МАТИЦА СРПСКА
1826

МАТИЦА СРПСКА
ОДЕЉЕЊЕ ЗА ПРИРОДНЕ НАУКЕ
ЗБОРНИК
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–)

Главни уредници / Editors-in-Chief

Miloš Jovanović (1951), Branislav Bukurov (1952–1969),
Lazar Stojković (1970–1976), Slobodan Glumac (1977–1996),
Rudolf Kastori (1996–2012), Ivana Maksimović (2013–)

141

Articales are available in full-text at the web site of Matica Srpska, Serbian Citation Index, CABI, EBSCO, Web of Science Master Journal List, AGRIS FAO (abstracts)

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

MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES

141

NOVI SAD
2021

CONTENTS / САДРЖАЈ

ARTICLES AND TREATISES / ЧЛАНЦИ И РАСПРАВЕ

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 141, 9—20, 2021

UDC 582.261.1(282 Mlava)
https://doi.org/10.2298/ZMSPN2141009J
ORIGINAL SCIENTIFIC ARTICLE

Olga S. JAKOVLJEVIĆ[*], *Slađana S. POPOVIĆ*[2], *Ivana M. ŽIVIĆ*[1], *Katarina Z. STOJANOVIĆ*[1], *Jelena Ž. KRIZMANIĆ*[1]

[1] University of Belgrade, Faculty of Biology
Studentski Trg 16, Belgrade 11000, Serbia
[2] University of Belgrade, Institute of Chemistry, Technology and Metallurgy
National Institute of the Republic of Serbia, Center of Ecology and Technoeconomics
Njegoševa 12, Belgrade 11000, Serbia

TEMPORAL DYNAMICS OF DIATOMS IN THE MLAVA RIVER

ABSTRACT: Temporal analysis of epilithic diatom community was studied within four seasons per year at five sampling sites in the Mlava River. A trout fish farm was built in the upper part of the river (between the first (ML1) and the second sampling site (ML2)). The highest diversity was noticed in spring (159 diatom taxa), while the lowest in the period of autumn (89). A total number of taxa that were dominant (relative abundance greater than 5% at least at one sampling site) during four seasons was 13. Conspicuous temporal dynamics was shown using canonical correspondence analysis (CCA). Large number of taxa was identified in more than two seasons; some were, however, specific to only one season. Many of them were found only in the winter, while a slightly smaller number of taxa were characteristic only for spring or summer.
KEYWORDS: diatom community, trout fish farm, diversity

INTRODUCTION

Diatoms (Bacillariophyta) are eukaryotic, photosynthetic, microscopic organisms and one of the most successful groups of algae. Insufficient study of their diversity has contributed to various estimates of the total species number (from 100,000 to 10 million species) (Kale and Karthick, 2015).

They are of great importance both for nature and for humans. It is estimated that these photosynthetic active organisms are responsible for 20–25%

[*] Corresponding author. E-mail: olga.jakovljevic@bio.bg.ac.rs

of total primary production on the Earth (Bozarth et al., 2009). Recently, they have attracted the attention of scientists due to their possibly great biotechnological potential (biofuels synthesis, food, chemical and pharmaceutical industries, nanotechnology) (Bozart et al., 2009; Medarević et al., 2016). Furthermore, diatoms have been widely used in biomonitoring of surface waters, being the basic tool for water quality assessment in many European countries (Szczepocka et al., 2014).

Despite their enormous importance, we know surprisingly little about the structure and dynamics of their communities (Lane et al., 2003) which is the basis for further research on their bioindicator role. First, it is necessary to study the diatom community structure along the entire river system, since it represents a continuum of interdependent habitats and many different microhabitats (Dalu et al., 2014). Research within large geographical areas (e.g. at the national level) indicated that topography (altitude, longitude and latitude) and climate are the most important factors influencing the variation in the composition of diatom communities (Tison et al., 2005).

Many anthropogenic activities can alter the water quality, which can drastically change the composition of diatom communities in rivers, but also make water unusable for drinking, recreation and irrigation (Potapova and Charles, 2003). Therefore, the study of diatom community composition is of great importance, which is the subject of this study. There are only a few studies on diatom communities of the Mlava River (Jakovljević et al., 2016; Simić et al., 2016), without a detailed study on their temporal and spatial dynamics. The study of temporal dynamics of the diatom community from the Mlava River was conducted by comparing diatom diversity at each sampling site during four seasons, determining dominant taxa in each season and examining the temporal persistence of diatom community in the Mlava River.

MATERIAL AND METHODS

The Mlava River, with a length of 78 km, is one of the longest rivers in the Eastern Serbia. The source of the Mlava River was placed under protection in 1979 as a “hydrological natural monument” (Gavrilović and Dukić, 2002). Diatom sampling of the Mlava River was performed during 2011–2012 in four seasons: spring (April 2011 and May 2012), summer (July and September 2011), autumn (December 2011) and winter (February 2012). For the purposes of this research, five sites were selected along the investigated part of the Mlava River. Between ML1 and ML2 there was built a trout fish farm. The ML1 is located at the source from which the farm is supplied with water. In relation to the farm, the location of sampling sites is as follows: ML1 – located 250 m upstream of the fish farm, ML2 – 20 m downstream from the fish farm, ML3 – 500 m downstream, ML4 – 2.6 km downstream, ML5 – 6.4 km downstream.

Chemical water parameters were measured and presented in Jakovljević et al. (2016). A total of 30 epilithic diatom samples were collected from April 2011 to May 2012. Samples were taken from medium-sized stones using a toothbrush, according to European and national standard (EN 13946, 2015). Cleaned diatom frustules (without organic content) were achieved using the hot H_2SO_4, $KMnPO_4$ and $C_2H_2O_4$ (Krammer and Lange-Bertalot, 1986). Permanent diatom slides were prepared by mounting the material in Naphrax®. Carl Zeiss AxioImager. M1 microscope, with AxioCam MRc5 camera and AxioVision 4.8 software was used for microscopic analysis of permanent slides. Qualitative and quantitative analysis was performed using the magnifications of 1000 x and 1600 x and the standard literature for diatom identification. Quantitative analysis has shown a relative abundance of identified taxa (%) which was determined by counting 400 valves of epilithic diatoms at each permanent slide. CANOCO program for Windows, Version 5.0 (Ter Braak and Šmilauer, 2012) was used for statistical analysis in order to represent the relationship of the recorded diatom taxa based on their presence/absence and the sampling seasons used as explanatory variable.

RESULTS

Concerning the taxonomic spectrum of diatoms from the Mlava River throughout four seasons, the highest diversity was recorded in spring, while the lowest in the autumn period (Figure 1). Twenty-four taxa were designated as dominant (their relative abundance was >5% at least at one site and one season).

By exploring diversity during the seasons in the Mlava River, spring (April 2011 and May 2012) stands out as the season during which the highest number of taxa were recorded (159 taxa) (Figure 1). Regarding sampling sites within this season, the highest number of taxa were identified at ML2 sampling site (82 taxa in April 2011), while the lowest was recorded at ML1 (45 taxa in April 2011) (Figure 1).

A total of 15 diatom taxa, with relative abundance greater than 5% at least at one sampling site, were identified in the Mlava River during the spring season (Table 1). *Achnanthidium minutissimum* was the dominant taxon at all sites with relative abundance of 15.4% to 44.8%. *Achnanthidium pyrenaicum* and *Amphora pediculus* were subdominant taxa during spring period.

In the summer period (July and September 2011), the presence of 140 diatom taxa was documented in the Mlava River (Figure 1). The highest number of taxa was found at the ML1 (67 in September 2011), and the lowest at the ML5 (30 in September 2011) (Figure 1).

The same number of dominant taxa was recorded in this season, as in the spring (15) (Table 2). *A. pyrenaicum* (34.7%), *A. pediculus* (30.5%), *Cocconeis lineata* (35.6%) and *Denticula tenuis* (40.1%) were the dominant taxa in summer period. *A. pyrenaicum* was dominant taxon at the ML1 in September, *A. pediculus* at the ML4 in July and the ML3 in September, and *C. lineata* at the ML5 in both July and September (Table 2). Subdominant taxa in the summer season were *A. minutissimum*, *Cocconeis pseudolineata* and *Gomphonema elegantissimum*.

Table 1. Relative abundance of diatom taxa (%) present in the epilithic diatom community in the Mlava River during the spring season (April 2011 and May 2012).

Taxa	Spring									
	April 2011					**May 2012**				
	Sampling sites									
	ML1	ML2	ML3	ML4	ML5	ML1	ML2	ML3	ML4	ML5
	Relative abundance of taxa (%)*									
Achnanthidium minutissimum	41.1	26.2	36	22.3	15.4	29.6	31.4	20.9	44.8	33
Achnanthidium pyrenaicum	9.4	20.5	8.5	2.7	4.4	36.5	6.6	2.7	4.6	0.2
Amphora pediculus	0.9	10.3	7	15.6	30.9	5.9	8.9	9.9	7.8	4.1
Cocconeis lineata	6.4	0.7	3.4	1.9	4.4	+	0.4	0.4	0.4	1.7
Denticula tenuis	+	6.7	4.1	7.9	1.7	1.2	17.2	16.7	3.6	10.8
Encyonema minutum	0.2	5.6	3.1	2.9	1.4	+	3.3	3.7	5.4	0.9
Gomphonema elegantissimum	1.7	1.2	2.6	1.2	6.9	0.5	+	1.9	1.4	1.4
Gomphonema micropus	13.4	1.9	3.9	1.4	0.9	0.2	+	0.4	5.1	0.4
Gomphonema olivaceum	+	0.7	2.9	6.7	7.9	+	/	3.2	15.2	1.9
Navicula tripunctata	+	/	/	0.2	0.2	+	0.4	2.7	+	11.3
Nitzschia fonticola	/	8.1	1.2	1.9	1.2	+	2.3	2.4	0.4	5.9
Planothidium dubium	5.4	0.4	/	/	0.4	4.9	0.9	0.4	+	0.9
Planothidium frequentissimum	13.3	0.7	0.4	+	0.9	0.5	1.4	0.7	0.4	0.9
Reimeria sinuata	3.9	2.2	1.9	12.9	9.7	+	3.7	8.2	3.9	1.9
Ulnaria ulna	/	+	+	+	+	7.2	7.3	2.7	+	0.4

(*) – Taxa with a relative abundance greater than 5% at least at one site, (+) – Relative abundance of taxon less than 5%, (/) – Taxon not recorded.

The autumn period is characterized as the period in which the lowest number of diatom taxa was recorded (89 taxa) (Figure 1). The ML1 sampling site is distinguished by the highest number of taxa (72 taxa), while the other four sampling sites do not vary much by the number of taxa. In the Mlava River, during the autumn, the presence of nine diatom taxa, with a relative abundance higher than 5% at least at one site, was determined (Table 3). Out of these nine taxa, five were found at all five sites. *A. minutissimum* (36.3%), *A. pediculus* (32.1%) and *D. tenuis* (28.9%) were dominant taxa.

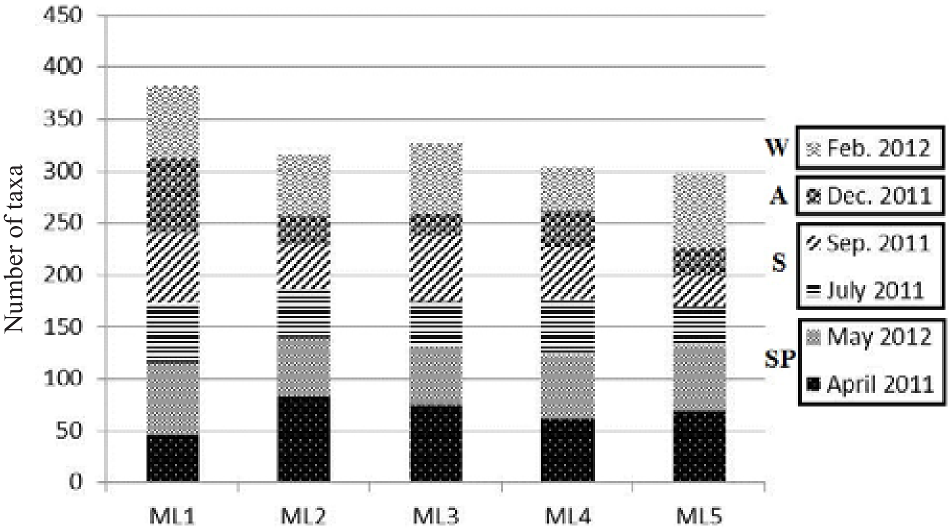

Figure 1. The number of recorded taxa in the Mlava River at 5 sampling sites (ML1-ML5) during 4 sampling seasons: spring (SP) – April 2011 and May 2012, summer (S) – July and September 2011, autumn (A) – December 2011 and winter (W) – February 2012. A total of 15 diatom taxa, with relative abundance greater than 5% at least at one sampling site, were identified in the Mlava River during the spring season (Table 1). *Achnanthidium minutissimum* was the dominant taxon at all sites with relative abundance of 15.4% to 44.8%. *Achnanthidium pyrenaicum* and *Amphora pediculus* were subdominant taxa during spring period.

Considering winter period (February 2012), a total of 131 diatom taxa were recorded in the Mlava River (Figure 1). The ML5 and ML4 sites are distinguished by the highest (72 taxa) and lowest diversity (42 taxa), respectively. During the winter, 13 taxa were recorded with relative abundance higher than 5% at least at one site (Table 3). The following taxa can be singled out as dominant: *A. minutissimum* (24.2%), *D. tenuis* (28.7%), *Gomphonema olivaceum* (40.5%) and *Nitzschia fonticola* (34.4%).

Figure 2 shows the relationship of the 45 best fitted diatom taxa from the Mlava River and the sampling seasons. In addition to the large number of taxa that are identified in more than two seasons (those placed in the center of the ordination diagram), there were some that were connected with only one season.

Table 2. Relative abundance of diatom taxa (%) present in the epilithic diatom community in the Mlava River during the summer season (July and September 2011).

Taxa	Summer									
	July 2011					September 2011				
	Sampling sites									
	ML1	ML2	ML3	ML4	ML5	ML1	ML2	ML3	ML4	ML5
	Relative abundance of taxa (%)*									
Achnanthidium minutissimum	14.7	20	10.5	17.2	8.4	13.6	11.2	7	4.2	2
Achnanthidium pyrenaicum	21.1	0.4	+	0.4	1.4	34.7	/	/	0.5	/
Amphora pediculus	29.6	13.5	9	24.4	6.4	14.3	6.5	30.5	14.1	9.7
Cocconeis neodiminuta	0.2	0.2	/	/	0.4	0.7	+	0.2	0.9	9.7
Cocconeis lineata	+	0.7	17	6.9	35.6	0.7	1.2	5.7	19.8	34.5
Cocconeis pseudolineata	4.2	+	0.5	0.2	2.9	6.9	0.5	0.2	1.4	14.2
Denticula tenuis	0.7	32.8	15.2	8.9	7.6	1.7	40.1	27.2	24.2	1.7
Diatoma vulgaris	/	2.9	10	1.4	0.7	/	3	1	2.7	/
Encyonema minutum	0.2	7.6	2	2.2	+	0.2	8.2	5.5	4.7	/
Gomphonema elegantissimum	2.4	0.7	2.7	12.4	10.3	9.4	+	+	5.9	4.5
Nitzschia fonticola	/	2.4	1	2.7	/	/	5.7	2	0.5	0.5
Planothidium dubium	7.9	0.4	/	1.4	/	9.4	0.5	1	+	/
Planothidium lanceolatum	0.4	0.7	1.5	0.9	3.7	+	+	+	+	6.7
Reimeria sinuata	0.7	1.9	2.7	7.9	6.9	2.9	1	1.2	5.9	4.2
Staurosirella pinnata	/	3.7	1.2	0.7	/	/	6	2.7	1.7	/

(*) – Taxa with a relative abundance greater than 5% at least at one site, (+) – Relative abundance of taxon less than 5%, (/) – Taxon not recorded.

Table 3. Relative abundance of diatom taxa (%) present in the epilithic diatom community in the Mlava River during the autumn (December 2011) and winter seasons (February 2012).

Taxa	Autumn					Winter				
	December 2011					February 2012				
	Sampling sites									
	ML1	ML2	ML3	ML4	ML5	ML1	ML2	ML3	ML4	ML5
	Relative abundance of taxa (%)*									
Achnanthidium catenatum	/	/	/	63	/	+	/	/	/	/
Achnanthidium minutissimum	28.4	8	36.3	15.5	4.8	16.3	19.1	12.9	2.9	24.2
Achnanthidium pyrenaicum	/	1.9	8.8	2.9	3	4.2	/	0.9	+	1.2
Amphora pediculus	21.9	22.7	3.2	4.1	32.1	7.1	5.7	0.9	1.7	7.6
Cocconeis lineata	0.5	1.4	3.4	1.9	14.2	1.4	0.4	0.9	1.9	14.7
Denticula tenuis	11.9	28.9	20.9	5.5	13.2	18.5	28.7	19.1	6.8	10.2
Encyonema minutum	0.5	2.4	2.9	+	0.7	0.4	8.1	5.8	1.4	1.2
Fragilaria rumpens	+	/	/	/	/	5.9	4.5	0.2	/	/
Gomphonema olivaceum	+	0.4	/	/	/	0.4	+	+	40.5	2.2
Melosira varians	+	+	/	/	/	14.3	/	/	/	/
Nitzschia dissipata	+	/	0.2	/	/	+	+	+	0.7	5.6
Nitzschia fonticola	1.7	4.1	4.6	0.7	/	3.4	11	21.3	34.4	6.8
Nitzschia linearis	0.5	/	/	+	9.1	+	+	0.4	+	0.4
Planothidium dubium	10.2	0.4	/	+	/	6.6	+	0.4	+	0.9
Staurosirella pinnata	8.7	21.8	11.3	1.2	7.9	0.4	6	8	1.4	0.2

(*) – Taxa with a relative abundance greater than 5% at least at one site, (+) – Relative abundance of taxon less than 5%, (/) – Taxon not recorded.

