infection from 1–3.5%. In the conditions of naturally infected plants such disease incidence indicates that there is no significant correlation between field stem and seed infection, despite the fact that these pathogen can be seed borne.

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PHOMA MACDONALDI НА СЕМЕНУ И ЊЕН ЗНАЧАЈ У ЕТИОЛОГИЈИ ЦРНЕ ПЕГАВОСТИ СУНЦОКРЕТА

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САЖЕТАК: *Phoma macdonaldi* Boerema; телеоморф *Leptosphaeria lindquistii* Frezzi један је од најраширенијих патогена сунцокрета. Циљ овог истраживања био је да се утврди присуство гљиве *P. macdonaldi* на семену различитих хибрида сунцокрета као и корелација између инфекције стабла у пољу и инфекције семена. Процена интезитета болести извршена је на три хибрида која су гајена на шест локалитета. За одређивање здравственог стања коришћено је нетретирано и дорађено семе хибрида испитиваних у пољу. Интензитет напада болести није се значајно разликовао међу локалитетима. Просечан индекс обољења за хибриде H7, H9 и H19 био је 14,01%, 13,25% и 11,83% што показује да не постоје значајне разлике у осетљивости хибрида према црној пегавости. Такође, добијени индекс указује на толерантност испитиваних хибрида према овом проузроковачу болести. Анализом здравственог стања семена сунцокрета утврђено је присуство гљива из следећих родова: *Phoma, Alternaria, Botrytis, Sclerotinia, Penicillium* и *Aspergillus*. Зараженост семена гљивом *P. macdonaldi* утврђена је на дорађеном и нетретираном семену и кретала се од 1,2–3,5%. Није утврђена статистички значајна корелација између инфекције стабла и семена у пољским условима. Ово се може објаснити ниским степеном напада патогена и присуством симптома само на стаблу, а не на главици сунцокрета.

КЉУЧНЕ РЕЧИ: *Phoma macdonaldi*, инфекција у пољу, семе сунцокрета

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INHERITANCE OF ECONOMIC TRAITS OF *MICROCERASUS TOMENTOSA THUNB.* INTERVARIETAL HYBRIDS

ABSTRACT: A hybrid fund of *Microcerasus tomentosa* comprising 6 families with a total of 287 plants has been created. The features of the inheritance of important economic traits in hybrid offspring intervarietal hybrids *Microcerasus tomentosa* are defined. The hybrid family and cross combinations with high features of macrocarpa, small fruit size, dry berry separation, vitamin C, immunity and precocity are defined. During the study period of controlled hybrid offspring of crosses a number of elite seedlings was identified – Натали х Юбилейная, Натали х Смуглянка восточная, and Натали х Розовая урожайная that combine high rates of fruit weight with other economic traits.

KEYWORDS: *Microcerasus tomentosa*, *Monilia cinerea*, intervarietal hybridization, reciprocal hybrids

INTRODUCTION

Plant breeding success is largely dependent on the source of material and the degree of scrutiny. Varied primary sources are a reliable reserve of the selective breeding process. Allocation from the created gene pool of economic traits, preparation of new genetic donors in the actual directions of selective breeding, and development of the effective methods of their involvement in programs of the selective breeding are of crucial importance.

The fruit crop *Microcerasus tomentosa* is a promising crop for cultivation in Belarus. It covers the territory from the Pacific Ocean to the Himalayan Mountains and mountainous Turkestan in Central Asia [Царенко, 2004] and has a range of ecological plasticity and sufficient frost hardiness. *Microcerasus tomentosa* is

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widely introduced in Japan, China, Korea and the Far East – Khabarovsk and Primorsky regions of Russia. *Microcerasus tomentosa* is found to be promising for cultivation in the harsh conditions of the Canadian Prairies and in the north of the United States [Еремин, 1996; Казьмин, 1975; Михеев, 1990].

Almost none of the varieties of *Microcerasus tomentosa* is found suitable for the ground and climatic conditions of the Republic of Belarus. Only some of them are recommended for home gardening. This is primarily due to the lack of varieties resistant to *Monilia cinerea* and a large percentage of plant death at the age of 8–12 years in commercial orchards, low transportability of berries (wet berry separation and juice loss), absence of self-fertile varieties, very early flowering (without pollinators), and damping-off root collar in the spring. However, the cultivation of this crop is justified because of its early appearance, high annual yield, and resistance to frost and coccomycosis [Бученков, 2000; Царенко, 2004].

Michuryn I. V. was the first who came across *Microcerasus tomentosa* and introduced it to the gardens of the European part of Russia in 1923. First scientifically based selective breeding of *Microcerasus tomentosa* started in the 1930s by Tikhonov N. N. and continued by Kazmin G. T in the 1940s–50s. Since the 1970s the work on cherry selective breeding was continued by Carenko V. P. and Carenko N. A. [Еремин, 1996; Казьмин, 1975; Михеев, 1990].

The aim of our work is to analyze *Microcerasus tomentosa* hybrid offspring of our breeding and the selection of the promising forms.

MATERIAL AND METHODS

Studies on intervarietal hybridization and analysis of morphological and biological characteristics were performed from 2004 to 2012. This article presents the average values after nine years of research.

The object of research is a hybrid offspring out of intervarietal crossing of 6 *Microcerasus tomentosa* varieties: Ранняя розовая, Хабаровчанка, Смуглянка восточная, Юбилейная, Розовая урожайная, and Натали.

Hybridization, field observations and experiments were performed [Седова, 1999]. The hybrid forms were evaluated by diameter and weight of the fruit, the ratio of fruit weight to stone mass, type of separation of fruit, the vitamin C content, resistance to *Monilia cinerea*, and the timing of entry into fruition.

Resistance to *Monilia cinerea* was scored on the general status of plants: 1 point bushes perfectly healthy; 2 points – slight damage (individual twigs), 3 points – average loss (about 30% of twigs), 4 points – severe loss (up to 50% of twigs), 5 points – lesions on more than 50% of twigs, no gain.

Berry weight was determined by weighing 100 randomly selected fruits. Ascorbic acid content in berries in the phase of full ripeness was determined by the indophenol method.

RESULTS AND DISCUSSION

As a result of intervarietal hybridization in 30 cross combinations, 4,064 flowers were pollinated, 2,227 seeds received, and 861 seedlings grown (Table 1). After culling, 287 plants were selected for further study.

Table 1. Some features of intervarietal hybrids of *Microcerasus tomentosa* varieties (aggregated data over 9 years)

Crossing combination	Pollinated flowers, pieces	Accounting of ovary		Collected fruits		Grown seedling	
		pieces	%	pieces	%	pieces	%
Ранняя розовая х Хабаровчанка	126	77	61.11	71	56.35	25	19.84
Ранняя розовая х Смуглянка восточная	141	80	56.74	73	51.75	27	19.15
Ранняя розовая х Юбилейная	136	75	59.15	64	47.06	18	13.24
Ранняя розовая х Розовая урожайная	128	73	57.03	68	53.13	23	17.97
Ранняя розовая х Натали	127	75	59.06	70	55.12	27	21.26
Хабаровчанка х Ранняя розовая	132	82	62.12	75	56.82	28	21.21
Хабаровчанка х Смуглянка восточная	121	85	70.75	79	65.29	31	25.62
Хабаровчанка х Юбилейная	146	86	58.90	80	54.79	36	24.66
Хабаровчанка х Розовая урожайная	134	83	61.94	77	57.46	39	29.10
Хабаровчанка х Натали	124	84	67.74	80	64.52	35	28.23
Смуглянка восточная х Хабаровчанка	145	77	53.10	71	48.97	27	18.62
Смуглянка восточная х Ранняя розовая	136	81	59.59	74	54.41	28	20.59
Смуглянка восточная х Юбилейная	125	78	62.40	72	57.60	32	25.60
Смуглянка восточная х Розовая урожайная	142	74	52.11	66	46.48	19	13.38
Смуглянка восточная х Натали	137	69	50.36	60	43.80	15	10.95
Юбилейная х Смуглянка восточная	144	72	50.00	67	46.53	20	13.89

Юбилейная х Хабаровчанка	126	68	53.97	62	49.21	17	13.49
Юбилейная х Ранняя розовая	131	67	51.15	61	46.56	14	10.69
Юбилейная х Розовая урожайная	147	71	48.90	67	45.58	19	12.93
Юбилейная х Натали	150	73	48.67	69	46.00	23	15.33
Розовая урожайная х Юбилейная	123	85	69.11	78	63.41	29	23.58
Розовая урожайная х Смуглянка восточная	135	88	65.19	82	60.74	40	29.63
Розовая урожайная х Хабаровчанка	149	86	57.72	79	53.02	28	18.79
Розовая урожайная х Ранняя розовая	132	84	63.64	76	57.58	30	22.73
Розовая урожайная х Натали	137	87	63.50	80	58.39	36	26.28
Натали х Розовая урожайная	125	92	73.60	86	68.80	41	32.80
Натали х Ранняя розовая	147	94	63.95	88	59.86	43	29.25
Натали х Хабаровчанка	145	91	62.76	83	57.24	35	24.14
Натали х Смуглянка восточная	133	90	67.68	84	63.16	37	27.82
Натали х Юбилейная	140	93	66.43	85	60.71	39	27.86

It is established that Натали and Хабаровчанка grades are characterized by higher rates of set of fruits at cross-pollination, where the maternal plant is Натали grade.

