МАТИЦА СРПСКА
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МАТИЦА СРПСКА
ОДЕЉЕЊЕ ЗА ПРИРОДНЕ НАУКЕ

З Б О Р Н И К
МАТИЦЕ СРПСКЕ ЗА ПРИРОДНЕ НАУКЕ

MATICA SRPSKA
DEPARTMENT OF NATURAL SCIENCES
MATICA SRPSKA J. NAT. SCI.

Покренут 1951. / First published in 1951

Until volume 10, the journal was published under the title *Научни зборник Матице српске: Серија природних наука* (Scientific Proceedings of Matica Srpska: Natural Sciences Series) (1951–1955). Volume 11 was released under the title *Зборник Матице српске: Серија природних наука* (Matica Srpska Proceedings: Natural Sciences Series) (1956), volumes 12–65 under the title *Зборник за природне науке* (Proceedings for Natural Sciences) (1957–1983), and from volume 66 the journal was published under the title *Зборник Матице српске за природне науке* (Matica Srpska Proceedings for Natural Sciences) (1984–). From volume 84 (1993) the journal was published in English under the title *Matica Srpska Proceedings for Natural Sciences* (1993–2012), and since volume 125 under the title *Matica Srpska Journal for Natural Sciences* (2013–)

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YU ISSN 0352-4906 | UDK 5/6 (05)

MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES

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NOVI SAD
2014

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UDC 633/634:575.854]:632.4
UDC 579.67
DOI: 10.2298/ZMSPN1427007F

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ITEM – AGRO-FOOD MICROBIAL CULTURE COLLECTION: THE IMPORTANCE OF TOXIGENIC FUNGI IN THE FIGHT AGAINST MYCOTOXINS

ABSTRACT: Fungal culture collections are important to biologists, microbiologists, epidemiologists and others involved in health and natural sciences. The improvement of techniques and methods for fungal isolation and preservation has contributed to maintain large microbial collections, which represent a rich source of biological sciences research, especially taxonomic, pathological and biodiversity studies as well as industrial applications. The collection centers are responsible for repository reference strains and for the maintenance of these microorganisms. The ITEM Microbial Culture Collection of ISPA (Institute of Sciences and of Food Production) includes more than 10,000 strains belonging to various agro-food microorganisms with phytopathological and toxicological significance. These microorganisms are mainly fungal pathogens belonging to toxigenic genera of *Fusarium, Aspergillus,Alternaria*, and *Penicillium.* This collection is a remarkable resource in the fight against mycotoxins: the increasing number of toxigenic fungi included in this collection ensures an original genetic source for biotechnological applications in several fields of research, contributing to knowledge improvement about fungal biology and strategies development for reducing mycotoxin contamination.

KEYWORDS: Culture collection, ITEM, fungi, mycotoxin, biodiversity

INTRODUCTION

Microorganisms are essential components of biological diversity, fundamental elements which guarantee the existence of sustainable ecosystems [Hawksworth 1991, 1992].

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Fifty percent of the living biomass on the planet is microbial [Center for microbial ecology, 1995] and microorganisms provide an important source of genetic information for molecular biology and biotechnology [Bull *et al.*, 1992].

The first report of a fungal culture collection on plant materials dated back to 1718 by Micheli [1729]. However, only one century later, sterile techniques started to be developed [Vittadini 1852]. The first independent center to endeavor preserving and supplying a wide range of fungal cultures was the Centraal bureau voor Schimmelcultures (CBS, Baarn, The Netherlands). Now located in Utrecht, the CBS has been a depository for patent strains since 1955.

The role of the first microbial culture collections, apart for teaching and pure research, was related to agriculture, brewing and medicine. In 1930, with the discovery of penicillin, the importance of these collections strongly raised together with the awareness that fungi are great sources of biological activities. The industrial and biotechnological applications of fungi include brewing and wine making, baking food processing, enzyme productions, antibiotics, organic acid and vitamin production, genetic engineering, pesticides and insecticides development [Smith *et al.,* 1983; Reed 1982; Onions *et al.*, 1981; Korzybsky *et al.*, 1979].

The increasing number of culture collections worldwide, private or institutional, mirrors the need to preserve this wealth protecting the microbial gene pool for biological researches, industrial applications and biodiversity preservation.

Roles and maintenance of culture collections

Culture collections may be considered as living libraries of our natural scientific heritage [Sly 1998]. The access to cultures of microorganisms is an essential requirement for the management of microbiology and related disciplines.

The main roles of culture collections are:

– to supply strains for research, teaching and industry in a timely and cost effective manner. In the case of pathogenic strains specific licenses may be needed for holding a strain in a laboratory,
– to safeguard genetic resources as an insurance policy through holding stocks in long-term storage for future needs,
– to make identifications and maintain type cultures for comparative purposes,
– to store patent and industrial strains of microorganisms,
– in education and training,

– for biodiversity conservation: the number of fungal strains currently maintained in collections throughout the world is more than 170,000, representing approximately 7,000 species [Hawksworth and Kirsop 1988].

The increasing demand of culture collections for authenticated, reliable biological material and associated information has paralleled the growth of biotechnology. Worldwide recognition of the need to preserve the microbial gene pool has highlighted the need for centers of expertise in culture isolation, maintenance, identification and taxonomy. For this purpose, organizations such as the World Federation for Culture Collection (WFCC) provided recommendations for good practice in culture collections and standards of operation for official guarantees acknowledgment.

Maintaining living microbial cultures requires specific conservation skills and quality assurance to ensure genetic stability. The number of strains maintained by a single culture collection is limited by 1) the equipment needed to guarantee an appropriate holding, 2) the representativeness of the strains, and 3) the specific aim of the collection.

The size and the methods for establishment and maintenance of a culture collection are strongly dependent on the means of the laboratory, both in term of space and economical resources. Nevertheless, whatever the size of the collection is, it must be properly maintained.

Traditionally, the isolates are cultured on agar slants of suitable media and then subcultured onto fresh slants at regular intervals. The subcultures are stored in a refrigerator until required, or until the next scheduled subculturing. This system, used for short term storage and routine procedures, has some serious drawbacks:

- risk of contamination – incorrect manipulation or conservation may lead to contamination of the culture,
- loss of viability – if subculturing is not carried out at the required intervals and the cultures are inadequately stored, sensitive isolates may lose viability and be irrecoverable,
- continued growth at chill temperatures – some organisms, such as *Listeria monocytogenes*, are capable of slow growth at 0 °C or even less,
- labeling mistakes – subculturing a large number of agar slants many times carries a significant risk of a culture being wrongly labeled,
- genetic drift and mutation – every subculture carries potential genotypic and phenotypic changes such as loss of virulence and resistance factors or reduced motility.

These inconveniences can make serious problems in a laboratory, inducing misleading results and loss of isolates. In order to avoid these drawbacks, The

American Type Culture Collection (ATCC) recommends that no more than five passages (subcultures) should be made from the original type strain.

To overcome these problems, methods such as cryogenic storage and freeze-drying are used for long term storage, which guarantee a backup of the collection.

The cryogenic storage, usually with liquid nitrogen, is the most common method used for long term storage of cultures. The suspensions of spores, prepared in a cryoprotectant medium generally containing 10–15% glycerol, is dispensed into suitable containers, which are then immersed in, or suspended above, liquid nitrogen. If the isolate survives this process, the strain viability is guaranteed for several years.

Another method for long term storage is the freeze-drying, also known as lyophilisation. A thick suspension of spores is first prepared in a suitable suspending medium, such as 10% skim milk or a specific lyophilisation buffer. This suspension is then dispensed into small glass vials and frozen. Once frozen, they are placed in the drying chamber of a freeze dryer and dried under vacuum for 2–24 hours to remove water in the frozen state. When drying is complete the vial is sealed and then stored in the dark at 8 °C or less. Many bacterial and fungal species will remain stable and viable for at least a year under these conditions and in some cases cultures have been successfully revitalized many years later.

Due to the availability of innovations, in the trade and transportation as well as in the communication, as well as a precise legislation that regulates this exchange, the access to culture collections is easier, faster and less expensive than in the past, thus enhancing the growth of a high quality biological material and scientific services.

ITEM COLLECTION

Since 2002, the ITEM Microbial Culture Collection belongs to ISPA (Institute of Sciences of Food Production, Bari, Italy, emerged from the IstitutoTossine e Micotossine) and hosts about 10,000 microorganism, including strains of different genera of noteworthy phytopathological and toxicological importance such as *Aspergillus*, *Alternaria*, *Fusarium*, *Penicillium*, etc.

In 1998 ITEM joined the European Culture Collection Organization (ECCO). The catalogue of the Collection was first published in 1997 and it is available online at www.ispa.cnr.it/Collection. For each fungal strain the following information are given on the website: a) name of the species, author/s, ITEM accession number; b) geographical origin, substrate/host, isolation, year, depositor, accession numbers of other Collections; c) biological, molecular, chemical and toxicological information; d) specific references.

The strains collected are related to the production of more than 100 bioactive metabolites with biologic interest (i.e. antibiotics, entomo-, phyto- and zoo-toxins). The strains are kept as single spore cultures, as fresh cultures on agar, or cryoconserved in liquid nitrogen. The collection offers services of safe deposit and preservation of cultures in the public collection, identification of fungal strains and selling service.

Importance of ITEM collection in the fight against mycotoxins

Mycotoxins [Bennett *et al.*, 2003] are secondary metabolites produced by toxigenic fungi that contaminate food and feed chain in pre- and post-harvest processes and represent a great concern worldwide both about the economic implications and the health of consumers, whether direct or indirect. Mycotoxins are responsible for many different toxic effects within a wide range of severity, including the induction of cancer and intestinal, blood, kidney and nerve defects.

International trade in agricultural commodities such as wheat, rice, barley, corn, sorghum, soybeans, groundnuts and oilseeds amounts to hundreds of millions of tonnes each year. Many of these commodities are subjected to a high risk of mycotoxin contamination. The FAO estimated that each year between 25% and 50% of the world's food crops are contaminated with mycotoxins [FAO, 1988; Mannon and Johnson 1985].

Mycotoxin contamination is now one of the most insidious challenges to overcome in food quality. The possibility of accessing the great biodiversity represented in the ITEM collection is fundamental to deepen the knowledge of toxigenic fungi.

The availability of a culture collection allows a wide range of research applications:

- Identification of toxigenic fungi and evaluation of mycotoxigenic risks,
- Research on reproduction and biodiversity, phylogenetics and population genetic studies,
- Development of new molecular probes to detect microbial contamination,
- Development of new diagnostic methods and biomarkers,
- Development of new methods to isolate, characterize and quantify metabolites,
- Development of detoxification strategies.

In recent years particular efforts have been made to analyze the etiology, epidemiology and ecophysiology of toxigenic fungi and their ability to produce and accumulate mycotoxin in pre- and post-harvest of the most important crops and agro-food chain, especially in the Mediterranean area.

The main commodities considered were cereals and cereal-based products, grape and wine, fruits and vegetables, while the mycotoxins of most concern from a food safety perspective include aflatoxins, ochratoxin, patulin, *Alternaria* toxins, citrinin and toxins produced by *Fusarium* spp. [Desjardins and Proctor 2007], including fumonisins, trichothecenes (principally nivalenol, deoxynivalenol, T-2 and HT-2 toxin) and zearalenone.

The results of multidisciplinary approaches have led to the biological, molecular and toxigenic characterization of *Fusarium* species (ITEM collection hosts more than 3700 *Fusarium* strains) mainly colonizing crops and showing different mycotoxicological profiles and specific toxicological risks. Epidemiological studies dealing with ochratoxin producing species showed that higher occurrence of strains contaminating grapes and wine is related to the species of *Aspergillus* section *Nigri*.

The success of these efforts is based on the correct isolation and collection of the isolates. The proper maintenance of the ITEM culture collection and its improvement is fundamental to achieving this goal.

Today, the ITEM collection is continuously growing in terms of strains number and species included. ITEM collection represents a wealth for the international research community. It provides an abundance of materials and information available for research and promotes the increase of knowledge about fungal biology, contributing to the development of strategies for reducing mycotoxin contamination worldwide.

ACKNOWLEDGMENT

This work was financially supported by EC KBBE-2007-222690-2 MYCORED. We are especially grateful to Vincenzo Ricci who provided dedication and expertise in establishing and maintaining ITEM culture collection throughout these years.

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ITEM – ПОЉОПРИВРЕДНО-ПРЕХРАМБЕНА ЗБИРКА МИКРОБИОЛОШКИХ КУЛТУРА: ЗНАЧАЈ ТОКСИКОГЕНИХ ГЉИВА У БОРБИ ПРОТИВ МИКОТОКСИНА

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РЕЗИМЕ: Збирке гљивичних култура важне су за биологе, микробиологе, епидемиологе и све друге који се баве здравственим и природним наукама. Унапређење техника и метода за изолацију и очување гљивица допринело је одржању великих микробних збирки, које представљају богат извор за истраживање у биолошким наукама, а посебно за проучавања у областима таксономије, патологије и биодиверзитета, као и за индустријску примену. Центри у којима се чувају те збирке одговорни су за репозиторијум референтних сојева као и за чување ових микроорганизама. ITEM – пољопривредно-прехрамбена збирка микробиолошких култура Института наука производње хране (ISPA - Institute of Sciences of Food Production) обухвата више од 10.000 сојева који припадају различитим пољопривредно-прехрамбеним микроорганизмима који су од фитопатолошког и токсиколошког значаја. Ови микроорганизми су углавном гљивични патогени који припадају токсигеним родови-

ма *Fusarium, Aspergillus,Alternaria*и *Penicillium*. Ова колекција је изузетан ресурс у борби против микотоксина: све већи број токсикогених гљива које су део ове збирке осигурава оригиналан генетски извор за примене у биотехнологији у неколико области истраживања, доприносећи тако обогаћивању знања о биологији гљива као и развоју стратегија за смањење контаминације микотоксинима.

КЉУЧНЕ РЕЧИ: збирка култура, ITEM, гљивице, микотоксин, биодиверзитет

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci., Novi Sad, № 127, 15—22, 2014

UDC 631.461:632.954
DOI: 10.2298/ZMSPN1427015H

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THE EFFECT OF MICROBIAL INOCULA ON THE GROWTH OF BLACK LOCUST, SIBERIAN ELM AND SILVER MAPLE SEEDLINGS

ABSTRACT: Growth and development of forest plants depend mostly on the soil microbial activity since no mineral or organic fertilizers are applied. Microbial processes can be activated and conditions for plants development improved with the introduction of selected microorganisms in the soil. With the aim of obtaining quality planting material in a shorter period of time, the effects of *Azotobacter chroococcum* and *Streptomyces* sp. on the early growth of black locust (*Robinia pseudoacacia*), Siberian elm (*Ulmus pumila*) and silver-leaf maple (*Acer dasycarpum*) were investigated in this study. Microorganisms were applied individually and in a mixture (1:1). Plant height was measured on the 90th, 120th and 180th day after planting. Plant diameter, as well as the number of actinomycetes and azotobacters was measured at the end of the vegetation period (180 days after planting). Applied microorganisms had a positive effect on the seedling height in all three plant species, with the best effect found in the black locust. Effectiveness of applied microorganisms on seedling diameter was the highest in the silver-leaf maple. The largest number of azotobacters was found in the rhizosphere of black locust. Number of microorganisms from both groups was increased in the inoculated variants.

KEYWORDS**:** black locust, Siberian elm, silver-leaf maple, *Azotobacter chroococcum*, *Streptomyces* sp., seedling growth, microorganisms

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INTRODUCTION

One of the basic functions of soil microbial biomass is the decomposition and transformation of organic materials, which are mostly derived from plant residues. Soil microorganisms start decomposition of soil organic matter and provide nutrients for plants [Barriuso and Solano 2008]. Rhizosphere microorganisms living near the root play a significant role in the plant nutrition. The root exudates influence chemical and microbiological properties of soil [Innes *et al.*, 2004; Gottel *et al.*, 2011]. Plant growth promoting microorganisms exert a positive influence on the plants through different, direct and indirect, mechanisms [Hayatsu *et al.*, 2008]. As opposed to the agricultural crops to which nutrients are supplemented through organic and mineral fertilizers, nutrition of forest and ornamental woody plants mainly depends on the microbiological activities [Li *et al.*, 2004]. Therefore, plant growth and development may be improved through activation of microbial processes. One of the ways to activate the microbial processes is via an introduction of effective microorganisms (biofertilizers) in the soil. These microorganisms may be introduced in the soil during pre-plant preparation of the soil, by application on the seed or root prior to planting. Biofertilizers are widely used in the production of field, vegetable and fruit crops for a considerable period of time, but are applied sporadically in the production of forest woody plants.

The effects of *Azotobacter* and actinomycetes on the early growth of black locust, Siberian elm and silver-leaf maple and the survival of introduced microorganisms in the rhizosphere of the selected plants were investigated with the aim of obtaining quality planting material in a shorter period of time.