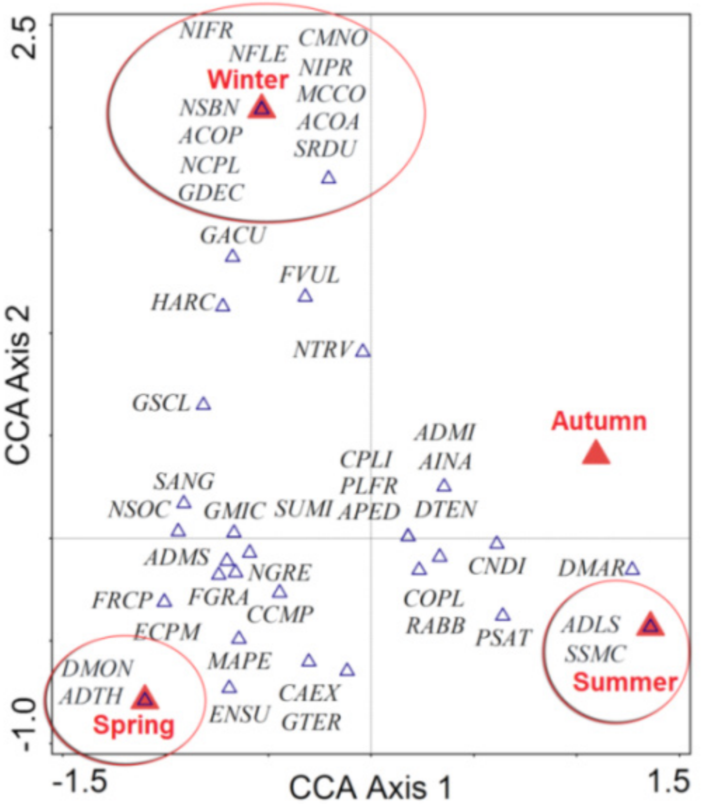

Figure 2. CCA showing the relationship of diatom taxa recorded in the Mlava River and sampling seasons.

Nitzschia frustulum (NIFR), *Nitzschia flexa* (NFLE), *Navicula subalpina* (NSBN), *Amphora copulata* (ACOP), *Nitzschia capitellata* (NCPL), *Geissleria decussis* (GDEC), *Craticula minusculoides* (CMNO), *Nitzschia pura* (NIPR), *Meridion circulare* var. *constrictum* (MCCO), *Achnanthes coarctaca* (ACOA), *Staurosira dubia* (SRDU), *Gomphonema acuminatum* (GACU), *Hannaea arcus* (HARC), *Frustulia vulgaris* (FVUL), *Navicula trivialis* (NTRV), *Gomphonema subclavatum* (GSCL), *Surirella angusta* (SANG), *Nitzschia sociabilis* (NSOC), *Gomphonema micropus* (GMIC), *Surirella minuta* (SUMI), *Cocconeis lineata* (CPLI), *Planothidium frequentissimum* (PLFR), *Amphora pediculus* (APED), *Adlafia minuscula* (ADMS), *Navicula gregaria* (NGRE), *Fragilaria gracilis* (FGRA), *Fragilaria recaptellata* (FRCP), *Cymbella compacta* (CCMP), *Encyonopsis minuta* (ECPM), *Mayamaea permitis* (MAPE), *Encyonema subminutum* (ENSU), *Cymbella affinis* (CAEX), *Gomphonema tergestinum* (GTER), *Achnanthidium minutissimum* (ADMI), *Amphora inariensis* (AINA), *Denticula tenuis* (DTEN), *Cocconeis neodiminuta* (CNDI), *Cocconeis pseudolineata* (COPL), *Rhoicosphenia abbreviata* (RABB), *Psammothidium subatomoides* (PSAT), *Diploneis marginestriata* (DMAR), *Adlafia suchlandtii* (ADLS), *Stauroneis smithii* (SSMC), *Diatoma moniliformis* (DMON), *Achnanthidium thienemannii* (ADTH).

Many taxa, as designated on diagram, were found only in the winter, while a slightly smaller number of taxa were characteristic only for spring or summer. No taxon stands out only in the autumn. Diatom taxa recorded in two seasons were *Gomphonema subclavatum* and *Hannaea arcus* (spring and winter), *Cymbella affinis* (summer and spring), as well as *Diploneis marginestriata* (autumn and summer).

DISCUSSION

A fluctuation of epilithic diatom diversity can occur due to environmental conditions, geological nature, grazing, resources limitation, habitat disturbance, substrate availability. Moreover, many pollutants can be a limiting factor for growth and dominance of epilithic diatom taxa (Al-Harbi, 2017). Dalu et al. (2014) stated that small-scale perturbations involving usable nutrients can stimulate functioning of ecosystems, while continued perturbations usually have a negative impact on community equilibrium and functioning. The average diversity values of epilithic diatom community were the highest in spring, which is also pointed out by Allan and Castillo (2007) studying freshwaters in temperate regions. Variations in water flow can cause significant changes in the taxonomic composition of diatom communities of the rivers (Martinez de Fabricius et al., 2003), which probably had a great influence on the diversity and composition of diatoms throughout the seasons in our study as well.

In the Mlava River, during the spring period, *Achnanthidium pyrenaicum*, beside *A. minutissimum*, is singled as dominant taxon. It is a species characteristic for rivers and streams with carbonate base and high flow velocity (Lange-Bertalot et al., 2017), which is confirmed by our results, as well. Namely, the highest relative abundance of this taxon was in the spring (Table 1), when the highest flow velocity was recorded (Jakovljević et al., 2016). A similar relative abundance (50%) of this species was also recorded in the San River in Poland, while the relative abundance of other dominant taxa varied in the range of 5–10% (Noga et al., 2014). In our study, the relative abundance of other taxa shown in Table 1 was higher. In the summer, the dominance of *Denticula tenuis* can be observed in the Mlava River (Table 2). According to van Dam et al. (1994), this species is widespread in oligosaprobic waters with high relative abundance. Having in mind our results on the water quality of the Mlava River based on phytobenthos (Jakovljević et al., 2016), its presence with a high relative abundance in the community confirms the literature data. *Achnanthidium catenatum* was one of the dominant taxa in the autumn, which is common in stagnant waters, while it occurs with low relative abundance in running waters (Lange-Bertalot et al., 2017). In our study, although it was found in only one site (ML4), it is represented with a relative abundance of 63% (Table 3). Furthermore, it is interesting that the site where this taxon was recorded is characterized by the highest velocity (Jakovljević et al., 2016). Thus, our results show that this taxon can be distributed with high relative abundance also in fast-flowing waters. During the winter period, the dominant members of the community were *A. minutissimum*, *D. tenuis* and *Nitzschia fonticola* (tolerant to high levels of organic pollution (Lange-Bertalot et al., 2017)). In the same period, the dominance of *Gomphonema olivaceum* with a community share of 40.5% can be noticed (Table 3). This species is one of the most common of the genus *Gomphonema* in Central Europe, often with a high share in the community. Toman et al. (2014) pointed out the ability to firmly attach to the substrate as the most probable reason for the high abundance of this species.

CONCLUSION

Studying of temporal dynamics and community composition of diatoms is very important for improving biomonitoring protocols of water bodies. Sampling time, as well as sampling site choice is crucial in such studies. We need to increase the number of these studies including more rivers in Serbia with the aim of creation diatom-based classification of rivers, thus better biomonitoring protocols.

ACKNOWLEDGMENT

This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2020-14/200178 and Grant No. 451-03-68/2020-14/200026).

REFERENCES

Al-Harbi SM (2017): Seasonal dynamics and community composition of epilithic microalgae in relation with environmental factors at northwest coast of Jeddah, The Red Sea. *J. Oceanogr. Mar. Sci.* 5: 1–8.

Allan JD, Castillo MM (2007): *Stream ecology: structure and function of running waters*. Netherlands: Springer.

Bozarth A, Maier UG, Zauner S (2009): Diatoms in biotechnology: modern tools and applications. *Appl. Microbiol. Biotechnol.* 82: 195–201.

Dalu T, Froneman P, Chari L, Richoux N (2014): Colonisation and community structure of benthic diatoms on artificial substrates following a major flood event: A case of the Kowie River (Eastern Cape, South Africa). *Water SA* 40: 471–480.

EN 13946 (2015): Water quality – Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers. Institute for Standardization of Serbia.

Gavrilović D, Dukić Lj (2002): *Reke Srbije*. Beograd: Zavod za udžbenike i nastavna sredstva.

Jakovljević O, Popović S, Vidaković D, Stojanović K, Krizmanić J (2016): The application of benthic diatoms in water quality assessment (Mlava River, Serbia). *Acta Bot. Croat.* 75: 199–205.

Kale A, Karthick B (2015): The diatoms: Big significance of tiny glass houses. *Resonance* 20: 919–930.
Krammer K, Lange-Bertalot H (1986): Bacillariophyceae. 1. Teil: Naviculaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (eds.), *Sübwasserflora von Mitteleuropa* 2/1, Jena: G. Fischer Verlag.
Lane CM, Taffs KH, Corfield JL (2003): A comparison of diatom community structure on natural and artificial substrata. *Hydrobiologia* 493: 65–79.
Lange-Bertalot H, Hofmann G, Werum M, Cantonati M (2017): *Freshwater Benthic Diatoms of Central Europe: Over 800 Common Species Used in Ecological Assessment*. Oberreifenberg: Koeltz Botanical Books.
Martinez de Fabricius AL, Maidana N, Gómez N, Sabater S (2003): Distribution patterns of benthic diatoms in a Pampean river exposed to seasonal floods: The Cuarto River (Argentina). *Biodivers. Conserv.* 12: 2443–2454.
Medarević ĐP, Lošić D, Ibrić SR (2016): Diatoms-nature materials with great potential for bioapplications. *Hemijska industrija* 70: 613–627.
Noga T, Stanek-Tarkowska J, Pajączek A, Kochman N, Peszek Ł (2014): Ecological assessment of the San River water quality on the area of the San Valley Landscape Park. *J. Ecol. Eng.* 15: 12–22.
Potapova M, Charles DF (2003): Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition. *Freshw. Biol.* 48: 1311–1328.
Simić S, Đorđević N, Mitrović N (2016): *Diversity of algae in protected natural habitats – the Mlava springhead and the Krupaj springhead.* 2nd International Symposium on Nature Conservation, 1–2 April 2016, Novi Sad, Abstract Book, 47 pag.
Szczepocka E, Szulc B, Szulc K, Rakowska B, Żelazna-Wieczorek J (2014): Diatom indices in the biological assessment of the water quality based on the example of a small lowland river. *Oceanol. Hydrobiol. Stud.* 43: 265–273.
Ter Braak CJ, Ńmilauer P (2012): Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer power, Ithaca.
Tison J, Park YS, Coste M, Wasson JG, Ector L, Rimet F, Delmas F (2005): Typology of diatom communities and the influence of hydro-ecoregions: A study on the French hydrosystem scale. *Water Res.* 39: 3177–3188.
Toman M, Grońelj A, Zelnik I (2014): The Influence of Selected Factors on the Distribution of Epilithic Diatoms in a Torrential River the Kamnińka Bistrica (Slovenia). *Acta Bot. Croat.* 73: 447–463.
Van Dam H, Mertens A, Sinkeldam J (1994): A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. *Neth. J. Aquat. Ecol.* 28: 117–133.

ОРИГИНАЛНИ НАУЧНИ РАД

ВРЕМЕНСКА ДИНАМИКА СИЛИКАТНИХ АЛГИ РЕКЕ МЛАВЕ

Олга С. ЈАКОВЉЕВИЋ[1], Слађана С. ПОПОВИЋ[2], Ивана М. ЖИВИЋ[1],
Катарина З. СТОЈАНОВИЋ[1], Јелена Ж. КРИЗМАНИЋ[1]

[1] Универзитет у Београду, Биолошки факултет
Студентски трг 16, Београд 11000, Србија
[2] Универзитет у Београду, Институт за хемију, технологију и металургију
Институт од националног значаја за Републику Србију
Центар за екологију и техноекономику
Његошева 12, Београд 11000, Србија

РЕЗИМЕ: Циљ овог истраживања је био да се анализира временска динамика заједнице силикатних алги реке Млаве током 4 сезоне на 5 локалитета. У горњем току реке изграђен је пастрмски рибњак (између првог и другог локалитета). Највећи диверзитет је утврђен у пролеће са 159 идентификованих таксона силикатних алги, а најмањи у јесењем периоду (89 таксона). Укупно 13 таксона силикатних алги могу се окарактерисати као доминантни (њихова релативна бројност је била већа од 5% на барем једном локалитету). Канонијска кореспондентна анализа (CCA) је показала изражену временску динамику заједнице силикатних алги. Велики број таксона је идентификован у више од две сезоне, док су неки били карактеристични за само једну сезону. Многи од њих су установљени само у зимском периоду, али и у пролећном и летњем (нешто мањи број таксона).

КЉУЧНЕ РЕЧИ: заједнице силикатних алги, пастрмски рибњак, диверзитет

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 141, 21—33, 2021

UDC 582.685.24(Novi Sad)
https://doi.org/10.2298/ZMSPN2141021D
ORIGINAL SCIENTIFIC ARTICLE

Sara Ž. ĐORĐEVIĆ[*], *Ivana M. SENTIĆ*, *Jelena D. ČUKANOVIĆ*, *Mirjana Ž. LJUBOJEVIĆ*, *Magdalena G. PUŠIĆ*

The University of Novi Sad, Faculty of Agriculture
Department of Fruit Growing, Viticulture, Horticulture and Landscape Architecture
Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia

INFLUENCE OF URBAN HEAT ISLAND ON *Tilia tomentosa* Moench. BLOOMING

ABSTRACT: Temperature increase in urban areas due to a high share of paved and impermeable surfaces affects indigenous woody species by making changes in their biological processes. Field research includes monitoring the blooming period of 18 individuals of *Tilia tomentosa* Moench. in one vegetation season located in three different habitats (green strip, paved surface, and park). The beginnings of four blooming phenophases are monitored (beginning of blooming, beginning of full blooming, end of full blooming, and end of blooming), as well as the total duration of blooming. Analyzed individuals are located in the densely built-up part of Novi Sad in Serbia which is under the influence of the urban heat island effect. The results show that the blooming period of individuals located in the park lasted longer and occurred later. Between individuals from green strips and paved surfaces there are no significant differences in the blooming period and the occurring of phases, but in relation to park individuals, blooming duration is shorter and occurs earlier. Besides age and hereditary traits, air temperature as a part of the microclimate of analyzed habitats is a very important factor when analyzing the blooming phenophase. In that way, the change in blooming phenophases can be an indicator of the adaptability of indigenous species to the urban heat effect and climate change.

KEYWORDS: blooming phenology, silver linden, *Tilia tomentosa* Moench., urban heat island, white linden

INTRODUCTION

Urbanization and climate change are creating many environmental problems in cities. The larger human population in urban areas contributes to the increase in impervious paved surfaces leading to urban heat island formation (UHI) (Vujović et al., 2021). This is a phenomenon when the urban areas are often several degrees warmer than the surrounding rural areas (Huang et al., 2019),

[*] Correspondent author. E-mail: sara.djordjevic@polj.uns.ac.rs

thus making them more vulnerable to climate change (Avashia et al., 2021). Climate change influences the changes in temperature, precipitation, and photoperiod that affect plant habitats. Therefore, the timing of annually biological events and the rhythm of plant phenology can be climate change indicators (Fitchett et al., 2015; Fagras, 2019). The blooming phenophase is a direct indicator of the adaptability of plants to new environmental conditions (Stojičić, 2014). Plants cannot always track rapid climate changes, so they result in phenological mismatches and deviations (Fox, 2019).

The dominant cause of phenological responses of plants to accelerated climate warming in urban areas, in the form of earlier blooming, is the urban heat island effect (Luo et al., 2007). A large number of paved surfaces in urban areas are often not heat-and water-permeable. As a result, the increased intensity of the urban heat islands accelerates the phenophases of plants (Jochner et al., 2013).

Savić et al. (2012) observed the appearance of an urban heat island in the City of Novi Sad that contributes to reduced cooling in the late afternoon and evening, resulting in higher temperatures in the city during the night. Such climate change has a great influence on all biological processes, among which is the blooming phenology. Another study Savić et al. (2018) showed that the most densely built-up areas in Novi Sad (Detelinara, Grbavica, Stari Grad, and Podbara) have very high or high-risk heatwave values which are influenced by the urban heat island effect. These values cause a higher rate of mortality of people in these areas (Savić et al., 2018), so a question arises how these urban heat islands affect plants and their phenophases.

Although urban flora consists of indigenous and non-indigenous species, urbanization can be a bigger threat for native species (Garrard et al., 2018) affecting their habitat and thus making them more vulnerable to climate change. One of them is silver linden (*Tilia tomentosa* Moench.), a valuable species that is frequently planted in urban areas (Weryszko-Chmielewska et al., 2019) and is widespread in the City of Novi Sad.

The aim of the research is to register the time in which the phenophases of the blooming of silver linden (*Tilia tomentosa* Moench.) appear, in three different habitat conditions in Novi Sad (within the paved area, on the green strip, and in the park) and to compare the blooming period of individuals from different habitat types. In addition, it aims to analyze the dynamics of blooming (beginning, duration, and end), which shows the impact of habitat conditions on the phenology of blooming and the possible threat that climate change or urban heat island effect has on this indigenous species.

MATERIAL AND METHODS

Study area

The analyzed individuals of silver linden (*Tilia tomentosa* Moench.) are located at the border of the settlements of Nova Detelinara and Banatić and in the settlement of Sajmište in Novi Sad (Figure 1).

Figure 1. Location of the study area

Individuals are located in different habitats – green strip along the road (individuals A1–A6), on a paved surface (individuals B1–B6), and in Futoški Park (individuals C1–C6) (Figure 2). Although located in the same climatic area, these habitats provide different ecological conditions for the development and phenology of species. The selection of individuals to be observed is based on field analysis. All trees are physiologically mature, aged between 20 and 40 years.