One of the main objectives of *Microcerasus tomentosa* selection is the increase in the size of fruits. The analysis of average values of diameter and mass of fruits has shown that their maximum values are typical of hybrid families where the maternal plant is Натали grade (Table 2). The analysis of hybrid progeny has revealed significant differences in combining ability of the original parental grades on the basis of the diameter of fruits. Most of the studied grades significantly exceeded their offsprings. Field evaluation of hybrid seedlings, conducted in different years showed that the highest yield of large-fruited plants is observed in those combinations where both or one of the parents had adequate high rate. Thus, in combinations of crossing Натали х Смуглянка восточная, Юбилейная х Смуглянка восточная, and Розовая урожайная х

Смуглянка восточная the share of seedlings with large fruits was 72.5–85.7%, and in combinations of Натали х Юбилейная, and Натали х Розовая урожайная – above 90%. The analysis of medium-sized fruits has shown that in a hybrid family where the parent variety is Ранняя розовая, diameter is 1.64 ± 0.15 cm; Хабаровчанка – 1.47 ± 0.12 cm; Смуглянка восточная – 1.69 ± 0.16 cm; Юбилейная – 1.77 ± 0.18 cm; Розовая урожайная – 1.73 ± 0.17 cm; and Натали – 1.82 ± 0.19 cm (Table 2). Maximum diameter of fruits recorded in reciprocal hybrids of Натали х Юбилейная is 1.94 ± 0.21 cm.

The analysis of hybrid offspring has shown that the greatest number of plants in the progeny of grades with different fruit weight usually occupies an intermediate position between the parental forms. However, in most combinations of individual seedlings some were superior to the best parent form of the analyzed lines.

The evaluation of a number of fruit *Microcerasus tomentosa* parental forms and intervarietal hybrids has identified a close relationship between the manifestation of this trait and the characteristics of the genotype. It was found that the source of increased mass of berries are Натали and Юбилейная grades. In all hybrid combinations involving these grades, obtained transgressive seedlings (from 6.5% to 12.8%) with fruit weight 3.9–4.6 g, and their output did not depend on the maternal or paternal form used.

It was found that the average fruit weight in the hybrid family with Ранняя розовая as the mother grade was 2.25 ± 0.17 g; Хабаровчанка – 2.05 ± 0.14 g; Смуглянка восточная – 2.55 ± 0.19 g . Юбилейная – 3.55 ± 0.21 g; Розовая урожайная – 3.05 ± 0.20 g; and Натали – 4.00 ± 0.23 g (Table 2).

Table 2. Quality of fruits of varieties of *Microcerasus tomentosa* (aggregated data over 9 years)

Hybrid family (maternal grade)	Diameter of a fruit, cm	Mass of a fruit, g.	Mass of a stone, g. (%)	Type of a separation of a fruit	Content of vitamin C, мg/ 100 g.
Ранняя Розовая	1.64±0.15	2.25±0.17	0.18±0.03 (8.00)	damp	19.9±1.7
Хабаровчанка	1.47±0.12	2.05±0.14	0.17±0.02 (8.29)	damp	18.7±1.5
Смуглянка восточная	1.69±0.16	2.55±0.19	0.19±0.04 (7.45)	semi-dry	25.4±1.9
Юбилейная	1.77±0.18	3.55±0.21	0.21±0.05 (5.92)	semi-dry	32.5±2.4
Розовая урожайная	1.73±0.17	3.05±0.20	0.20±0.04 (6.56)	damp	23.5±1.8
Натали	1.82±0.19	4.00±0.22	0.21±0.05 (5.25)	semi-dry	30.6±2.1

The value of *Microcerasus tomentosa* grades is also defined by a ratio of mass of edible part to the mass of a stone. The volume of waste in many respects depends on this indicator. According to Мегердичев Е. Я. the stone has to be small, not comprising more than 7%. The average stone mass in the hybrid family with Ранняя розовая as the mother grade was 0.18 ± 0.03 g; Хабаровчанка – 0.17 ± 0.02 g; Смуглянка восточная – 0.19 ± 0.04 g; Юбилейная – 0.21 ± 0.05 g; Розовая урожайная – 0.20 ± 0.04 g; and Натали – 0.21 ± 0.05 g. Our research showed that smaller stones are typical of the reciprocal hybrids of Натали х Юбилейная (5.76%) and Натали х Розовая урожайная (6.28%), and larger ones for Хабаровчанка х Ранняя розовая (8.17%) and Хабаровчанка х Смуглянка восточная (7.86%) (Table 2). Minimal stone mass is fixed in reciprocal hybrids Натали х Юбилейная. In hybrid families Натали х Юбилейная and Натали х Розовая урожайная the share of transgressive seedlings for this indicator was 9.5% and 18.7 %, respectively.

The study of vitamin C content in the period of biological ripeness of fruits showed that in hybrid families this indicator changes, ranging from 18.7 to 32.5 mg/100 g. In fruit hybrids, where the mother plant is Ранняя розовая, the average content of vitamin C is 19.9 ± 1.7 mg/100 g; Хабаровчанка – 18.7 ± 1.5; Смуглянка восточная – 25.4 ± 1.9; Юбилейная – 32.5 ± 2.4; Розовая урожайная – 23.5 ± 1.8; Натали – 30.6 ± 2.1 (Table. 2). Selection estimation of intervarietal *Microcerasus tomentosa* hybrids indicates the possibility of obtaining forms with high vitamin C content by excision in the hybrid offspring of transgressive genotypes. The most effective donors of these traits were Юбилейная and Натали grades. However, the determination of the degree of dominance revealed that inheritance of vitamin C inclines to a worse parent, and even depression in most of the studied families. The heterotic effect was only found in Натали х Юбилейная family.

The study of hybrid forms on the basis of separation of fruits showed that the dry berry separation is typical of hybrids where maternal plants are Смуглянка восточная, Юбилейная, and Натали grades (Table 2).

Offspring of varieties crossed with a wet margin fruit was mainly characterized by low rate of the studied trait, and a significant portion of seedlings had a negative transgression. In combination with the best of these parents, there were identified transgressive seedlings superior to parents. Thus, in Натали х Юбилейная and Натали х Смуглянка восточная families there was found 40.7% and 56.5% respectively of plants with dry margin.

As a result of the analysis of *Microcerasus tomentosa* hybrids stability to *Monilia cinerea* it is established that hybrid families significantly differ due to this indicator. Absolutely steady forms have not been revealed. Hybrid families where maternal plants were Смуглянка восточная, Юбилейная, and Натали grades were put into relatively stable group (loss 2 points); with average

steadiness (3 points) – Розовая урожайная; unstable (4 points) – Ранняя розовая, and Хабаровчанка (Table 3).

The assessment of hybrid fund on the basis of an early maturing of fruits allowed to define that the earliest introduction in fructification is typical of hybrid families where maternal plants were Натали and Юбилейная grades (Table 3).

Early introduction in fructification is particularly noted in the reciprocal combinations of Натали х Юбилейная where more than 50% of seedlings enter a fructification period on the 3rd year of vegetation.

Table 3. Stability to *Monilia cinerea* and early maturing of fruits of *Microcerasus tomentosa* varieties (aggregated data over 9 years)

Hybrid family (maternal grade)	Stability to *Monilia cinerea*, point	Introduction in fructification, year
Ранняя розовая	4	4
Хабаровчанка	4	5
Смуглянка восточная	2	4
Юбилейная	2	3
Розовая урожайная	3	4
Натали	2	3

CONCLUSION

The hybrid fund of 6 families and 287 plants by the way of inter-high quality hybridization of *Microcerasus tomentosa* has been created.

Hybrid families, where maternal plants are Смуглянка восточная, Юбилейная, and Натали grades, have high features of macrocarpa, a small fruit size, dry berry separation, and vitamin C.

The hybrid forms of families, where maternal plants are Смуглянка восточная, Юбилейная, and Натали grades, can be used as an initial material in the selection of *Monilia cinerea* stability.

The early introduction in fructification is typical of hybrid families with Юбилейная and Натали grades.

During the study period of controlled hybrid offspring of crosses a number of elite seedlings was identified – Натали х Юбилейная, Натали х Смуглянка восточная, and Натали х Розовая урожайная, that combine high rates of fruit weight with other economic traits.

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НАСЛЕЂИВАЊЕ ЕКОНОМСКИХ ОСОБИНА КОД *MICROCERASUS TOMENTOSA THUNB.* МЕЂУСОРТНИХ ХИБРИДА

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РЕЗИМЕ: Направљен је хибридни фонд *Microcerasus tomentosa* који се састоји од шест породица са укупно 287 биљака. Дефинисане су карактеристике наслеђивања важних економских особина хибридног потомства *Microcerasus tomentosa* међусортних хибрида. Одређена је хибридна породица као и унакрсне комбинације са високим обележјима великог и малог плода, сепарацијом сувих бобица, витамином Ц, имунитетом и превременим развојем. Током периода истраживања контролисаног укрштеног хибридног потомства, установљен је број елитних садница − Натали х Юбилеиная, Натали х Смуглянка восточная и Натали х Розовая урожаиная које комбинују високу стопу тежине плода са другим економским особинама.

КЉУЧНЕ РЕЧИ: *Microcerasus tomentosa*, *Monilia cinerea*, међусортна хибридизација, реципрочни хибриди

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VERTEBRATE FAUNA AT THE NEOLITHIC AND ENEOLITHIC SITES IN VOJVODINA (SERBIA)

ABSTRACT: Based on current research results, a total of 40 vertebrate species from 4 classes have been registered at 10 archaeological sites from the **Neolithic period** in Vojvodina (Serbia). The most numerous one is the mammal class (Mammalia) with 25 species, then bird class (Aves) with 9 species, osteichthyes (Osteichthyes) are represented by 5 species, while reptiles (Reptilia) are the poorest class with only one species. For the **Eneolithic period**, at 7 archaeological sites, a total of 11 species members of Mammalia class have been registered.