MATERIAL AND METHODS

The trial was conducted on the soil with the following chemical properties: pH in H_2O 7.8; pH in KCl 7.4; N 0.13%; $CaCO_3$ 4.55%; humus 3.1%; 18.4 mg P_2O_5 in 100 g of soil; 20.5 mg K_2O in 100 g of soil. Seeds of black locust *(Robinia pseudoacacia)*, Siberian elm *(Ulmus pumila)* and silver-leaf maple *(Acer dasycarpum)* were taken from the collection of the Institute of Lowland Forestry and Environment in Novi Sad, Serbia. Bacteria *Azotobacter chroococcum* and actinomycete *Streptomyces sp*. were used for inoculation (both from the collection of the Faculty of Agriculture in Novi Sad, Serbia). *Azotobacter chroococcum* was grown in a mannitol medium (Hi Media Laboratories Pvt. Limited Mumbai, India), and actinomycetes in a synthetic agar (Hi Media Laboratories Pvt. Limited Mumbai, India). Microorganisms were applied individually and in a mixture (1:1). On 50 seeds of investigated plant species 50 ml of grown culture medium, cell density 10^8 cells in 1 ml, was applied, i.e. 50 ml of inoculum was added next to the seedling. Inoculation was performed in three ways: in the first group of plants microorganisms were applied immediately before planting; in the second group of plants microorganisms were added next to the root 30 days after planting; in the third group of plants microorganisms were applied to seeds before planting, and next to the root 30 days after; in the fourth group of plants microorganisms were not applied (control group). Planting was carried out at the beginning of May. Plant height was measured three times during the vegetation period (90, 120 and 180 days after planting). Plant diameter was measured at the end of the vegetation period (180 days after planting). The number of azotobacters and actinomycetes in the rhizosphere soil was determined at the end of the vegetation period with the method of agar plates [Benson 2002]. For this purpose the same nutrient media as for inoculum multiplication were used.

Statistical analysis of variance was performed (Fisher LSD) using STATISTICA 12,0 software (Hamburg, Germany).

RESULTS AND DISCUSSION

Mineral fertilizers and appropriate plant protection measures are used in the production of the woody plants seedlings. However, it is often forgotten that microorganisms also provide the plant with large quantities of nutrients. The use of mineral fertilizers may be reduced to minimum or completely omitted through activation of microbial processes in the soil [Higa and Parr 1994]. In this study, microorganisms were used with the aim to improve early growth and development of plants by applying nitrogen fixing bacterium (*Azotobacter chroococcum*) and actinobacterium promoting mineralization of soil organic matter (*Streptomyces sp.*). Applied microorganisms had a positive effect on the seedling height in all three plant species, with the best effect found in the black locust (Table 1). Effectiveness of inoculation on seedling diameter was the highest in the silver-leaf maple (Table 2).

Table 1. The effect of inoculation on the height of seedlings

Vari-ants	*Inoculated*	*Height of plant(cm) Black locust*				*Height of plant(cm) Silver maple*				*Height of plant(cm) Siberian elm*			
		Days after seeding				*Days after seeding*				*Days after seeding*			
		90	120	180	*avg.	90	120	180	*avg.	90	120	180	*avg.
1.	*Seed*	124.7	166.4	186.1	159.1	60.6	77.1	86.0	74.6	96.4	129.4	142.6	122.8
	Root	114.6	163.5	184.2	154.1	52.0	71.0	82.0	68.3	94.5	122.9	138.5	118.6
	Seed and root	128.8	176.3	191.5	165.5	53.2	73.4	88.4	71.7	92.6	133.5	143.3	123.1
2.	*Seed*	118.6	153.6	176.4	149.5	35.1	57.5	68.4	53.7	92.0	123.4	136.3	117.2
	Root	117.8	161.5	177.2	152.2	28.0	42.2	52.2	40.8	94.2	129.1	142.1	121.8
	Seed and root	124.4	158.2	178.6	153.7	59.6	76.3	83.5	73.1	90.6	111.9	136.8	113.1

3.	*Seed*	126.4	168.2	187.9	160.8	55.5	85.4	90.7	77.2	95.2	133.6	145.0	124.6
	Root	125.5	165.1	186.9	159.2	51.0	72.5	84.0	69.2	97.0	126.1	139.6	119.9
	Seed and root	126.3	164.8	186.6	159.2	44.3	70.3	85.0	66.5	93.5	116.9	139.3	116.6
4.	*Control*	99.1	116.9	149.8	121.9	17.7	32.0	40.5	30.1	88.6	111.4	134.1	111.4
	A	13.30	26.08	25.82		36.38	39.65	48.75		7.07	16.33	8.11	
	B	55.41	106.6	76.99		76.00	93.38	96.96		13.45	34.48	14.70	
	AXB	11.63	17.54	11.58		34.70	33.53	30.42		4.24	23.21	10.89	

Note: *1. Azotobacter chroococcum, 2. Streptomyces sp., 3. Azotobacter chroococcum + Streptomyces sp., 4. control*
$LSD_{0,05}$ test: Variants - treatment A, Inoculation - treatment B; *avg. - average values

Table 2. The effect of inoculation on the diameter of plants 180 days after sowing

Variants	*Inoculated*	*Diameter of plants (mm)*		
		Black locust	*Siberian elm*	*Silver maple*
1.	*Seed*	10.0	7.70	8.20
	Root	10.6	11.6	11.0
	Seed and root	11.9	8.80	6.80
2	*Seed*	12.4	9.40	8.20
	Root	11.9	10.2	5.30
	Seed and root	12.2	9.80	8.40
3	*Seed*	10.1	9.50	8.00
	Root	12.7	9.10	9.50
	Seed and root	14.9	8.80	8.30
4.	*Without inoculation*	9.80	8.10	4.50
	A	0.41	0.11	0.36
	B	0.61	0.41	0.79
	AXB	0.39	0.32	0.53

Note: *1. Azotobacter chroococcum, 2. Streptomyces sp., 3. Azotobacter chroococcum + Streptomyces sp., 4. control*
$LSD_{0,05}$ test: Variants - treatment A, Inoculation - treatment B

Method of inoculant application may influence its efficiency. In soybean and other leguminous plants better efficiency is obtained by seed inoculation [Redžepović *et al.*, 2007; Jarak *et al.*, 2006] whereas in this study there were no significant differences between methods of inoculation. The efficiency of application of single or multi-strain inoculants depends on the type of microorganisms applied and their interrelationships. In this study, application of *Azotobacter* and multi-strain inoculation had the same effects on the seedling height.

Different effects of applied microorganisms are also due to the composition of root exudates [Frostegard *et al.*, 1993; Marschner *et al.*, 2001]. Organic and amino acids are predominant in the rhizosphere of beech, and hydrate carbons in the rhizosphere of birch [Smith 1976]. Great differences in the composition of root exudates as well as in the composition of microbial communities were found between birch on the one hand, and pine or fir tree on the other hand [Priha *et al.*, 1999].

In this study, azotobacters and streptomyces were less abundant than in the rhizosphere of agricultural crops [Jarak *et al.*, 2011] which is probably due to the differences in the composition of root exudates [Nihorimbere *et al.*, 2011]. In the control variants, the largest number of actinomycetes was found in the rhizosphere of black locust, while lower but similar number was determined in other two plant species (Table 3). In contrast, the number of azotobacters was the lowest in the rhizosphere of Siberian elm, and the highest in the rhizosphere of black locust. However, in the inoculated variants, the number of investigated microorganisms increased as expected. A large quantity of live cells of microorganisms was introduced via inoculation, and they adapted well due to favourable conditions and increased in number. Likewise, a considerable amount of easily-decomposed fractions of soil organic matter was left after a part of introduced microorganisms was destroyed, thus favouring multiplication of actinomycetes and azotobacters.

Table 3. The number of actinomycetes and azotobacters in rhizospheric soil

Variants	*Inoculated*	*Number of actinomycetes($10^4 g^{-1}$)*			*Number of azotobacters($10^2 g^{-1}$)*		
		Black locust	*Siberian elm*	*Silver maple*	*Black locust*	*Siberian elm*	*Silver maple*
1.	*Seed*	26	50	17	39	20	36
	Root	28	57	18	163	59	60
	Seed and root	30	55	18	29	16	10
2.	*Seed*	36	136	113	19	15	16
	Root	43	63	33	45	34	37
	Seed and root	30	60	23	53	11	30
3.	*Seed*	25	163	93	29	16	84
	Root	20	160	43	49	14	27
	Seed and root	23	93	50	53	29	55
4.	*Without inoculation*	26	46	17	15	3	7
LSD 5%	A	28,71	193,71	106,42	108,36	50,51	89,58
	B	4,98	95,86	114,80	128,70	47,93	31,02
	A x B	12,81	94,22	86,76	122,44	47,11	75,86

Note: *1. Azotobacter chroococcum, 2. Streptomyces sp., 3. Azotobacter chroococcum* + *Streptomyces sp., 4. control*
$LSD_{0,05}$ test: Variants - treatment A, Inoculation - treatment B

Microbial biomass also acts as a small but labile reservoir of nutrients that contributes to maintaining long-term soil sustainability. The use of microorganisms in plant production has also been confirmed by many other research studies. Actinomycetes are known to be highly efficient mineralizers due to their ability to degrade lignin, pectin and the most resistant compounds that accumulate as components of humus [Seong *et al.*, 2001]. Therefore, their use in plant production can give positive results, as confirmed by this study. Bacteria from the genus *Azotobacter* exploit free nitrogen in/from the atmosphere and increase nitrogen balance in the soil for 40–60 kg ha^{-1} per year [Rajaee *et al.*, 2007]. For that reason, *Azotobacter* is used as inoculant in the production of field and vegetable crops. With the application of *Azotobacter* in the maize production, more uniform seed germination, yield higher by 3% and increased soil microbiological activity are obtained. Therefore, the use of *Azotobacter* as an alternative to nitrogen mineral fertilizers is warmly recommended [Hajnal *et al.*, 2005]. This study suggests that with the application of *Azotobacter* good effects can be achieved in the production of woody plant seedlings.

CONCLUSION

This study has reported positive effects of *Azotobacter* on the woody plant seedlings. The best effect on the seedling height was found in the black locust, and the poorest in the Siberian elm. Effectiveness of applied microorganisms on seedling thickness (diameter) was the best in the silver-leaf maple. Application of *Azotobacter* alone as well as combination of *Azotobacter* and actinomycetes had the same effect on the seedling height, while application of actinomycetes alone had poorer effect. In the control variants, the largest number of actinomycetes was found in the rhizosphere of Siberian elm, while the largest number of azotobacters was found in the rhizosphere of black locust. Number of actinomycetes and azotobacters increased in the inoculated variants.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support from the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project III 43002, 2011–2014).

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УТИЦАЈ МИКРОБНИХ ИНОКУЛАНАТА НА РАСТ САДНИЦА БАГРЕМА, СИБИРСКОГ БРЕСТА И СРЕБРНОЛИСНОГ ЈАВОРА

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РЕЗИМЕ: У исхрани биљака посебно су важни ризосферни микроорганизми који живе непосредно уз корен. Интродукцијом одабраних врста микроорганизама у земљиште могу се активирати микробиолошки процеси и тако побољшати услови за развој биљака. С циљем добијања квалитетног садног материјала за краће време, у овим истраживањима испитан је утицај примене *Azotobacter chroococcum* и *Streptomyces sp*. на почетни пораст багрема (*Robinia pseudoacacia*), сибирског бреста (*Ulmus pumila*) и сребрнолисног јавора (*Acer dasycarpum*). Микроорганизми су примењени појединачно и у смеши (1:1). Висина биљака мерена је 90, 120 и 180 дана након сетве. Пречник стабла биљака, број актиномицета и азотобактера мерен је на крају вегетационог периода (180 дана након сетве). Ефекат примењених микроорганизама на висину садница био је позитиван код све три биљне врсте при чему су најбољи резултати утврђени код багрема, а најслабији код сибирског бреста. Ефективност инокуланата на дебљину садница била је најбоља код сребрнолисног јавора. Начин инокулације није имао утицаја на пораст садница. На висину садница подједнак утицај имала је примена азотобактера и здружена инокулација. Број азотобактера је био највећи у ризосфери багрема. На варијантама где је примењена инокулација, број обе групе микроорганизама био је повећан.

КЉУЧНЕ РЕЧИ: багрем, сибирски брест, сребрнолисни јавор, *Azotobacter chroococcum*, *Streptomyces sp.*, раст садница, микроорганизми

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci., Novi Sad, № 127, 23—33, 2014

UDC 626.1(497.113):574.64
DOI: 10.2298/ZMSPN1427023J

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WATER QUALITY ASSESSMENT OF THE DTD CANAL SYSTEM BY DIATOM INDICES

ABSTRACT: The main objective of this paper is to evaluate the water quality based on diatom indices in the study area of the Danube–Tisa–Danube (DTD) canal system. We used four diatom indices: Watanabe's index (DAIpo), biological diatom index (BDI or IBD), the trophic diatom index (TDI) and index of pollution sensitivity (IPS). Benthic samples were collected in the spring and autumn 2002 and 2003 from nine sampling sites. The standard method with concentrated sulfuric acid was used for treatment of the algological samples [Krammer and Lange-Bertalot 1986] and then permanent slides of diatoms were made. The abundance was estimated by counting 400 valves of each taxa present on slide [Round 1991, 1993]. Investigation of the DTD canal system resulted in description of 145 diatom taxa. Based on the indicator values of identified taxa we calculated four diatom indices (DAIpo, BDI, TDI and IPS) and estimated water quality in the study area of the DTD canal system.

KEYWORDS: diatom indices, diatoms, DTD canal system, water quality

INTRODUCTION

Assessment of the human impact on aquatic organisms has been carried out for more than a century and the first studies assessing the effect of pollution on freshwater diatom communities appeared approximately 60 years ago. After these first approaches, benthic diatoms in rivers became obligatory bioindicators in several European and American countries in the late 1990s [Rimet 2012].

The Water Framework Directive (2000/60/EC), established in December 2000 by the European Commission, requires the monitoring and classification of all surface and ground waters physically, chemically and biologically

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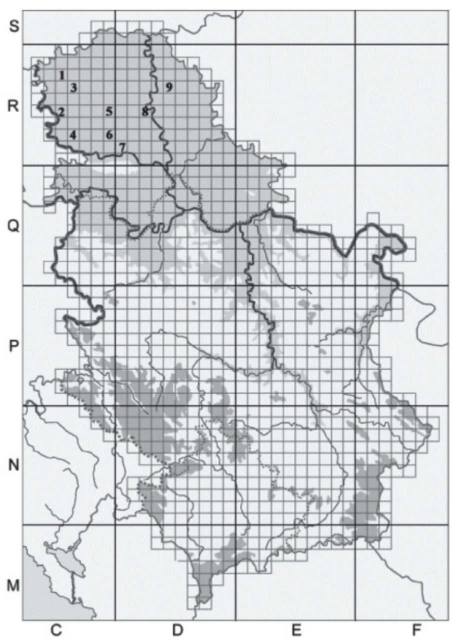
[Żelazowski *et al.*, 2004]. The best way to express the relationship between diatom indicator species and water quality in rivers and streams are diatom indices. Indices are simple arithmetic expressions that are based on some form of counting species.

The study of algae in the Danube–Tisa canal started in the 1960s [Szabados 1966]. During 1980s and 1990s, the authors studied the plankton composition and saprobiological characteristics of the DTD canal system [Pujin *et al.*, 1986; Obušković 1989, 1991, 1992; Kojčić *et al.*, 1995; Pujin 1998]. Then follows a series of papers about the seasonal dynamics of phytoplankton and ecological potential of diatoms in the DTD canal system [Nemeš 2009; Nemes and Matavuly 2004, 2005a,b, 2006; Nemeš and Matavulj 2007a,b; Nemeš *et al.* 2003, 2004, 2005, 2006a,b, 2007a,b, 2008].

MATERIALS AND METHODS

Algological samples were collected in the spring and autumn 2002 and 2003 from nine sampling sites (Fig. 1)



1	Sombor
2	Srpski Miletić
3	Mali Stapar
4	Bač
5	Vrbas I
6	Bački Petrovac
7	Novi Sad
8	Bačko Gradište
9	Novo Miloševo

Fig. 1. Distribution of sampling sites along the DTD canal system

Benthic samples were collected depending on the type of substrate. As a fixative and preservative 4% solution of formaldehyde was used. The standard method with concentrated sulfuric acid was used for treatment of the algological samples [Krammer and Lange-Bertalot 1986] which were then mounted in a synthetic medium Naphrax®. Microscopic examination of the permanent slides was done using the light microscope Zeiss AxioImager M1 with digital camera AxioCam MRc5 and AxioVision 4.8 software.

The relative abundance of taxa within the diatom community in the samples was determined by the valve percentage representation of each taxon relative to 400 numbered valve at every permanent slide [Round 1991, 1993]. According to the indicator values of identified taxa, we calculated four diatom indices (DAIpo, BDI, TDI and IPS) and estimated water quality in the study area of the DTD canal system.

Watanabe and his co-workers in Japan devised DAIpo index in relation to organic pollution of river waters [Watanabe *et al.*, 1988]. Classification of water based on this index is as follows: DAIpo < 50 - polysaprobic water; 50–70 - α-mesosaprobic water; 70–85 - β-mesosaprobic water; 85–100 - oligosaprobic water.

At BDI, species profiles were calculated according to the presence probability values of the species along the seven water quality classes [Coste *et al.*, 2009]. Classification of water based on this index is as follows: 1–4 - poor water quality; 5–8 - low water quality; 9–12 - moderate water quality; 13–16 - good water quality; 17–20 - very good water quality.

Concern over the increasing influx of nutrients into rivers of Europe initiated the creation of the TDI [Kelly and Whitton 1995]. Classification of water based on this index is as follows: 0–19.99 - very low nutrient concentration; 20–39.99 - low nutrient concentration; 40–59.99 - intermediate concentrations of nutrients; 60–79.99 - high concentrations of nutrients; 80–100 - very high concentrations of nutrients.