Phenological observations

Field research includes monitoring the blooming period of 18 individuals of *Tilia tomentosa* Moench. in one vegetation season – 6 of them located on a green strip along the road or parking lot with the width of 5 to 1.4 m, 6 located within a paved area with an opening up to 1.4 m wide, and 6 located in a park area (Futoški Park) (Figure 2). Phenological observations are performed

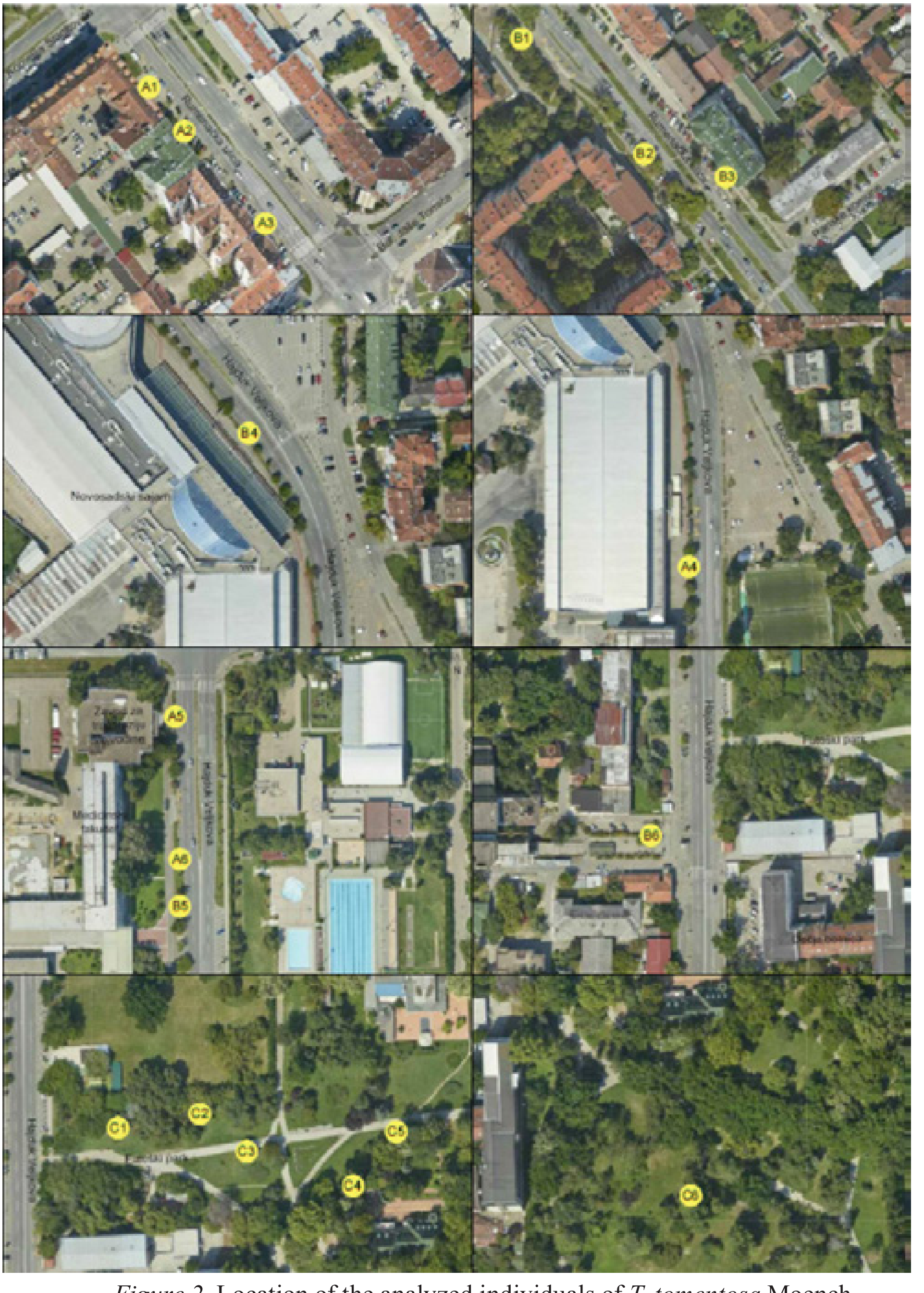

Figure 2. Location of the analyzed individuals of *T. tomentosa* Moench.
(A – Individuals on green strips, B – Individuals on a paved surface, C – Individuals in Futoški Park)

by visual observation in sunny weather on the same day in the second half of the day. Observations of the bloom phenophase of the selected individuals were performed in the period from June 11th to July 5th, 2021.

In this paper, four phases are modeled by the methodology of Tomić et al. (2014) and Stojičić (2014): 1. The beginning of blooming 2. The time of full blooming 3. The end of full blooming 4. The end of blooming (Figure 3). The beginning of blooming is the day when 25% of inflorescences are open in relation to the volume of the canopy, full blooming with over 50% of fully open inflorescences, the end of full blooming when 80% of inflorescences are open and 20% of flowers are dried, and the end of blooming when 80% of the flowers fall off (the flowers are without perianth).

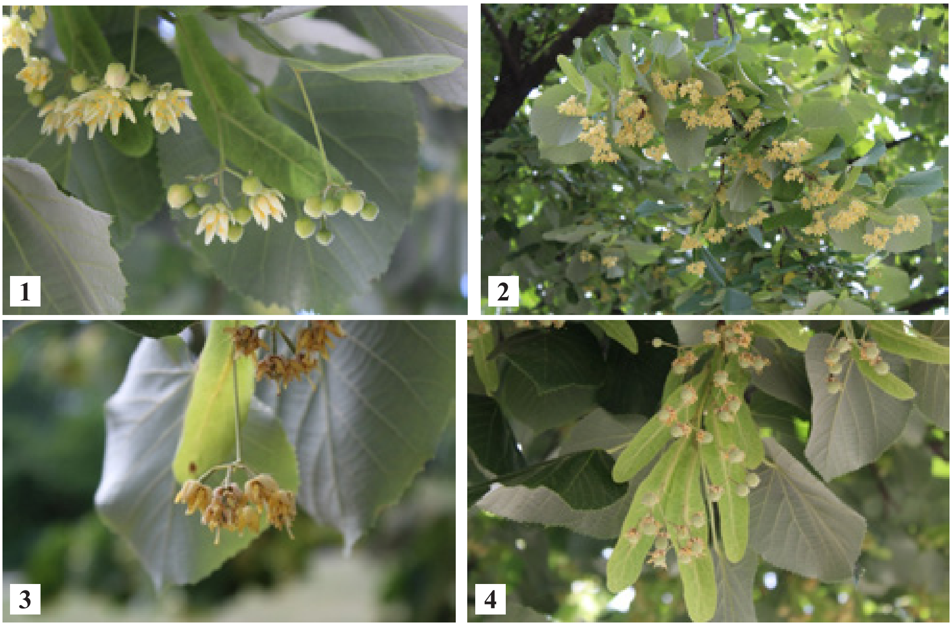

Figure 3. The phenophases of blooming of *T. tomentosa* Moench.
(1 – The beginning of blooming, 2 – The time of full blooming,
3 – The end of full blooming, 4 – The end of blooming)

Since there are no meteorological stations at the studied locations, the climatic elements of the meteorological station Novi Sad – Rimski Šančevi (RHMZ site) are used in the analysis of the influence of temperature on the flowering phenology. It should be noted that Rimski Šančevi are located outside the urban environment and the temperatures are lower than in the city. To determine the mean daily air temperatures, the data are read daily at 7 am, 2 pm, and 9 pm between June 11th and July 5th. In addition, due to the study of the urban heat island effect, daily minimal air temperatures are specially noted, as values most similar to the night temperature values, when the process of the urban heat island is the most intense (Savić et al., 2012). The collected data are statistically processed using Statistica 14 software package (StatSoft, Inc., Tulsa, OK, USA). Investigation of multi-character variation is conducted by Principal Component Analysis (PCA). The minimum temperatures and the beginnings of the phases of individuals from different habitats in the observed period are graphically shown to determine the relationship between blooming and temperature factors, i.e. the urban heat island effect.

RESULTS AND DISCUSSION

In the first phase, the beginning of blooming was first noticed on June 11th on individual A5 located on the green strip in Hajduk Veljkova Street along the road, and the last noticed was on June 22th on individual C4 in Futoški Park (Table 1). The end of blooming occurred the earliest on June 21st on individual B5, which is located on a paved surface, and the latest on July 5th on individual C4, which is located in Futoški Park. Individual B5 on the paved surface had the shortest blooming period (9 days), while individual C3 on the park surface had the longest blooming period (18 days).

Table 1. Phenophases of silver linden blooming (A – individuals on green strip, B – individuals on paved surface, C – individual in Futoški Park)

Individual	First phase	Second phase	Third phase	Fourth phase	Total blooming duration (days)
A1	15.6.	17.6.	22.6.	26.6.	12
A2	17.6.	19.6.	23.6.	29.6.	13
A3	14.6.	16.6.	22.6.	27.6.	14
A4	18.6.	20.6.	22.6.	27.6.	10
A5	11.6.	14.6.	19.6.	25.6.	15
A6	16.6.	19.6.	22.6.	25.6.	10
B1	15.6.	17.6.	23.6.	30.6.	16
B2	13.6.	15.6.	21.6.	26.6.	14
B3	15.6.	17.6.	22.6.	29.6.	15
B4	12.6.	15.6.	20.6.	25.6.	14
B5	13.6.	15.6.	19.6.	21.6.	9
B6	18.6.	19.6.	25.6.	28.6.	11
C1	14.6.	16.6.	23.6.	30.6.	17
C2	17.6.	20.6.	22.6.	1.7.	15
C3	16.6.	18.6.	25.6.	3.7.	18
C4	22.6.	24.6.	2.7.	5.7.	14
C5	18.6.	21.6.	26.6.	2.7.	15
C6	20.6.	22.6.	28.6.	3.7.	13

The principal component analysis (Figure 4) showed that the individuals from Futoški Park (C) stand out because they are characterized by a longer duration of blooming (from 13 to 18 days). Individuals on paved surfaces and green strips are grouped together, so they are not grouped solely on the basis of habitat. Compared to park individuals they perform blooming in a shorter period and the beginning of blooming occurs earlier. So, what can be noticed is that individuals are grouped mainly based on the total duration of blooming. The longer period of blooming of individuals in the park is conditioned not only by the age of individuals but also by ecological and habitat conditions because on the park surface individuals have enough space to develop the underground and aboveground parts and thus perform their phenophases normally.

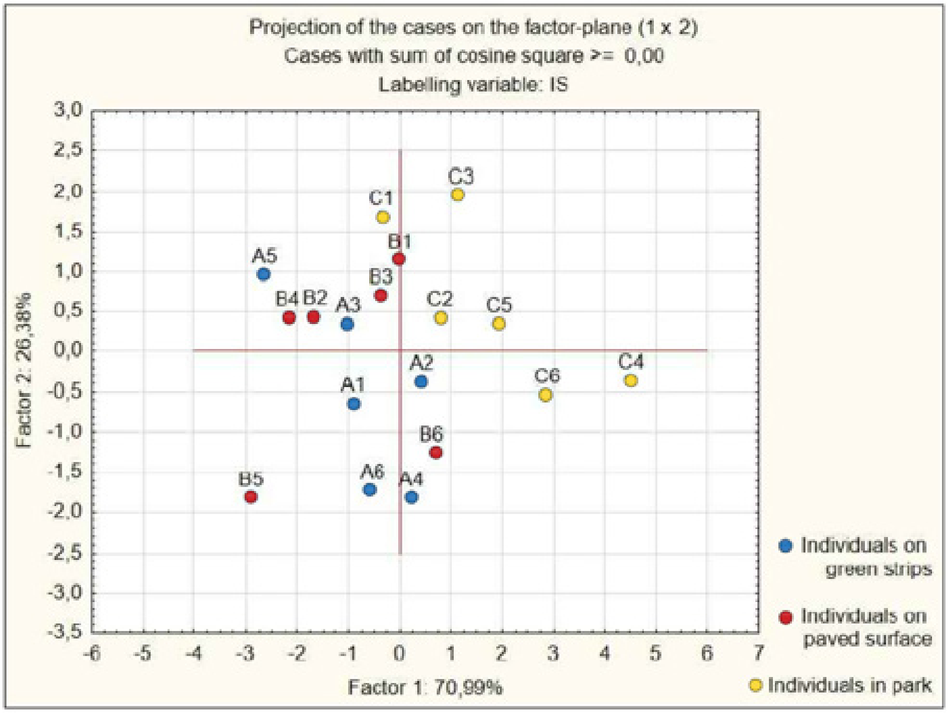

Figure 4. Principal component analysis of blooming phenology data of *Tilia tomentosa* Moench.

The difference between the individuals on paved surfaces and green strips and park individuals is also conditioned by microclimatic conditions, because unlike natural environments like parks, impervious paved surfaces may negatively affect urban trees through enhanced extreme soil and air temperatures (Sand et al., 2018). Their vitality is endangered when the permeable surface area around the tree is small (Sand et al., 2018). The high share of impermeable paved surfaces around the individuals on paved surfaces and the insufficient width of green stripes cannot create environmental conditions similar to parks, which affect their biological processes including the blooming phenophase.

Figures 5, 6, and 7 show examples of individuals from each habitat type. These individuals are chosen to show minimum and maximum extent of phenophases (A6 as the individual that has the earliest first blooming phase and the shortest duration of blooming among the 6 individuals on green strips, B5 as the individual that has the shortest blooming period among all 18 individuals, and C4 that has the latest first blooming phase and the longest duration of blooming among 18 individuals) and to show graphically how habitat, minimum temperature, and blooming phenophases are related. It can be seen in the figures that the individual from the paved surface (B5) starts blooming the earliest and has the shortest blooming, while the individual from the park surface (C4) starts blooming the latest and has the longest blooming. Also, on the example of a park individual, it can be noticed that when the minimum temperatures decrease, the blooming phases lengthen, while the increase in the minimum temperature in the period from June 14th to 23rd accelerates the blooming of individuals on the green strip (A6) and paved surface (B5). In addition to the high temperature, a small amount of precipitation and lower relative humidity in the observed period cause a shorter course of blooming of individuals.

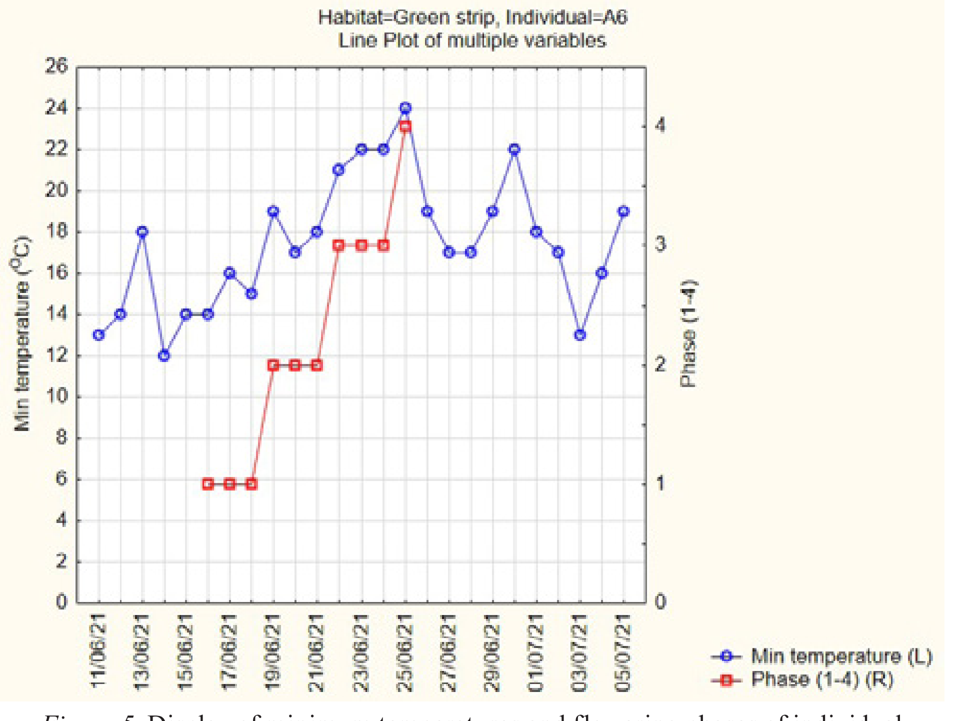

Figure 5. Display of minimum temperatures and flowering phases of individual A6 located on the green strip

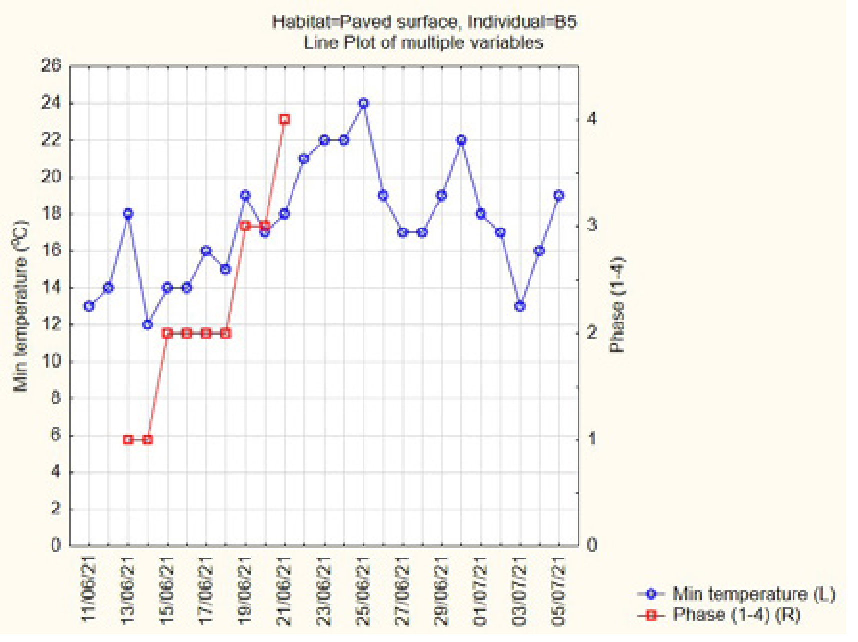

Figure 6. Display of minimum temperatures and flowering phases of individual B5 located on a paved surface

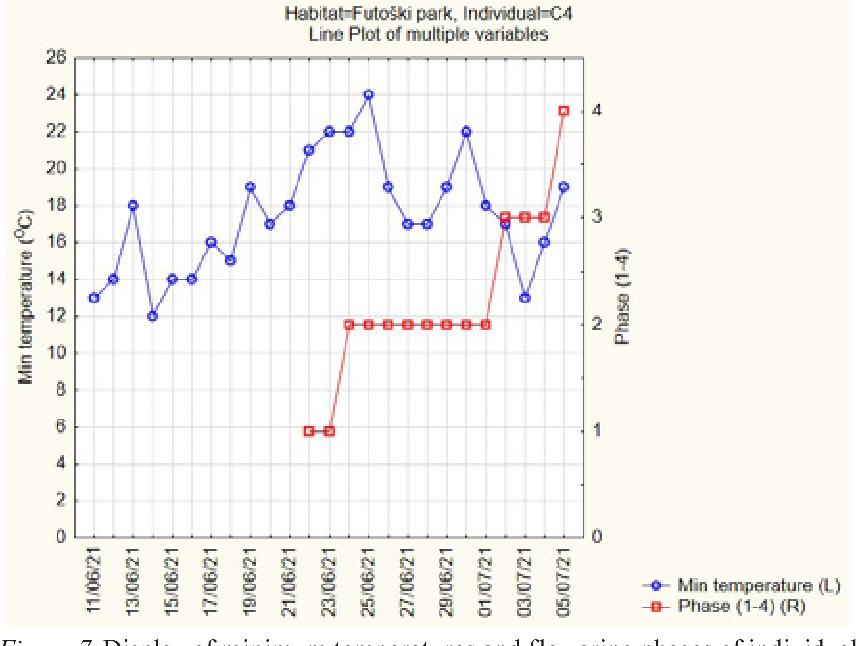

Figure 7. Display of minimum temperatures and flowering phases of individual C4 located in Futoški Park

The meteorological data are taken from the nearest meteorological station Rimski Šančevi, but since it is located outside the urban environment, the real temperatures at the locations are certainly higher. The differences in the duration of phenophases of individuals in different habitats are conditioned by the microclimatic conditions prevailing at the study area. Individuals have the earliest and shortest blooming on a paved surface because the area around the tree is impermeable and retains heat for the longest time in the evening, which is why the impact of the urban heat island effect is then the strongest. The situation is similar with individuals on the green strip because it is of insufficient width and is surrounded by impermeable concrete or asphalt surfaces. Individuals on the park surface have the most favorable microclimate, with water- and heat-permeable surfaces that enable cooling and water penetration, so their blooming is longer and more uniform.