KEYWORDS: Archaeological sites, Neolithic period, Eneolithic period, vertebrate fauna, Vojvodina (Serbia)

INTRODUCTION

Over the past eighty years or so, archaeozoological researches of various periods have been done at dozens of sites in Vojvodina. The collected sample is immense, and it predominantly consists of vertebrate bones (Vertebrata), seashells and snail shells (Mollusca). The paper shows data from 10 sites from the **Neolithic** and 7 sites from the **Eneolithic period**. Bones were collected for the first time in 1932 at **Starčevo** Neolithic site, where the research continued between 1969 and 1970 [Clason, 1980]. Sites **Nosa-Biserna Obala**, where works started in 1957 [Bőkőnyi, 1974], and **Ludaš-Budžak** where the archaeological digs were done in 1965 [Bőkőnyi, 1974] also belong to this period. **Golokut** near Vizić was researched in 1973 and 1976 [Blažić, 1984], and **Donja Branjevina** near Deronje in 1987 [Blažić, 1992a]. Osteological

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materials at multilayered **Gomolava** archaeological site, where the digs initially started in 1953 [Petrović, 1984], have been sistematically collected since 1971. There are 4 and 6 sites from the **Neolithic** and **Eneolithic period** respectively along the highway through Srem (Neolithic sites: **Malo Kuvalovo**-Krnješevci, **Prosine**-Pećinci, **Zlatara-Ruma** and **Kudoš**-Šašinci, and Eneolithic sites: **Zlatara**-Ruma, **Žirovac**-Ruma, **Kudoš**-Šašinci, **Livade**-Sremska Mitrovica, **Mitrovačke livade**-Sremska Mitrovica and **Erem**-Sremska Mitrovica) [Blažić, 1992b].

Faunal communities, which have been successively changing in the territory of Vojvodina during the Pleistocene and the early Holocene, are indicators of climatic conditions. They help the reconstruction of environments and are significant for studying periods and human cultures [Nedeljković, 1993]. Current thorough archaeozoological researches in the territory of Vojvodina show that present fauna is just the remnant of a far richer fauna from the early Holocene.

MATERIAL AND METHODS

This paper shows results of the vertebrate (Vertebrata) fauna research from 10 archaeological sites in Vojvodina from the **Neolithic** and 7 sites from the **Eneolithic period** (Map 1). Osteological material comes from the settlements and necropoleis. Determination has been done according to the keys by Driesch [1976], Schmid [1972] and comparative osteological collections.

RESULTS AND DISCUSSION

The period that the collected and processed material from archaeological sites in Vojvodina originates from, is divided into nine phases, of which the **Neolithic** (6000–3200 BC) and the **Eneolithic period** (3200–2000 BC) can be singled out. The most important archaeological site in Vojvodina is **Gomolava**-Hrtkovci, where eight cultural layers have been recorded. For the purposes of this paper, the following periods receive special attention: **late Neolithic – early Eneolithic** (3800–3400 BC) (marked with "I" in the text); **middle Eneolithic** (3400–2800 BC) and **late Eneolithic** (2800–2000 BC) [Petrović, 1984]. The second and third layer in this paper are marked with "II–III".

Map 1. Map of Vojvodina with marked sites (1–14) with dating given for each site (I for the Neolithic and II–III for the Eneolithic).
1. Gomolava-Hrtkovci (I, II–III), 2. Golokut-Vizić (I), 3. Donja Branjevina-Deronje (I), 4. Nosa-Biserna obala (I), 5. Ludaš-Budžak (I), 6. Starčevo (I), 7. Malo Kuvalovo-Krnješevci (I), 8. Prosine-Pećinci (I), 9. Zlatara-Ruma (I, II–III), 10. Žirovac-Ruma (II–III); 11. Kudoš-Šašinci (I, II–III); 12. Livade-Sremska Mitrovica (II–III); 13. Mitrovačke livade-Sremska Mitrovica (II–III); 14. Erem-Sremska Mitrovica (II–III)

Based on current research results, a total of 40 vertebrate species from 4 classes have been registered at 10 archaeological sites in Vojvodina (Serbia) from the **Neolithic period**. The most numerous one is the mammal class (Mammalia) with 25 species classified in 5 orders, of which Carnivora and Artiodactyla are present with 10 and 8 species respectively. Bird class (Aves) is represented by 9 species classified into 4 orders, while from the Accipitriformes order determination could be done only to genus. Osteichthyes (Osteichthyes) are present with 5 species classified into 3 orders, while reptiles (Reptilia) are the poorest class with only one species (Table 1).

Table 1. Fauna of some archaeological sites in Vojvodina during the **Neolithic** (I) and **Eneolithic** (II–III)

TAXON	DATING	SITE AND AUTHOR
Classis MAMMALIA		
Ordo Rodentia		
Castor fiber L. 1758	I	1[2],[14]; 3[3]; 5[9]; 6[15]
Ordo Lagomorpha		
Lepus europaeus Pall. 1778	I II–III	1[2],[14]; 2[1]; 3[3]; 4[9]; 5[9]; 9[4] 11[4]
Lepus capensis L. 1758	I	1[2],[14]
Ordo Carnivora		
Canis familiaris L.	I II–III	1[2],[14]; 2[1]; 3[3]; 5[9]; 6[15]; 7,9[4] 1[2]; 9,11[4]
Canis lupus L. 1758	I	1[2],[14]; 2[1]; 5[9]; 6[15]
Vulpes vulpes (L. 1758)	I	1[2],[14]; 3[3]; 5[9]; 6[15]; 9[4]
Ursus arctos L. 1758	I	1[2],[14]; 6[15]
Mustela nivalis L. 1766	I	1[2],[14]
Martes martes (L. 1758)	I	3[3]; 9[4]
Meles meles (L. 1758)	I	1[2],[14]; 4[9]; 5[9]; 6[15]
Lutra lutra (L. 1758)	I	1[2],[14]; 3[3]; 6[15]
Felis silvestris Schreber 1777	I	1[2],[14]; 5[9]; 6[15]
Lynx lynx (L. 1758)	I	1[2],[14]
Ordo Perissodactyla		
Equus hydruntinus Regalia 1907	I	4[9]; 5[9]; 6[15]
Equus ferus Boddaert 1785	I	1[2],[14]
Equus przewalski Poliakov, 1881	I	6[15]
Equus cabalus L. 1758	I II–III	1[2],[14] 11,14[4]
Ordo Artiodactyla		
Sus scrofa domestica L. 1758	I II–III	1[2],[14]; 2[1]; 3[3]; 5[9]; 6[15]; 7,8,9[4] 1[2]; 9-14[4]
Sus scrofa L. 1758	I II–III	1[2],[14]; 2[1]; 3[3]; 4[9]; 5[9]; 6[15]; 9,11[4] 1[2]; 10,11,14[4]
Sus sp.	I	1[2],[14]; 6[15]
Cervus elaphus L. 1758	I III–III	1[2],[14]; 2[1]; 3[3]; 4[9]; 5[9]; 6[15]; 7,8,9[4] 1[2]; 9,10,11,13,14[4]

Capreolus capreolus (L. 1758)	I II–III	1[2],[14]; 2[1]; 3[3]; 4[9]; 5[9]; 6[15]; 7,8,9[4] 1[2]; 9,13[4]
Bos taurus L.	I II–III	1[2],[14]; 2[1]; 3[3]; 4[9]; 5[9]; 6[15]; 7,8,9,11[4] 1[2]; 9-14 [4]
Bos primigenius (Bojanus 1827)	I II–III	1[2],[14]; 2[1]; 3[3]; 4[9]; 5[9]; 6[15] 10,11[4]
Bos sp.	I	6 [15]
Ovis aries L. 1758	I II–III	1[2],[14]; 2[1]; 3[3]; 4[9]; 5[9]; 6[15]; 7,9,11[4] 1[2]; 9–14 [4]
Capra hircus L.1758	I II–III	1[2],[14]; 2[1]; 3[3]; 4[9]; 5[9]; 6[15]; 7,9,11[4] 1[2]; 9–14 [4]
Classis AVES		
Ordo Anseriformes		
Anas clypeata L. 1758	I	6[15]
Anser anser (L. 1758)	I	1[2],[14]; 6[15]
Anser fabalis (Latham 1787)	I	6[15]
Cygnus olor (Gmelin 1789)	I	6[15]
Cygnus cygnus (L. 1758)	I	6[15]
Ordo Accipitriformes		
Milvus sp.	I	6[15]
Aquila sp.	I	6[15]
Circus sp.	I	6[15]
Ordo Galliformes		
Gallus domesticus (L. 1758)	I	6[15]
Ordo Gruiformes		
Grus grus (L. 1758)	I	6[15]
Otis tarda L. 1758	I	3[3]; 4[9]; 6[15]
Ordo Charadriiformes		
Numenius arquata (L. 1758)	I	6[15]
Classis REPTILIA		
Ordo Testudines		
Emys orbicularis (L. 1758)	I	1[2],[14]; 3[3]; 5[9]
Classis OSTEICHTHYS		
Ordo Salmoniformes		
Esox lucius L. 1758	I	1[2],[14]; 3[3]; 5[9]; 6[15]

Ordo Cypriniformes		
Aspius aspius (L. 1758)	I	6[15]
Abramis brama (L. 1758)	I	6[15]
Cyprinus carpio L. 1758	I	1[2],[14]; 3[3]; 5[9]; 6[15]
Ordo Siluriformes		
Silurus glanis L. 1758	I	1[2],[14]; 3[3]; 5[9]; 6[15]

N.B. The number in the square brackets is the reference number; the number outside the square brackets is the site number

After the analysis of the fauna at archaeological sites, it can be concluded that, when it comes to mammals, the most diverse fauna is at **Gomolava** (site no. 1) where 22 species of this class have been registered, together with only one bird species, one reptile species and 3 fish species [Clason, 1979[14]; Blažić, 1986[2]]. The next in line is **Starčevo** (site no. 6) where 18 mammal species, 9 bird species (which is the greatest diversity of this vertebrate class at the Neolithic sites in Vojvodina) and 5 fish species have been registered [Clason, 1980[15]]. Similar situation is at **Donja Branjevina** (site no. 3) where 14 mammal species, 3 fish species, one reptile species and one bird species have been registered [Blažić, 1992a[3]]. With 16 mammal species, 3 fish species and one reptile species, **Ludaš-Budžak** (site no. 5) is not far behind [Bőkőnyi, 1974[9]]. At **Golokut** (site no. 2), as well as at sites along highway though Srem: **Malo Kuvalovo**-Krnješevci (site no. 7), **Prosine**-Pećinci (site no. 8), **Zlatara**-Ruma (site no. 9) and **Kudoš**-Šašinci (site no. 11) only mammals have been registered, while the greatest number of species (11) has been recorded at **Golokut** and **Zlatara** [Blažić, 1984[1]; 1992b[14]] (Table 1).