IPS is related to organic pollution, ion concentration and eutrophication. Class boundaries for the IPS were not set by the original authors, but generally the classification of the related IBD is used [Nieuwenhuis *et al.*, 2005].

RESULTS AND DISCUSSION

Diatoms community composition

The investigation of the algological samples from DTD canal system resulted in description of 145 diatom taxa. They were divided into 3 classes, 4 subclasses, 11 orders, 21 families and 39 genera.

The most common were the representatives of the *Bacillariophyceae* class with 116 taxa (80.00%), then *Fragilariophyceae* class (22 taxa, 15.17%) and finally *Coscinodiscophyceae* class (7 taxa, 4.82%). *Bacillariophycideae* subclass had the highest number of taxa (116 taxa, 80.00%), while the lowest number of taxa was recorded in the *Coscinodisophycideae* subclass (2 taxa, 1.37%). As for the orders, the most common were the representatives of the *Naviculales* order with 39 taxa (26.89%), and then the representatives of the *Cymbellales* order with 30 taxa (20.68%). The lowest number of taxa was recorded in *Aulacoseirales* and *Coscinodiscales* orders (1 taxon, 0.68%). *Fragilariaceae* was the most species-rich family (22 taxa, 15.17%), then *Bacillariaceae* (21 taxa, 14.48%), and *Naviculaceae* (20 taxa, 13.79%). *Navicula* was the most species-rich genus (19 taxa, 13.10%), then *Fragilaria* (16 taxa, 11.03%) and *Nitzschia* (12 taxa, 8.27%).

Cyclotella meneghiniana Kützing (Fig. 2, d) and *Stephanodiscus hantzschii* Grunow (Fig. 2, e) were the taxa that occur at all investigated sites of the DTD canal system, and in all samples. *Cocconeis placentula var. lineata* (Ehr.) van Heurck (Fig. 2, b) and *Fragilaria biceps* (Kütz.) Lange-Bertalot (Fig. 2, a) were taxa that occur at most of the studied sites of the DTD canal system. *Aulacoseira granulata* (Ehr.) Simonsen (Fig. 2, c), *C. meneghiniana* and *S. hantzschii* were the most dominant taxa.

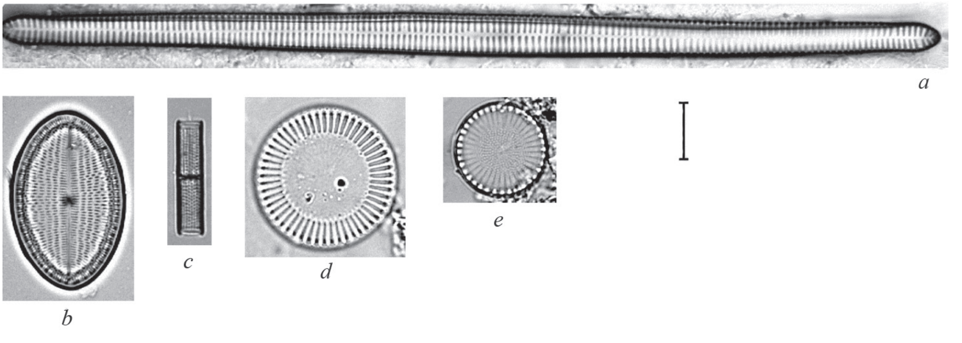


Fig. 2. LM micrographs. a) *Fragialria biceps*; b) *Cocconeis placentula* var. *lineata*; c) *Aulacoseira granulata*; d) *Cyclotella meneghiniana;* e) *Stephanodiscus hantzschii*. Scale bar = 10 μm.

In most samples the proportion of planktonic centric diatoms was high. This is not surprising considering the flow rate of the DTD canal system water. Ács *et al.* [2003] had reported similar results in the Danube River. In previous studies, *C. meneghiniana* was also the dominant species in the DTD canal system [Nemeš *et al.*, 2005a,b, 2006a,b]. Also, *Cyclotella* and *Stephanodiscus* species (usually abundant in lowland, eutrophic conditions) were found in high numbers in benthic diatom films from some rivers in England. 32% of the benthic diatom film from the River Wear was composed of *Cyclotella* because of the long period of low flows [Kelly 1998].

Water quality assessment using diatom indices

Four diatom indices for the 16 samples were calculated on the basis of the determined diatom flora at 9 sampling sites (Fig. 3).

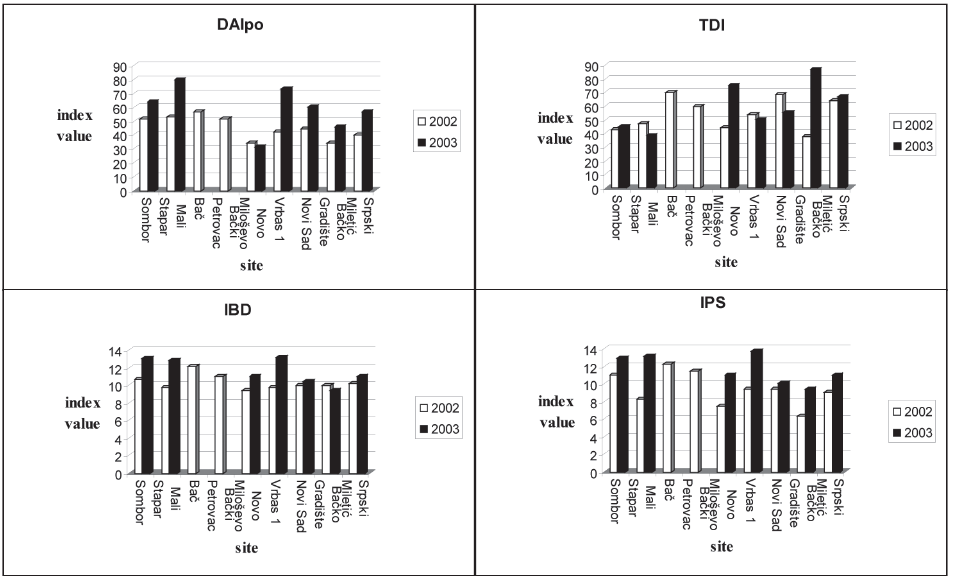


Fig. 3. Diatom indices values at the sampling sites of the DTD canal system

According to the results obtained by calculating diatom indices, it was evident that the DTD canal system water was of moderate quality at most sampling sites. It belongs to the group of α-mesosaprobic water with intermediate nutrient concentrations. Previous studies show similar results [Obušković 1991, 1992; Nemeš *et al.*, 2006a,b, 2007a,b]. At most sites there was an improvement of water quality in 2003 comparing to 2002, which can be concluded from the results of all four diatom indices. From two sites (Bač and Bački Petrovac) sampling was performed only in 2002, so we could not determine the trend of water quality at these sites.

According to the DAIpo values that were in the range of 31.66 to 79.48, water quality of the DTD canal system was in the range of the polysaprobic to the β-mesosaprobic water depending on the locality, year of sampling and sampling period of the year (Fig. 3).

Since water can be characterized as β-mesosaprobic water at only two sites (Vrbas I and Mali Stapar), water of the DTD canal system during the spring and autumn 2002 and 2003 can be characterized as transitive between α-mesosaprobic and polysaprobic water. DAIpo values were probably affected by many water-quality factors. High nitrite and phosphate concentrations and low dissolved oxygen have been suggested to reduce those values, unlike the high electric conductivity and nitrate concentrations [Uchikoshi *et al.*, 2008]. The best water quality in relation to organic pollution was determined at Mali Stapar sampling site and the worst at Novo Miloševo, which can probably be attributed to the effect of the settlement wastewater.

According to the TDI values, we can conclude that the water of the DTD canal system was characterized by low, intermediate and high nutrient concentrations at different sampling sites, depending on the year of sampling and season of the year (Fig. 3). However, the index values for the majority of sites were in the range of 40.22 to 56.61, which means that intermediate concentrations of nutrients in the water of the DTD canal system were present. The largest change in nutrient concentrations occurred at Bačko Gradište site (from low to very high concentration of nutrients in only 5 months time). In Serbia, water quality of the Nišava River was assessed according to the TDI. The results showed that most of the sites on the Nišava River in investigated period had eutrophic character of water (high value of TDI) [Andrejić 2012].

As for the TDI, water quality was found different considering various sampling sites. Very high TDI values were recorded at one site (Bačko Gradište, February, 2003), which indicates high degree of eutrophication. Low values were recorded at two sites (Mali Stapar, 2003 and Bačko Gradište, 2002). Similar to this study, when considering the TDI, some sampling sites of Upper Porsuk Creek (Turkey) were under eutrophication threat while others were eutrophic, very polluted [Solak 2011]. Juttner *et al.* [2003] found the TDI to be significantly correlated with orthophosphate concentrations when %PTV (% of motile algae) values were < 20% [Bellinger *et al.*, 2006]. Since in all cases %PTV were < 20% it could be concluded that the organic pollution had very little impact on water eutrophication. TDI showed no significant correlation with percentages of "eutrophic" species. This may occur because of various factors affecting diatom composition. Some of these are not related directly to water quality (e.g. current speed, grazing) [Eassa 2012].

Values obtained by calculating the BDI clearly indicated the moderate quality of the DTD canal system water. Calculating the index for 14 samples (out of 16) from seven sites (out of 9), the values obtained were in the range of 9.41 to 12.15 indicating the moderate water quality (Fig. 3). Calculating the index for the other two samples from two different sites (Vrbas I, 2003 and Sombor, 2003) the values obtained indicated good water quality. Vrbas I site was characterized by good water quality calculating BDI and DAIpo indices. Values obtained by calculating the IPS are very similar with values obtained by BDI (Fig. 3).

According to the research conducted in the Pilica River (Poland), the BDI shows great similarity to the IPS [Szulc and Szulc 2013]. In our study, the situation was the same. It was expected, as both studied were based on the same Zelinka and Marvan formula. Also, according to the other research conducted in the same river, both indices significantly positively correlated with COD-CR and conductivity, while negatively correlated with total nitrogen, total phosphorus and phosphates [Szulc 2009]. Similar correlations have been observed in French rivers [Coste *et al.*, 2009].

CONCLUSION

The main objective of this paper was to evaluate the water quality in the study area of the DTD canal system based on four diatom indices (DAIpo, BDI, TDI and IPS).

The abundance was estimated by counting 400 valves of each taxa present on slide (Round, 1991, 1993). The investigation of the algological samples from the DTD canal system resulted in description of 145 diatom taxa. They were divided into 3 classes, 4 subclasses, 11 orders, 21 family and 39 genera. The most common were the representatives of the *Bacillariophyceae* class with 116 taxa (80%). *Bacillariophycideae* subclass had the highest number of taxa (116 taxa, 80%). As for the orders, the most common were representatives of the *Naviculales* order, with 39 taxa (26.89%). *Fragilariacea* was the most species-rich family (22 taxa, 15.17%). *Navicula* was the most species-rich genus (19 taxa, 13.10%).

Cyclotella meneghiniana and *Stephanodiscus hantzschii* were the taxa that occur at all investigated sites of the DTD canal system, and in all samples. *A. granulata*, *C. meneghiniana* and *S. hantzschii* were the most dominant taxa.

According to the results obtained by calculating diatom indices, the water quality of the DTD canal system at most sites was moderate, belonging to the group of α-mesosaprobic water with intermediate nutrient concentrations.

ACKNOWLEDGMENTS

Financial support was provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. TR 037009).

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ПРОЦЕНА КВАЛИТЕТА ВОДЕ КАНАЛСКЕ МРЕЖЕ ДТД НА ОСНОВУ ДИЈАТОМНИХ ИНДЕКСА

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РЕЗИМЕ: Главни циљ овог рада је процена квалитета воде на основу дијатомних индекса у испитиваном делу каналске мреже ДТД. Користили смо четири дијатомна индекса: DAIpo, BDI, TDI I IPS. Узорци бентоса су сакупљени са девет локалитета у пролеће и јесен 2002. и 2003. године. Алголошки узорци су третирани стандардним лабораторијским поступком са концентрованом сумпорном киселином [Krammer and Lange-Bertalot 1986], а потом су направљени трајни препарати. Бројањем 400 валви за сваки узорак процењено је укупно богатство врста [Round 1991, 1993]. Истраживањем каналске мреже ДТД утврђено је укупно 145 таксона силикатних алги. На основу индикаторских вредности идентификованих таксона израчуната је вредност четири дијатомна индекса (DAIpo, BDI, TDI и IPS) и одређен је квалитет воде у испитиваном делу каналске мреже ДТД. Резултати су показали да је вода каналске мреже ДТД на већини испитиваних локалитета средњег квалитета, да спада у групу алфа-мезосапробних вода и да је са умереном концентрацијом нутријената.

КЉУЧНЕ РЕЧИ: дијатомни индекси, силикатне алге, каналска мрежа ДТД, квалитет воде

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci., Novi Sad, № 127, 35—42, 2014

UDC 639.311:582.232(497.11)
DOI: 10.2298/ZMSPN1427035T

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MICROCYSTIN CONCENTRATION IN FISHPOND WATERS

ABSTRACT: Cyanobacterial blooming is a worldwide problem which sometimes results in cyanotoxin production. Most commonly produced cyanotoxins are microcystins (MCs), potent inhibitors of protein phosphatases. Protein phosphatase 1 (PP1) is known to be involved in the regulation of a variety of cellular processes. In this study, MC concentration was assessed via inhibition of protein phosphatase 1 (PP1 assay) in water samples taken from 14 lakes of Mužlja fishpond, Vojvodina, Serbia. During the summer of 2011, cyanobacterial growth occured in the fishpond lakes and small, planktonic crustacean *Daphnia* sp. was used to control or/and prevent further development of cyanobacteria. Different MC concentrations (calculated as microcystin-LR equivalents) were detected, mostly depending on the occurrence and grazing of *Daphnia* sp. More thorough monitoring of fishponds should be conducted, both in Serbia and around the world, in order to gain more precise estimation of cyanotoxin concentrations and their accumulation in organisms used for human consumption and thus prevent possible negative health effects.

KEYWORDS: *Daphnia,* fishpond, Serbia, cyanobacteria, microcystin (MC)

INTRODUCTION

Cyanobacteria are a ubiquitous group of prokaryotes which produce a large number and variety of bioactive substances. These substances have a diverse range of biological activities and chemical structures, and can affect many biochemical processes within cells [Smith and Doan 1999; Singh *et al.*, 2005]. Cyanobacterial blooms can occur as a result of increasing eutrophication of

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water bodies [Pearl *et al.*, 2005], during which cyanotoxins could be produced and released into water.

Some of the most common cyanotoxins in fresh waters are microcystins (MCs), cyclic heptapeptides which comprise a diverse group containing around 90 structurally similar congeners [Pearson *et al.*, 2010]. These hepatotoxins are potent inhibitors of protein serine/threonine phosphatases (types 1 and 2A) in eukaryotic organisms [Runnegar *et al.*, 1995]. Protein phosphatase 1 (PP1) is known to be involved in the regulation of a variety of cellular processes, such as cell division, glycogen metabolism, muscle contractility, protein synthesis, and HIV-1 viral transcription.

Cyanobacterial toxins and other secondary metabolites produced by cyanobacteria during blooming in fishponds present a potential health threat to aquatic life, and consequently, humans. The aim of this paper was to assess MC concentration in water samples from Mužlja fishpond in Serbia using PP1 assay.

MATERIALS AND METHODS

Water samples were collected from 14 different lakes (Maja, Veliko Klosi, Joca, Novo Mišino, Beba, Daphnia, Malo Klosi, Miša, Magda, Veliko lake, Lake 2, 3, 4 and 7) of Mužlja fishpond located in Vojvodina (Serbia) during 2011. Lakes were grouped as lakes of early introduction (Maja lake, Veliko Klosi lake, Joca lake, Novo Mišino lake, Lake 3 and 4), lakes of late introduction (Beba lake, Daphnia lake, Malo Klosi lake and Miša lake), and control lakes (Magda lake, Veliko lake, Lake 2 and 7). Early and/or late introduction reffers to the time of *Daphnia* introduction into the lakes: early - before cyanobacterial blooms, and late - during or after cyanobacterial blooms. In control lakes, there was no introduction of daphnids. To compare the data, water samples were collected in shallow waters from piers or docks from the same sampling sites during September and November, 2011. About 1 liter of sample water was collected in plastic containers. The water samples were transferred to the laboratory within 6h from the sampling time.

In order to differentiate between potential intracellular and extracellular content of cyanotoxin, 100 ml of water samples were filtrated and the extracellular filtrate was stored at 4 °C until assay. Filters with cyanobacterial biomass (intracellular filtrate) have been dried over night. The next day 75% methanol has been used for extraction of cyanotoxins for 24 hours, then sonicated and centrifuged. Supernatants were stored at 4 °C until assay.

Method applied by An and Carmichael [1994] was optimized [Simeunović 2009] and used for MCs detection in water samples. This method is a colorimetric assay of inhibition of the enzyme protein phosphatase 1 (PP1). MCs inhibit PP1 enzyme activity and the detection of inhibition was performed by measuring the inhibiting effect of MCs on the activity of the enzyme, or to be more precise, separation of the phosphate groups of para-nitrophenylphosphate substrate (pNPP). Measuring the resulting product para-nitrophenol pNP (yellow), it is possible to determine the level of activity, and consequently, the level of enzyme inhibition from tested MCs and examined water samples. In the wells of microtiter plates 10 µl of the enzyme was placed, and 10 µl of standard or sample were added. Pure toxin MC-LR (Sigma-Aldrich) was used as a standard. After 5 min of preincubation, 180 µl of pNPP substrate was added. Inhibiting effect of MC-LR standard in the samples (level of pNP production) was measured after 2 hours of incubation at 37 ± 1 °C, at a wavelength of 405 nm using photometer microplate reader (MULTISCAN EX Termo Labsystems).