Tomić et al. (2014), in their research on silver linden in Banja Luka, confirmed that their blooming period is shorter due to higher air temperatures. Also, they add less precipitation and low air humidity as climatic elements that can shorten the blooming period of silver linden. The same authors also state that the duration of the linden blooming period largely depends on hereditary traits because they find intraspecies variability within their researched individuals. So, it should be emphasized that, besides different environmental conditions, hereditary traits can influence the variations in blooming phenophases. Thus, further research should investigate how much the hereditary traits of silver linden affect the shift of the blooming phases. On the other hand, besides hereditary traits, author Stojičić (2014) highlighted that from all environmental conditions climatic parameters have the greatest influence on the phenology. Due to current problems with climate change, research of other climatic parameters, besides air temperature, could be a next step which will show better how the urban heat island effect affects and endangers not only the phenophases, but other processes of indigenous woody plants like silver linden.

CONCLUSION

The research results after comparing the blooming period between individuals on different habitats from June 11th to July 5th, 2021 show that individuals located in the park have a longer blooming period (13–18 days) than individuals on green strips (10–15 days) and paved surface (9–16 days). Also, the blooming phenophases occur earlier in individuals in the park than in those on green strips and paved surfaces. In the first phase, the beginning of blooming was first noticed on June 11th on individual A5 located on the green strip in Hajduk Veljkova Street along the road, and last on June 22th on individual C4 in Futoški Park. The end of blooming occurred the earliest on June 21st on individual B5, which is located on a paved surface, and the latest on July 5th on individual C4, which is located in Futoški park. Individual B5 on the paved surface had the shortest blooming period (9 days), while individual C3 on the park surface had the longest blooming period (18 days).

In paved areas and green strips, the share of impermeable paved surfaces around the individuals is high. This enhances soil and air temperatures, enables root and canopy development, and affects biological processes of urban trees including the blooming phenophase. Different microclimatic conditions in the analyzed habitats are among the major reasons for the difference in duration and start of blooming.

One of the elements of microclimate noticed during the blooming period, as a value most similar to the night temperature when the process of the urban heat island is the most intense, is minimum temperature. The increase of minimum temperatures in the observed period is directly related to the shorter duration of phenophases, especially within individuals on green strips and paved surfaces whose blooming lasts significantly shorter than within park individuals. This indicates that an increasing number of paved, impermeable surfaces that cool slowly, negatively affect the blooming phenophase of these indigenous species, accelerating them and thus disrupting their basic role and function. Given that the research is conducted only in one vegetation season and that the sites are relatively close, no general conclusions can be drawn about the differences and causes of changes in the phenophase of silver linden blooming, but this research can serve as a starting point for future research on similar issues.

To fulfill the ecological, sanitary, and aesthetic function of indigenous species such as silver linden (*Tilia tomentosa* Moench.), it is necessary to provide more green and permeable and less paved impermeable surfaces, favorable microclimatic conditions, and sufficient space for the root system and canopy development. Proper and careful planning of the urban landscape through ecological approaches, preservation of indigenous species, increasing the number of green areas, careful selection of planting sites, and good care of seedlings, especially in the post-planting period, can mitigate the effects of climate change. In this way, by creating a favorable microclimate and reducing the urban heat island effect, the blooming phenophases of indigenous species would take place normally and be undisturbed.

REFERENCES

Avashia V, Garg A, Dholakia H (2021): Understanding temperature related health risk in context of urban land use changes. *Landsc. Urban Plan.* 212: 104–107. [DOI: 10.1016/j.landurbplan.2021.104107]

Fagras S (2019): *Changes in Plant Phenology in the Central United States in Response to Climate Change*. Environmental Studies Undergraduate Student Theses, University of Nebraska, Lincoln, Nebraska, 233.

Fitchett JM, Grab SW, Thompson DI (2015): Plant phenology and climate change: Progress in methodological approaches and application. *Prog. Phys. Geogr.* 39: 460–482.

Fox N (2019): *Plant phenology and climate change: possible effect on the onset of various wild plant species first flowering day in the UK*. Master Thesis in Geographical Information Science, Lund University, Lund, Sweden.

Garrard GE, Williams NS, Mata L, Thomas J, Bekessy SA (2018): Biodiversity sensitive urban design. *Conserv. Lett.* 11: e12411.

Huang K, Li X, Liu X, Seto KC (2019): Projecting global urban land expansion and heat island intensification through 2050. *Environ. Res. Lett.* 14: 114037.

Jochner S, Alves-Eigenheer M, Menzel A, Morellato LPC (2013): Using phenology to assess urban heat islands in tropical and temperate regions. *Int. J. Climatol.* 33: 3141–3151.

Luo Z, Sun OJ, Ge Q, Xu W, Zheng J (2007): Phenological responses of plants to climate change in an urban environment. *Ecol. Res.* 22: 507–514.

Sand E, Konarska J, Howe AW, Andersson-Sköld Y, Moldan F, Pleijel H, Uddling J (2018): Effects of ground surface permeability on the growth of urban linden trees. *Urban Ecosystem* 21: 691–696.

Savić S, Marković V, Šećerov I, Pavić D, Arsenović D, Milošević D, Dolinaj D, Nagy I, Pantelić M (2018): Heat wave risk assessment and mapping in urban areas: case study for a midsized Central European city, Novi Sad (Serbia). *Nat. hazards* 91: 891–911.

Savić S, Mitrović M, Lazić L (2012): Analiza novosadskog urbanog ostrva toplote. *Zbornik radova*, Univerzitet u Novom Sadu, PMF, Departman za geografiju, turizam i hotelijerstvo 41: 18–28.

Stojičić Đ (2014): *Uticaj faktora spoljašnje sredine na cvetanje drvenastih vrsta iz pododeljka Magnoliophyta u Beogradu*. Doktorska disertacija. Šumarski fakultet, Univerzitet u Beogradu.

Tomić B, Daničić V, Mataruga M, Cvjetković B (2014): Fenologija cvjetanja tri vrste lipe (*Tilia* sp.) na području grada Banja Luke. *Glasnik Šumarskog fakulteta Univerziteta u Banjoj Luci*, 20: 27–40.

Vujovic S, Haddad B, Karaky H, Sebaibi N, Boutouil M (2021): Urban Heat Island: Causes, Consequences, and Mitigation Measures with Emphasis on Reflective and Permeable Pavements. *Civil Eng.* 2: 459–484.

Weryszko-Chmielewska E, Piotrowska-Weryszko K, Dąbrowska A (2019): Response of *Tilia* sp. L. to climate warming in urban conditions – Phenological and aerobiological studies. *Urb. For. Urb. Green.* 43: 126369.

ОРИГИНАЛНИ НАУЧНИ РАД

УТИЦАЈ УРБАНОГ ОСТРВА ТОПЛОТЕ НА ЦВЕТАЊЕ *Tilia tomentosa* Moench.

Сара Ж. ЂОРЂЕВИЋ, Ивана М. СЕНТИЋ,
Јелена Д. ЧУКАНОВИЋ, Мирјана Ж. ЉУБОЈЕВИЋ, Магдалена Г. ПУШИЋ

Универзитет у Новом Саду, Пољопривредни факултет
Департман за воћарство, виноградарство, хортикултуру и пејзажну архитектуру
Трг Доситеја Обрадовића 8, Нови Сад 21000, Србија

РЕЗИМЕ: Повећање температуре у урбаним подручјима, због велике количине поплочаних и непропусних површина, утиче на аутохтоне дрвенасте врсте мењајући њихове биолошке процесе. Теренска истраживања обухватала су праћење периода цветања 18 индивидуа *Tilia tomentosa* Moench., које се налазе на три различита станишта (зелена трака, поплочана површина и парк) у једној вегетационој сезони. Праћени су почеци четири фенофазе цветања (почетак цветања, почетак пуног цветања, крај пуног цветања, крај цветања), као и укупно трајање цветања. Анализиране индивидуе налазе се у густо изграђеном делу Новог Сада (АП Војводина, Србија) који је под утицајем ефекта урбаног острва топлоте. Резултати показују да је период цветања индивидуа у парку трајао дуже и догодио се касније. Између индивидуа са зелених трака и поплочаних површина није било значајних разлика у периоду цветања и појављивању фаза, али у односу на парковске индивидуе, цветање је трајало краће и почело је раније. Осим старости и наследних особина, температура ваздуха (која је део микроклиме) анализираних станишта веома је важан фактор при анализи фенофазе цветања. На тај начин промене фенофаза цветања могу бити показатељи прилагођавања аутохтоних врста на ефекат урбаног острва топлоте и на климатске промене.

КЉУЧНЕ РЕЧИ: бела липа, сребрнолисна липа, *Tilia tomentosa* Moench., урбано острво топлоте, фенологија цветања

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 141, 35—47, 2021

UDC 582.998.1(497.113)
https://doi.org/10.2298/ZMSPN2141035S
REVIEW SCIENTIFIC ARTICLE

Aleksandra M. SAVIĆ[*]

Institute for Plant Protection and Environment, Department of Herbology
Teodora Drajzera 9, Belgrade 11000, Serbia

Ambrosia trifida L. (GIANT RAGWEED)

ABSTRACT: *Ambrosia trifida* L. belongs to the group of invasive weeds, which in colonized areas cause great damage to the biodiversity of autochthonous flora and vegetation as well as in plant production. It originates from North America wherefrom it has spread to many parts of Europe. In Serbia it is currently locally present in the area of Bačka (the northern region of the country). Early and rapid growth rate, vegetative and generative production, high degree of morphological and reproductive plasticity have given it a competitive advantage over many other weeds, hence in many countries it is considered one of the most problematic weeds in agricultural production. *A. trifida* could cause great damage in root crops, vegetable gardens and orchards and its harmfulness is measured by the negative impact on biodiversity by suppressing indigenous and other non-indigenous species. With its allergens, *A. trifida* negatively affects human health. Observing its vegetative and generative potential and climate change on the other hand, recent research indicates the potential for the spread of *A. trifida* in our country and in Europe, which could be a serious risk for agrophytocenoses and the ecosystem as a whole. In 2019, it was added to the EPPO A2 List of quarantine pests recommended. It can be controlled with the use of mechanical, biological and chemical measures.

KEYWORDS: *Ambrosia trifida* L., Ambrosia spp., allergenic species, competition, invasive weeds, secondary metabolites, weed control, yield losses

INTRODUCTION

Invasive plant species may be a serious threat to crop fields and natural habitats (Pyšek et al., 2009; Vilà et al., 2011; Essl et al., 2009; 2015). Since they do not have their particular natural agent of control in the new area, they can grow fast and compete for resources. If establishment is effectual, invasive species generate noticeably (two to five times) more biomass than indigenous species and better exploit the available resources (Szymura et al., 2018). Considering their conspicuous competitive potential, they suppress indigenous populations and gravely disturb biodiversity (Gioria and Osborne, 2014).

[*] Corresponding author. E-mail: aleksandra.m.savic@gmail.com

The species of genus Ambrosia (family Asteraceae) are widespread and heavily deleterious invasive plants. The genus has around 40 species, originating mostly from North America and they have been introduced into new countries in the 19th century (Makra et al., 2005; Gerber et al., 2011; Smith et al., 2013). In North America and Europe, it started causing grave trouble in the past decades, contributing to an evident increase in respiratory allergic reactions (D'Amato and Spieksma, 1992; D'Amato, 2007). Pronounced adaptability to new conditions makes them able of surviving and generating colony of an invasive species. Beside *Ambrosia artemisiifolia* L., which is the most damaging species of this genus (Kazinczki et al., 2008), *Ambrosia trifida* L. also causes considerable damage in agricultural production (Weaver, 2001; Harrison et al., 2001). It has been present in numerous countries in Europe (Follak et al., 2013, www.cabi.org), and its harmfulness in crop has been remarked in Serbia (Malidža and Vrbničanin, 2006). In recent years, *A. trifida* has been a species that has attracted the attention of many researchers (Harrison et al., 2001; Follak et al., 2013; Page and Nurse, 2015; Savić et al., 2019a, b; 2020a, b; 2021).

TAXONOMIC HIERARCHY

Domain: Eukaryota
 Kingdom: Plantae
  Phylum: Spermatophyta
   Subphylum: Angiospermae
    Class: Dicotyledonae
     Order: Asterales
      Family: Asteraceae
       Genus: Ambrosia
        Species: *Ambrosia trifida*
         Sub-species: *Ambrosia trifida* var. *texana* and *Ambrosia trifida* var. *trifida*
          EPPO code: AMBTR

DISTRIBUTION

Ambrosia trifida originates from North America and it is distributed from Western to Central Europe. Currently, it inhabits all continents except Africa and Oceania. It is assumed that it was introduced by imports of commercial grain and oilseed and during World War II, when military movements were also vectors of introduction for this species in Europe (Lawalree, 1947; Follak et al., 2013; Ardenghi and Polani, 2016). At this time, it is present in many parts of Europe (Slovak, Serbia, Germany, Switzerland, Italy, Slovenia, Bulgaria, Austria, Romania, Czech Republic, etc.). Furthermore, this species it has been introduced in Asia, Japan, China, Mongolia and South Korea, Georgia, Israel, etc.

(Follak et al., 2013; www.cabi.org). In 2019, *A. trifida* was added to the EPPO A2 List of quarantine pests.

Follak et al. (2013) researched the rate of spread of *A. trifida* in Central and Eastern Europe. The number of reports peaked in the periods 1951–1990 and thereafter reduced suddenly. For a relatively long time, the percentage of established populations was rare. It is supposed that the decrease in the spread rate was impacted by the fact that *A. trifida* mostly inhabits ruderal habitats and thus its distribution is less intense than invasive species present in rural and urban areas (Follak et al., 2013; Chauvel et al., 2015). Seeds are rich in fat and protein and Harrison et al. (2003) found that predators (moths, mice and birds) feed on seeds of *A. trifida* and in that way they reduce the number and limit its spread to some extent. In addition, it is determined that seeds of *A. trifida* are a food for species from the order *Diptera*, *Lepidoptera* and *Coleoptera* (Harrison et al., 2001). The percentage of identified populations did not change for a relatively long period. However, it is recently reported that the population of *A. trifida* is permanently increasing and this fact could be connected to climate changes which are affecting almost all European countries (Johnson et al., 2005; Follak et al., 2013; Mora et al., 2018). Since it is well adjusted to warm habitats with more light and nutrient-rich soils (Dinelli et al., 2013), it is assumed that climate change has affected its re-spread.

On the Balkan Peninsula, it was first found in the 1980s in Banat (Serbia) (Koljadžinski and Šajinović, 1982). A few years later, it was recorded in Slovenia (Vasić, 1990). After that, Malidža and Vrbničanin (2006) recorded a large population of *A. trifida* in Central Bačka (Despotovo, Kucura, Savino Selo and Ravno Selo). Thereafter, reports on its incidence in the crop fields have arisen in Serbia, specifically in Pannonia Plain region (along the roads in villages, between settlements, and on field edges, as well as in sunflower, maize, soybean, and sugar beet crops) (Malidža and Vrbničanin, 2006; Vrbničanin et al., 2012; 2015).

BIOLOGY AND ECOLOGY

Ambrosia trifida is a broadleaf annual plant (therophyte, T4) which is only propagated from seeds. Drier, warm habitats, with more light, and the nutrient-rich soils suit this species (Vrbničanin et al., 2015). Taking into account different growth conditions, it can display substantial plasticity in height, degree of branching, number and size of leaves, amount of reproduction (Abul-Fatih and Bazzaz, 1980). It forms an upright, strong and branched stem, covered in short, white hairs in its upper part, while in the lower part the stem is bare. Root is very strong, with a dense system of lateral roots. *A. trifida* is also characterized by leaf variability, where individuals often form a leaf plate with three to five lobes, or without lobes, and sometimes the lobes absence is a result of varied environmental conditions during the plants development. *A. trifida* is a diploid species (2n = 24; Payne, 1964). In nature, there has been observed the formation of hybrid forms between *A. artemisiifolia* and *A. trifida* (a new taxon, *A*. x *helenae*) which affects the creation of morphological and genetic diversity (Wagner, 1958; Vincent et al., 1988; Strother, 2006).