Bos taurus is the only species present at all ten above-mentioned sites from the Neolithic period. The proportion of this species in mammal fauna at several Neolithic sites in Romania is over 50% and its presence in relation to domesticated mammal species is over 70% [Stanc et al., 2010]. Domination of ox is also discussed by Blažić et Radmanović [2011] in Kolubara basin in Serbia. As opposed to these findings, for domestic species, beside *Bos taurus*, Susi [2007] also mentiones *Canis familiaris, Ovis aries, Capra hircus* and *Sus scrofa domestica* present at several Neolithic sites in the territory of Romanian Banat and Transylvania.

The aforementioned composition of wild fauna is, above all, a result of the fact that at the end of the Pleistocene and the beginning of the Holocene there was a **drastic climactic changes** that affected flora and fauna of Europe [Sommer et Benecke, 2006], and therefore of the Pannonian Plain. The climactic changes were the aftermath of lowering of glaciers all the way to Pannonian Plain and their subsequent retreat. During the glacial (Gűnz, Mindel,

Riss, Wűrm) Pannonia was almost completely surrounded by glaciers. Melt downs occurred in certain time intervals when interglacial periods appeared (Gűnz/Mindel, Mindel/Riss, Riss/ Wűrm) and when glaciers retreated far to the north [Nedeljković, 1993]. As it can be seen from the Table 1, at Gomolava, Donja Branjevina, Nosa-Biserna Obala, Ludaš-Budžak, Starčevo and Zlatara sites, the presence of the following species has been registered: *Meles meles, Martes martes, Mustela nivalis* and *Lutra lutra* (Carnivora order). This is very significant because, after the last glaciation, the populations of the first three species were separated in Iberian, Italian and Balkan peninsula, and the species *Lutra lutra* is considered to be the Holocene immigrant. *Felis silvestris* also re-colonised Europe during the last glaciation from the above-mentioned refugiums [Sommer et Benecke, 2004; 2006]. *Cervus elaphus* appeared in the territory of Serbia during the Riss/Wűrm interglacial. It continued to exist in the Holocene, therefore it frequently occured at archaeological sites in Vojvodina, mostly because it was an important game. *Bos primigenius* is a form of Bison typical for a warmer climate, and it appeared in the Pleistocene (Wűrm) interstages. It was also widespread during the Holocene [Nedeljković, 1993]. *Equus hydruntinus* survived climactic changes at the end of the Pleistocene and came to the fore at the beginning of the Neolithic, characterising thus the wild fauna of the Kőrőš culture (Nosa-Biserna obala and Ludaš-Budžak) [Lazić, 1988].

Characteristics of sites also affect the fauna composition. **Gomolava** was erected on the Sava River left bank [Petrović, 1984]. During the middle Neolithic, the area around this site, as well as the Fruška Gora Mountain, were covered in mixed deciduous oak forests in which there were small glades covered with grass and underbrush. Apart from this, there was also a wetland area in the old abandoned branch of the Sava River. This environment affected the richness of the vertebrate fauna [Classon, 1979]. Remains of wild animals from the archaeological site **Golokut**, to a certain extent, also represent the image of fauna of the Fruška Gora Mountain, which has been changed today [Blažić, 1984]. In the flat part of Srem, between southern slopes of the Fruška Gora Mountain and Sava River, in fertile plain rich in water streams, the following archaeological sites are located: **Malo Kuvalovo, Prosine, Zlatara** and **Kudoš**. In the natural environment that offered good life conditions, flora and fauna were rich [Blažić, 1992b]. Settlement **Starčevo** was on the old Danube bank, at the border of river valley near Pančevo [Classon, 1980]. In prehistoric times, river valley was a wetland, intersected with meanders, streams and marshes overgrown with reed. The valley was most likely combination of forests and open areas. The river was much closer to the settlement than it is today. *Cervus elaphus, Sus scrofa* and *Bos taurus* are the signs of the forest-steppe surroundings of the site, while the remaining wild species are the signs of a wetland terrain [Lazić, 1988]. **Donja Branjevina** site is characteried by

the vicinity of the Danube, rich forests, and low banks with wetland habitats [Blažić, 1992a]. In the northern part of Vojvodina there are **Nosa-Biserna Obala** and **Ludaš-Budžak** Neolithic sites, where first proofs of ox domestication have been found [Bőkőnyi, 1974]. The presence of *Equus hydruntinus* and *Capreolus capreolus* at Nosa-Biserna Obala site are signs of the forest-steppe surroundings of the site.

Apart from the climactic changes and characteristics of the areas, fauna composition of the archaeological sites from the Neolithic period was also influenced by the fact that the basic characteristics of this period were the economy based on agriculture and animal husbandry, as well as the beginning of the animal domestication process [Blažić, 1997]. It can be stated that domestic species have been regularly found at corresponding sites in Pannonia and South-Eastern Europe. Concerning the wild species, there are certain differences between the sites in Vojvodina, other regions in Serbia, as well as neighbouring countries. Bőkőnyi [1974], at Polgar-Csőszhalom site in Hungary, apart from the species registered in Serbia, lists also findings of *Ardea purpurea* and *Bubo bubo*, and at Röszke-Lůdvár site 9 more bird species not registered in Vojvodina. The same author drew attention to the Rebensteiner Mauer site in Austria from the same period, where the presence of *Erinaceus europaeus* and *Sciurus vulgaris* has been registered. In comparison with Neolithic sites of Crkvine and Belež in Kolubara basin [Blažić and Radmanović, 2011] and Divostin near Kragujevac in Serbia [Bőkőnyi, 1988], then Anza near Štip in FYR Macedonia [Bőkőnyi, 1976], Obre I and Obre II near Kakanj in Bosnia [Bőkőnyi, 1977] and Sitagroi in Greece [Bőkőnyi, 1986], greater diversity of vertebrate fauna has been registered at sites in Vojvodina from the same period. Nevertheless, it should be stated that, in comparison with the last-mentioned site, mammals *Rupicapra rupicapra*, Dama sp. and *Erinaceus europaeus*, and bird species *Anas platyrhynchos, Mergus merganser* and *Coturnix coturnix have* not been registered in Vojvodina. Absence of *Gyps fulvus* in Vojvodina is in relation with the zoogeographical distribution of these species. In comparison with Obre I site, squirrel - *Sciurus vulgaris* has not been registered in Vojvodina. The absence of *Rupicapra rupicapra* in Vojvodina has been registered when compared with the Neolithic sites of Lepenski Vir III [Bőkőnyi, 1969] and Padina [Clason, 1980], while at Padina *Erinaceus europeus* has also been present, as well as a greater number of bird species. In comparison with Petnica site, where only mammals have been registered [Greenfield, 1986], there is a greater diversity of this vertebrate class in Vojvodina. Vertebrate fauna of the Neolithic sites in Vojvodina does not significantly differ from the fauna of Vinča-Belo Brdo site [Dimitrijević, 2006], noting that *Lutra lutra* has not been registered in Vojvodina.

These differences were caused by geographical location, habitat conditions, social-economic organisation of settlements, and span of archaeological research.

From the **Eneolithic period**, at 7 archaeological sites, a total of 11 species members of Mammalia class classified into 4 orders have been registered. The greatest number of species (8) belongs to the Artiodactyla order (Table 1). The number of species goes from 4 at **Livade**-Sremska Mitrovica (site no. 12) to 10 at **Kudoš** (site no. 11) [Blažić, 1992b[4]]. *Bos taurus*, which dominated animal husbandry in this period, is present at all sites [Blažić, 1997], and apart from this species, *Sus scrofa domestica*, *Ovis aries* and *Capra hircus* are also present (Table 1). After comparing findings from the territory of Vojvodina with neighbouring countries, it can be stated that significant diversity of ornithofauna has been recorded at two sites in south-east Romania and at several sites in Bulgaria dating from the Eneolithic period [Gal et Kessler, 2002; Boev, 1993], while at Bodrogzsadány archaeological site in Hungary, Bőkőnyi [1974] also registered *Esox lucius*. In contrast to these statements, vertebrate fauna of the Eneolithic sites in Vojvodina is almost identical to the one at the sites from the same period in the territory of Romania, also mentioned by Susi [1983]. However, in relation to the statements by this author from 1993, regarding the Eneolithic sites in Romania, there have been no *Meles meles, Martes martes, Ursus arctos, Vulpes vulpes, Canis lupus, Lynx lynx* and *Castor fiber* in Serbia.

The above-mentioned differences are most likely caused by the research span.

CONCLUSIONS

Based on current research results from 10 archaeological sites in Vojvodina (Serbia) from the **Neolithic** and 7 sites from the **Eneolithic period**, the following can be concluded:

- 40 vertebrate species belonging to 4 classes have been registered from the **Neolithic period**. The most numerous one is the mammal class (Mammalia) with 25 species, then bird class (Aves) with 9 species, osteichthyes (Osteichthyes) are present with 5 species, while the poorest class is reptiles (Reptilia) with only one species.
- From the **Eneolithic period**, a total of 11 species members of Mammalia class have been registered.
- The most diverse vertebrate fauna from the **Neolithic period** is at Gomolava, while the greatest diversity of birds has been recorded at Starčevo.
- *Bos taurus* is the only species present at all 10 above-mentioned sites from the **Neolithic period**.
- From the **Eneolithic period**, at all 7 sites, the following species have been registered: *Bos taurus Sus scrofa domestica*, *Ovis aries* and *Capra hircus*.