Pure toxin MC-LR was used for formation of standard curve which was the base for determination of MCs concentration (expressed as MC-LR equivalents) in the tested water samples. Detection of MCs was performed in three replicates and the results were expressed as mean values of three measurements.

RESULTS

Results of mean MC concentration in water samples from Mužlja fishpond in Serbia obtained via PP1 assay are presented in Table 1.

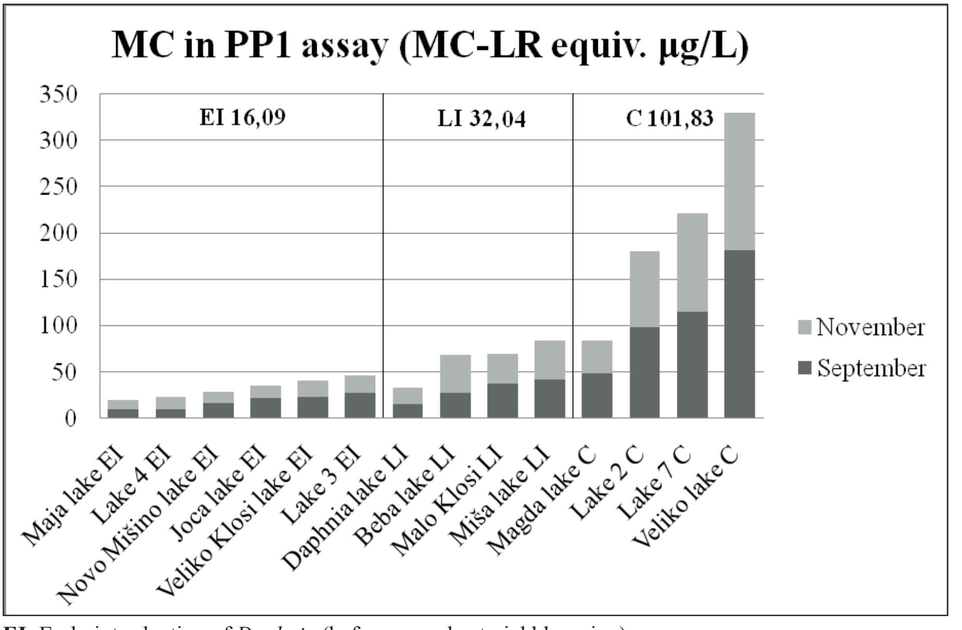
Table 1. Results of mean MC concentration in water samples from Mužlja fishpond in Serbia obtained via PP1 assay

WATER SAMPLE	**MICROCYSTIN CONCENTRATION IN PP1 ASSAY (MC-LR equiv. µg/L)**			
	September 13, 2011		November 17, 2011	
	intracellular	extracellular	intracellular	extracellular
EI	10.783	7.283	8.05	6.066
LI	28.525	1.655	23.375	10.525
C	108.2	2.2	85.825	7.45

EI: Early introduction of *Daphnia* (before cyanobacterial blooming) in Maja lake, Veliko Klosi lake, Joca lake, Novo Mišino lake, Lake 3 and 4;
LI: Late introduction of *Daphnia* (during or after cyanobacterial blooming) in Beba lake, Daphnia lake, Malo Klosi lake and Miša lake;
C: Control lakes − Magda lake, Veliko lake, Lake 2 and 7.

Mean MC concentrations in three groups of Mužlja lakes were higher in September than in November, and intracellular toxicity was higher than extracellular. Lowest mean extracellular MC concentration was recorded in late introduction lakes, and the highest mean intracellular concentration in control lakes during September (1.655 and 108.2 µg/L, respectively).

Concentrations of total MC, intracellular and extracellular, in all the lakes of Mužlja fishpond during two investigated periods (September and November) are presented in Figure 1.



EI: Early introduction of *Daphnia* (before cyanobacterial blooming)
LI: Late introduction of *Daphnia* (during or after cyanobacterial blooming)
C: Control lakes

Figure 1. Concentrations of total MC in all the lakes of Mužlja fishpond during September and November of 2011.

Figure 1 also shows rising mean values of total MC (16.09 µg/L, 32.04 µg/L, 101.83 µg/L) in three groups of lakes (early introduction, late introduction and control lakes) during both investigated periods. Lowest concentrations were recorded in early introduction lakes (from 10–27.6 µg/L). Higher values were detected in late introduction lakes (15–41.4 µg/L), and the highest concentrations were found in control lakes (48.1–180.9 µg/L) in September, 2011. In November of the same year the trend continued, with lowest concentration detected in early introduction lake (Maja lake: 9.4 µg/L), and the highest in control lake (Veliko lake: 149.2 µg/L).

DISCUSSION

Intracellular and extracellular concentrations of MC in water samples collected from the three groups of lakes of Mužlja fishpond during two investigated periods were detected using PP1 assay. During the summer all lakes bloomed and the presence of MCs in water samples from both investigated periods was confirmed.

Mean values of PP1 assay showed differences between intracellular and extracellular concentrations. In September, intracellular concentrations of MC were higher than in November. Also, it is obvious that in almost every sample group the detected intracellular concentrations were higher, which can be explained with the fact that 50% to 95% of the cyanotoxins is intracellular during the growth stage of the bloom [Chorus and Bertram 1999; Griffiths and Saker 2003].

Furthermore, mean intracellular concentrations of MC in September and November were ten times higher in control than in early introduction lakes. The differences among the three groups of lakes were evident and the observed MC concentrations dependend on the occurrence of *Daphnia* sp. in fishpond lakes. To be more precise, in lakes where the introduction of daphnids was done before cyanobacterial bloom, total MC concentration was the lowest (18.066 μg/L). Detected total concentration of MC in lakes where introduction of daphnids was realized during or after bloom was higher. The highest concentrations were detected in control lakes (110.4 μg/L). A total of MC concentration, both intracellular and extracellular, was the lowest in early introduction lake (Maja lake), and the highest in control lake (Veliko lake) for both investigated periods. Therefore, there is a noticeable impact of *Daphnia* species on MC concentration in water samples from Mužlja lakes.

Our findings are in accordance with previous studies by Pogozhev and Gerasimova [2001], where it was shown that *Daphnia longispina* can control phytoplankton development and thus increase the transparency of blooming water and recovery of highly-eutrophic water bodies. Furthermore, Mohamed [2001] indicated that *Daphnia* feeds facultatively on toxic cyanobacteria under the conditions of depletion of edible food. Accumulation of MCs in Daphnia was also documented in the same study. These results should be taken into consideration when primary consumers such as *Daphnia* are used in the biomanipulation of toxic phytoplankton [Mohamed 2001]. Studies have shown that cyanobacteria have strong adverse effects on *Daphnia* and other zooplankton, such as increased mortality, decreased growth rate, delayed maturation and decreased offspring production [e.g. Lampert 1981; Hietala *et al.*, 1995; de Bernardi and Giussani 1990; Lotocka 2001; Deng *et al.*, 2008]. However, there is an evidence of behavioral adaptations of zooplankton which enhance their ability to coexist with toxic cyanobacteria [DeMott *et al.*, 1991]. Moreover, in some cases the survival of daphnids was improved and size of the animals augmented when continuously exposed to toxic cyanobacteria [Gustafsson and Hansson 2004]. A recent research has shown that high concentrations of MC do not prevent *Daphnia* from strongly suppressing phytoplankton biomass in nature [Chislock *et al.*, 2013].

Fish can get in contact with cyanobacteria and their toxins, which may affect their growth, development, reproduction and survival. Some exposure routes are: active introduction by oral consumption of prokaryotic cells (especially in the case of phytoplanktivorous species) or other organisms that have accumulated cyanotoxins (e.g. zooplankton) [Li *et al.*, 2004; Xie *et al.*, 2004]; and passive entry, through direct contact with the gill epithelium and the surrounding water containing toxins. However, it is frequently a combination of both of these types [Malbrouck and Kestemont 2006]. Therefore, cyanobacterial cells and daphinds with accumulated MC in fishponds can present a possible health risk for fish and the end consumers − humans.

CONCLUSION

During the summer of 2011, cyanobacterial growth occured in the lakes of Mužlja fishpond in Vojvodina, Serbia. Crustacean *Daphnia* sp. was used to control or/and prevent further development of cyanobacteria in fishpond. PP1 assay was used to give valuable information about the MC concentration of the sampled fishpond water. Depending on the occurrence of *Daphnia* sp. in fishpond lakes, different concentrations of MCs were obtained. Highest values were detected in control lakes and the lowest in lakes where introduction of daphnids was done before blooming. These results indicate the significance of timely introduction of phytoplankton grazing organisms, such as daphids, as a possible method for mitigation of blooming effects on aquatic life. More thorough monitoring of fishponds should be conducted, both in Serbia and around the world, in order to gain more precise estimation of cyanotoxin concentrations and their accumulation in organisms used for human consumption and thus prevent possible negative health effects.

ACKNOWLEDGEMENT

The authors would like to acknowledge funding from the Ministry of Education, Science and Technological Development of the Republic of Serbia (project number: 176020).

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КОНЦЕНТРАЦИЈЕ МИКРОЦИСТИНА У ВОДИ РИБЊАКА

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РЕЗИМЕ: Цветање цијанобактерија светски је проблем који понекад резултира стварањем цијанотоксина. Од цијанотоксина најчешћи су микроцистини (МЦ), снажни инхибитори протеин фосфатазе. Познато је да је протеин фосфатаза 1 (ПП1) укључена у регулацију различитих ћелијских процеса. У овој студији, концентрација МЦ процењена је преко инхибиције протеин фосфатазе 1 (ПП1 тест) у узорцима са 14 језера рибњака Мужља у Војводини. Током лета 2011. године, догодио се раст цијанобактерија у језерима рибњака, и мали планктонски рачићи *Daphnia* sp. искоришћени су да би се контролисао и/или спречио даљи развој цијанобактерија. Утврђене су различите концентрације МЦ (израчунате као микроцистин-LR еквиваленти), углавном у зависности од појаве и испаше *Daphnia* sp. Требао би се спровести појачани надзор рибњака, како у Србији тако и широм света, да би се добиле прецизније процене концентрације цијанотоксина и њиховог нагомилавања у организмима који се користе за исхрану људи, и на тај начин спречили могући негативни ефекти.
КЉУЧНЕ РЕЧИ: *Daphnia,* рибњак, Србија, цијанобактерија, микроцистин (МЦ)

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci., Novi Sad, № 127, 43—56, 2014

UDC 633.2(497.113 Iđoš)
DOI: 10.2298/ZMSPN1427043K

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PASTURE VEGETATION NEAR THE VILLAGE OF IĐOŠ

ABSTRACT: A pasture on the solonchakic solonetz soil in the vicinity of the village of Iđoš (Banat, Serbia) was found to harbor 137 plant taxa (129 species, 7 subspecies and 1 variety). The plant cover they formed was specific from the ecological, phytogeographical and phytocoenological points of view. The specific ecological feature of the surveyed plant cover was that 47 or 34.31% of the recorded taxa were rated with the ecological index $\mathbf{S}_+$ due to their ability to grow in saline soil. The specific phytogeographical feature of the surveyed plant cover was the presence of two Pannonian endemics, *Plantago schwarzenbergiana* Schur and *Statice gmelini* subsp. *hungaricum* (Klokov) Soó, and two subendemics, *Puccinellia limosa* Holmb. and *Roripa kerneri* Menyh. The specific phytocoenological feature of the surveyed plant cover was the presence of two phytocoenoses from the class *Phragmitetea* Tx. et Prsg. 1942 (ass. *Scirpo-Phragmitetum medioeuropaeum* and ass. *Bolboschoenetum maritimi continentale*), one phytocoenose from the class *Molinio-Arrhenatheretea* Tx.1937 p.p., Br.-Bl. et Tx. 1943 p.p. (ass. *Trifolio-Lolietum perennis*) and ten phytocoenoses from the class *Festuco-Puccinellietea* Soó 1968 (ass. *Puccinellietum limosae*, ass. *Pholiuro-Plantaginetum tenuiflorae*, ass. *Hordeetum histricis*, ass. *Agrostio-Alopecuretum pratensis*, ass. *Agrostio-Beckmannietum*, ass. *Halo-Agropyretum repentis*, ass. *Poeto-Alopecuretum pratensis halophyticum*, ass. *Artemisio-Festucetum pseudovinae*, ass. *Trifolio-Festucetum pseudovinae* and ass. *Achilleo-Festucetum pseudovinae*). The presence of 34.31% of taxa rated with the ecological index S_+, the presence of two Pannonian and two sub-Pannonian floristic elements and the predominance of stands from the class *Festuco-Puccinellietea* Soó 1968 led us to conclusion that the pasture near the village of Iđoš (Banat, Serbia) is a part of the halobiome of the Pannonian Plain.

KEYWORDS: flora, halobiome, Iđoš (Banat - Serbia), pasture, vegetation

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INTRODUCTION

Iđoš is a village in the region of Banat, Serbia (Fig. 1). It was built at the edge of the alluvial plain formed in the lower course of the Zlatica River, on a small elevation of the chernozem soil on sandy loess. The location is surrounded by the saline and alkalized hydromorphic smonitza soil from the north and northwest, the hydromorphic smonitza soil from the west and south, and the calcareous black meadow soil on the loess terrace from the east.

A characteristic feature of the area around the village, i.e. of the alluvial plain of the Zlatica River, is the presence of large expanses of saline soils. These soils had become saline as the result of the alluvial type of salinization that occurred due to surface flooding by waters from Carpathian basin. However, flood waters pressure on the alluvial plain of the Zlatica River gradually decreased, as a consequence of the melioration of the Moriš River, subsequent melioration of the Zlatica River, and the construction of Kikinda canal with a network of smaller drainage canals attached to it. This explains why the saline areas in the alluvial plain of the Zlatica River are presently in the process of salt leaching.

The investigated natural pasture is located southwest of the village of Iđoš and it had formed on the high grounds composed of the solonchakic solonetz soil. The surrounding low grounds include a belt of the saline hydromorphic smonitza soil, which is about half a kilometer wide and borders the pasture from the west and north, and a belt of the hydromorphic smonitza soil, which is between one and two kilometers wide and surrounds the pasture from the south and east. Drainage canals have been dug through both of these belts as well as through a part of the pasture itself.

According to the data obtained from the nearest meteorological station located in the town of Kikinda, the average annual precipitation in the studied location is 555 mm and the average annual temperature is 11.1 °C. The Walter's climogram indicated that the beginning of the vegetation period is characterized by a significant increase in precipitation and considerable but gradual increases in temperature. The rainfall reaches its peak in June and considerably drops afterwards. In contrast to the rainfall, the temperatures rise gradually at the beginning of the vegetation period, stabilize in the middle of this period, and start notably to decline only in October. Because of such combination of rainfall and temperatures, the studied location is under the impact of an unfavorable semi-arid period, which lasts from mid July to late September and negatively affects the vegetation cover.

Due to a limited organic production of the solonchakic solonetz soil, the area southwest of the village of Iđoš lies fallow. A natural vegetation cover formed there and it is used by village residents for cattle grazing and mowing.

Our aim was to study the flora and vegetation cover of the natural pasture on the solonchakic solonetz soil located in the vicinity of the village of Iđoš and to describe its characteristics.

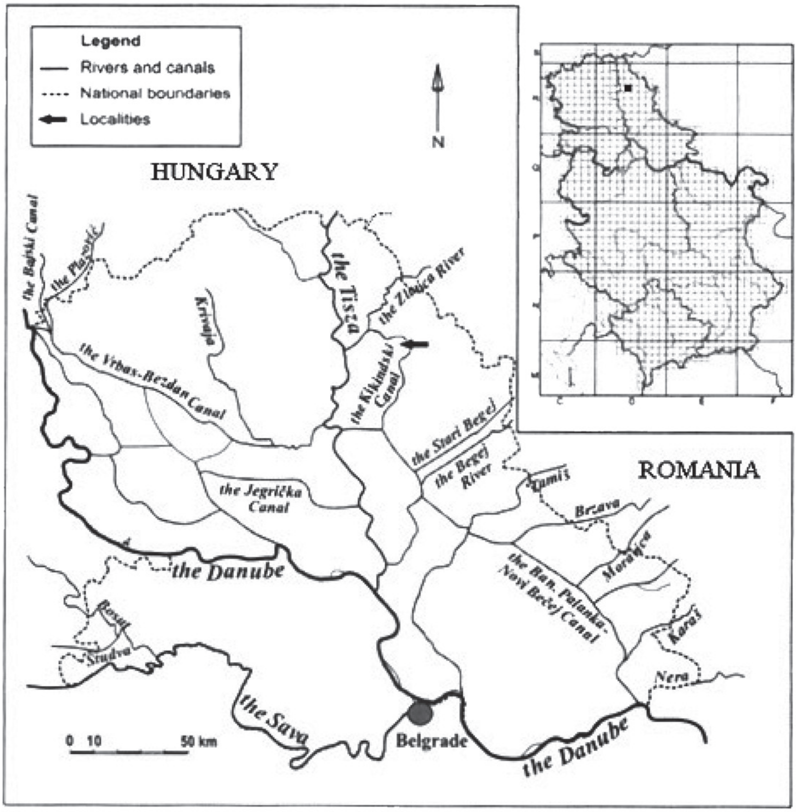

Fig. 1. Map of the Province of Vojvodina (Northern Serbia) with the location of the investigated site

MATERIAL AND METHODS

Soil data for the studied location are taken from Benka and Salvai [2005]. Climatic data for the studied location are taken from Ljevnaić-Mašić [2010]. The flora and vegetation of the natural pasture near the village of Iđoš were collected during field trips conducted over a period of several years. The collected plant materials were determined and their names conformed to the nomenclature according to Josifović [1970–1976], Săvulescu [1952–1976] and Tutin *et al.* [1964–1980]. The ecological value of the taxa identified as adapted to the saline habitat was assessed according to the criteria of Landolt [Landolt, 1977; Knežević, 1994]. The place of the identified taxa among endemics or sub-endemics of the Pannonian Plain was determined according to the publications by Soó [1964–1985]. The syntaxonomic position of the plant communities observed in the various sites of the investigated pasture was determined according to the publication "Halophytic vegetation of the Yugoslav part of the Banat region" [Knežević *et al.*, 1998].