The final plant height may reach 6 m. Their leaves (20–30 cm in length) are mainly placed in the terminal part of the plant and depending on their size and position; the available light is notably reduced to the other species (Basset and Crompton, 1982). This species has high photosynthetic (C3 photosynthesis system) and net assimilation rates (Abul-Fatih and Bazzaz, 1979). It is a monoecious plant which flowers and produces seeds from July to September. An allometric fruit distribution is typical for this species (Harrison et al., 2001). The flowers are unisexual, with males flowers producing pollen and females flowers producing seeds. Anthers in male flowers form clusters, located on the terminal part of the plant and contain 10–15 flowers grouped in inflorescences up to 30 cm long (Bassett and Crompton, 1982). Female heads are 6–10 mm in size and are located in the axils of the upper leaves. The pistils are located in groups at the leaf base below the anthers. One plant can produce about 10 million pollen grains a day. Pollen grains (tricolpate, three-celled pollen) (16–27 µm in diameter) are spherical with spines and spinules (Basset and Terasmae, 1962; Curtis and Lersten 1995; Liu et al., 2012). The pollen grain is round with spikes and contains about 50 proteins (antigens) that act as allergens. During the day, the concentration of pollen is highest from five to ten o'clock in the morning. Pollen stays in the air for more than 100 days, with the highest concentrations in August and September (Johnson et al., 2007). Pollination in this species is anemophilous and is more successful between than within individual plants (Bassett and Crompton, 1982). The fruit is achenia (seed) and characterized by 6–8 blunt teeth at the top, without papules, grey-green to light dark in colour. The achenes are 6–11 mm wide and 7–14 mm long (Bassett and Crompton, 1982). *A. trifida* produces up to 5,000 seeds/plant (Abul-Fath and Bazzas, 1979). Seeds of this species can germinate at a depth of 0.5 cm, but active soil seed bank is typically up to 5 cm of soil depth, however, large seed size makes it able to germinate from deep soil of 16 cm (Abul-Fath and Bazzas, 1979). Depending on the depth of seed burial, it can establish a seed bank of soil for up to 21 years (Toole and Brown, 1946; Stoller and Wack, 1974; Harrison et al., 2007).

Seeds are polymorphic (of different dimensions and colours). Larger seeds have a higher ability to germinate. The seeds fall from the plant in the period of full physical maturity (autumn). The incorporation of seeds into the soil takes place with the help of precipitation, the activity of earthworms and other predators or during tillage (Harrison et al., 2003). In addition, the germination rate of this species could also be affected by other weed species which, if their competitive characteristics enable it, hinder germination, growth and development (Savić, 2019a, b; 2020a, b; Savić et al, 2021). However, *A. trifida* germinates in early spring, so it has a potentially much better chance than other species to occupy a given area and achieve its vegetative and generative production (Savić, 2020b). *A. trifida* germinates and emerges from early spring (March/April). It germinates at wide range of temperatures (from 4 to 41 °C), with an optimum between 10 and 24 °C. It is preferable if soil moisture conditions are suitable (17% to 55% soil moisture, with an optimum at 20% to 30%)

(Ballard et al., 1996). Opposite that, Schutte et al. (2008) found that emergence occurs during relatively dry periods. Additionally, Cui et al. (2007) showed that *A. trifida* is a good accumulator of heavy metals (Pb, Zn, Cu, Cd) at the root level and it can grow successfully in the soil where the concentration of metals is quite high.

COMPETITIVE ABILITY

Many scientists have paid special attention to studying the competitive abilities of *A. trifida* (Abul-Fatih and Bazzaz, 1979; Webster, 1994; Williams and Masiunas, 2006). It is a highly competitive species that can dominate the annual plant community due to a rather early germination and emergence and high rates of growth (Webster, 1994; Malidža and Vrbničanin, 2006; Follak et al., 2013; Harrison et al. 2001; Page and Nurse, 2015). As a strong competitor, it efficiently draws water and nutrients from the soil and thus reduces the natural resources needed for the growth and development of other plants, which can lead to significant losses in crop yields. When it occurs on agricultural land, it quickly conquers space and thus hinders the growth and development of crops, especially wheat, corn, soybeans, sunflowers, beans and other crops (Weaver, 2001; Williams and Masiunas, 2006; Vrbničanin et al., 2012). *A. trifida* is more competitive and causes greater yield losses in soybeans than in maize. Some authors have reported yield losses of 13% in maize and 50% in soybean with as few as one plant of *A. trifida*/m^2 (Baysinger and Sims, 1991; Harrison et al., 2001). Additionally, Harrison et al. (2001) found yield loss in maize of 60% with 14 plants of *A. trifida*/10 m^2. According to these results, Harrison et al. (2001) predicted yield losses of maize as high as 90% if *A. trifida* density was 14 plants/m^2. Similar study determines that 1.7 *A. trifida* plant/10 m^2 reduces yield of maize by 18% (Webster et al., 1994). If we compare the influence of two different species of ragweed on maize yield, one plant/m^2 of *A. artemisiifolia* and *A. trifida* reduced maize yield by 6% and 14% (Weaver, 2001; Harrison et al., 2001). Unlike *A. artemisiifolia*, *A. trifida* reduced twice the maize yield, so compared to *A. artemisiifolia* it can be characterized as more harmful to crops (Weaver, 2001; Harrison et al., 2001). According to Vrbničanin et al. (2012) 2 plants of *A. trifida*/m^2 reduced dry mass of sunflower by 25.3%. Webster et al. (1994) found that one plant of *A. trifida*/m^2, can reduce soybean yield by 77%, unlike other species at the same conditions (*Abutilon theophrasti*, *Amarantus retroflexus*, *A. artemisiifolia*, *Chenopodium album* and *Datura stramonium*) that reduce the yield soybeans by 9, 18, 12, 14 and 15%, respectively (Rathmann and Miller, 1981; Kirkpatrick et al., 1983; Weaver, 2001; Bensch et al., 2003). According to other authors, only a few plants of *A. trífida*/m^2 reduced soybean yield by 70%, while by the similar density *Xanthium strumarium* and *A. artemisiifolia* can reduce soybean yield by 30% and 15% (Coble et al., 1981; Bloomberg et al.,1982). *A. trifida* has relative strong interspecific competitive ability (Montagnani et al., 2017). Liebman and Nichols (2020) modelled *A. trifida* population dynamics in different crop rotation, where rotations of crops (two-year corn-soybean; corn-soybean-rye-alfalfa system) have a higher probability of controlling *A. trifida* populations.

Although it can cause grave damages to the crops, it can also negatively affect biodiversity by suppressing other species (Abul-fatih and Bazzaz, 1979; Follak et al., 2013). There are not much data on studying the competition of weeds. However, some literature sources cite the results of inter/intraspecies competition between *A. trifida* and other weeds species. Savić et al. (2019a; 2020a, b; 2021) revealed that the different ratio of *A. trifida* and *A. artemisiifolia*/m^2 was reflected in all vegetative parameters. With an increase in the number of *A. trifida*/m^2 in different treatments (20/80%, 40/60%, 60/40%, 80/20%, 100/0% *A. trifida/A. artemisiifolia* /m^2) where the total numbers were 10 and 100 plant/m^2, values of vegetative parameters (height, plant width, number of leaves and dry mass) of *A. trifida* decreased. Its highest dry weight was recorded in treatments with its lowest abundance *A. trifida* (20%/m^2) compared to *A. artemisiifolia* (80%/m^2) ,while its lowest dry weight was recorded in monoculture treatment of *A. trifida* (100%/m^2), which indicates a more pronounced intraspecific competition. Taking into account the vegetative production of *A. trifida*, especially at a lower number of plants/m^2 compared to *A. artemisiifolia*, its competitive strength is more pronounced. Moreover, the large vegetative production of *A. trifida* enabled greater dominance in relation to other weed species *Chenopodium album*, *Polygonum aviculare*, *Setaria viridis*, *Bilderdykia convolvulus*, *Echinochloa crus-galli*, *Sorghum halepense* (Savić, 2020; Savić et al., 2021). Savić et al. (2021) found that due to more pronounced intraspecific competition, with a high population *A. trifida* suppressing itself, harmful effects on other species and ecosystem occurs only with a smaller number of plants per unit area (up to 40, maximum 50 plants/m^2). At high numbers, intraspecific competition occurs; the number of *A. trifida*/m^2 decreases, and this favours the growth and development of other plant species.

IMPACT OF SECONDARY METABOLITES OF *A. trifida* ON OTHER ORGANISMS

Many weed species have been characterized as highly recognizable for their secondary metabolites that influence the germination of other plants in nature (Todaria et al., 2005). In species of the genus Ambrosia, allelopathic influence on neighbouring plants has been confirmed. Several studies have shown that these species can synthesize various secondary metabolites including flavonoids, sesquiterpenes, lactones, phenolic acids, ambrosine, isabelin, psilostachine, and others. These compounds have been found to have a broad spectrum of biological activity, primarily inhibiting or stimulating the growth of other species (Beres et al., 2002; Wang et al., 2005; Kong et al., 2010).

The allelopathic effects of *A. trifida* on other plants are also well documented. Root exudates, leaf leachate, and decaying leaves produce allochemical compounds that inhibit germination and growth of other species, both in natural and agricultural environments (Kong et al., 2007). Secondary metabolites (carotenesquiterpenes, thiarubrins and thioephenes, etc.) isolated from this species show biological activities on microorganisms and nematodes and some of them inhibit the growth of other plants (Wang et al., 2006; Kong et al., 2007). Sarić-Krsmanović et al. (2020) determined components of an essential oil produced from leaves of *A. trifida* (monoterpene hydrocarbons 25%; oxygenated monoterpenes 35%; sesquiterpene hydrocarbons 22%; oxygenated sesquiterpenes 13%; phenylpropanoids 0.8%). According to these authors, an increase in essential oil concentration leads to decrease in seed germination of watermelon, lettuce, tomato and cucumber. Wang et al. (2005) revealed that the volatile oil of *A. trifida* significantly inhibited the seed germination and seedling growth of maize and wheat. Kong et al. (2007) confirmed that the high invasiveness of *A. trifida* resulted in side effects on wheat growth and yield. *A. trifida* produces sesquiterpenes of the carotene type (1 α-angelo-yloxy-carotol and 1 α-2-methyl-butyro-yloxy-carotol) and their low concentrations (20 mg^{-1}) can inhibit wheat growth by more than 10% (Williams and Masiunas, 2006; Kong et al., 2007). Wang et al. (2006) confirmed the main essential oil components of *A. trifida* (bornyl acetate, borneol, caryophyllene oxide, α-pinene, germacrene D, β-caryophyllene, trans-carveol β-myrcene, camphor, limonene) and determined strong bactericidal and fungicidal activity against *Staphylococcus aureus*, *Candida albicans*, *Klebsiella pneumoniae*, while *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Asperigillus niger* were less sensitive. In addition, *A. trifida* was recorded as a host of the *Xylella fastidiosa* (Black et al., 2004), aster yellows, tobacco mosaic, tobacco ring spot and tobacco streak viruses (Royer and Dickinson, 1999).

Interestingly, the presence of *A. trifida* may contribute to a lower number of parasitic nematodes. The study revealed a tendency that the number of nematodes in the soybean rhizosphere with the presence of *A. trifida* was lower than without *A. trifida*. Populations of several parasitic nematodes in soybean rhizospheres were suppressed by the presence of *A. trifida* and in particular, the number of *Aphelenchoides*, *Filenchus* and *Tylenchus* nematodes was significantly reduced. Additional experiments showed that the root of *A. trifida* secreted allelochemicals, such as acetylenes and their sulfur derivatives, which show high biological activity against parasitic nematodes in neighbouring soybean rhizospheres (Wang et al., 1998).

Contrary to the negative influence of this species on the ecosystem, Ahmad et al. (2013) state the positive characteristics of this species and possibilities converting of *A. trifida* biomass to biochar and its use as an adsorbent for the depuration of trichloroethylene contaminated water. Furthermore, Yakkala et al. (2013) confirmed that biochar derived from vegetative tissues of this species can be used to remove heavy metal ions from aqueous solutions.

PREVENTION AND CONTROL OF *A. trifida*

As one of the plants with strong allergenic properties, which grow in our climate, it must be systematically controlled in order to protect and improve human health and the environment, which includes the application of various measures: preventive, agrotechnical, mechanical, chemical and administrative measures. In order to control the spread of *A. trifida,* it is necessary to carry out its constant destruction. The main goal of the control programme of *A. trifida* should be based on the attempt to reduce seed dispersal and the formation of new populations in new habitats, as well as to reduce their numbers below the harmfulness threshold. The choice of measures depends on the crop in which this species is located, the method of cultivation as well as the presence of other weed species. The application of integral control measures of *A. trifida*, as in the case of other weeds, implies the application of all measures (crop rotation, tillage, planting of quality planting material, crop care, proper fertilization, application of biological control measures and herbicide application). Mowing will effectively reduce generative production. Morover, control of *A. trifida* using electrical discharges proved to be good to excellent (Rasmusson et al., 1980). In general, in the conditions of intensive agricultural production, agrotechnical measures and herbicides are mostly used. There is a little research on the control of newer weed species such as *A. trifida*.

Many publications have reported results of potential biocontrol agents of *A. trifida* (insects, fungi, bacteria). They have been identified as biological agents for suppressing ambrosia species: *Stobaera concinna*, *Trigonorhinus tomentosus*, *Tarachidia condefacta*, *Euaresta bella* and *E. festiva* (Sheppard et al., 2006), *Zygogramma suturalis*, *Epiblema strenuana* (Zhou et al., 2014). Additionally, some microorganisams can be used for biocontrol of *A. trifida*. For example, *Puccinia xanthii* forma *specialis ambrosid-trifidae* (Batra, 1981) and the bacterial pathogen *Pseudomonas syringae* pv. *tagetis* are the causal agents of a disease characterized by apical chlorosis on several members of Asteraceae. In many countries, introductions of biological control agents have been made against Ambrosia spp. in China, Australia, Russia, Georgia (Julien and Griffiths, 1998). The most of biocontrol agents are specific only to the genus of ragweed, and no doubt some of the successfully introduced agents also attacked *A. trifida* if it was present.

Registered active substances that can be used to control of *A. trifida* are: 2, 4-D, bentazone, glyphosate, chlorimuron, dicamba, diflufenzopyr, glyphosate imazaquin, acifluorfen, imazethapyr, isoxaflutole, mesotrione, prosulfuron, rimsulfuron (Weed Science Society of America, 2003). In addition to growth traits, the species propensity to develop resistance contributes to the successful survival of *A. trifida* populations. As one of the most competitive weeds in row crops, it has evolved resistance to multiple herbicide biochemical sites of action within the plant, necessitating the development of new and integrated methods of weed control. Glyphosate-resistant of *A. trifida* was first reported in 2004 (Stachler, 2008) and as of 2016 has been confirmed in 12 US states (Heap, 2016; Vink et al., 2012). Moreover, populations of this species have been reported to be resistant to (ALS) – inhibitors acetolactate synthase, which raises concerns about future chemical control choices (Patzoldt and Tranel, 2002).

ACKNOWLEDGEMENT

Ministry of Education, Science and Technological Development of the Republic of Serbia for supporting this investigation (Projects No. 451-03-9/ 2021-14/ 200116; III46008) and COST Action CA17122.

REFERENCES

Abul-Fatih HA, Bazzaz FA (1979): The biology of *Ambrosia trifida* L. II. Germination, emergence, growth and survival. *New Phytol*. 83: 817–827.

Abul-Fatih HA, Bazzaz FA (1980): The biology of *Ambrosia trifida* L. IV. Demography of plant leaves. *New Phytol*. 84: 107–111.

Ahmad M, Moon DH, Vithanage M, Koutsospyros A, Lee SS, Yang JE (2014): Production and use of biochar from buffalo weed (*Ambrosia trifida* L.) for trichloroethylene removal from water. *J. Chem. Technol. Biotechnol*. 89: 150–157.

Ardenghi NMG, Polani F (2016): La flora Della provincial di Pavia (Lombardia, Italia settentrionale). *Nat. His. Sci*. 3: 51–79.

Ballard TO, Foley ME, Bauman TT (1996): Germination, Viability, and Protein Changes during Cold Stratification of Giant Ragweed (*Ambrosia trifida* L.) seed. *J. Plant Physiol.* 149: 229–232.

Bassett IJ, Crompton CW (1982): The Biology of Canadian weeds: 55.: *Ambrosia trifida* L. *Can. J. Plant Sci*. 62: 1003–1010.

Bassett IJ, Terasmae J (1962): Ragweeds, Ambrosia species, in Canada and their history in postglacial time. *Can. J. Bot*. 40: 141–150.

Batra SWT (1981): Puccinia xanthii forma specialis ambrosia-trifidae. *Mycopathologia* 73: 61–64.

Baysinger JA, Sims BD (1991): Giant ragweed (*Ambrosia trifida*) interference in soybeans (*Glycine max*). *Weed Sci*. 39: 358–362.

Bensch CN, Horak MJ, Peterson D (2003): Interference of redroot pigweed (*Amaranthus retroflexus*), *Palmer amaranth* (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. *Weed Sci*. 51: 37–43.

Béres I, Kazinczi G, Narwal SS (2002): Allelopathic plants. Common ragweed (*Ambrosia elatior* L. Syn *A. artemisiifolia*). *Allelopathy J*. 9: 27–34.

Black MC, Sánchez MA, Davis JL (2004): Supplemental plant hosts for *Xylella fastidiosa* near four Texas hill country vineyards. *Texas Pierce's Disease*. 25: 167–170.

Bloomberg JR, Kirkpatrick BL, Wax LM (1982): Competition of common cocklebur with soybean. *Weed Sci*. 30: 507–513.