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ФАУНА КИЧМЕЊАКА НЕОЛИТСКИХ И ЕНЕОЛИТСКИХ ЛОКАЛИТЕТА У ВОЈВОДИНИ (СРБИЈА)

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САЖЕТАК: На основу досадашњих резултата истраживања, са 10 археолошких локалитета из неолита у Војводини (Србија), детерминисано је укупно 40 врста кичмењака припадника четири класе. Најбројнија је класа сисара (Mammalia) са 25 врста, следи класа птица (Aves) са девет врста, класа коштуриба (Osteichthyes) са пет врста, док је најсиромашнија класа гмизаваца (Reptilia) са само једном врстом. У периоду енеолита на седам археолошких локалитета регистровано је укупно 11 врста припадника класе сисара (Mammalia). У неолиту фауна сисара најразноврснија је на Гомолави, док је највећи диверзитет птица забележен на локалитету Старчево.

На свих 10 приказаних локалитета из периода неолита присутан је једино *Bos taurus*. У енеолиту на свих седам локалитета регистровани су: *Bos taurus*, *Sus scrofa domestica*, *Ovis aries* и *Capra hircus*.

КЉУЧНЕ РЕЧИ: Археолошки локалитети, фауна кичмењака, енеолит, неолит, Војводина (Србија)

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VERTEBRATE FAUNA OF THE ROMAN PERIOD, MIGRATIONS PERIOD AND MEDIEVAL PERIOD IN VOJVODINA (SERBIA)

ABSTRACT: Based on current published and unpublished research results, a total of 16 vertebrate species members of mammal (Mammalia), bird (Aves) and osteichthyes (Osteichthyes) classes have been registered at 11 archaeological sites from the **Roman Period** in Vojvodina. Mammals dominate with 12 species and one genus, birds are present with 3 species, and osteichthyes with one. From the **Migration Period**, at 9 sites, 22 vertebrate species have been registered, of which 13 species and one genus of mammals, 4 species and one genus of birds, and 5 species from the Osteichthyes class. At 8 sites from the **Medieval Period**, 16 vertebrate species have been registered. Mammals are the most numerous class with 10 species and one genus, while birds are present with 4 species and one genus. Furthermore, two species of osteichthyes have also been registered.

KEYWORDS: Roman Period, Migration Period, Medieval Period, archaeological sites, vertebrate fauna, Vojvodina (Serbia).

INTRODUCTION

At research sites in Vojvodina, the Roman Period, Migration Period and Medieval Period are characterised by somewhat smaller vertebrate fauna diversity in comparison with previous periods, but they are still interesting and deserve attention. At archaeological sites from the Roman Period (I–IV centuries AD), the number of bones of domesticated animals is far greater than that of wild animals. The reason for this is because in this period, when great changes in animal husbandry occurred, breeding animals had a very important

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role in providing enough food [Blažić, 1992; 1993; 1997; Nedeljković, 2008]. Furthermore, the Romans brought larger races of oxen, pigs, goats, sheep, horses, dogs and poultry to the newly conquered provinces [Bökönyi, 1974; Blažić, 1992; 1993; Nedeljković, 2008].

From the second half of the IV and until the end of the IX century, in the vast space of Europe, there was a great migration of people during which various tribes had passed through and lived in the territory of the present Vojvodina. During the Migration Period, indigenous fauna was not greatly changed, but the improved Roman races of domestic animals were almost completely lost. Therefore, from this period we can encounter specimens of small primitive races of domestic animals which had lower withers [Bökönyi, 1974; Blažić, 1997; Nedeljković, 2008].

Primitive but developed animal husbandry is typical for the sites from the Medieval Period shown in this paper [Blažić, 1997]. Therefore, bone analysis from that period can offer basic data on animal species used both in diet and everyday life.

MATERIAL AND METHODS

This paper features both published and unpublished results of the vertebrate fauna researches from 11 archaeological sites from the Roman Period in Vojvodina, 9 sites from the Migration Period and 8 sites from the Medieval Period (Map 1). Osteological material comes from the settlements and necropoleis. Determination has been done according to the keys by Driesch [1976] and Schmid [1972] and comparative osteological collections.

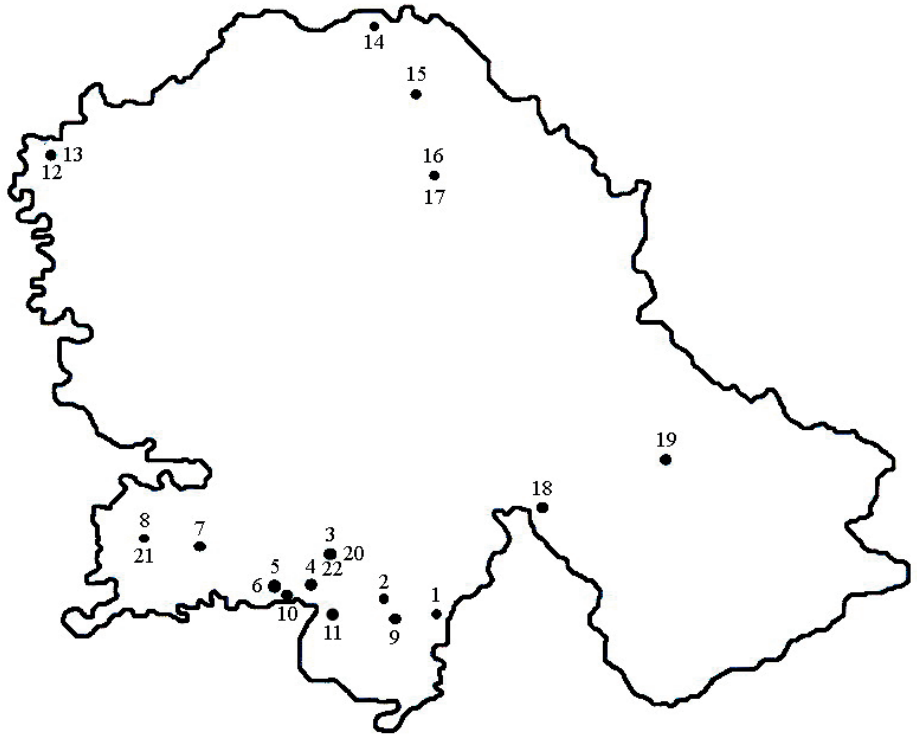
RESULTS AND DISCUSSION

The period, from which the collected and processed material from archaeological sites in Vojvodina originates, is divided into nine phases: the Neolithic, the Middle Eneolithic, the Late Eneolithic, the Bronze Age, the Early Iron Age, the Late Iron Age, the **Roman Period** (I–IV centuries AD), the **Migration Period** (IV–IX centuries AD) and the **Medieval Period** (IX century to 1526), of which the last three phases are the subject of this paper.

At 11 archaeological sites from the **Roman Period** in Vojvodina, 16 vertebrate species have been registered. They belong to the following classes: mammal (Mammalia), bird (Aves) and osteichthyes (Osteichthyes). Mammals dominate with 12 species and one genus; birds are present with 3 species: *Aquila heliaca, Gallus domesticus* and *Anas domestica*, and only carp-*Cyprinus carpio* has been recorded for osteichthyes. Mammals are classified into 5 orders, of which the most numerous one is Artiodactyla with 7 species. Within this order, the presence of Camelus genus should be pointed out (Table 1).

Map 1. Map of Vojvodina with marked sites (1–22) with dating given for each site (VII for the Roman Period, VIII for the Migration Period and IX for the Medieval Period).

1. Malo Kuvalovo-Krnješevci (VII, IX); 2. Prosine-Pećinci (VII, IX); 3. Zlatara-Ruma (VII); 4. Kudoš-Šašinci (VII, IX); 5. Livade-Sremska Mitrovica (VII); 6. Mitrovačke livade-Sremska Mitrovica (VII); 7. Bregovi Atovac-Kuzmin (VII); 8. Gajić-Adaševci (VII, IX); 9. Prosine-Prhovo (VII); 10. Sirmijum 85-Sremska Mitrovica (VII, VIII, IX); 11. Vranj (VII); 12. Baćan-Kolut (VIII); 13. Ritska dolina-Kolut (VIII); 14. Stub 76-Horgoš (VIII); 15. Kopovo-Sanad (VIII); 16. Ciglana-Padej (VIII); 17. Višnjevača-Padej (VIII); 18. Ciglana-Baranda (VIII); 19. Velike njive-Dobrica (VIII); 20. Žirovac-Ruma (IX); 21. Vračić-Adaševci (IX); 22. Humka kod Vrcalove vodenice (IX).



After the analysis of vertebrate fauna at the sites, it can be concluded that the greatest diversity have **Sirmium site no. 85**-Sremska Mitrovica (site no. 10) [Nedeljković, 2008[15]] and **Vranj** (site no. 11) where most of the representatives from this period have been registered, including Camelus genus [Blažić, 1993[4]; Vuković et Blažić, in press[25]]. At **Malo Kuvalovo**-Krnješevci (site no. 1), **Kudoš**-Šašinci (site no. 4) and **Prosine**-Prhovo (site no. 9), apart from mammals, *Gallus domesticus* was also registered, and at the remaining sites from this period only mammals were registered, the number of which varies between 3 and 9 [Blažić, 1992[3]]. The above-mentioned differences can

be explained by the span of archaeological research and differences between settlements.

At all above-mentioned sites from the Roman Period that analysed in this paper, the following species have been registered: *Bos taurus, Ovis aries* and *Capra hircus,* whose dominance is mentioned by Blažić [1997].

From the **Migration Period**, 22 vertebrate species have been registered at 9 sites, of which 13 species and one genus of mammals. Concerning the bird species, the following have been registered: *Gallus domesticus, Anser domestica, Anas domestica, Columba domestica*, as well as Corvus genus. The diversity of Osteichthyes is also important, because the representatives of all 5 orders found at archaeological sites in Vojvodina have been registered. Mammals are also classified into 5 orders, of which the most numerous one is Artiodactyla with 7 species. Within the Rodentia order, the presence of Ratus genus should be mentioned, because it has not been registered in the previous periods (Table 1).