RESULTS AND DISCUSSION

On the pasture on the solonchakic solonetz soil in the vicinity of the village of Iđoš (Banat, Serbia) 137 taxa were found (Tab. 1).

Table 1. A pasture flora on the solonchakic solonetz soil in the vicinity of the village of Iđoš (Banat, Serbia)

N°	Taxon	Ecological index for salinity
1.	*Achillea millefolium* L.	S_-
2.	*A. millefolium* L. subsp. *collina* (Becker) Weiss	S_-
3.	*Agrimonia eupatoria* L.	S_-
4.	*Agropyrum repens* (L.) Beauv.	S_+
5.	*Agrostis alba* L.	S_-
6.	*Alisma plantago-aquatica* L.	S_-
7.	*Allium vineale* L.	S_-
8.	*Alopecurus pratensis* L.	S_-
9.	*Andropogon ischaemum* L.	S_-
10.	*Artemisia maritima* L. subsp. *monogyna* (W. et K.) Gams.	S_+
11.	*Asclepias syriaca* L.	S_-
12.	*Asperula cynanchica* L.	S_-
13.	*Aster tripolium* L. var. *pannonicus* (Jasq.) Beck	S_+
14.	*Astragalus cicer* L.	S_-
15.	*Atriplex hastata* L.	S_+
16.	*A. litoralis* L.	S_+
17.	*A. tatarica* L.	S_+
18.	*Beckmannia eruciformis* (L.) Host	S_+
19.	*Bolboschoenus maritimus* (L.) Palla	S_+
20.	*Bromus commutatus* Schrad.	S_-
21.	*B. mollis* L.	S_-
22.	*Bupleurum tenuissimum* L.	S_+
23.	*Calystegia sepium* (L.) R. Br.	S_-
24.	*Capsella bursa pastoris* (L.) Medik.	S_-

25.	*Carduus nutans* L.	S_-
26.	*Carex distans* L.	S_+
27.	*C. vulpina* L.	S_-
28.	*Centaurea cyanus* L.	S_-
29.	*Cerastium caespitosum* Gilib.	S_-
30.	*C. dubium* (Bast.) Schwarz.	S_-
31.	*Chenopodium album* L.	S_-
32.	*Ch. glaucum* L.	S_+
33.	*Cichorium intybus* L.	S_-
34.	*Cirsium arvense* (L.) Scop.	S_-
35.	*Conium maculatum* L.	S_-
36.	*Convolvulus arvensis* L.	S_-
37.	*Cynodon dactylon* (L.) Pers.	S_-
38.	*Dactylis glomerata* L.	S_-
39.	*Daucus carota* L.	S_-
40.	*Dipsacus laciniatus* L.	S_-
41.	*Eryngium campestre* L.	S_-
42.	*Euclidium syriaca* (L.) R. Br.	S_-
43.	*Euphorbia cyparisias* L.	S_-
44.	*Festuca vallesiaca* Sch. subsp. *pseudovina* (Hack.) A. et G.	S_+
45.	*Filipendula hexapetala* Gilib.	S_-
46.	*Fragaria viridis* Duchense	S_-
47.	*Galium aparine* L.	S_-
48.	*G. pedemontanum* All.	S_-
49.	*G. verum* L.	S_-
50.	*Geranium columbinum* L.	S_-
51.	*Gratiola officinalis* L.	S_+
52.	*Gypsophila muralis* L.	S_-
53.	*Heleocharis palustris* (L.) R. Br.	S_-
54.	*Helminthia echioides* (L.) Gärtn.	S_-
55.	*Hordeum maritimum* Stokes subsp. *gussoneanum* (Parl.) A. et G.	S_+
56.	*Hypericum perforatum* L.	S_-
57.	*Inula britannica* L.	S_+
58.	*Juncus atratus* Krock.	S_-
59.	*J. compressus* Jacq.	S_+
60.	*J. gerardi* Lois.	S_+
61.	*Knautia arvensis* (L.) Coult.	S_-
62.	*Lactuca saligna* L.	S_+

63.	*Lathyrus hirsutus* L.	S_-
64.	*L. tuberosus* L.	S_-
65.	*Lemna minor* L.	S_-
66.	*Lepidium draba* L.	S_-
67.	*L. perfoliatum* L.	S_-
68.	*L. ruderale* L.	S_-
69.	*Lolium perenne* L.	S_-
70.	*Lotus angustissimus* L.	S_+
71.	*L. corniculatus* L.	S_-
72.	*L. tenuis* Kit.	S_+
73.	*Lycopus europaeus* L.	S_-
74.	*Lysimachia nummularia* L.	S_-
75.	*Lythrum virgatum* L.	S_-
76.	*Matricaria chamomilla* L.	S_+
77.	*M. inodora* L.	S_+
78.	*Medicago falcata* L.	S_-
79.	*M. lupulina* L.	S_-
80.	*Melilotus officinalis* (L.) Pallas	S_-
81.	*Mentha pulegium* L.	S_+
82.	*Myosurus minimus* L.	S_-
83.	*Oenanthe silaifolia* M. B.	S_+
84.	*Ononis spinosa* L.	S_-
85.	*Onopordon acanthium* L.	S_-
86.	*Ornithogalum gussonei* Ten.	S_-
87.	*Panicum crus-galli* L.	S_-
88.	*Pastinaca sativa* L.	S_-
89.	*Pholiurus pannonicus* (Host) Trin.	S_+
90.	*Phragmites communis* Trin.	S_+
91.	*Pimpinella saxifraga* L.	S_-
92.	*Plantago lanceolata* L.	S_-
93.	*P. maior* L.	S_+
94.	*P. schwarzenbergiana* Schur	S_+
95.	*P. tenuiflora* W. et K.	S_+
96.	*Poa pratensis* L.	S_-
97.	*P. pratensis* L. subsp. *angustifolia* (L.) Sm.	S_-
98.	*Podospermum canum* C. A. Mey.	S_+
99.	*Polygonum aviculare* L.	S_-
100.	*P. lapathifolium* L.	S_-

101.	*Potentilla argentea* L.	S_-
102.	*Prunus spinosa* L.	S_-
103.	*Puccinellia limosa* (Schur) Holmb.	S_+
104.	*Ranunculus lateriflorus* DC	S_+
105.	*R. repens* L.	S_-
106.	*R. sardous* Cr.	S_+
107.	*Roripa kerneri* Menyh.	S_+
108.	*Rosa canina* L.	S_-
109.	*Rubus caesius* L.	S_-
110.	*Rumex crispus* L.	S_+
111.	*Salvia nemorosa* L.	S_-
112.	*Schoenoplectus lacuster* (L.) Palla	S_-
113.	*Sinapis arvensis* L.	S_-
114.	*Sonchus arvensis* L.	S_+
115.	*Statice gmelini* Willd. subsp. *hungaricum* (Klokov) Soó	S_+
116.	*Stenactis annua* (L.) Ness.	S_-
117.	*Taraxacum officinale* Weber	S_+
118.	*Taraxacum serotinum* (W. et K.) Poir. subsp. *bessarabicum* (Hor.) Hand.-Maz.	S_+
119.	*Thymus marschallianus* Willd.	S_-
120.	*Torilis arvensis* (Huds.) Link.	S_-
121.	*Trifolium angulatum* W. et K.	S_+
122.	*T. arvense* L.	S_-
123.	*T. campestre* Schreb.	S_-
124.	*T. filiforme* L.	S_-
125.	*T. ornithopodioides* (L.) Sm.	S_+
126.	*T. parviflorum* Ehrh.	S_+
127.	*T. pratense* L.	S_-
128.	*T. repens* L.	S_+
129.	*T. striatum* L.	S_+
130.	*Typha angustifolia* L.	S_+
131.	*T. latilatifolia* L.	S_-
132.	*Urtica dioica* L.	S_-
133.	*Verbascum blattaria* L.	S_+
134.	*Verbena officinalis* L.	S_-
135.	*Veronica anagallis-aquatica* L.	S_-
136.	*Vicia angustifolia* L.	S_-
137.	*Xanthium italicum* L.	S_+

The specific ecological feature of the surveyed plant cover was that 47 or 34.41% of the recorded taxa were rated with the Landolt's ecological index $\mathbf{S_+}$ (Fig. 2).

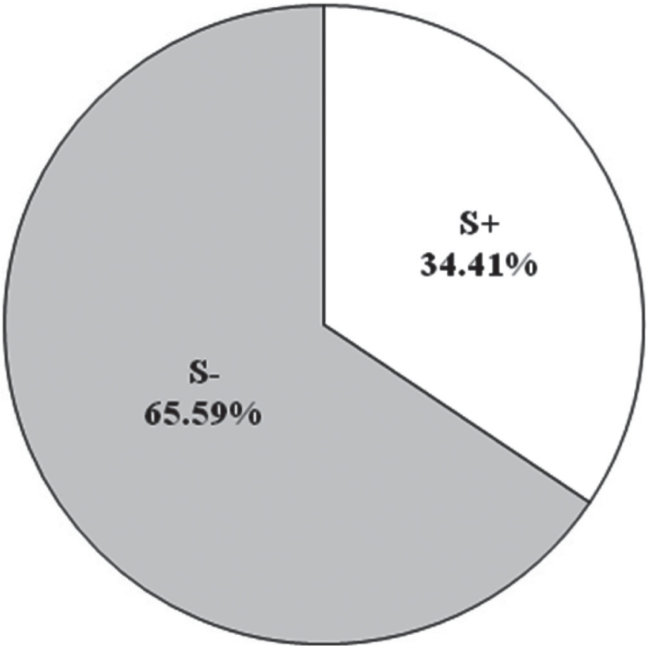

Fig. 2 Graphic representation of taxa marked by S_- and S_+ on the solonchakic solonetz soil in the vicinity of the village of Iđoš

The flora of the studied pasture had a lower percentage of taxa marked by the ecological index S_+ than the pasture on the banks of Slano Kopovo oxbow lake located near the town of Novi Bečej [Knežević *et al.*, 2005]. However, the percentage of taxa marked by the ecological index S_+ was higher than that found for the flora of the pasture on the banks of Okanj oxbow lake located in the vicinity of the village of Elemir [Knežević *et al.*, 2012]. The specific plant-geographic feature of the studied plant cover was the presence of two endemics of the Pannonian Plain, *Plantago schwarzenbergiana* Schur and *Statice gmelini* subsp. *hungaricum* (Klokov) Soó, and two sub-endemics of the Pannonian Plain, *Puccinellia limosa* Holmb. and *Roripa kerneri* Menyh.

The taxa recorded on the studied pasture formed a complex mosaic of stands. Their syntaxonomic position is as follows:

Phragmitetea Tx. et Prsg. 1942
  Phragmitetalia W. Koch1926 emend. Pign 1953

Phragmition communis W. Koch 1926 emend. Soó 1947
Ass. *Scirpo-Phragmitetum* W. Koch 1926 ***medioeuropaeum*** Tx. 1941 p.p. emend. Soó 1971

Bolboschoenetalia maritimi Hejný 1967 p.p. (*Bolboschenetea maritimi* Tx. 1969,
Scirpetalia maritimi Borhidi 1970 p.p.)
Bolboschoenion maritimi continentale Soó (1945) 1947 emend. Borhidi 1970
Ass. *Bolboschoenetum maritimi continentale* Soó (1927) 1957 (*Scirpetum maritimi* Tx. 1937)

Molinio-Arrhenatheretea Tx.1937 p.p., Br.-Bl. et Tx. 1943 p.p.
Arrhenatheretalia Pawl. 1926
Cynosurion cristati Tx. 1947
Ass. *Trifolio-Lolietum perennis* Kripp. 1967

Festuco-Puccinellietea Soó 1968
Festuco-Puccinellietalia Soó 1968
Puccinellion limosae (Klika 1937) Wendel. 1943
Ass. *Puccinellietum limosae* (Rapcs. 1927) Soó 1930
Ass. *Pholiuro-Plantaginetum tenuiflorae* (Rapcs. 1927) Wendel. 1943
Ass. *Hordeetum histricis* (Soó 1933) Wendel. 1943
Halo-Agrostion albae pannonicum Knežević 1990
Ass. *Agrostio-Alopecuretum pratensis* Soó (1933) 1947
Ass. *Agrostio-Beckmannietum* (Rapcs. 1916) Soó 1933

Artemisio-Festucetalia pseudovinae Soó 1968
Festucion pseudovinae Soó 1933
Meso-Festucenion pseudovinae Vučković 1985
Ass. *Halo-Agropyretum repentis* Vučković 1985
Ass. *Poeto-Alopecuretum pratensis halophyticum* Vučković 1982
Halo-Festucenion pseudovinae Vučković 1985
Ass. *Artemisio-Festucetum pseudovinae* (Magyar 1928) Soó 1945
Xero-Festucenion pseudovinae Vučković 1985
Ass. *Trifolio-Festucetum pseudovinae* Vučković 1985
Ass. *Achilleo-Festucetum pseudovinae* (Magyar 1928) Soó 1945.

Plant communities that inhabit these stands are described hereafter.

The stands of the communities from the class ***Phragmitetea,*** i.e. the stands of ass. *Scirpo-Phragmitetum medioeuropaeum* and ass. *Bolboschoenetum maritime continentale*, grow in fragments. They cover spots in the lower, peripheral edges of the pasture and the banks of drainage canals that run through the pasture. They make the weedy plant cover of the pasture, which cattle avoid to graze and farmers do not mow.

The stands of the communities from the class ***Molinio-Arrhenatheretea***, i.e. the stand of ass. *Trifolio-Lolietum perennis*, grow on low grounds, in places which are flooded for a long time in the spring and which are slightly saline. These plants make a fairly thick cover, but their stands are floristically poor. Their floristic composition includes plant species characteristic for lowland meadows of the class *Molinio-Arrhenatheretea* (*Trifolium repens*, *T. pratense*, *Lolium perenne*, *Lotus corniculatus*, *Taraxacum officinale* and others), while in the later part of the vegetation period there occur numerous plant species that belong to the meadow-steppe vegetation of continental Salinas from the class *Festuco-Puccinellietea* (*Festuca vallesiaca* subsp. *pseudovina*, *Hordeum maritimum* subsp. *gussoneanum*, *Podospermum canum* and others) and to the arid meadows from the class *Festuco-Brometea* (*Achillea millefolium* L. subsp. *collina*, *Cynodon dactylon*, *Plantago lanceolata* and others). The stands with the floristic composition described above were formed as a consequence of intensive anthropogenic activities. The construction of canals impeded the development of primary lowland meadows while the intensive grazing lowered the rate of soil aeration while increasing the erosion of the topsoil. In some parts of the pasture, those that harbored stands of ass. *Trifolio-Lolietum perennis*, there were attempts to grow wheat, which resulted only in the destruction of the natural vegetation cover.

The stands of the communities from the class ***Festuco-Puccinellietea*** were found to make the dominant vegetation cover in the studied pasture. The vegetation cover consists of plant communities from the alliances *Puccinellion limosae*, *Halo-Agrostion albae pannonicum* and *Festucion pseudovinae*.

Regarding the alliance *Puccinellion limosae*, stands of ass. *Puccinellietum limosae*, ass. *Pholiuro-Plantaginetum tenuiflorae* and ass. *Hordeetum histricis* have developed in the studied pasture. The stands of ass. *Puccinellietum limosae* and ass. *Pholiuro-Plantaginetum tenuiflorae* are rare, small in size and floristically very poor. They are insignificant from the points of grazing and mowing. The stands of ass. *Hordeetum histricis* have developed as a result of prolonged flocking of grazing animals around the pens. Pasture species from the alliance *Agropyro-Rumicion crispi* and weed species (*Agropyrum repens*, *Poa pratensis* L. subsp. *angustifolia*, *Trifolium pratense, T. repens*, *Cirsium arvense*, *Conium maculatum*, *Mentha pulegium*, *Onopordon acanthium, Plantago maior*, *Xanthium italicum* and others) are frequent in these stands. These species are significant because they suggest that animal pens need to be frequently relocated in order to avoid trampling and occurrence of weed species in the pasture.

Regarding the alliance *Halo-Agrostion albae pannonicum*, stands of ass. *Agrostio-Alopecuretum pratensis* and ass. *Agrostio-Beckmannietum* have developed in the studied pasture. Their sites are typically spacious, water impermeable and slightly saline depressions in which rainwater is collected. It is interesting that the plant cover from these sites is exploited economically. Namely, as this cover type is not subject to intensive grazing, there is no waterlogging due to livestock trampling. The mowing by hand or machines during the most vigorous plant development provides a considerable quantity of hay with a relatively good quality.