Centre for Agriculture and Bioscience International (CABI). Available to: www.cabi.org

Chauvel BA, Rodriguez C, Moreau Q, Martinez RB, Fried G (2015): Spread of *Ambrosia trifida* L. in France: historical and ecological knowledge for the eradication of the species. *J. Bot.* 71: 25–38.

Coble HD, Williams FM, Ritter, RL (1981): Common ragweed interference in soybeans. *Weed Sci*. 29: 339–342.

Cui S, Zhou Q, Chao L (2007): Potential hyperaccumulation of Pb, Zn, Cu and Cd in endurant lants distributed in an old smeltery, Northeast China. *Environ. Geol*. 51: 1043–1048.

Curtis JD, Lersten N (1995): Anatomical aspects of pollen release from staminate flowers of *Ambrosia trifida* (Asteraceae). *Int. J. Plant Sci*. 156: 29–36.

D'Amato G, Spieksma FTM (1992): European allergenic pollen types. *Aerobiologia* 8: 447–450.

D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Liccardi G, Popov T, Van Cauwenberge P (2007): Allergenic pollen and pollen allergy in Europe. *Allergy* 62: 976–990.

Dinelli G, Marotti I, Catizone P, Bosi S, Tanveer A, Abbas R, Pavlović D (2013): Germination ecology of *Ambrosia artemisiifolia* L. and *Ambrosia trifida* L. biotypes suspected of glyphosate resistance. *Open Life Sci*. 8: 286–296.

Essl F, Bir K, Brandes D, Broennimann O, Bullock JM, Chapman SD, Chauvel B, Dullinger S, Fumanal B, Guisan A, Karrer A, Kazinczi G, Kueffer C, Laitung C, Lavoie C, Leitner M, Mang T, Moser D, Muller-Scharer H, Petitpierre B, Richter R, Schaffner U, Smith M, Starfinger U, Vautard R, Vogl G, Von der Lippe M, Follak S (2015): Biological Flora of the British Isles: *Ambrosia artemisiifolia* L. *J. Ecol*. 103: 1069–1098.

Essl F, Dullinger S, Kleinbauer I (2009): Changes in the spatio-temporal patterns and habitat preferences of *Ambrosia artemisiifolia* during its invasion of Austria. *Preslia*. 81: 119–133.

Follak S, Dullinger S, Kleinbauer I, Moser D, Essl F (2013): Invasion dynamics of three allergenic invasive *Asteraceae* (*Ambrosia trifida*, *Artemisia annua*, *Iva xanthiifolia*) in central and eastern Europe. *Preslia* 85: 41–61.

Gerber E, Schaffner U, Gassmann A, Hinz HL, Seier M, Müller-Schärer H (2011): Prospects for biological control of *Ambrosia artemisiifolia* in Europe: learning from the past. *Weed Res*. 51: 559–573.

Gioria M, Osborne BA (2014): Resource competition in plant invasions: emerging patterns and research needs. *Front. Plant Sci*. 5: 501.

Harrison SK, Regnier EE, Schmoll JT, Webb JE (2001): Competition and fecundity of giant ragweed in corn. *Weed Sci*. 49: 224–229.

Harrison SK, Regnier EE, Schmoll JT (2003): Postdispersal predation of giant ragweed (*Ambrosia trifida*) seed in no-tillage corn. *Weed Sci*. 51: 955–964.

Harrison SK, Regnier EE, Schmoll JT, Harrison JM (2007): Seed size and burial effects on giant ragweed (*Ambrosia trifida*) emergence and seed demise. *Weed Sci*. 55: 16–22.

Heap I (2016): The international survey of herbicide resistant weeds.

Johnson B, Loux M, Nordby D, Sprague C, Nice G, Westhoven A, Stachler J (2007): Biology and management of giant ragweed. *The Glyphosate, Weeds, and Crops Series*. West Lafayette 98: 979–984.

Johnson WG, Gibson K, Weller S (2005): Late season weed escapes in Indiana soybean fields. *WSSA* Abstracts. 45: 57–58.

Julien MH, Griffiths MW (1998): *Biological Control of Weeds: a World Catalogue of Agents and their Target Weeds*. Fourth Edition. Wallingford, UK: CAB International.

Kazinczi G, Béres I, Novák R, Bíró K, Pathy Z (2008): Common ragweed (*Ambrosia artemisiifolia*): a review with special regards to the results in Hungary. I. Taxonomy, origin and distribution, morphology, life cycle and reproduction strategy. *Herbologia* 9: 55–91.

Kirkpatrick BL, Wax LM, Stoller EW (1983): Competition of jimsoweed in soybean. *Agron. J.* 75: 833–836.

Koljadžinski B, Šajinović B (1982): Prisustvo još jednog predstavnika roda Ambrosia L. vrste *Ambrosia trifida* L. u Vojvodini. *Fragmenta herbol. Jugosl.* 11: 5–10.

Kong CH (2010): Ecological pest management and control by using allelopathic weeds (*Ageratum conyzoides*, *Ambrosia trifida,* and *Lantana camara*) and their allelochemicals in China. *Weed Biol. Manag.* 10: 73–80.

Kong CH, Wang P, Xu XH (2007): Allelopathic interference of *Ambrosia trifida* with wheat (*Triticum aestivum*). *Agric. Eco. Environ.* 119: 416–420.

Lawalree A (1947): Les Ambrosia adventices en Europe Occidentale. *Bull. Jard. Bot. Etat Brux.* 18: 305–315.

Liebman M, Nichols VA (2020): Cropping system redesign for improved weed management: a modeling approach illustrated with giant ragweed (*Ambrosia trifida*). *Agronomy* 10: 262.

Liu JX, Wang M, Chen BX, Jin P, Li JY, Zeng K (2012): Microsporogenesis, microgametogenesis, and pollen morphology of *Ambrosia artemisiifolia* L. in China. *Plant Syst. Evol.* 298: 43–50.

Makra L, Juhász M, Béczi R, Borsos EK (2005): The history and impacts of airborne Ambrosia (Asteraceae) pollen in Hungary. *Grana* 44: 57–64.

Malidža G, Vrbničanin S (2006): Novo nalazište alohtone korovske vrste *Ambrosia trifida* L. na području Vojvodine. *VIII savetovanje o zaštiti bilja*, Zbornik rezimea, 44–45.

Montagnani C, Gentili R, Smith M, Guarino MF, Citterio S (2017): The worldwide spread, success, and impact of ragweed (Ambrosia spp.). *Crit. Rev. Plant Sci.* 36: 139–178.

Mora C, Spirandelli D, Franklin EC, Lynham J, Kantar MB, Miles W, Barba EW (2018): Broad threat to humanity from cumulative climate hazards intensified by greenhouse gas emissions. *Nat. Clim. Change.* 8: 1062–1071.

Page ER, Nurse RE (2015): Cropping systems and the prevalence of giant ragweed (*Ambrosia trifida*): From the 1950's to present. *Field Crops Res.* 184: 104–111.

Patzoldt WL, Tranel PJ (2002): Molecular analysis of cloransulam resistance in a population of giant ragweed. *Weed Sci.* 50: 299–305.

Payne WW (1964): A re-evaluation of the genus Ambrosia Compositae. *J. Arnold Arbor.* 45: 401–438.

Pyšek P, Lambdon PW, Arianoutsou M, Kühn I, Pino J, Winter M (2009): Alien vascular plants of Europe. In: *Handbook of Alien Species in Europe*, 43–61.

Rasmusson DD, Dexter AG, Warren H (1980): *The use of electricity to control weeds.* Volume 34.

Rathmann DP, Miller SD (1981): Wild oat (*Avena fatua*) competition in soybean (*Glycine max*). *Weed Sci.* 29: 410–414.

Royer F, Dickinson R (1999): *Weeds of Canada the Northern United States – A guide for identification.* Edmonton: The University of Alberta Press.

Sarić-Krsmanović M, Umiljendić JG, Radivojević L, Rajković M, Šantrić L, Đurović-Pejčev R (2020): Chemical composition of *Ambrosia trifida* essential oil and phytotoxic effect on other plants. *Chem. Biodivers.* 17: e1900508.

Savić A, Lazarević J, Vrbničanin S (2019a): Interspecijska i intraspecijska kompeticija vrsta *Ambrosia trifida* i *Ambrosia artemisiifolia*. *Acta herb.* 28: 65–75.

Savić A, Pavlović D, Božić D, Vrbničanin S (2019b): Negativan uticaj *Ambrosia artemisiifolia* i *A. trifida* na poljoprivrednu proizvodnju. *Biljni lekar* 47: 317–329.

Savić A, Mileusnić A, Pavlović D, Božić D, Vrbničanin S (2020a): The influence of *Ambrosia trifida* on vegetative production of *A. artemisiifolia*. *Pesticide and Phytomed.* 35: 105–115.

Savić A (2020b): *Kompeticija vrsta Ambrosia trifida L. i Ambrosia artemisiifolia L. u prirodnom ekosistemu.* Doktorska disertacija, Univerzitet u Beogradu, Poljoprivredni fakultet.

Savić A, Oveisi M, Božić D, Saulić M, Pavlović D, Müller-Schärer H, Vrbničanin S (2021): Competition between *Ambrosia artemisiifolia* and *Ambrosia trifida*: Is there a threat of a stronger competitor? *Weed Res*. 1–9. [https://doi.org/10.1111/wre.12479]

Schutte BJ, Regnier EE, Harrison SK (2008): The association between seed size seed longevity among maternal families in *Ambrosia trifida* L. populations. *Seed Sci. Res*. 18: 201–211.

Sheppard AW, Shaw RH, Sforza R (2006): Top 20 environmental weeds for classical biological control in Europe: a review of opportunities, regulations and other barriers to adoption. *Weed Res*. 46: 93–117.

Smith M, Cecchi L, Skjoth CA, Karrer G, Sikoparija B (2013): Common ragweed: a threat to environmental health in Europe. *Environ. Int*. 61: 115–126.

Stachler JM (2008): Characterization and management of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) and horseweed [*Conyza canadensis* (L.) Cronq.]. Ph.D.

Stoller EW, Wax LM (1974): Dormancy changes fate of some annual weed seeds in the soil. *Weed Sci*. 22: 151–155.

Strother JL (2006): *Flora of North America, North of Mexico. Magnoliophyta: Asteridae*, part 8: Asteraceae, part 3. Oxford University Press. New York, 10–18.

Szymura M, Szymura TH, Wolski K, Świerszcz S (2018): Can native grass species outcompete invasive goldenrods? Results of a replacement series experiment. *Weed Res*. 58: 304–317.

Todaria NP, Singh B, Dhanai CS (2005): Allelopathic effects of trees extract, on germination and seedling growth of field crops. *Allelopathy J.* 15: 285–293.

Toole EH, Brown E (1946): Final results of the Duvel buried seed experiment. *J. Agricul. Res.* 72: 201–210.

Vasić O (1990): *Ambrosia trifida* L. (Asteraceae) new adventive plant in Slovenia. *Razpr. Razr. Naravosl*. Vede/SAZU, 31: 391–396.

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P (2011): Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecol. Lett*. 14: 702–708.

Vincent G, Lauzer D, Cappadocia M (1988): Characterization of reciprocal hybrids of common ragweed, *Ambrosia artemisiifolia*, and giant ragweed, *A. trifida*. *Weed Sci.* 36: 574–576.

Vink JP, Soltani N, Robinson DE, Tardif FJ, Lawton MB, Sikkema PH (2012): Glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) control with preplant herbicides in soybean [*Glycine max* (L.) Merr.]. *Can. J. Plant Sci.* 92: 913–922.

Vrbničanin S, Gavrić M, Malidža G (2015): Kriterijumi, metode i rezultati kartiranja alohtonih invazivnih korova na području Srbije. U: Vrbničanin, S. (Ed.), *Invazivni korovi: invazivni procesi, ekološko-genetički potencijal, unošenje, predviđanje, rizici, širenje, štete i kartiranje*, Beograd: Herbološko društvo Srbije, 233–316.

Vrbničanin S, Malidža G, Božić D, Rajković M, Pavlović D, Sarić M, Elezović I (2012): Kompetitivni odnosi između suncokreta (*Helianthus annuus* L.) i ambrozije trolisne (*Ambrosia trifida* L.). *XIV Simpozijum o zaštiti bilja i IX Kongres o korovima*. Zbornik rezimea radova, Zlatibor, 128–129.

Wagner WH (1958): The hybrid ragweed *Ambrosia artemisiifolia trifida*. *Rhodora* 60: 309–316.

Wang Wei, Zhu Xin Ru, Liu Wei Zhi (1998): Influence of ragweed (*Ambrosia trifida*) on plant parasitic nematodes. *J. Chem. Ecol*. 24: 1707–1714.

Wang P, Liang WJ, Kong CH, Jiang Y (2005): Allelopathic potential of volatile allelochemicals of *Ambrosia trifida* L. on other plants. *Allelopathy J.* 15: 131–136.

Wang P, Kong CH, Zhang CX (2006): Chemical composition and antimicrobial activity of the essential oil from *Ambrosia trifida* L. *Molecules* 11: 549–555.

Weaver SE (2001): Impact of lamb's-quartes, common ragweed and green foxtail on yeld of corn and soybean in Ontario. *Can. J. Plant Sci.* 81: 821–828.

Webster TM, Loux MM, Regnier EE, Harrison SK (1994): Giant ragweed (*Ambrosia trifida*) canopy architecture and interference studies in soybean (*Glycine max*). *Weed Technol.* 8: 559–564.

Weed Science Society of America (2003). Available to: https://wssa.net/

Williams MM, Masiunas J B (2006): Functional relationships between giant ragweed (*Ambrosia trifida*) interference and sweet corn yield and ear traits. *Weed Sci.* 54: 948–953.

Yakkala K, Yu RM, Roh H, Yang JK, Chang YY (2013): Buffalo weed *Ambrosia trifida* L. var. *trifida* biochar for cadmium II lead II adsorption in single mixed system. *Desalin Water Treat*. 51: 7732–7745.

Zhou ZS, Chen HS, Zheng XW, Guo JY, Guo W, Li M, Wan FH (2014): Control of the invasive weed *Ambrosia artemisiifolia* with *Ophraella communa* and *Epiblema strenuana*. *Biocontrol Sci. Technol*. 24: 950–964.

ПРЕГЛЕДНИ НАУЧНИ РАД

Ambrosia trifida L. (АМБРОЗИЈА ТРОЛИСНА)

АЛЕКСАНДРА М. САВИЋ

Институт за заштиту биља и екологију
Одсек за хербологију
Теодора Драјзера 9, Београд 11000, Србија

РЕЗИМЕ: *Ambrosia trifida* L. припада групи инвазивних корова који у колонизованим подручјима наносе велику штету биодиверзитету аутохтоне флоре и вегетације, као и биљној производњи. Пореклом је из Северне Америке, одакле се раширила у многе делове Европе. У Србији је тренутно локално присутна на подручју централне Бачке. Рана и брза стопа раста, вегетативна и генеративна производња, висок степен морфолошке и репродуктивне способности, омогућили су јој конкурентску предност у односу на многе друге корове, па се у многим земљама сматра једним од најпроблематичнијих корова у пољопривредној производњи. *A. trifida* би могла проузроковати велике штете у окопавинама, повртњацима и вочњацима, а њена штетност се мери и негативним утицајем на биодиверзитет потискујући алохтоне и аутохтоне врсте. Својим алергенима негативно утиче на здравље људи. Имајући у виду њен вегетативни и генеративни потенцијал и узимајући у обзир климатске промене (које су захватиле многе земље), новија истраживања указују могућност ширења ове врсте у нашој земљи и Европи, што би могло представљати озбиљан ризик за агрофитоценозе и екосистем у целини. Од 2019. године додат је на EPPO A2 листу препоручених карантинских штеточина. Може се сузбијати механичким, биолошким и хемијским мерама.

КЉУЧНЕ РЕЧИ: *Ambrosia trifida* L., алергене врсте, губици приноса, инвазивни корови, компетиција, секундарни метаболити, сузбијање корова

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 141, 49—57, 2021

UDC 66.095.268:630
https://doi.org/10.2298/ZMSPN2141049G
ORIGINAL SCIENTIFIC ARTICLE

*Milica M. GALIĆ, Mirjana M. STAJIĆ, Jasmina LJ. ĆILERDŽIĆ**

University of Belgrade, Faculty of Biology
Takovska 43, Belgrade 11000, Serbia

"GREEN" APPROACH IN UTILIZATION OF COMMON AGROFORESTRY RESIDUES BY *Laetiporus sulphureus* ENZYMES' COCKTAIL

ABSTRACT: *Laetiporus sulphureus* BEOFB 1040 has shown the capacity to produce ligninolytic enzymes and consequently degrade eight common agroforestry residues. The highest activities of Mn-dependent and Mn-independent peroxidases (2079.55 U/L and 492.42 U/L, respectively) were detected after treatment of plum and oak sawdust, while laccases synthesis was inhibited by all tested residues. However, despite laccase absence this brown-rot species was not only a good depolymerizer of cellulose and hemicellulose but also an effective delignifier of selected residues. The highest level of lignin content reduction of 43.09% along with high degradation of holocellulose (35.24% of cellulose and 38.42% of hemicellulose) was noted in raspberry sawdust. On the other hand, a selectivity index of even 4.55 was achieved on wheat straw where only 20.27% of lignin was degraded. Consequently, the highest and the lowest dry matter loss (21.25% and 5.83%) were noted on raspberry sawdust and wheat straw, respectively. These results indicate that *L. sulphureus* BEOFB 1040 is a mushroom with a strong potential for different biotechnological applications and certainly deserves further studies.

KEYWORDS: agroforestry residues, *Laetiporus sulphureus*, ligninolytic enzymes, lignocellulose depolymerization

INTRODUCTION

In line with the constant growth of an already large human population, increased industrialization, climate changes and ecosystem disturbance, natural resources exhaustion is bound to happen.

On the other hand, numerous persistent pollutants harmful to the environment are generated and accumulated in enormous amounts. These facts raise

* Corresponding author. E-mail: simonicj@bio.bg.ac.rs

serious concerns that initiate many studies and focus on finding effective solutions (Bumbac et al., 2010; Hyde et al., 2019; Ferreira et al., 2020; Morsi et al., 2020). Nowadays, the emphasis is on the development of a bio-based economy, i.e. on the processes which improve the utilization of resources with by-products that could be further used as resources in some new processes (Scarlat et al., 2015). There is a growing need for optimization of biomass treatment that could contribute to the sustainability of agriculture and many industry branches.