Sirmium site no. 85-Sremska Mitrovica, where members of all 3 classes have been registered, is characterised by the greatest diversity of vertebrate fauna [Nedeljković, 2008[15]], while the other sites, with the exception of **Ciglana**-Baranda (site no. 18), feature only mammals. The number of mammal species is between 5 and 7 [Blažić, 1999–2000[7]].

Out of 13 recorded mammal species from the Migration Period at all research sites, 5 have been registered: *Equus cabalus*, *Sus scrofa domestica*, *Bos taurus, Ovis aries* and *Capra hircus* [Blažić, 1999–2000; Nedeljković, 2008].

From the **Medieval Period**, at 8 archaeological sites in Vojvodina, 16 species from Mammalia, Aves and Osteichthyes classes have been registered. Mammals from this period also dominate, with 10 species and one genus. Concerning birds, there have been registered the same species as from the Migration Period, while from Osteichthyes class, carp-*Cyprinus carpio* and catfish-*Silurus glanis* have been registered. Mammals are classified into the following orders: Rodentia, Carnivora, Perissodactyla and Artiodactyla, the last being the most numerous in terms of species (7) (Table 1).

Table 1. Vertebrate fauna of some archaeological sites in Vojvodina during the **Roman Period** (VII), **Migration Period** (VIII) and **Middle Ages** (IX)

TAXON	DATING	SITE AND AUTHOR
Classis MAMMALIA		
Ordo Rodentia		
Cricetus cricetus (L. 1758)	VII	9[3]
Castor fiber L. 758	VII-IX	10[15]

Ratus sp.	VIII IX	10[15] 10[15]
Ordo Lagomorpha		
Lepus europaeus Pall. 1778	VII VIII	10[15] 10[15]
Ordo Carnivora		
Felis domestica Fischer 1829	VIII IX	10[15] 10[15]
Canis familiaris L.	VII VIII IX	1,2,3,4,7,8,9[3]; 10[15]; 11[4],[25] 10[15]; 12-18 [7] 1,2,4,8,21,22[3]; 10[15]
Canis lupus L. 1758	VII VIII	11[4],[25] 10[15]
Meles meles (L. 1758)	VII-IX	10[15]
Ordo Perissodactyla		
Equus cabalus L. 1758	VII VIII IX	1,2,3,4,6,7,8,9[3]; 10[15]; 11[4],[25] 10[15]; 12-19[7] 1,2,4,8,21,22[3]; 10[15]
Equus asinus L. 1758	VIII	14[7]
Ordo Artiodactyla		
Sus scrofa domestica L. 1758	VII VIII IX	1,2,3,4,6,7,8,9[3]; 10[15]; 11[4],[25] 10[15]; 12-19[7] 1,2,4,8,20,21,22[3]; 10[15]
Sus scrofa L. 1758	VII VIII IX	1,2,3,4,7,8,9[3]; 10[15]; 11[4],[25] 18[7] 1,2,8,21[3]; 10[15]
Cervus elaphus L. 1758	VII VIII IX	1,2,3,4,7,8,9[3]; 10[15]; 11[4],[25] 10[15] 1,2,4,8,20,21,22[3]; 10[15]
Camelus sp.	VII	11[4],[25]
Capreolus capreolus (L. 1758)	VII VIII IX	2,4[3]; 10[15]; 11[4],[25] 10[15] 1,2,8,20,21[3]; 10[15]
Bos taurus L.	VII VIII IX	1–9[3]; 10[15]; 11[4],[25] 10[15]; 12-19[7] 1,2,4,8,20,21,22[3]; 10[15]
Ovis aries L. 1758	VII VIII IX	1–9[3]; 10[15]; 11[4],[25] 10[15]; 12-19[7] 1,2,4,8,20,21,22[3]; 10[15]
Capra hircus L.1758	VII VIII IX	1–9[3]; 10[15]; 11[4],[25] 10[15]; 12-19[7] 1,2,4,8,20,21,22[3]; 10[15]

Classis AVES		
Ordo Accipitriformes		
Aquila heliaca Savigny 1809	VII	11[4],[25]
Ordo Galliformes		
Gallus domesticus (L. 1758)	VII VIII IX	1,4,9[3]; 10[15]; 11[4],[25] 10[15] 1,8,21[3]; 10[15]
Meleagris gallopavo L.1758	VII-IX	10[15]
Ordo Anseriformes		
Anser domestica L. 1758	VIII IX	10[15] 10[15]
Anas domestica L. 1758	VII VIII IX	10[15] 10[15] 10[15]
Ordo Columbiformes		
Columba domestica Gmelin 1799	VIII IX	10[15] 10[15]
Ordo Passeriformes		
Corvus sp.	VIII IX	10[15] 10[15]
Classis OSTEICHTHYS		
Ordo Acipenseriformes		
Acipenser ruthenus L. 1758	VIII	10[15]
Ordo Salmoniformes		
Esox lucius	VIII	10[15]
Ordo Cypriniformes		
Cyprinus carpio L. 1758	VII VIII IX	11[4],[25] 10[15] 10[15]
Ordo Perciformes		
Sander lucioperca L. 1758	VIII	10[15]
Ordo Siluriformes		
Silurus glanis L. 1758	VIII IX	10[15]; 18[7] 10[15]

N.B. The number in the square brackets is the reference number; the number outside the square brackets is the site number

Sirmium site no. 85-Sremska Mitrovica from the Medieval Period, as it was the case with previous two periods, has the greatest diversity and it is the only one where the representatives of 3 vertebrate classes have been recorded.

At **Malo Kuvalovo**-Krnješevci, **Gajić** and **Vračić**-Adaševci (sites no. 8 and 21), mammals and domestic hen-*Gallus domesticus* have been registered, while the remaining sites feature only mammals. The number of mammal species is between 6 and 10 per site [Blažić, 1992[3]; Nedeljković, 2008[15]]. At Sirmium site no. 85-Sremska Mitrovica from the Medieval Period, the development of animal husbandry, which still experiences regressive changes, has been recorded. Due to great migrations and wars, almost entire result of the Roman selection in animal husbandry was destroyed. Animal husbandry was also more important than hunting in this period [Nedeljković, 2008].

Sus scrofa domestica, Cervus elaphus, *Bos taurus, Ovis aries* and *Capra hircus* are the species found at all 8 above-mentioned sites from this period. Their dominance in the Medieval Period is also pointed out by Blažić [2005].

For two mammal species (*Castor fiber*-beaver and *Meles meles*-badger), as well as for one bird species (*Meleagris gallopavo*-turkey), all from the Sirmium site no. 85, it has not been possible to determine whether they originate from the Roman Period, Migration Period or Medieval Period [Nedeljković, 2008].

In the territory of Serbia, the research from the **Roman period** has been done around the walls of Felix Romuliana [Dimitrijević et Medović, 2007]. Domesticated mammals have been mainly analysed, but the wild ones have also been present. In relation to these researches, at sites in Vojvodina, the presence of fox-*Vulpes vulpes* has not been registered. Concerning fauna research from the **Medieval Period** in the territory of Serbia, Ras-Gradina site [Blažić, 1999] should be mentioned, because 6 domestic and 3 wild mammal species have been registered there. Sites in Vojvodina have a greater diversity.

After comparing data from the territory of Vojvodina with other countries from the region, several significant facts should be noted. Concerning ichtyofauna, at sites in Vojvodina from the Roman Period, Migration Period and Medieval Period, a total of 5 fish species have been registered, in comparison to the territory between the Danube and Black Sea where 13 fish species from these periods have been registered [Stanc et Bejenaru, 2008]. As it is already mentioned, at sites in Vojvodina, during these periods, 6 bird species and one genus have been noted, in contrast to the territory of Bulgaria where, at 18 sites from the Roman Period and Medieval Period, 57 species have been registered [Boev, 1993]. Table 1 shows that domestic hen-*Gallus domesticus* has been noted in Vojvodina at 5 sites from **the Roman Period** and at 4 sites from the Medieval Period. Presence of this bird species at sites in Hungary and Romania, also from the Roman Period, Migration Period and Medieval Period, is discussed by Gal [2008]. Furthermore, it has been pointed out that the dominant, or one of the dominant species at sites in Vojvodina is ox-*Bos taurus*. Its presence at archaeological sites in Romania of various periods is discussed by Stanc et al. [2010], noting that in the Medieval Period its presence in the mammal fauna

was between 35% and 65%, and in domesticated mammal fauna between 45% and 65%. Vertebrate fauna of the **Roman Period** in the territory of Romania is also discussed by Susi [1988, 1993, 1996 and 1999]. In comparison to these data, during the Roman Period in Vojvodina the following species have not been registered: *Equus asinus* [Susi, 1988], *Ursus arctos* and *Vulpes vulpes* [Susi, 1993], *Bos primigenius* [Susi, 1996], while the same author [1999], for this period, explicitly mentions the presence of *Castor fiber*. Concerning the Medieval Period, unlike the sites in the territory of Romania mentioned by Susi [1990, 1998], at archaeological sites in Vojvodina the following species have not been registered: *Lepus europaeus, Martes martes, Ursus arctos* and *Bos primigenius*.