Regarding the alliance *Festucion pseudovinae*, stands of communities from the suballiances *Meso-Festucenion pseudovinae*, *Halo-Festucenion pseudovinae* and *Xero-Festucenion pseudovinae* have developed in the studied pasture. This is the evidence of the ecological diversity of the studied sites.

Shallow depressions in which rainwater collects in the spring are the sites where stands of the communities from the suballiance *Meso-Festucenion pseudovinae*, i.e. the stands of ass. *Halo-Agropyretum repentis* and ass. *Poeto-Alopecuretum pratensis halophyticum* have developed. Because of increased humidity, plant species characteristic for the vegetation of lowland meadows from the class *Molinio-Arrhenatheretea* have developed in addition to the halophytes. The essential conditions that promoted the development of the stands of ass. *Halo-Agropyretum repentis* were intensive grazing and farming efforts undertaken in these sites. Therefore, these sites had acquired characteristics of ruderal vegetation developing on a slightly saline soil. The essential conditions for the development of the stands of ass. *Poeto-Alopecuretum pratensis halophyticum* were repeated Salina and the lack of intensive grazing. These sites have acquired characteristics of the mesophilic meadow vegetation developing on a slightly saline soil.

The sites where stands of the communities from the suballiance *Halo-Festucenion pseudovinae*, i.e. stands of ass. *Artemisio-Festucetum pseudovinae* have developed, occupy a small part of the studied pasture. These are in fact fairly saline strips of land, which had developed due to topsoil erosion. The vegetation cover formed on them is floristically poor, making short and sparse canopy. This explains why the studied pasture was found to provide poor grazing.

The sites that had given raise to the stands of the suballiance *Xero-Festucenion pseudovinae*, i.e. the stands of ass. *Trifolio-Festucetum pseudovinae* and ass. *Achillea-Festucetum pseudovinae*, are the highest parts of the pasture and they are moderately to slightly saline. The stands of ass. *Trifolio-Festucetum pseudovinae* are small in size. Their mowing provides substantial quantities of hay in early spring, but later in the season the pasture is sparse. The stands of ass. *Achillea-Festucetum pseudovinae* are the predominant plant cover in the investigated pasture. Their floristic composition, in addition to the species that are typical of the meadow-steppe vegetation of the continental Salinas from the class *Festuco-Puccinellietea*, includes plant species that comprise the humid meadows from the class *Molinio-Arrhenatheretea*, which are present in the early spring, and the species that comprise the dry meadows from the class *Festuco-Brometea*, which develop later in the season. These sites are good hayfields early in the spring, and the best pasture afterwards.

CONCLUSION

A pasture on the solonchakic solonetz soil in the vicinity of the village of Iđoš (Banat, Serbia) was found to harbor 137 plant taxa (129 species, 7 subspecies and 1 variety). The plant cover they formed was specific from the ecological, phytogeographical and phytocoenological points of view. The specific ecological feature of the surveyed plant cover was that 47 or 34.41% of the recorded taxa were rated with the ecological index S_+ due to their ability to grow in saline soil. The specific phytogeographical feature of the surveyed plant cover was the presence of two Pannonian endemics, *Plantago schwarzenbergiana* Schur and *Statice gmelini* subsp. *hungaricum* (Klokov) Soó, and two subendemics, *Puccinellia limosa* Holmb. and *Roripa kerneri* Menyh. The specific phytocoenological feature of the surveyed plant cover was the presence of two phytocoenoses from the class *Phragmitetea* Tx. et Prsg. 1942 (ass. *Scirpo-Phragmitetum medioeuropaeum* and ass. *Bolboschoenetum maritimi continentale*), one phytocoenose from the class *Molinio-Arrhenatheretea* Tx.1937 p.p., Br.-Bl. et Tx. 1943 p.p. (ass. *Trifolio-Lolietum perennis*) and ten phytocoenoses from the class *Festuco-Puccinellietea* Soó 1968 (ass. *Puccinellietum limosae*, ass. *Pholiuro-Plantaginetum tenuiflorae*, ass. *Hordeetum histricis*, ass. *Agrostio-Alopecuretum pratensis*, ass. *Agrostio-Beckmannietum*, ass. *Halo--Agropyretum repentis*, ass. *Poeto-Alopecuretum pratensis halophyticum*, ass. *Artemisio-Festucetum pseudovinae*, ass. *Trifolio-Festucetum pseudovinae* and ass. *Achilleo-Festucetum pseudovinae*). The stands of the communities from the class *Festuco-Puccinellietea* Soó 1968 were found to make the dominant vegetation cover in the studied pasture. The presence of 34.31% of taxa rated with the ecological index S_+, the presence of two Pannonian and two sub-Pannonian floristic elements and the predominance of stands from the class *Festuco-Puccinellietea* Soó 1968 led us to conclusion that the pasture near the village of Iđoš (Banat, Serbia) is a part of the halobiome of the Pannonian Plain.

ACKNOWLEDGEMENT

This study is part of the project TR31016 titled „Improvement of field forage crops agronomy and grassland management“ supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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ВЕГЕТАЦИЈА ПАШЊАКА У ОКОЛИНИ НАСЕЉА ИЂОШ

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РЕЗИМЕ: У близини насеља Иђош, на пашњаку, на солоњецу солончакастом, констатовано је 137 биљних таксона (129 врста, седам подврста и један варијетет). Биљни покривач који они формирају је специфичан у еколошком, фитогеографском и фитоценолошком смислу. Специфичне еколошке карактеристике испитаног биљног покривача су 47 или 34,31% констатованих таксона окарактерисаних са S_+ због њихове способности да расту на сланом земљишту. Специфична фитогеографска карактеристика испитаног биљног покривача је присуство два панонска ендема, *Plantago schvarzenbergiana* Schur и *Statice gmelini* subsp. *hungaricum* (Klokov) Soó, и два субендема, *Puccinellia limosa* Holmb. и *Roripa kerneri* Menyh. Специфична фитоценолошка карактеристика испитаног биљног покривача је присуство две заједнице из класе *Phragmitetea* Tx. et Prsg. 1942 (ass. *Scirpo-Phragmitetum medioeuropaeum* и ass. *Bolboschoenetum maritimi continentale*), једне заједнице из класе *Molinio-Arrhenatheretea* Tx.1937 p.p., Br.-Bl. et Tx. 1943 p.p. (ass. *Trifolio-Lolietum perennis*) и десет заједница из класе *Festuco-Puccinellietea* Soó 1968 (ass. *Puccinellietum limosae*, ass. *Pholiuro-Plantaginetum tenuiflorae*, ass. *Hordeetum histricis*, ass. *Agrostio-Alopecuretum pratensis*, ass. *Agrostio-Beckmannietum*, ass. *Halo-Agropyretum repentis*, ass. *Poeto-Alopecuretum pratensis halophyticum*, ass. *Artemisio-Festucetum pseudovinae*, ass. *Trifolio-Festucetum pseudovinae* и ass. *Achilleo-Festucetum pseudovinae*). На основу присуства 34,31% таксона окарактерисаних еколошким индексом S_+, присуство два панонска и два субпанонска флорна елемента и доминацију састојина класе *Festuco-Puccinellietea* Soó 1968, закључили смо да је пашњак, у близини насеља Иђош (Банат, Србија), део халобиома Панонске низије.

КЉУЧНЕ РЕЧИ: флора, халобиом, Иђош (Банат – Србија), пашњак, вегетација

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci., Novi Sad, № 127, 57—64, 2014

UDC 632.954(497.11)
UDC 633.912(497.11)
DOI: 10.2298/ZMSPN1427057V

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EUPHORBIA DAVIDII – AN INVASIVE WEED SPECIES IN THE FIELDS OF SERBIA

ABSTRACT: *Euphorbia davidii* Subils (*Euphorbiaceae*) or toothed spurge is a plant native to North America, but in Europe it is an alien weed. The populations of this weed were recorded in the Province of Vojvodina (Serbia) for the first time in 2007 in the arable fields in two localities: between the villages of Aleksa Šantić and Pačir and also between the villages of Pačir and Đurđin. There were no previous published data about the occurrence of this species in Serbia, nor about management measures in crops to suppress this agricultural invader. In this paper, we present experiences with several herbicide treatments applied to suppress populations of toothed spurge from the crop fields in Serbia during the last six years. The most effective was treatment with a high concentration of glyphosate in the early phases of toothed spurge growing. The populations of this invasive weed spread and formed more or less dense patches in the crop field, the area of distribution increased from 3 ha to 7 ha. Observations and experiences with treatments suggest that *Euphorbia davidii,* as an invasive plant, has significant impact on crop fields, therefore further investigation of suppression measures and monitoring of its population is needed.

KEYWORDS**:** *Euphorbia davidii*, herbicide, invasive plants, toothed spurge, weeds

Short running title: Vajgand et al.: *Euphorbia davidii* - experience with herbicide

INTRODUCTION

Introduction and naturalization of new plant species are taking place worldwide and the number of alien plant species being introduced is steadily increasing [Weber and Schrade 2006]. Some of the introduced alien plants

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have become invasive weeds. Early confirmation and subsequent containment of new weed threats are required to substantially reduce their long-term costs of impact and control [Virtue 1996]. Measures towards mitigating the problems associated with new weeds include regular monitoring and risk assessments [Weber and Schrade 2006].

Euphorbia davidii Subils (Euphorbiaceae), toothed spurge is a relatively new alien in Europe's flora. It is native to North Mexico, USA, Canada, but introduced to South America (Argentina), Australia and Europe [Geltman 2012]. The data from European studies and herbarium materials about known populations of *E. davidii* in Russia, Ukraine, Moldavia, Romania, Bulgaria, Hungary, Italy, Belgium, Switzerland and France were summarised by Barina *et al.* [2013].

Euphorbia davidii is an annual plant (Fig. 1), 20–50 cm tall, stem is oppositely branched, pubescent, with two layers of hairs: upper with sparse, more or less patent longer hairs and lower which is denser with shorter hairs. Leaves are opposite, petiolate; the lamina is up to 6(-8) cm long, lanceolate to elliptical, sometimes red-spotted; the base is attenuate; the margin is crenate-serrate; the apex is acute to acuminate, hairy on both surfaces; the hairs have a wide basal cell. Inflorescence is terminal; cyathia app. 2.5 mm long, bracteoles inside cyathium with non-glandular lacinia; staminate flowers up to 15 per cyathium; pistillate flower exceeding cyathia, the ovary usually glabrous, the stigma two-lobed; capsule glabrous; seeds a little longer than wide, on cross section tetragonal, on its surface there are relatively few irregularly formed tubercula, with a large yellow reniform caruncle [Geltman 2012; Oprea *et al.,* 2012; Barina *et al.,* 2013] (Fig 1).

Fig 1. A detail of *Euphorbia davidii* plant

There were no previous published data about the occurrence of this species in Serbia [Vrbničanin *et al.,* 2004; Anačkov *et al.,* 2013]. Very little is known about management measures in crops to suppress this agricultural invader [Stoorie and Cook 1996]. In this paper, we present results of empirical studies of treatments by different herbicide applied to suppress populations of the toothed spurge in crop fields in Serbia during the last six years.

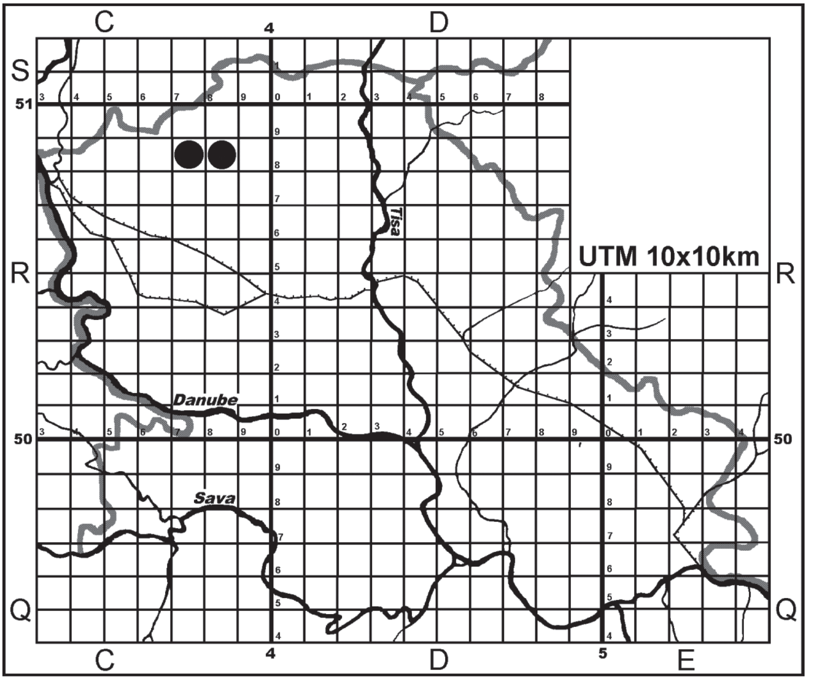
MATERIAL AND METHODS

The distribution map of *E. davidii* in Serbia was made using the Universal Transverse Mercator (UTM) 10x10 km grid system [Survey 1973]. From 2007, when the toothed spurge was first recorded in Serbia, to 2013 different herbicides were applied, depending on crop. In maize fields: isoxaflutole + cyprosulfamide; isoxaflutole + acetochlor; nicosulfuron + prosulfuron; tembotrione + isoxadifen-etil; foramsulphurone + isoxadifen-etyl; and foramsulfuron + isoxadifen-etyl + iodosulfuron-methyl-sodium were applied. In sunflower fields: flurochloridone; acetochlor + terbutylazine and fluazifop-p-butyl. After winter wheat harvest and the occurence of toothed spurge in wheat stubble in summer, there was an attempt of suppression with glyphosate (3.5 l/ha). On the edge of a maize field, a high concentration of glyphosate (2 liter glyphosate in 1 liter of water) was applied using hand-held sprayer. Herbicide effects were visually assessed using 0 (no effect) to 5 (total death) rating system [McMillan and Cook 1992; Stoorie and Cook 1996].

RESULTS

Distribution of *Euphorbia davidii* in Serbia was first recorded in 2007, in crop fields in Vojvodina (Serbia), between the villages of Aleksa Šantić and Pačir (CR78 according to 10x10 km UTM) (Map 1). Populations of this plant were also recorded between the villages of Pačir and Đurđin (CR88), approx. 10–12 km from the first location. The populations were distributed in several scattered small patches on a total area of c. 3 ha. In the last six years up to 2013, the area of distribution of toothed spurge was c. 7 ha and the abundance of species also increased (Fig. 2). According to farmers' experience, row cultivation and herbicide treatments generally applied against other common weeds were unsuccessful in suppression of the toothed spurge. According to visual assessments performed one week and one month after treatment, several applied herbicide had no visible effect on the toothed spurge plants (Tab. 1). The most effective (2–3 on the rating scale) was treatment with high concentration of glyphosate (2 litre glyhosate in a 1 litre of water), applied using a hand-held sprayer, which caused black spots on toothed spurge leaves. Nevertheless, these weed plants were not killed by this herbicide.

Fig. 2. *Euphorbia davidii* in a maize field in Serbia in 2013.



Map 1. Distribution of *Euphorbia davidii* marked with ● on UTM map of the Province of Vojvodina (Serbia)

Table 1. Effect of herbicide treatment on toothed spurge according to visual assessment and empirical observations, using 0 (no effect) to 5 (total death) rating system scale

Crop	Herbicide active ingredients	Effect
Maize	isoxaflutole + cyprosulfamide	0
	isoxaflutole + acetochlor	1
	nicosulfuron + prosulfuron	0
	tembotrione + isoxadifen-etil	0
	foramsulphurone + isoxadifenv-etyl	1
	foramsulfuron + isoxadifen-etyl + iodosulfuron-methyl-sodium	1
Sunflower	flurochloridone	1
	acetochlor + terbutylazine	1
	fluazifop-p-buthyl	0
Wheat (stubble)	glyphosate	2
	glyphosate (high concentration)	2–3

According to observations the populations of *Euphorbia davidii* probably had some effect on different crops: in field patches with dense populations of toothed spurge maize plants were smaller and maize ears were also smaller. Sunflower ripened approx. 15–20 days earlier than plants in the same field without toothed spurge and sunflower heads were much smaller. There is no evidence for these observations because experimental studies and measurements of crop plants were not performed yet. Further survey is needed to measure the potential impact of this weed on different crops.

Invasiveness of toothed spurge in Serbia can be assessed according to the fact that during the six years observations of populations of this species, which easily spread and formed more or less dense patches in the crop fields (Fig. 1), the area of distribution more than doubled. The majority of the weed populations were destroyed by row cultivation. Nevertheless, a lot of individuals remained between crops and are spreading further.

DISCUSSION

E. davidii has been discovered in Serbia, but the origin of this introduction is unknown. It has also been introduced to other European countries, spreading mainly along railways. However, the species which can also invade agricultural fields was probably introduced by crop seeds imported from North America [Geltman 2012; Barina *et al.,* 2013]. Most populations of toothed spurge in Europe are small, but due to its wide distribution, the growing number of recently established populations, and its invasive ability, special attention should be paid to changes in distribution and population sizes in Europe. Toothed spurge is a weed of onions and cereals in southern Russia and Uzbekistan [Kudryavtseva and Chernetsova 1993]. In Russia and Ukraine this species is on the list of quarantine weeds [Vladimirov and Petrova 2009]. *E. davidii* was found in Hungary on a single locality in an arable field [Molnár *et al*., 2012; Pinke *et al.,* 2012]. In France it occurs in agricultural fields, mostly maize or soybean and a rapid assessment indicate that the species present an intermediate risk requiring further observations [Girod and Fried 2011]. The occurrence of the new invasive weed in Serbia also confirms the importance of agricultural fields as potential introduction entrances for biological invasion [Girod and Fried 2011].