Lignocellulosic biomasses, such as crop and forest residues, municipal and food industry solid waste, are generated in enormous annual amount worldwide and could be either serious environmental ballast or perspective eco-friendly resource for the production of biofuels, various chemicals, biofibers, array of enzymes, and many other products (Ghaffar et al., 2015; Sharma et al., 2019). Thus, in addition to the huge amount of globally produced wheat straw and corn stalks as the most abundant crop residues, the quantity of locally important ones is not negligible. For example, according to Food and Agriculture Organization Corporate Statistical Database, average annual amounts of raspberry and blackberry only in Serbia as the leading producer of these fruits are 60,000 tons and 25,440 tons, respectively, which imply remarkable quantity of their cuttings. However, lignocellulose consists of a cellulose-hemicellulose matrix immersed in a net of lignin and because of this complex structure, high lignin persistent and strong lignin-holocellulose bond, its transformation represents a great challenge (Isroi et al., 2011; Ćilerdzić et al., 2017; Sharma et al., 2019). The main challenge is a degradation of lignin, the most recalcitrant natural polymer, with physico-chemical depolymerization having several harmful effects on the environment, and ecologically justified and sustainable conversion can be performed by only a few bacterial species and mushrooms, primarily white-rot ones (Sanchez, 2009; Hyde et al., 2019; Sharma et al., 2019).

White-rot species are excellent producers of various ligninolytic enzymes and therefore play a crucial role in pretreatment, i.e. the first phase of lignocelluloses utilization. However, brown-rot fungi could also be a biotechnologically interesting group because of their ability to primarily successfully depolymerize hemicellulose and cellulose but also modify lignin to some extent (Singh et al., 2014; Ghaffar et al., 2015). *Laetiporus sulphureus* (Bull.) Murrill is a member of this group and its unique taste led to intensive studies of nutritional value and medicinal effects as well as to the development of a method for cultivation at the industrial scale. However, knowledge on its enzymatic systems and degradation potential, especially ligninolytic ones, is scarce. Considering the mentioned significance of this species, we defined the goals of the study, determination of ability to synthesize ligninolytic enzymes and their activity profiles as well as the extent of depolymerization of common agroforestry residues.

MATERIALS AND METHODS

Organism and cultivation conditions

The culture of *Laetiporus sulphureus* BEOFB 1040 was isolated from fruiting bodies collected from *Fraxinus* sp. in Belgrade (Serbia) and maintained on Malt agar medium at 4 °C, in the culture collection of the Institute of Botany, Faculty of Biology, University of Belgrade (BEOFB).

The inoculum was obtained by inoculation of synthetic medium (glucose, 10.0 g/L; NH_4NO_3, 2.0 g/L; K_2HPO_4, 1.0 g/L; $NaH_2PO_4 \cdot H_2O$, 0.4 g/L; $MgSO_4 \cdot 7H_2O$, 0.5 g/L; yeast extract, 2.0 g/L; pH 6.5) with a mycelium of 7-day old culture, incubation at room temperature (22 ± 2 °C) on a rotary shaker for 7 days, washing of harvested biomass with sterile distilled water (dH_2O) and its homogenization with dH_2O in a laboratory blender (Waring, USA) (Stajić et al., 2010).

Selected agro-forestry residues (apple-, blackberry-, grapevine-, oak-, plum- and raspberry sawdust, corn stalks and wheat straw, in the amount of 6.0 g) were soaked with 30.0 mL of the modified synthetic medium (without glucose) into 250 mL-flasks, inoculated with 9.0 mL of the homogenized inoculums and cultivated at 25 °C during 21 days (Stajić et al., 2010).

Determination of enzyme activity

Extracellular ligninolytic enzymes were extracted by stirring of 21-day old samples with 50.0 mL dH_2O a magnetic stirrer at 4 °C for 10 min. Thus obtained extracts were centrifuged (at 4 °C and 3000 rpm, for 15 min), and resulting supernatants were used for spectrophotometrically (BioQuest CECIL CE2501, UK) determination of activities of Mn-oxidizing peroxidases [Mn-dependent peroxidases (MnP, EC 1.11.1.13) and Mn-independent peroxidases (MnIP, EC 1.11.1.16)] and laccase (Lac, EC 1.10.3.2).

The activities of Mn-oxidizing peroxidases and laccases were determined according to the methods of Ćilerdžić et al. (2017) using 3mM phenol red (ε_{610} = 22000 $M^{-1}cm^{-1}$) and 2,2'-azino-bis-[3-ethyltiazoline-6-sulfonate] (ABTS) (ε_{436} = 29300 M^{-1} cm^{-1}), respectively, as the substrates. Enzymatic activity was expressed in U/L, and activity of 1U presents the amount of enzyme that transforms 1 µmol of substrate per min.

All the experiments were done in triplicate and the results were expressed as mean ± standard error.

Determination of lignin, cellulose and hemicellulose contents

The loss of substrate dry matter (%) was determined by equation

$$(Mi - Mf)/Mi \times 100$$

where M*i* presents the initial lignocellulosic mass and M*f* the mass after fermentation.

The contents of lignin, cellulose, and hemicellulose were determined by modified methods of Kirk and Obst (1988) and Van Soest et al. (1991). Dried ground sample (1.0 g) was treated with neutral detergent/Na_2SO_3 mixture under refluxing conditions to remove soluble sugars, proteins, lipids, and vitamins. The obtained biomass presented neutral detergent fibers (NDF). Acidic detergent fibers (ADF) were obtained by the treatment of the samples with acidic detergent solution, and the difference between NDF and ADF presented hemicellulose amount. After sample incubation with 72% H_2SO_4 at 30 °C and hydrolysis at 120 °C, lignin content (LC) was determined and expressed as the percentage of quantity presented in the initial sample. Cellulose content was calculated as the difference between ADF and LC.

The selective capability of lignin degradation was expressed as a selectivity index (SI), which presents a ratio between amounts of removed lignin and cellulose.

RESULTS AND DISCUSSION

The obtained results clearly showed that the activity of Mn-oxidizing peroxidases depends on the carbon source *i.e.* the type of substrate. Thus, the studied *L. sulphureus* BEOFB 1040 showed considerable variability in enzymes' activities after cultivation on selected agroforestry residues (Figure 1). In the case of MnP, tested species secreted its highly active isoforms on plum sawdust (2079.55 U/L). The moderate activity was noted after blackberry sawdust fermentation (691.92 U/L), oak sawdust, and wheat straw induced significantly weaker activity of 125.00 U/L, while only 32.20 U/L was detected on corn stalks (Figure 1).

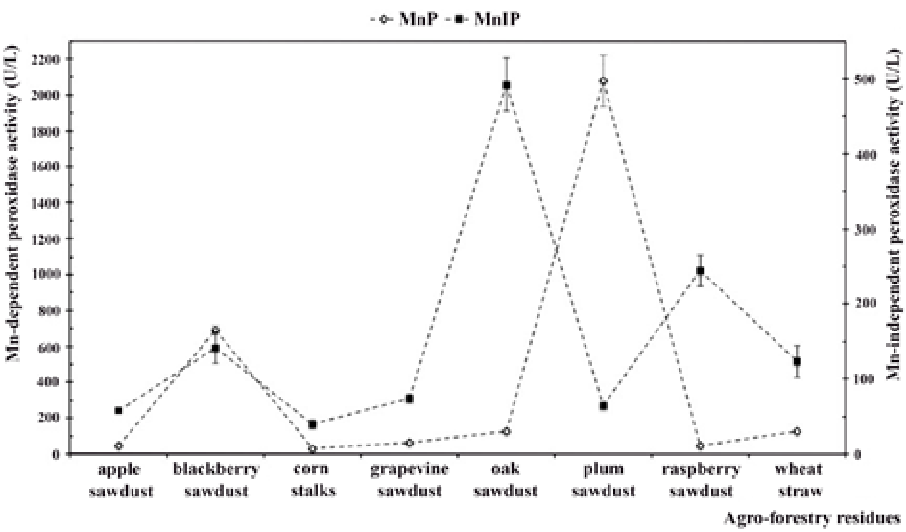

Figure 1. Mn-oxidizing peroxidases activity of *L. sulphureus* BEOFB 1040 during agro-forestry residues fermentation

Different profile of MnIP activity was observed on tested agro-forestry residues (Figure 1). Generally, regarding MnP, *L. sulphureus* BEOFB 1040 showed lower values of MnIP activity and slightly weaker variation depending on substrates type. Namely, the highest level of the enzyme activity (492.42 U/L) was induced by oak sawdust. Furthermore, MnIP production was also well induced by raspberry sawdust (244.32 U/L). However, contrary to MnP, plum sawdust caused synthesis of MnIP isoform which activity was only 64.39 U/L, while similarly to MnP, corn stalks were the weakest inducer of production active MnIP isoform (30.00 U/L) (Figure 1). Laccase activity was not detected after fermentation of all tested plant residues by studied species reason of which could be in the period of cultivation of 21 days.

Generally, in comparison to white-rot species, not much data is available about lignin-degrading enzymes of brown-rot fungi. Comparing to our results, Thakur and Tripathi (2020) reported extremely low MnP activity (~5.00 U/L) for *Postia placenta* after 10 to 15 days of cultivation in the nutrient-rich and nutrient-poor medium under static and shaking conditions. However, this species produced a slightly higher active MnIP isoform (55.00 U/L) after 10 days of cultivation, which was similar to our results obtained after apple sawdust fermentation. Contrary to *L. sulphureus* BEOFB 1040, four strains of brown-rot species *Coniophora puteana*, studied by Lee et al. (2004), synthetized laccase with activity peak of 3.5 U/mL detected on day 5^{th} of submerged fermentation of oak sawdust.

Despite lacking active laccase on the 21^{st} day of cultivation, *L. sulphureus* BEOFB 1040 showed significant efficiency and selectivity in tested lignocellulose depolymerization (Table 1). Although the correlation between enzyme activity and delignfication level was not achieved, this strain was not only a good depolymerizer of cellulose and hemicellulose but also an effective delignifier of the plant residues (Figure 1; Table 1).

The highest level of lignin content reduction (43.09%) was noted after raspberry sawdust fermentation, but high degradation of holocellulose (35.24% of cellulose and 38.42% of hemicellulose) along with lignin resulted in a low selectivity index (1.22). On the other hand, *L. sulphureus* weakly depolymerized cellulose (4.45%) in wheat straw which in combination with high delignification percentage (20.27%) resulted in the highest selectivity index of even 4.55. However, this species was an even better delignifier of blackberry and oak sawdust (32.09% and 24.23%, respectively) but its high consumption of cellulose (20.07% and 12.06%, respectively) resulted in selectivity indices of 1.60 and 2.01. The lowest selectivity in lignocellulose degradation of 0.66 and 0.83 was observed after fermentation of plum and grapevine sawdust, respectively (Table 1).

Results on lignocelluloses depolymerization obtained in the present study are in accordance with data on *Coniophora puteana* and its great capacity to degrade all cell wall layers despite low enzymes' activities (Lee et al., 2004). On the other hand, our results are also consistent with the results of Nurika et al. (2020) who pointed out that the differences in the lignocellulose composition certainly affect the extent of depolymerization. An interesting fact is that some brown-rot species, such as *Serpula lacrymans*, which is considered not

Table 1. The level of agro-forestry residues depolymerization (%) by *Laetiporus sulphureus*

Agro-forestry residues	**Samples**	**Sample weight** (g)	**Polymers composition of samples** (mg)			**Dry matter loss** (%)	**Extent of polymers degradation** (%)			SI
			Lignin	**Cellulose**	**Hemicellulose**		**Lignin**	**Cellulose**	**Hemicellulose**	
Apple sawdust	Control*	6.00	1158.00	2808.00	1176.00	/	/	/	/	/
	BEOFB 1040	4.81	889.48	2202.06	822.17	19.83	23.19	21.58	30.09	1.07
Blackberry sawdust	Control*	6.00	1218.00	2712.00	1038.00	/	/	/	/	/
	BEOFB 1040	4.75	827.20	2167.82	798.67	20.83	32.09	20.07	23.06	1.60
Corn stalks	Control*	6.00	594.00	2796.00	1860.00	/	/	/	/	/
	BEOFB 1040	5.49	395.14	2014.10	1602.00	8.50	33.48	27.97	13.89	1.20
Grapevine sawdust	Control*	6.00	1421.41	2652.00	887.08	/	/	/	/	/
	BEOFB 1040	4.96	1184.24	1973.68	674.42	17.35	16.97	25.58	23.97	0.66
Oak sawdust	Control*	6.00	1530.00	2808.00	1159.00	/	/	/	/	/
	BEOFB 1040	5.42	1159.24	2470.15	980.48	9.70	24.23	12.06	15.47	2.01
Plum sawdust	Control*	6.00	1837.49	2544.00	1368.00	/	/	/	/	/
	BEOFB 1040	5.01	1432.29	1872.99	761.22	16.53	22.05	26.38	44.36	0.83
Raspberry sawdust	Control*	6.00	1200.00	2160.00	1308.00	/	/	/	/	/
	BEOFB 1040	4.71	682.95	1398.87	805.41	21.25	43.09	35.24	38.42	1.22
Wheat straw	Control*	6.00	666.00	2418.00	1692.00	/	/	/	/	/
	BEOFB 1040	5.65	531.01	2310.44	1587.37	5.83	20.27	4.45	6.18	4.55

to utilize lignin in metabolism, possess the ability to degrade it to a considerable extent (Nurika et al., 2020). Singh et al. (2014) reported that wheat straw was not delignified by *L. sulphureus* DSM11211, while the percentage of removed lignin from digestate remaining from maize silage by *Gleoeophyllum trabeum* was <10% after even 28 days of their fermentation, which showed higher ligninolytic potential of *L. sulphureus* strain researched in the present study.

Differences in lignocellulose depolymerization extent reflected on the level of total dry mass reduction. Thus, the highest dry matter loss was detected for raspberry sawdust (21.25%) and slightly lower for blackberry and apple sawdust (20.83% and 19.83%, respectively), while the smallest percentage of the reduction was observed after wheat straw fermentation (5.83%) (Table 1). Nurika et al. (2020) also showed that *S. lacrymans* possessed various capacities to reduce the weight of agricultural residues during 35 days, from 16% in sugarcane bagasse to 32% in corn leaves. Comparing the results of the weight loss of corn residues between this and our study after the same period of fermentation, *S. lacrymans* achieved a higher drop in mass.

CONCLUSIONS

It has long been thought that all brown-rot fungi use the same mechanism for lignocellulose decay and that their ligninolysis is limited to minor oxidative modifications. However, numerous studies, including this one, showed that degrading mechanisms differ among them, which could be of great significance because they can offer a novel approach for the pretreatment of various residues and thus increasing the potential of the biorefinery. We have successfully screened lignocellulolytic potential of *L. sulphureus* BEOFB 1040 and reported its good efficiency in breaking up the lignin together with significant capacity to degrade cellulose and hemicellulose of most frequent agro-forestry residues in Serbia.

ACKNOWLEDGEMENT

This study was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2020-14/200178).

REFERENCES

Bumbac C, Pena-Leonte E, Dumitrescu C, Ghita I, Stefanescu M (2010): Heavy metals removal using residual fungal biomass. *J. Environ. Prot. Ecol.* 11: 822–829.

Ćilerdžić J, Galić M, Vukojević J, Brčeski I, Stajić M (2017): Potential of Selected Fungal Species to Degrade Wheat Straw, the Most Abundant Plant Raw Material in Europe. *BMC Plant Biol.* 17: 75–81.

Ćilerdžić J, Galić M, Vukojević J, Stajić M (2019): *Pleurotus ostreatus* and *Laetiporus sulphureus*–Possible Agents Against Alzheimer and Parkinson Diseases. *Int. J. Med. Mushrooms* 21: 275–289.

Ferreira JA, Varjani S, Taherzadem MJ (2020): A critical review on the ubiquitous role of filamentous fungi in pollution mitigation. *Curr. Pollut. Rep.* 6: 295–309.

Ghaffar SH, Fan M, McVicar B (2015): Bioengineering for utilisation and bioconversion of straw biomass into bio-products. *Ind. Crop. Prod.* 77: 262–274.

Hyde KD, Xu J, Rapior S, Jeewon R, Lumyong S, Niego AGT, Chaiyasen A et al. (2019): The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Divers*. 97: 1–136.

Isroi I, Millati R, Niklasson C, Cayanto C, Taherzadeh MJ, Lundquist K (2011): Biological treatment of Lignocelluloses with white-rot fungi and its applications. *BioResources* 6: 5224.

Kirk TK, Obst JR (1988): Lignindetermination. In: Colowick, SP, Kaplan NO (eds.), *Methods in Enzymology* 161, SanDiego: AcademicPressInc. 87–101.

Lee KH, Wi SG, Singh AP, Kim YS (2004): Micromorphological characteristics of decayed wood and laccase produced by the brown-rot fungus *Coniophora puteana*. *J. Wood Sci.* 50: 281–284.

Morsi R, Bilal M, Iqbal HMN, Ashraf SS (2020): Laccases and peroxidases: The smart, greener and futuristic biocatalytic tools to mitigate recalcitrant emerging pollutants. *Sci. Total Environ.* 714: 136572.

Nurika I, Suhartini S, Barker GC (2020): Biotransformation of tropical lignocellulosic feedstock using the brown rot fungus *Serpula lacrymans*. *Waste Biomass Valori*. 11: 2689–2700.

Sánchez C (2009): Lignocellulosic residues: biodegradation and bioconversion by fungi. *Biotechnol. Adv.* 27: 185–194.

Scarlat N, Dallemand JF, Monforti-Ferrario F, Nita V (2015): The role of biomass and bioenergy in a future bioeconomy: Policies and facts. *Environ. Develop.* 15: 3–34.

Sharma HK, Xu C, Qin W (2019): Biological pretreatment of lignocellulosic biomass for biofuels and bioproducts: an overview. *Waste Biomass Valori*. 10: 235–251.