However, the largest amount of data on fauna diversity at archaeological sites from all research periods, therefore also from the Roman Period and Medieval Period, was given by Bőkőnyi [1974] for the territory of Hungary. Concerning the Roman Period, differences in the composition of vertebrate fauna between Vojvodina and Hungary, according to the data given by this author, exist for 12 sites in Hungary. Bőkőnyi [1974] states that, at Tokod-Erzébetakna site, *Grus grus* has been recorded as a member of bird species, while at Tác archaeological site, 14 wild and 2 domestic bird species have been registered. The mammal fauna at sites from the Roman Period in Hungary is also significantly more diverse, because, apart from the species registered in Vojvodina, the following species are also present: *Vulpes vulpes, Ursus arctos, Felis domestica* and *Bos primigenius*, and at the above-mentioned Tác site *Equus asinus*, *Esox lucius* and *Emys orbicularis.* Nineteen archaeological sites from the Medieval Period in the territory of Hungary are also characterised by richer vertebrate fauna, because, in addition to the species registered in Vojvodina, **7 mammal species**: *Sciurus vulgaris, Lepus europeus, Felis silvestris, Canis lupus, Vulpes vulpes, Ursus arctos, Bos bubalis*, as well as Camelus genus; **16 bird species**: *Ciconia ciconia, Buteo buteo, Haliaetos albicilla, Milvus migrans, Pavao cristatus, Perdix perdix, Phasianus coclchicus, Grus grus, Otis tarda, Bubo bubo, Strix aluco, Columba palumbus, Corvus frugilegus, Turdus pilaris, Turdus viscivorus, Upupa epops*; **1 reptile species**: *Emys orbicularis*, as well as **4 fish species**: *Blicca bjoerkna, Carassius carassius, Esox lucius* and *Sander lucioperca* are also present. Bőkőnyi [1976] further discusses wild and domesticated mammal, bird and reptile species at several archaeological sites in Hungary, also from the Medieval Period, and except the ones noted in Vojvodina, he additionally mentions: *Bos primigenius*, *Lepus europaeus* and *Emys orbicularis*. Archaeozoological researches in the territory of Hungary were continued by Bartosiewitz [1996, 1998], who, at several medieval sites, also noted the presence of the above-mentioned species like *Lepus europaeus*, then

Vulpes vulpes, *Urusus arctos* and *Esox lucius*. It has already been noted that the above-mentioned species from this period are not registered in Vojvodina.

The identified differences between archaeological sites from the Roman Period, Migration Period and Medieval Period in Vojvodina and sites of the same dating in neighbouring countries can be explained by the span of archaeological research.

CONCLUSIONS

Based on current published and unpublished research results from 11 archaeological sites from the **Roman Period** in Vojvodina, 9 sites from the **Migration Period**, and 8 sites from the **Medieval Period**, the following can be concluded:

– From the **Roman Period**, a total of 16 vertebrate species that are members of mammal (Mammalia), bird (Aves) and osteichthyes (Osteichthyes) classes have been registered. Mammals dominate with 12 species and one genus; birds are present with 3 species, and osteichthyes with one.

– From the **Migration Period**, 22 vertebrate species have been registered, of which 13 species and one genus of mammals, 4 species and one genus of birds, and 5 species from the Osteichthyes class.

– At sites from the **Medieval Period**, 16 vertebrate species have been registered. Mammals are also the most numerous class with 10 species and one genus, while birds are present with 4 species and one genus. Furthermore, two species of osteichthyes have been registered.

– For all three periods, the greatest vertebrate fauna diversity has been recorded at **Sirmium site no. 85**-Sremska Mitrovica**.**

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ФАУНА КИЧМЕЊАКА ИЗ РИМСКОГ ПЕРИОДА, СЕОБЕ НАРОДА И СРЕДЊЕГ ВЕКА У ВОЈВОДИНИ (СРБИЈА)

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РЕЗИМЕ: На основу досадашњих објављених и необјављених резултата истраживања на 11 археолошких локалитета у Војводини из римског периода регистровано је укупно 16 врста кичмењака припадника класа сисара (Mammalia), птица (Aves) и коштуриба (Osteichthyes). Са 12 врста и једним родом доминирају сисари, птице су заступљене са три врсте, а коштурибе са једном. Током периода сеобе народа на девет налазишта забележено је 22 врсте кичмењака од чега су 13 врста и један род сисари, регистроване су четири врсте и један род птица, док је пет врста припадало класи Osteichthyes. На осам локалитета из средњевековног периода констатовано је 16 врста кичмењака. И у овом периоду најбројнији су сисари са 10 врста и једним родом, док је од птица забележено четири врсте и један род. Такође су детерминисане и две врсте коштуриба. Током ова три периода највећим диверзитетом фауне кичмењака одликовао се локалитет Сирмијум локалитет 85-Сремска Митровица.

КЉУЧНЕ РЕЧИ: Археолошки локалитети, фауна кичмењака, римски период, сеоба народа, средњи век, Војводина (Србија)

INSTRUCTION TO AUTHORS
(www.maticasrpska.org.rs)

This version of Instruction to Authors is valid starting from the year 2012 and the volume number 122

1. General remarks

1.1. ***JOURNAL FOR NATURAL SCIENCES*** (short title: J. Nat. Sci. Matica Srpska) publishes manuscripts and review articles as well as brief communications from all scientific fields as referred to in the title of the journal. Review articles are published only when solicited by the editorial board of the journal. Manuscripts that have already been published *in extenso* or in parts or have been submitted for publication to other journal will not be accepted. The journal issues two numbers per year.

1.2. The manuscripts should be written in correct English language regarding the grammar and style. The manuscripts should be submitted electronically as a separate file to **vnikolic@maticasrpska.org.rs** and enclosed with the author's written consent for the publishing of the manuscript.

1.3. Upon the reception of the manuscript, the author shall be assigned with a manuscript code, which has to be referred to in any further correspondence. The authors will be notified about the manuscript reception within seven days and about the reviewers' opinion within two months from submission. All submitted manuscripts are reviewed and proofread.

2. Planning and preparing of the manuscript

2.1. Type the manuscripts electronically on A4 (21 x 29.5 cm) format with 2.5 cm margins, first line indent, and 1.5 line spacing. When writing the text, the authors should use *Times New Roman* size 12 font and when writing the abstract, key words, summary, and footnotes use font size 10.

2.2. First name, middle initial and last name should be given for all authors of the manuscript and their institutional affiliations, institution name, and mailing address. In complex organizations, a full hierarchy should be mentioned (e.g. University of Novi Sad, Faculty of Sciences – Department of Biology and Ecology). The institution of employment of each author should be stated below the author's name. The position and academic degrees should not be cited. If there is more than one author, indicate separately institutional affiliation for each of the authors. Put the name and mailing address (postal or e-mail address) of the author responsible for correspondence at the bottom of the first page. If there is more than one author, write the address of only one author, usually the first one.

2.3. Structure the text of the original articles into Abstract, Key Words,

Introduction, Material or Methods, or Material and Methods, Results or Results and Discussion, Discussion, Conclusion, References, Summary and Key Words in Serbian language, and Acknowledgement (if there is one). Original articles should not be longer than 10 pages, including the references, tables, legends, and figures.

2.4. Titles should be informative and not longer than 10 words. It is in the best interest of the authors and the journal to use words in titles suitable for indexing and electronic searching of the article.

2.5. The authors should submit the title of the article with last name and the initials of the first author.

(if the article has more than one author, *et al*. should be used for other authors) and running title of not more than five words.

2.6. List up to 10 key words using words and phrases that describe the content of the article in the best way and that allow indexing and electronic searching of the paper. List the key words alphabetically and divided by commas.

2.7. The Abstract in English language and Summary in Serbian language should be a short and informative presentation of the article. Depending on the length of the article, the Abstract may have from 100 to 250 words. Summary written in Serbian language can be 1/10 length of the article and should contain the title of the article, first, middle initial, and last names of the authors, authors' institutional affiliation and address, and key words.

2.8. Write the information about financial support, advices, and other forms of assistance, if necessary, at the end of the article under the Acknowledgement. Financial support acknowledgement should contain the name and the number of the project, i.e. the name of the program from which the article originated, and the name of the institution that provided the financial support. In case of other forms of assistance the author should submit the first name, middle initial, last name, institutional affiliation, and the address of the person providing the assistance or the full name and the address of the assisting institution.

3. Structure the Review articles in Abstract, Key Words, Text of the manuscript, Conclusion, and References; submit Summary and Key Words in Serbian language. Review articles should not be longer than 12 pages, including references, tables, legends, and figures.

4. Write brief communication according to the instructions for original articles but not be longer than five pages.

5. References

5.1. List the References alphabetically. Examples:

(a) Articles from journals: Last name CD, Last name CD (2009): Title of the article. Title of the journal (abbreviated form) 135: 122-129.

(b) Chapters in the book: Last name ED, Last name AS, Last name IP (2011): Title of the pertinent part from the book. In: Last name CA, last name IF (eds.), Title of the book, Vol.4, Publisher, City

(c) Books: Last name VG, Last name CS (2009): Title of the cited book. Publisher, City
(d) Dissertations: Last name VA (2009): Title of the thesis. Doctoral dissertation, University, City
(e) Unpublished articles: designation "in press" should be used only for papers accepted for publishing. Unpublished articles should be cited in the same way as published articles except that instead of journal volume and page numbers should write "in press" information.
(f) Articles reported at scientific meetings and published *in extenso* or in a summary form: Last name FR (2011): Proceedings, Name of the meeting, Meeting organizers, Venue, Country, 24-29
(g) World Wide Web Sites and other electronic sources: Author's last name, Author's initial. (Date of publication or revision). Title, In: *source in Italics*, Date of access, Available from: <Available URL>. Use n.d. (no date) where no publication date is available. Where no author is available, transfer the organization behind the website or the title to the author space.

5.2. References in the text should include author's last name and the year of publishing. When there are two authors both should be cited, but in case of three or more authors, cite the first author only and follow with et al.

5.3. If two or more articles of the same author or authors published in the same year are cited, designate the publishing years with letters a, b, c, etc., both in text and reference list.

5.4. The names of the periodicals should be abbreviated according the instructions in the *Bibliographic Guide for Authors and Editors* (BIOSIS, Chemical Abstracts Service, and Engineering Index, Inc.).