There is no published data about herbicide treatment of toothed spurge in European countries. However, agronomists and farmers should know which herbicides could be used to control it. Thorough survey of herbicide effects had been performed in Australia, where the toothed spurge is of concern because many of the commonly used herbicides, such as glyphosate, had little effect [Stoorie and Cook 1996]. According to herbicide trials applied in Australia good efficacy (not excellent) in toothed spurge control can be expected by applying herbicides in wheat based on metsulfuron-methyl and fluroxypyr-meptyl [Stoorie and Cook 1996]. Some of the active ingredients tested in Australia are not available on the Serbian market, therefore in this article more attention was paid to those herbicides which can be applied in Serbia. In maize fields it can be recommended fluroxypyr-meptyl + terbutylazine, and bromoxynil octanoate + terbutylazine. Paraquat-dichloridee alone or in mixture with terbutyazine is recommended for the stubble. Early application of herbicides on small toothed spurge plants gives better results and their efficiency will be higher [Stoorie and Cook 1996].

CONCLUSION

The observations suggest that the distribution and frequency of *Euphorbia davidii* in Serbia increased during the past six years. It continues spreading and is very likely to become a more troublesome weed than it is at present. This plant has the potential to become a noxious agricultural weed in summer-sown crops. We, therefore, stress the importance of permanent monitoring of this species in Serbia. Only high concentration of glyphosate on stubble was efficient. Further evaluation will be required to refine the effects of herbicides.

ACKNOWLEDGEMENTS

We are grateful to Radomir Rakić for providing information about the occurrence of the toothed spurge in his crop fields and to Jenő J. Purger for his help in preparing map.

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EUPHORBIA DAVIDII – ИНВАЗИВНА КОРОВСКА ВРСТА НА ПОЉИМА СРБИЈЕ

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РЕЗИМЕ: *Euphorbia davidii* Subils (Euphorbiaceae), давидијева млечика, пореклом је из Северне Америке. Пронађена је као нова врста инвазивног корова у Војводини (Србија) на два локалитета: имеђу села Пачира и Алексе Шантића као и између Пачира и Ђурђина. Ова врста је први пут примећена 2007. године као коров у окопавинама. Од тада су проведени бројни покушаји сузбијања ове инвазивне врсте, али је ширење њених популација настављено. У раду је дат морфолошки опис врсте, приложене су и фотографије биљака и карта распрострањења у Србији. Досадашња искуства показала су да већина хербицида које обично примењују за заштиту појединих усева немају никакав утицај на давидијеву млечику. Најбољи, мада слаби резултати у сузбијању овог инвазивног корова постигнути су применом јачих доза глифосата у почетним развојним фазама млечике. С обзиром на инвазивни карактер ове врсте и потенцијално значајан утицај на усеве и природна станишта, неопходно је стално праћење популација. Потребно је провести и даља систематска истраживања на њеном сузбијању, јер су неке хербицидно активне материје показале задовољавајуће резултате када се примене на млечику у најосетљивијим фазама развоја.

КЉУЧНЕ РЕЧИ: *Euphorbia davidii*, давидијева млечика, хербициди, инвазивне биљке, коров

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci., Novi Sad, № 127, 65—75, 2014

UDC 581.526.3:504.5(497.6)
DOI: 10.2298/ZMSPN1427065M

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SEASONAL DYNAMICS OF COPPER AND ZINC ACCUMULATION IN SHOOTS OF *PHRAGMITES AUSTRALIS* (CAV.) TRIN EX STEUD., *TYPHA LATIFOLIA* L. AND *TYPHA ANGUSTIFOLIA* L.

ABSTRACT: The concentrations of Cu and Zn were measured in shoots of plants *Phragmites australis* (Cav.) Trin ex Steud., *Typha latifolia* L. and *Typha angustifolia* L. at four locations in the area Bardača (Necik – neglected fishpond, Lug – fishpond used for recreation activities, Sinjak – active fishpond and Matura – river connected to some of the fishponds). In all these types of water bodies, Zn and Cu concentrations were the highest in young plants (May–June) and then declined until the end of the season, especially in September. *Phragmites australis* has accumulated higher amounts of Zn than *Typha latifolia* and *Typha angustifolia*, whereas for Cu the difference between species was not so clear. In relation to the site, the largest concentrations of Zn and Cu were recorded in plants from the sites Sinjak and Matura, followed by Necik and Lug respectively. Such site specific differences are related to specific ecological conditions at each habitat.

KEYWORDS: aquatic macrophytes, translocation of heavy metals, phytoremediation

Running title: Maksimović et al., Zinc and copper accumulation in macrophytes

INTRODUCTION

Aquatic macrophytes are essential components of each aquatic freshwater ecosystem, with a number of important functional roles [Elis *et al.*, 1994; Stojanović *et al.*, 1994; Janković *et al.*, 1988; DeBusk *et al.*, 2001; Wetzel 2001;

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Stanković *et al.*, 2009]. Aquatic plants can accumulate high amounts of heavy metals in some tissues, reaching up to $10–10^6$ times higher concentrations than in the surrounding water or sediment. Bioaccumulation of heavy metals depends on plant species, plant organ and a number of abiotic parameters [Boyd 1970; St-Cyr *et al.*, 1994; Pajević *et al.*, 2008; Maksimović 2007; Grisey *et al.*, 2012]. In biological monitoring of aquatic ecosystems aquatic macrophytes are important indicators providing further insight into the habitat status. Analyses of variations in aquatic vegetation composition and quality provide reliable information regarding the quality of water, sediment and coastal area of the water body [Tremp and Kohler 1995; Melzer 1999; Madsen *et al.*, 2001].

Water Framework Directive [European Union 2000] defines the necessity of use and application of biological indicators for more precise determination of ecological status, clasiffication and monitoring of surface and underground waters [Stelzer *et al.*, 2003].

Heavy metals, Zn and Cu included, are increasingly becoming a global environmental pollution issue. Species *Phragmites australis*, *Typha latifolia* and *Typha angustifolia* had proven to be dominant species in many aquatic freshwater habitats. These species can serve as secondary sources of food to some animal species for which they also represent important microhabitats. They also have an economical value because of their potential use as construction materials or thermal energy sources. In recent decades, these species have frequently been analysed and used for thorough scientific monitoring as indicators of environmental pollution or as phytoremediators [De Busk *et al.*, 2001; Fedice and Etdet 2002; Chandra and Yadav 2011; Grisey *et al.*, 2012; Uka *et al.*, 2013].

Previous analyses of mineral composition in *Phragmites australis*, *Typha latifolia* and *Typha angustifolia* in the area of marshy complex of Bardača (Republic of Srpska, Bosnia and Herzegovina) showed high variations in accumulation of different macro and microelements during the season, depending on the location of the sampled plant material in the habitat [Maksimović and Stanković 2005; Maksimović 2007]. Continuing these investigations, this paper gives further insight into the ecological status of the area analysing Zn and Cu accumulation during the vegetational season at different locations, for the three above-mentioned species of water vascular plants. The aim of this study was to discern significant variations in heavy metal content during the season in order to give more precise assesment of the bioindicative role and remediaton potential of these three species.

MATERIALS AND METHODS

Swamp ecosystem Bardača is located in the north east part of Lijevča polja, sorrounded by several rivers (Sava, Brzaja, Vrbas, Matura) and one canal (Osorna-Borna-Ljevčanica). The elevation of the area is arround 100 meters above see level, at the foot of Motajica mountain, in the center of moderate climate belt (45^{o} $08^{'}$ north latitude and 17^{o} $25^{'}$ east latitude).

The research was conducted at the following points in the area: 1) Necik (it used to be a fishpond complex, but is neglected today; surface arround 40 hectares; average depth 110 cm), 2) Dugopoljski Lug (a fishpond pool used mostly for recreation and sport fishing, located on the west side of the canal Stublaja from which it receives water; surface 60 hectares; average depth 160 cm), 3) Sinjak (it used to be abandoned, but today it is an active fishpond located eastward from the Summer pool; surface 40 hectares; average depth 180 cm), 4) Matura (the river that originates from the spring at the locality Razboj and flows into the Sava River near the fishpond Bardača; its water is used for some of the fishponds in the area).

On each of the four described points in the Bardača area, shoot samples (leaves and stems) of three species were collected during the vegetational season: common reed (*Phragmites australis* (Cav.) Trin. ex Steud.), broadleaf cattail (*Typha latifolia* L.) and narrowleaf cattail (*Typha angustifolia* L.). After initial drying, plant material was mixed and heated with H_2O_2, followed by heating at 450 ºC and treatment with 25% HCl. Conentrations of Cu and Zn in prepared solutions were determined by employing atomic apsorption spectrophotometry (Varian, AAS240FS). All analyses were performed in three independent technical replications. Obtained results were processed by ANOVA variance analyses, followed by Duncan Multiple Range Test. Average values followed by the same letter are not significantly different for $p<0.05$.

RESULTS AND DISCUSSION

The highest Zn content, in all three analysed species, is determined at the beginning of the vegetation season (May–June), followed by a decrease during the second part of the season, with lowest values determined in September (Tables 1–3). In general, such results are in accordance with previously published results regarding macro and micro elements at the same locality in the same plants [Maksimović and Stanković 2005; Maksimović 2007] and with results published by Grisey *et al* [2012]. Such results indicate higher rate of plants metabolic activity in the first part of the vegetation season (May and June), and in some cases during the mid summer month of July (and August in just a few localities), resulting in stronger uptake rate and correlated heavy metal accumulation. According to many researchers, the peak of the macrophyte vegetational growth in similar aquatic habitats is mid summer, starting early in March [Karunaratne *et al.*, 2003; Windham *et al.*, 2003; Sollie and Verhoven 2008; Quan *et al.*, 2007]. Eid *et al.* [2012] also determined that the *Typha dominigensis* shoot content of most nutrients and heavy metals (Zn and Cu included) increased rapidly in the early parts of the vegetation season, reaching maximal values in July and then decreased again. It seems that early development of stems and leaves at the beginning of the season, determines higher nutrient mobility in the aboveground translocation system, resulting in high nutrient activation and translocation from the rhizome to upper plant parts. In the second part of the growing season, concentration of nutrients, along with heavy metals, can gradually decrease, also as the result of "dilution effect", by which mineral elements are diluted in the large stem and leaves biomass. Most of the shoot biomass is being formed in the April–July period, and shoot growth significantly decreases in August. Also, as the end of the season approaches, transfer of some elements from leaves to rhizome slowly starts to increase, leading to general accumulation of organic matter in the rhizome, providing essential amounts of nutrients for the new growth in the following year [Zhao *et al.*, 2013]. Senescence process attributes to decrease in nutrients and heavy metal concentrations in the plant shoot at the end of the vegetation season.

Table 1. Zn content in shoots of *Phragmites australis* at four localities of Bardača area (µg/g of dry weight)

Localities	Month of the year					
	5	6	7	8	9	average
Necik	32.50 a	23.24 b	16.69 b	6.47 d	8.61 b	17.50
Lug	27.20 b	18.22 c	13.89 b	13.01 c	13.93 a	17.25
Sinjak	33.26 a	39.98 a	36.53 a	32.51 a	16.27 a	31.71
Matura	22.81 b	42.15 a	17.39 b	16.80 b	8.72 b	21.58
average	28.94	30.90	21.13	17.20	11.88	

Values followed by different letters in a same column were significantly different at $p<0.05$

Table 2. Zn content in shoots of *Typha latifolia* at four localities of Bardača area (µg/g of dry weight)

Localities	Month of the year					
	5	6	7	8	9	average
Necik	20.36 a	22.58 a	22.06 a	16.02 a	13.14 a	18.83
Lug	18.56 a	16.58 b	15.10 b	13.12 b	13.30 a	15.33
Sinjak	16.08 a	19.93 ab	9.31 c	8.24 c	7.69 c	10.21
Matura	19.63 a	23.83 a	15.32 b	17.27 a	8.86 b	16.98
average	22.95	25.74	15.45	13.66	10.75	

Values followed by different letters in a same column were significantly different at $p<0.05$

Table 3. Zn content in shoots of *Typha angustifolia* at four localities of Bardača area (μg/g of dry weight)

Localities	Month of the year					
	5	6	7	8	9	average
Necik	23.70 a	22.34 a	15.35 b	15.60 a	13.93 a	18.18
Lug	22.41 a	22.34 a	21.43 a	15.68 a	10.89 b	18.55
Sinjak	15.40 b	11.70 b	8.54 c	8.37 c	4.79 d	9.76
Matura	16.03 b	22.66 a	9.09 c	11.03 b	8.43 c	13.45
average	19.39	19.76	13.60	12.67	9.51	

Values followed by different letters in a same column were significantly different at $p<0.05$

Some significant differences were determined between analysed species and localities. Zn content in *Phragmites australis* (Table 1), at locality Sinjak, was higher in relation to other localities. However, the same relation was not indicated for Zn accumulation in *Typha latifolia* and *Typha angustifolia* (Tables 2 and 3). The highest Zn uptake and accumulation level was determined in *Phragmites australis*, followed by *Typha latifolia* and than *Typha angustifolia*. Similar differences between *Phragmites australis* and *Typha angustifolia* have been determined by Nikolić *et al.* [2003] and Chandra and Yadav [2011]. Higher Zn accumulation in *Phragmites australis* was previously found in relation to *Typha latifolia* [Grisey *et al.*, 2012], which is of importance for selection of more suitable phytoremediators.

Significantly higher Zn concentrations were determined in *Phragmites australis* at Sinjak locality. Same relations were not confirmed by *Typha latifolia* and *Typha angustifolia*, having the lowest Zn accumulations at the same locality. Such variations might be indicated by specific conditions at this habitat. As an active fishpond, Sinjak has specific water regime, water quality, and occasional removal of macrophyte biomass, related to the application of technological measures during its commercial supervision. The circulation of different elements in fishponds can especially be affected by fish food, fish biotic interactions, and fish excrements along with plant decomposition [Van Donk and Otte 1996; Petr 2000; Wetzel 2001].

Similar to Zn, the content of Cu during the vegetational period in all three species (Tables 4–6) was the lowest at the end of the season, in September. If these species are to be used as phytoremediation tools, the harvesting of shoots should take place in the middle of the season, when the heavy metals content is high and the biomass quantity comes to its seasonal peak. This is especially important because large amounts of decomposing leaf and stem litter are returned to the aquatic system at the end of the vegetation season, affecting the cycle and transition of heavy metals present in the ecosystem [Unamuno *et al.*, 2007]. Bragato *et al.* [2009] determined that at the end of the season significantly higher amounts of heavy metals are found in senescing leaves of *Phragmites australis*, suggesting an adaptation strategy by which plants redistribute toxic heavy metals from all plant parts to used leaves. They determined higher Zn and Cu content only in leaves dry weight, whereas in rhizome and shoot, accumulation levels where similar during the whole season, with small increase in the first two months of growth.

Table 4. Cu content in shoots of *Phragmites australis* at four localities of Bardača area (µg/g of dry weight)

Localities	Month of the year					
	5	6	7	8	9	average
Necik	4.64 b	3.78 c	3.57 b	2.45 a	1.86 b	3.26
Lug	2.92 c	2.87 c	3.87 b	2.97 a	2.45 a	3.02
Sinjak	7.52 a	39.91 a	5.78 a	3.25 a	2.22 a	11.74
Matura	3.10 c	31.91 b	2.57 c	2.26 a	1.39 c	8.25
average	4.55	19.62	3.95	2.74	1.98	

Values followed by different letters in a same column were significantly different at $p<0.05$

Table 5. Cu content in shoots of *Typha latifolia* at four localities of Bardača area (µg/g of dry weight)

Localities	Month of the year					
	5	6	7	8	9	average
Necik	5.16 ab	5.03 b	5.95 a	6.08 a	3.43 a	5.40
Lug	6.55 a	4.65 b	5.08 b	4.86 ab	2.98 b	4.82
Sinjak	4.99 b	18.40 a	4.44 c	3.91 b	2.51 c	6.85
Matura	4.70 b	16.76 a	3.41 d	5.11 ab	1.63 d	6.33
average	5.35	11.21	5.05	4.99	2.64	

Values followed by different letters in a same column were significantly different at $p<0.05$

Table 6. Cu content in shoots of *Typha angustifolia* at four localities of Bardača area (µg/g of dry weight)

Localities	Month of the year					
	5	6	7	8	9	average
Necik	5.75 b	5.25 d	4.90 b	5.51 a	3.38 b	4.96
Lug	5.74 b	7.55 b	5.66 a	5.53 a	3.64 a	5.63
Sinjak	7.22 a	6.34 c	4.46 c	3.78 b	1.98 c	4.76
Matura	3.66 c	13.99 a	3.40 d	4.16 b	1.75 c	5.40
average	5.59	8.28	4.61	4.75	2.69	

Values followed by different letters in a same column were significantly different at $p<0.05$

One extremely elevated peak of Cu accumulation was determined in *Phragmites australis* and *Typha angustifolia* during May and June at localities Sinjak and Matura, as well as in *Typha angustifolia* in June at locality Matura. Such high concentrations of Cu indicate some additional Cu release to the ecosystem at these localities. As expected in most plant species, determined concentrations of Cu in investigated plants were, in general, lower when compared to Zn. However, available reference data indicate that the degree of Zn uptake can significantly differ from the degree of Cu uptake, resulting in different bioconcentration factors between *Phragmites australis, Typha angustifolia* and *Typha latifolia*. Chandra and Yadav [2011] recorded higher Zn than Cu accumulation, in *Phragmites australis, Typha angustifolia* and *Cyperus esculentus*, highlighting that *Phragmites australis* is a Zn shoot accumulator, whereas *Typha angustifolia* is a Cu root accumulator. Higher accumulation of Zn and Cu in roots than in shoots of *Typha latifolia* was determined by Ye *et al*. [1997] and Klink *et al.* [2013]. In *Phragmites karka* Uka *et al.* [2013] also found higher potential in Zn accumulation. Alfadul and Al-Fredan [2013] found that Zn and Cu, among other heavy metals, are accumulated in higher concentrations in shoots than in roots of *Phragmites australis*, whereas Bonanno and Giudice [2010] determined reversed ratio, where Zn and Cu concentrations were higher in rhizome and roots. These variations in results obtained by different researchers probably exist because of the complexity of the heavy metal uptake process. Bioavailability of heavy metals depends on several parameters of water and sediment quality, especially pH and redox potential [Ye *et al.*, 1997; Sundareshwar *et al.*, 2003]. The uptake and translocation of heavy metals inside the plant can also differ depending on the antagonistic and synergistic reactions between different concentrations of available elements, presence of organic matter and clay fractions and oxygen concentrations. Specific biotic conditions of each site can have significant impact on the availability and uptake capacity of each heavy metal. Since these parameters are rarely balanced in the same way at different localities, it is hard to predict the precise accumulation potential of different plant parts, even in the same species, and all environmental parameters and physiological plant traits must be taken into consideration.

CONCLUSION

Analysing the accumulation of Zn and Cu in *Phragmites australis*, *Typha latifolia* and *Typha angustifolia* during the season (by observing both individual localities and general average values per each month), higher concentrations were determined during the first part of the vegetational season. These findings indicate that these species had higher metabolic rates, vegetational growth and more optimal conditions in the first part of the season, followed by generally reduced uptake and translocation of nutrients to the rhizome and the beggining of the senescence process in September. Zn accumulation in shoots was higher when compared to Cu accumulation in all three analysed species. Specific increase of Zn content in shoots of *Phragmites australis* at Sinjak locality indicate that specific conditions of the particular habitat have great influence on accumulation and distribution of Zn in this species. Increased Cu content in both *Phragmites australis* and *Typha latifolia* in May and June suggest that aditional release of Cu occured at Sinjak and Matura localities.

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СЕЗОНСКА ДИНАМИКА АКУМУЛАЦИЈЕ ЦИНКА И БАКРА У НАДЗЕМНИМ ДЕЛОВИМА ВРСТА *PHRAGMITES AUSTRALIS* (CAV.) TRIN EX STEUD., *TYPHA LATIFOLIA* L. И *TYPHA ANGUSTIFOLIA* L.

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РЕЗИМЕ: У надземном делу врста *Phragmites australis* (Cav.) Trin ex Steud., *Typha latifolia* L. и *Typha angustifolia* L. одређиване су сезонске промене концентрација цинка (Zn) и бакра (Cu) на четири станишта области Бардаче (Нецик – запуштени рибњак, Луг – рибњак који се користи за рекреативно-спортске активности, Сињак – активни рибњак и Матура – река која напаја неколико рибњака у овој области). У сва четири водена станишта, концентрације цинка и бакра биле су највеће у младим биљкама у првом делу вегетационе сезоне (мај–јун), након чега је утврђено постепено опадање у другом делу вегетационе сезоне, са најмањим измереним вредностима у септембру. Врста *Phragmites australis* акумулирала је веће концентрације цинка у односу на врсте *Typha latifolia* и *Typha angustifolia*, док за бакар није утврђена јасна разлика у акумулираним концентрацијама између испитиваних врста. У односу на локалитет, највеће концентрације цинка и бакра су утврђене у биљкама са станишта Сињак и Матура, а затим на стаништима Нецик и Луг, што указује на специфичан утицај различитих услова у испитиваним стаништима на њихов особен еколошки статус.

КЉУЧНЕ РЕЧИ: акватичне макрофите, транслокација тешких метала, фиторемедијација

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci., Novi Sad, № 127, 77—83, 2014

UDC 637.54'65:615.281.842
DOI: 10.2298/ZMSPN1427077S

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THE IMPORTANCE OF LEGISLATION AND FORENSIC FINDINGS FROM THE ANALYSIS OF TURKEY MEAT

ABSTRACT: The aim of this study was to investigate the importance of legislation regarding the process of testing the presence of Salmonella spp. in turkeys for slaughter and forensic investigation of the presence of antimicrobial drugs residues in turkey meat. The investigation was performed on a fattening farm, just before the delivery of turkeys for slaughter. Two pooled faecal samples were taken from turkeys and sent for analysis. Both samples were tested positive for Salmonella spp. Antibiogram was performed after that and the drug of choice for treatment was enrofloxacine. After turkeys were treated with antibiotics, again two pooled faecal samples were sent for analysis and now both were negative. Turkeys were sent for slaughter, but at the same time there were some suspicions that the owner did not comply with the time of the withdrawal period for the antibiotic used and a forensic investigation was performed in order to determine the presence of antimicrobial drug residues in slaughtered turkeys. Samples of liver, kidney and fat of turkeys were taken at the slaughter line and were sent for analysis for the presence of antimicrobial drugs residues. All of the samples were free of antimicrobial drug and the suspicions were rejected. Recommendations were made regarding the improvement of biosecurity and hygiene measures on farms and good animal husbandry practices in order to limit the use of antimicrobial agents.

KEYWORDS: antimicrobial drugs, residue, *Salmonella* spp., turkey

INTRODUCTION

Infections with Salmonella spp. cause gastrointestinal disorders in humans, causing morbidity, hospitalization, economic burden, and may lead to a lethal

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outcome [Pieskus *et al.*, 2006]. The most common route of infection is faecal-oral route, where people can become infected by ingesting the bacteria through contaminated food or water, or by direct or indirect contact with the faeces of infected humans and animals [Fearnley *et al.*, 2011]. Various studies have shown that foods of animal origin, especially poultry and pigs, represent major sources of Salmonella infections in humans [Erol *et al.*, 2013; Stojanac *et al.*, 2013]. Among them, turkey meat and products are attributed to be the important sources of food-borne salmonellosis [EFSA and ECDC, 2012].

Infection of turkeys with Salmonella spp. is usually asymptomatic and detection of the bacterium emerges by random monitoring of the farm [Danguy des D'eserts *et al.*, 2010]. In Serbia, the testing for presence of Salmonella in turkeys ready for slaughtering is regulated by the Ordinance Establishing Programs of Animal Health Protection Measures for 2013 [*Sl. glasnik RS*, 91/13].

Antimicrobial resistance in food-borne pathogens is of global concern because of the impact on public health. The use of antimicrobial drugs in food producing animals is associated with the emergence of resistant strains of some pathogens in humans [EFSA, 2008]. In turkey production, antimicrobial drugs are used in the treatment of infections in individual animals, but more frequently in the entire flock of turkeys. These antimicrobial drugs are used prophylactically and metaphylactically to prevent spreading of infection from diseased to healthy turkeys in the same building. Also, outside the European Union (EU), antimicrobial drugs are used as growth promoters.

Uncritical use of antibiotics and disregard of the withdrawal period in turkeys, lead to an emergence of antimicrobial drugs residues in meat. Regarding humans, the presence of antimicrobial drugs residues in meat leads to: allergic reactions; toxic, teratogenic and mutagenic effects; resistance of pathogenic bacteria and reducing the therapeutic effect of antimicrobial drugs; disorder of saprophytic intestinal flora and other things. The aim of this study was to investigate the importance of the legislation regarding the process of testing the presence of Salmonella spp. in turkeys for slaughter and forensic investigation of the presence of antimicrobial drugs residues in turkey meat.

MATERIAL AND METHODS

Testing on the farm

At a small turkey fattening farm with capacity of 600 animals in Vojvodina, the fattening was conducted in the period from September to December, 2013. Raising turkeys is performed using the floor system with deep litter of straw, which is added occasionally and the cleaning is carried out at the end of fattening period. Small turkeys, 3–4 weeks old and weighing 750–850 grams, are brought to the facility. After 90 days of fattening, when female animals weigh 11 kg and male animals 17 kg, turkeys are sent to slaughter.

In accordance with the Law on Veterinary Matters [*Sl. glasnik RS*, 30/10, čl. 93] animals in transport must be accompanied by the Animal health state certificate issued by the competent veterinary organization according to the Program of measures, and based on the evidence of the preventive measures and diagnostic trials performed. According to the Ordinance Establishing Programs of Animal Health Protection Measures for 2013 [*Sl. glasnik RS*, 91/13], turkeys intended for slaughter must be free of *Salmonella* spp., and a finding must not be older than 14 days.

Owner of turkeys filed a request for the issuance of the Animal health state certificate. Two pooled faecal samples were collected on the farm from the turkeys and were sent to the relevant institution for analysis. The test was performed using ISO 6579:2002 [ISO 2002].

After 6 days positive results were received, which revealed the presence of *Salmonella* spp. in the faeces of turkeys. Antibogram was performed on the isolated Salmonella spp. and the appropriate antimicrobial drug therapy with enrofloxacin was recommended. After the therapy was implemented and the prescribed 7 days withdrawal period was over, re-sampled faeces of turkeys was sent for analysis. The results were negative, i.e. *Salmonella* spp. was not identified in the faeces of turkeys and the Animal health state certificate was issued.

Immediately after the turkeys were slaughtered, a suspicion was raised that the owner of the animals had not complied with the withdrawal period regarding the applied antimicrobial drugs (7 days), i.e. he used it for longer period of time than recommended, and thus shortened the period between the cessation of the use of antimicrobial drugs and the moment of sending turkeys to slaughtering.

Investigation in a slaughterhouse

Three samples of liver, kidney and fat tissue, were taken from the slaughtered turkeys and were sent for analysis for the presence of antimicrobial drugs residues. Sampling and testing was performed in accordance with the Ordinance determining the program of systematic tracking of the residues of pharmacological, hormonal and other harmful substances in animals, products of animal origin, food of animal origin and animal feed [*Sl. glasnik RS*, 91/09]. The presence of residues of antibiotics was determined using five plates [Gaudin *et al*., 2010].

RESULTS AND DISCUSSION

Both tested pooled samples of faeces were positive for *Salmonella* spp. The incidence of *Salmonella* spp. in turkeys has been investigated in recent years around the world [Antunes *et al.*, 2003; Arsenault *et al.*, 2007; Cetinkaya *et al.*, 2008; Fearnley *et al.*, 2011; Padungtod and Kaneene 2006] and the results show that *Salmonella* spp. is often present in turkeys, as well as in our study. Turkeys infected with *Salmonella* spp. represent a source of human infection, and for this reason mandatory testing of turkeys before slaughter is very important [*Sl. glasnik RS*, 91/13]. Upon isolation, an antibiogram was performed for both samples (Table 1) and the drug of choice was enrofloxacine. The 5-days treatment was recommended, after which two pooled faecal samples were collected again from turkeys and were examined for the presence of *Salmonella* spp. The results obtained were negative, two pooled faecal samples were free from *Salmonella* spp., and a competent veterinarian issued the Animal health state certificate.

Table 1. Antimicrobial resistance of *Salmonella* in two faecal samples from turkeys

Antimicrobial	Sample 1	Sample 2
Amoxicillin	I	I
Ceftriaxone	S	I
Enrofloxacin	S	S
Gentamicin	I	R
Colistin	I	S
Neomycin	R	I
Penicillin	I	S
Streptomycin	R	R
Tetracycline	R	I
Doxycycline	I	R
Flumequin	S	S

S-sensitive, I – intermediate, R-resistant.

While the turkeys were in the slaughterhouse for slaughtering, suspicions were raised that the owner of turkeys treated them with antimicrobial drugs just before the slaughtering, i.e. he did not complied with the withdrawal period. At that moment, forensic examination was performed in order to identify potential suspects or the presence of antimicrobial drugs in meat and organs of turkeys. The samples of liver, kidney and adipose tissue of turkeys were taken immediately from the slaughtering line in accordance with legislation [*Sl. glasnik RS*, 91/09] and were sent for testing for presence of antimicrobial drugs residues. The presence of residues of antibiotics was determined using five plates [Gaudin *et al.*, 2010]. The presence of residues was tested for sulfonamides, amoxicillin, ampicillin, benzyl penicillin G, ceftriaxone, cloxacillin, dicloxacillin, erythromycin, lincomycin, oxacillin, tylosin, phenicols, quinolones, tetracyclines, and aminoglycoside antibiotics. None of the tested antimicrobial drugs were found in the samples. Widespread use of antimicrobial drugs, especially as prophylaxis and growth promoters, leads to resistance of *Salmonella* spp., and this is especially important because people get sick from salmonellosis via food-borne pathogen. *Salmonella* spp. in the meat is already resistant to most antimicrobial drugs, which is particularly acute in developing countries, where there is a wide and uncontrolled use of antimicrobial drugs [EFSA, 2008; Iossifidou *et al*., 2012; Rahimi 2012].

CONCLUSION

Detection of *Salmonella* spp. in the faeces of turkeys ready for slaughter confirmed the importance of the legislation regarding the control of health of animals and humans. Suspicion of the presence of antimicrobial drugs in turkey meat was eliminated forensically, but overall results from this study indicate the need for improved farming practice including more hygienic measures and good animal husbandry practices in order to limit the use of antimicrobial agents.

ACKNOWLEDGEMENT

This research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project No. TR31034.

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Pravilnik o utvrđivanju Programa sistematskog praćenja rezidua farmakoloških, hormonskih i drugih štetnih materija kod životinja, proizvoda životinjskog porekla, hrane životinjskog porekla i hrane za životinje. *Službeni glasnik RS*, 91/2009.

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ЗНАЧАЈ ЗАКОНСКЕ РЕГУЛАТИВЕ И ФОРЕНЗИЧКОГ НАЛАЗА У КОНТРОЛИ ЋУРЕЋЕГ МЕСА

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РЕЗИМЕ: Циљ овог рада био је да се испита значај законске регулативе у контроли *Salmonella* spp. код ћурака за клање и форензички истражи присуство резидуа антимикробних лекова у ћурећем месу. Испитивање је урађено на фарми за тов, непосредно пред испоруку ћурака на клање. Узета су два збирна узорка фецеса од ћурака и послата на анализу. Оба узорка била су позитивна на *Salmonella* spp. Затим је урађен антибиограм и лек избора за терапију био је *енрофлоксацин*. Након третмана ћурака са антимикробном терапијом, поново су послата два збирна узорка фецеса на анализу и тада су оба била негативна. Ћурке су послате на клање, а истовремено се појавила сумња да власник није поштовао време каренце за употребљени антибиотик и извршено је форензичко истраживање присуства резидуа антибиотика код закланих ћурака. На линији клања су узети узроци јетре, бубрега и масног ткива ћурака и послати на анализу на присуство резидуа антимикробних лекова. Сви испитивани узорци су били слободни од антимикробних лекова и сумња је одбачена. Препорука је да се повећају биосигурносне и хигијенске мере на фарми и добра произвођачка пракса са ограниченом употребом антимикробних препарата.

КЉУЧНЕ РЕЧИ: антимикробни лек, резидуе, *Salmonella* spp., ћурке

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1. Опште напомене

1.1 Зборник Матице српске за природне науке / Matica Srpska Journal for Natural Sciences (скраћени наслов: Matica Srpska J. Nat. Sci.) 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: vnikolic@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.

Зборник Матице српске за природне науке издаје Матица српска
Излази двапут годишње
Уредништво и администрација: Нови Сад, Улица Матице српске 1
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Matica Srpska Journal for Natural Sciences
Published twice a year
Editorial and publishing office: Novi Sad, 1 Matica Srpska Street
Phone: +381 21/6615798
E-mail: vnikolic@maticasrpska.org.rs
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www.maticasrpska.org.rs

The editors of the *Matica Srpska Journal for Natural Sciences*
completed the selection for Issue 127 (2/2014) on June 18, 2014
Editorial Staff Secretary: Vladimir M. Nikolić
Managing editor: Slavka Gajin
English text proofreader: Olivera Krivošić
Technical design: Vukica Tucakov
Published in september 2014
Computer set: Slađana Novaković, S&N book, Sremska Kamenica
Printed by: SAJNOS, Novi Sad

Публиковање овог *Зборника* помогли су
Министарство просвете, науке и технолошког развоја Републике Србије
Publication of this volume was supported by the Ministry of Education,
Science and Technological Development of the Republic of Serbia

CIP – Каталогизација у публикацији
Библиотека Матице српске, Нови Сад

5/6(082)

ЗБОРНИК Матице српске за природне науке – Matica Srpska Journal for Natural Sciences/ главни и одговорни уредник Ивана Максимовић. – 1984, св. 66–. – Нови Сад : Матица српска, Одељење за природне науке, 1984– , – 24 cm

Два пута годишње. – Наставак публикације Зборник за природне науке. – Текст на енг. језику, резимеи на енг. и на срп. језику.

ISSN 0352-4906

COBISS.SR-ID 5845250