5.5. Do not translate references to the language of the article. Write the names of cited national periodicals in their original, shortened form. For example, for the reference in Serbian language, put (Sr) at the end of the reference.

6. Units, names, abbreviations, and formulas

6.1. SI units of measurement (Système international d'unités) should be used but when necessary use other officially accepted units.

6.2. Write the names of living organisms using *Italics* font style.

6.3. Abbreviated form of a term should be put into parenthesis after the full name of the term first time it appears in the text.

6.4. Chemical formulas and complex equations should be drawn and prepared for photographic reproduction.

7. Figures

7.1. Authors may use black-and-white photographs and good quality drawings.

7.2. A caption with the explanation should be put below each figure.

8. Tables

8.1. Type tables on separate sheet of papers and enclosed them at the end of the manuscript.

8.2. Number the tables using Arabic numerals.

8.3. Above each table, write a capture with table explanation.

8.4. On the left margin, indicate the place of the tables in the text.

9. Electronic copy of the article

9.1. After the acceptance of the article, send a CD with final version of the manuscript and a printed copy to facilitate technical processing of the text. Articles should be written in Microsoft Word format and sent to the Editorial office of the *Matica Srpskai JOURNAL FOR NATURAL SCIENCES, Matica Srpska*, 1 Matica Srpska Street, 21000 Novi Sad (Uredništvo Zbornika Matice srpske za prirodne nauke, Matice srpske 1, 21000 Novi Sad).

9.2. Before printing, the manuscripts shall be sent to the authors for the approval of final version. Corrections of the text prepared for printing should be restricted to misspelling and printing errors as much as possible. For major changes of the text, a fee will be charged. Corrected manuscript should be returned to the Editorial office as soon as possible.

УПУТСТВО АУТОРИМА*
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1. Опште напомене

1.1 Зборник Матице српске за природне науке / Matica Srpska Journal for Natural Sciences (скраћени наслов: J. Nat. Sci. Matica Srpska) објављује оригиналне научне радове и прегледне чланке као и кратка саопштења из свих области које обухвата назив часописа. Прегледни радови се објављују само на позив редакције. Радови који су већ објављени у целости или у деловима или су понуђени другом часопису не могу бити прихваћени. Часопис објављује два броја годишње.

1.2. Прихватају се рукописи писани на енглеском језику. Језик мора бити исправан у погледу граматике и стила. Рукопис се доставља електронском поштом као посебан документ на адресу: zmspn@maticasrpska.org.rs, уз обавезну потписану изјаву аутора у вези са пријавом рада за штампу.

1.3. По примању рукописа, аутор ће добити шифру свог рада, коју треба увек наводити у даљој преписци. Уредништво ће обавестити аутора о приспећу рукописа у року од седам дана, а о мишљењу рецензената у року од два месеца од пријема. Сваки рад се рецензира и лекторише.

2. Припрема рукописа

2.1. Текст рада пише се електронски на страни А4 (21x29,5 cm), с маргинама од 2,5 cm, увлачењем првог реда новог пасуса, и размаком међу редовима 1,5. Текст треба писати у фонту *Times New Roman* словима величине 12 а сажетак, кључне речи, резиме и подножне напомене словима величине 10 pt.

2.2. Наводе се име, средње слово и презиме свих аутора рада као и назив установе (без скраћеница) у којој су аутори запослени, заједно са пуном поштанском адресом. У сложеним организацијама наводи се укупна хијерархија (на пример: Универзитет у Новом Саду, Природноматематички факултет – Департман за биологију и екологију). Место запослења наводи се непосредно испод имена аутора. Функције и звања аутора се не наводе. Ако је аутора више, мора се, посебним ознакама, назначити из које од наведених установа потиче сваки од наведених аутора. Контакт адреса аутора (поштанска или електронска) даје се у напомени при дну прве странице чланка. Ако је аутора више, даје се само адреса једног, обично првог аутора.

2.3. Рукопис оригиналног научног рада треба поделити на: Сажетак, Кључне речи, Увод, Материјал или Метод или Материјал и метод,

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* Ово упутство важи од 2012. године од броја часописа 122.

Резултати или Резултати и дискусија, Дискусија, Закључак, Литература, Сажетак и Кључне речи на српском језику и Захвалност (уколико за то постоји потреба). Оригинални научни радови не смеју бити дужи од 10 страна, укључујући литературу, табеле, легенде и слике.

2.4. Наслов рада треба да буде информативан, али не дужи од десет речи. У интересу је часописа и аутора да се користе речи прикладне за индексирање и претраживање.

2.5. Аутори треба да доставе и текући наслов који треба да садржи презиме и иницијале првог аутора (ако је аутора више, преостали се означавају са "et al.") и наслов рада у скраћеном облику, не више од пет речи.

2.6. За кључне речи треба користити термине или фразе које најбоље описују садржај чланка за потребе индексирања и претраживања. Број кључних речи не може бити већи од 10. Треба их навести абецедним редом и одвојити зарезима.

2.7. Апстракт на енглеском и резиме на српском треба да представљају кратак информативни приказ чланка. Апстракт у зависности од дужине чланка треба да има од 100 до 250 речи. Резиме на српском језику може бити до 1/10 дужине чланка и треба да садржи наслов рада, имена аутора, средње слово и презимена, назив и место у којима су аутори запослени и кључне речи.

2.8. Податке о финансијској помоћи, саветима и другим врстама помоћи, уколико за то постоји потреба, треба навести на крају рада, под насловом Захвалност. У захвалници за финансијску помоћ треба навести назив и број пројекта, односно назив програма у оквиру којег је чланак настао, као и назив институције која је финансирала пројекат или програм. У случају других видова помоћи треба навести име, средње слово и презиме, установу и седиште лица које је пружало помоћ, а ако је помоћ пружала установа пун назив и адресу.

3. Прегледни рад треба да садржи: Апстракт, Кључне речи, Закључак, Литературу, као и Резиме и Кључне речи на српском. Прегледни радови не смеју бити дужи од 12 страна, укључујући литературу, табеле, легенде и слике.

4. Кратко саопштење се пише по упутствима за оригиналан научни рад, али не сме да буде дуже од 5 страна.

5. Литература

5.1. Литературне наводе треба сложити абецедним редом на следећи начин:

(а) Чланци из часописа: Презиме CD, Презиме SP (2009): Назив рада. Име часописа (скраћени облик) 135: 122-129.

(б) Поглавља у књизи: Презиме ED, Презиме AS, Презиме, IP (2011): Наслов цитираног дела у књизи. In: Презиме CA, Презиме IF (eds.), Назив књиге, Вол. 4, Издавач, Град, 224-256.

(в) Књиге: Презиме VG, Презиме CS (2009): Наслов цитиране књиге. Издавач, Град.
(г) Дисертације: Презиме VA (2009): Назив тезе. Докторска дисертација, Универзитет, Град.
(д) Необјављени радови: Навод „у штампи" треба да се односи само на радове прихваћене за штампу. Необјављени радови: цитирати као да се ради о објављеном раду осим што се уместо волумена часописа и броја страна наводи „у штампи".
(ђ) Радови саопштени на научним скуповима штампани у целини или у изводу: Презиме FR. (2011): Зборник, Назив скупа, Организатор скупа, Место одржавања, Држава, 24-29.
(е) Електронски извори:

World Wide Web Sites and Other Electronic Sources
Author last name, Author initial. (Date of publication or revision). Title, In: *source in Italics,* Date of access, Available from: <Available URL>

Use n.d. (no date) where no publication date is available.
Where no author is available, transfer the organisation behind the website, or the title, to the author space.

5.2. Референце у тексту треба да укључе презиме аутора и годину издања. Ако има два аутора, треба навести обојицу, а у случају три или више аутора треба навести првог аутора и назначити "et al.".

5.3. Ако се наводе два или више радова истог или истих аутора, објављених у истој години, потребно је у тексту и списку литературе ставити а, б, ц, итд. иза године објављивања.

5.4. Имена часописа треба скраћивати према "Bibliographic Guide for Authors and Editors" (BIOSIS, Chemical Abstracts Service and Engineerings Index, Inc.,).

5.5. Референце се не преводе на језик рада. Наслови цитираних домаћих часописа дају се у оригиналном, скраћеном облику. Ако је референца нпр. на српском језику на крају се стави (Sr).

6. Јединице, имена, скраћенице и формуле

6.1. Треба користити SI ознаке за јединице (SI Systeme International d'Un.); изузетно се могу користити и друге званично прихваћене јединице.

6.2. Називе живих организама на латинском треба писати италиком.

6.3. При коришћењу скраћеница у тексту, пун термин треба навести приликом првог спомињања, а скраћеницу додати у загради.

6.4. Хемијске структурне формуле и сложене једначине треба нацртати и припремити за фотографску репродукцију.

7. Илустрације

7.1. За илустрације могу се користити црно беле фотографије и цртежи доброг квалитета.

7.2. Свака илустрација треба да има текст (легенду) који објашњава садржај прилога (испод слике).

8. Табеле

8.1. Табеле треба куцати на одвојеним страницама и приложити их на крају рада.

8.2. Табеле се означавају арапским бројевима.

8.3. Свака табела треба да почне насловом који објашњава њен садржај (изнад табеле).

8.4. Места табела у тексту треба означити на левој маргини.

9. Копија рада у електронској форми

9.1. После прихватања рада потребно је доставити CD са коначном верзијом рада. Приложити и једну копију одштампаног рада ради лакше техничке обраде. Рукопис треба слати на адресу: Уредништво Зборника Матице српске за природне науке, Матица српска, Ул. Матице српске, 21000 Нови Сад. Рукописи се шаљу у Word формату.

9.2. Пре уласка рада у штампу ауторима се доставља рукопис за коначну ревизију. Исправљање текста припремљеног за штампу треба ограничити на штампарске грешке. Значајне промене текста ће се наплаћивати. Кориговани текст треба вратити Уредништву у најкраћем могућем року.

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Излази двапут годишње
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