Sillo F, Gianchino C, Giordano L, Mari M, Gonthier P (2018): Local epidemiology of the wood decay agent *Laetiporus sulphureus* in carob stands in Sicily. *Forest Pathol*. 48: e12414.

Singh S, Harms H, Schlosser D (2014): Screening of ecologically diverse fungi for their potential to pretreat lignocellulosic bioenergy feedstock. *Appl. Microbiol. Biotechnol*. 98: 3355–3370.

Stajić M, Kukavica B, Vukojević J, Simonić J, Veljović-Jovanović S, Duletić-Laušević S (2010): Wheat Straw Conversion by Enzymatic System of *Ganoderma lucidum*. *BioResources* 5: 2362.

Thakur N, Tripathi A (2020): Comparative analysis of extracellular ligninolytic enzymes in wild white-rot fungi *Stereum hirsutum* and brown-rot fungi *Postia placenta* collected from Shilly forest of Himachal Pradesh. *Asia Life Sci.* 12: 16–27.

Van Soest PV, Robertson JB, Lewis BA (1991): Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci*. 74: 3583–3597.

ОРИГИНАЛНИ НАУЧНИ РАД

„ЗЕЛЕНИ" ПРИСТУП У КОРИШЋЕЊУ ЧЕСТИХ ОСТАТАКА ИЗ ПОЉОПРИВРЕДЕ И ШУМАРСТВА ПОМОЋУ ЕНЗИМСКОГ КОКТЕЛА *Laetiporus sulphureus*

Милица М. ГАЛИЋ, Мирјана М. СТАЈИЋ, Јасмина Љ. ЋИЛЕРЏИЋ

Универзитет у Београду, Биолошки факултет
Таковска 43, Београд 11000, Србија

РЕЗИМЕ: *Laetiporus sulphureus* BEOFB 1040 је показао способност да продукује лигнинолитичке ензиме и тако деградује осам честих остатака из пољопривреде и шумарства. Највеће активности Mn-зависне и Mn-независне пероксидазе (2079,55 U/L односно 494,42 U/L) су детектоване после третмана пиљевине шљиве и храста, док је синтеза лаказа била инхибирана свим тестираним остацима. Међутим, упркос одсуству лаказе ова врста изазивач браон труљења није била само добар деполимеризатор целулозе и хемицелулозе већ и ефикасан делигнификатор одабраног биљног отпада. Највиши ниво редукције садржаја лигнина (43,09%) заједно са значајном деградацијом холоцелулозе (35,24% целулозе и 38,42% хемицелулозе) је био забележен код пиљевине малине. Са друге стране, индекс селективности од чак 4,55 је постигнут на пшеничној слами где је само 20,27% лигнина било деградовано. Очекивано, највећи и најмањи губитак суве масе (21,25% и 5,83%) су забележени на пиљевини малине, односно пшеничној слами. Ови резултати указују да је *L. sulphureus* BEOFB 1040 врста са снажним потенцијалом за примену у различитим биотехнолошким процесима и да заслужује даља истраживања.

КЉУЧНЕ РЕЧИ: деполимеризација лигноцелулозе, *Laetiporus sulphureus*, лигнинолитички ензими, остаци из пољопривреде и шумарства

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 141, 59—67, 2021

UDC 633.34:631.15
https://doi.org/10.2298/ZMSPN2141059G
ORIGINAL SCIENTIFIC ARTICLE

Jelena M. GOLIJAN[*1], *Dušica JOVIČIĆ*[2], *Slavoljub S. LEKIĆ*[1], *Mile D. SEČANSKI*[3]

[1] University of Belgrade, Faculty of Agriculture
Department of Genetics, Plant Breeding and Seed Production
Nemanjina 6, Belgrade 11080, Serbia
[2] Institute of Field and Vegetable Crops
Maksima Gorkog 30, Novi Sad 21000, Serbia
[3] Maize Research Institute "Zemun Polje"
Slobodana Bajića 1, Belgrade 11085, Serbia

EFFECTS OF PRODUCTION METHODS ON SEED VIGOUR OF SOYBEAN SEEDS

ABSTRACT: The seed accelerated ageing test is one of the most important tests for testing seed vigour, which provides the determination of the degree of preservation for germination and the determination of the duration of the seed storage. The aim of this study was to observe effects of two different production methods (organic and conventional) on seed vigour of soybean cultivar *Kaća*, by the application of the seed accelerated ageing test. The seeds were exposed to stress conditions for 72 h (temperature of 45 ºC and air humidity of 100%). After the test was applied, the number of non-germinated seeds of organically produced soybean increased, which resulted in the reduction of the germination percentage. Compared to the standard laboratory method, after the seed accelerated ageing test was applied, the length of the seedling above-ground part (121.63 mm), fresh weight of the seedling above-ground part (8.9 g) and dry weight of the seedling above-ground part (1,05 g) were higher. Moreover, the length (100.25 mm), fresh (1.26 g) and dry weight (0.1 g) of the root were also higher. After the test was applied, the percentage of the off-type seedlings (10.75%) and non-germinated seeds (26%) was higher in conventionally produced soybean seeds, while the length of the seedling above-ground part (100.63 mm), root length (106.75 mm) and root fresh weight (1.39 g) were lower.

KEYWORDS: accelerate ageing test, conventional production, organic production, soybean

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) belongs to the family of legumes and is one the oldest cultivated species in the world. The mature soybean grain

[*] Corresponding author. E-mail: golijan.j@agrif.bg.ac.rs

typically contains about 40% proteins, 20% oil, 17% cellulose and hemicellulose, 7% sugar, 5% solid fibres and approximately 6% dry weight-based ash (Ciabotti et al., 2016). Such a favourable grain chemical composition makes soybean one of the most important sources of vegetable oils and proteins in the world, due to which it ranks highly in the proper diet (Hoffman and Falvo, 2004). The soybean production in Serbia has been growing. Since 2008, its favourable price has been contributing to the increased production. Moreover, the demand for soybean is high both in international and national markets. According to Kalentić et al. (2014), organic protein crops, primarily soybean, used as feedstuff of organically raised livestock, are highly imported in Germany and thus can be a significant export item of Serbian producers. The analysis of the agricultural production index indicates that a cyclical phenomenon of extreme weather conditions during the last decade strongly influenced the plant production, and these changes were especially obvious in the areas cultivated with soybean (Živanović and Popović, 2016). The production and processing of organically produced soybean have been increasing. Organic farming maintains and improves soil biodiversity, controls and increases soil fertility, protects the environment and applies the highest standards for the protection of plant and animal health (Ugrenović and Filipović, 2012).

The seed accelerating ageing test is characterised by the seed exposure to two changeable environmental factors, high temperature and high relative humidity, in a short period, which affect seed deterioration. High-vigour seeds endure these stress conditions and deteriorate more slowly with maintained high germination even after ageing in contrast to low-vigour seeds. Several already performed studies have shown that this test provided a very precise assessment of seed vigour in soybean, maize and some other crops. Quite a few studies have confirmed that the results of this test provided better predictability of field seed germination under stress conditions than standard germination tests (TeKrony, 2005). During the seed accelerating ageing test, seeds absorb water from the wet environment; hence there is a moisture increase in seeds, which together with a high temperature to which seeds are exposed, results in the process of accelerated ageing and deterioration of seeds (ISTA, 2014).

MATERIALS AND METHODS

Samples

Seeds of organically and conventionally grown soybean cultivar NS *Kaća* originated from the experimental plot of the Institute of Field and Vegetable Crops in Novi Sad (Bački Petrovac). A sown plot size amounted to three hectares. Prior to primary tillage, fertilisation with 120 kg NPK ha^{-1} at the ratio of 16:16:16 was applied in the conventional production, while fertilisers were not applied in the organic production. Appropriate herbicides based on imazamox and quizalofop-P-tefuryl were applied in the conventional production, while mechanical weed control, by manual hoeing was applied in the organic production.

Accelerated ageing test

Soybean seed vigour was determined by the accelerated ageing test. Seeds were exposed to double stress conditions – high temperature (41 °C) and high relative air humidity (100%) for 72 hours. After that, seeds were sown in the sand to determine seed germination.

The standard laboratory test, with four replications of 100 seeds, was applied to determine soybean seed germination and it was expressed as percentages (ISTA Rules, 2016). The germination first count was done on the fifth day, while germination was read on the eighth day (ISTA Rules, 2016). The seed germination percentage was determined by calculating the proportion of normal seedlings in the working sample. When seed incubation was completed, the following growth parameters were determined: the length of above-ground parts of seedlings and roots (mm), fresh and dry weights of the seedling above-ground parts and the root fresh and dry weights (g). The length of above-ground parts of seedlings and roots was determined by measuring the mean values of 10 seedlings from each replication, using a ruler. The fresh weights of the above-ground parts and roots were established by measuring mean values of 10 seedlings in four replications, in which, after drying in a thermostat at the temperature of 80 °C for 24 hours, the seedling dry weight was measured.

Statistical analysis

Results of all analyses were expressed as the mean of three measurements ± standard deviation (SD), while the significance of differences between the means ($p < 0.05$) was determined by using Tukey's test, software Statistica, version 12.0 (StatSoft Inc., USA). The correlation analysis between observed parameters in the accelerated ageing test and the standard germination test was performed by calculating the Pearson's correlation coefficient (r) ($p < 0.05$).

RESULTS AND DISCUSSION

The results of the vigour test of organically produced soybean seeds are presented in Table 1.

After the application of the accelerated ageing test, a statistically significant decrease in the first count and germination (26.75% and 29.25%, respectively) was recorded compared to the standard germination test (52.00% and 54.25%, respectively). Moreover, the percentage of non-germinated seeds statistically significantly increased to 61.75%. The percent of off-type seedlings did not statistically significantly differ between these two tests. Such results indicate that double stress conditions of the high temperature and high relative air humidity in the accelerated ageing test increased the number of non-germinated organic soybean seeds and thus lowered the germination percentage, which affected seed deterioration. The length of the seedling above-ground part (121.63 mm), fresh (8.93 g) and dry weights (1.05 g) of the seedling above-ground part were higher after the accelerated ageing test, while the root length (100.25 mm), and root fresh (1.25 g) and dry (0.12 g) weights were greater in the standard germination test. However, these differences were not statistically significant.

Table 1. Results of soybean seed accelerated ageing test

Parameter	Production Method			
	Organic		Conventional	
	Test		Test	
	Germination standard test	Accelerated ageing test	Germination standard test	Accelerated ageing test
Germination first count (%)	52,00±5,35aA	26,75±6,18bA	59,25±5,8aA	60,00±2,16aB
Germination (%)	54,25±6,02aA	29,25±2,22bA	73,00±4,24aB	63,25±2,63bB
Off-type seedlings (%)	7,00±1,83aA	9,00±1,83aA	4,75±0,96aA	10,75±0,96bA
Non-germinated seeds (%)	38,75±5,56aA	61,75±2,5bA	22,25±4,03aB	26,00±2,94aB
Length of seedling above-ground part (mm)	112,75±12,87aA	121,63±4,78aA	114,75±4,05aA	100,63±11,96aB
Root length (mm)	100,25±20,16aA	74,13±7,44aA	176,63±13,37aB	106,75±10,02bB
Fresh weight of seedling above-ground part (g)	8,76±0,55aA	8,93±0,97aA	7,96±0,38aA	8,27±0,61aA
Root fresh weight (g)	1,25±0,37aA	1±0,22aA	2,24±0,3aB	1,39±0,38bA
Dry weight of seedling above-ground part (g)	1,01±0,07aA	1,05±0,15aA	0,97±0,04aA	1,02±0,05aA
Root dry weight (g)	0,12±0,03aA	0,1±0,02aA	0,15±0,01aA	0,11±0,02bA

* Lowercase letters (a, b) indicate the statistical significance between the standard germination test and the accelerated ageing test (**n=4**, **SV±SD**), ***p<0.05*** (*Tukey's* test). Capital letters (A, B) indicate the statistical significance between organic and conventional production methods (**n=4**, **SV±SD**), ***p<0.05*** (*Tukey's* test).

After the application of the accelerated ageing test to determine vigour of conventionally produced soybean seeds (Table 1), a statistically significant reduction in seed germination (to 63.25%) was established, and also a slight difference in the first count in comparison to the standard laboratory test (60.00%). On the other hand, compared to the standard germination test, there was a statistically significant increase in the percentage of the off-type seedlings (10.75%), while the root length (106.75 mm), root fresh (1.39 g) and dry (0.11 g) weights were decreased. The exposure of seeds to the high temperature and high relative air humidity resulted in the increase in the number of non-germinated seeds and the occurrence of the off-type seedlings, thus seed germination was reduced. Compared to the standard germination test, the higher values of fresh (8.27 g) and dry (1.02 g) weights of the above-ground parts did not differ significantly after the application of the accelerated ageing test.

From the aspect of differences in the standard germination test between organically and conventionally produced soybean seeds, the statistical significance was recorded for germination (higher in conventionally produced seeds – 73.00%), non-germinated seeds (higher in organically produced seeds – 38.75%), root length (higher in conventionally produced seeds – 176.63 mm) and the root fresh weight (higher in conventionally produced seeds – 2.24 g). Comparing differences in the accelerated ageing test between organically and conventionally produced soybean seeds, the statistical significance was determined for the fist count (twice as large in conventionally produced seeds – 60%), germination (twice as large in conventionally produced seeds – 63.25%), non-germinated seeds (twice as large in organically produced seeds – 61.75%), length of the seedling above-ground part (longer in organically produced seeds – 121.63 mm) and the root length (longer in conventionally produced seeds – 106.75 mm).

Table 2 shows the values of correlation coefficients among the measurands. Mean values calculated from four replications, both production methods, both tests and both seed types, were used to determine the correlation coefficient. The highest positive correlation was recorded between the root length and the root fresh weight (r = 0.998), root length and the root dry weight (r = 0.969), as well as the first count and germination (r = 0.964). The highest negative correlations were determined between germination and non-germinated seeds (r = -0.991), first count and non-germinated seeds (r = -0.981), and also between the dry weight of the seedling above-ground part and the root dry weight (r = -0.981).

During the soybean seed ageing, biochemical changes occur and have a very strong impact on seed quality and vigour (Tatić, 2007). In addition, a specific chemical composition of seeds with 20–22% oil is suitable for degrading processes. Lipid autoxidation and the increase in free fatty acids during storage are the most common reasons for accelerated seed damages in oil plants (Lekić, 2003), while the accumulation of active oxygen species and free radicals is considered one of the most important factors of seed ageing (Bailly, 2004). Different storage conditions, primarily temperatures and air relative humidity, significantly affect soybean seed germination (Nkang and Umoh, 1997). According to Nkang and Umoh (1997), the optimal seed storage conditions are the temperature not higher than 25 °C and the relative air humidity ranging from 55% to 65%.

Balešević-Tubić et al. (2011) studied effects of ageing on vigour and biochemical changes in soybean seeds and established that extreme conditions of the temperature of 40 °C and the relative air humidity of 100% caused biochemical changes in seeds and reduced seed germination. After the 3-day accelerated ageing, the obtained seed germination was at the level of six-month natural ageing, both under controlled and conventional storage conditions. Seed germination after the 5-day accelerated ageing was equal to seed germination stored for 12 months under conventional storage conditions. According to Rastegar et al. (2011) the average germination period of deteriorated soybean seeds increased, which agreed with results obtained by Khaje Hoseini et al. (2003), who proved that deteriorated seeds needed more time to germinate.

Table 2. Correlation matrix of analysed parameters

Correlations	First count, %	Germination, %	Off-type seedling, %	Non-germinated seed, %	Length of seedling above-ground part, mm	Root length, mm	Fresh weight of seedling above-ground part, g	Root fresh weight, g	Dry weight of seedling above-ground part, g	Root dry-weight, g
First count, %	1.000	**0.964**	-0.232	**-0.981**	-0.758	0.687	-0.807	0.668	-0.773	0.636
Germination %		1.000	-0.418	**-0.991**	-0.595	0.854	**-0.913**	0.841	-0.896	0.800
Off-type seedling, %			1.000	0.295	-0.459	-0.734	0.371	-0.712	0.775	-0.865
Non-germinated seed, %				1.000	0.692	-0.792	**0.907**	-0.781	0.831	-0.716
Length of seedling above-ground part, mm					1.000	-0.122	0.471	-0.118	0.181	0.006
Root length, mm						1.000	**-0.903**	**0.998**	**-0.962**	**0.969**
Fresh weight of seedling above-ground part, g							1.000	**-0.913**	0.831	-0.779
Root fresh weight, g								1.000	**-0.945**	**0.955**
Dry weight of seedling above-ground part, g									1.000	**-0.981**
Root dry weight, g										1.000

The hypocotyl length is an important seed property, on which the emergence of the entire plant depends. This trait can be crucial for deeper sowing (Prijić and Jovanović, 1989). In addition, the root length also reflects the intensity of the initial growth of the seedling. According to Srebrić et al. (2010), the best germination of soybean seeds was achieved on chernozem, then on eutric cambisol, and the lowest on pseudogley. The variation in the length of primary roots and hypocotyls was present on different types of soils. The shortest, i.e. longest (8.3 cm) seedling hypocotyls were recorded on pseudogley, i.e. sand, respectively, under optimal conditions (20/30 °C). Significant differences in the primary root length were determined among all treatments within each genotype (genotype S1, S2, S3, S4), and then among genotypes within each treatment. Precipitation sums and distribution, and especially the occurrence of drought, reduce quality and yield of soybean seeds. Vujaković et al. (2006) stated that by applying the standard laboratory method, soybean seeds produced under irrigation conditions had higher germination than seeds produced by dry land farming conditions. These authors tested vigour by the application of Hiltner test, cold test and the accelerated ageing test, and established that the highest values of this parameter were obtained by the accelerated ageing test. According to the study carried out by Maksimović et al. (2004), germination of all observed soybean genotypes (Proteinka, Novosađanka and Vojvođanka) was higher than the minimum value (75%) prescribed by the Regulation of Seed Testing Quality of Agricultural Crops (Official Gazette of RS, issue 47/87). On that occasion, using the standard laboratory method, seed germination under rainfed conditions amounted to 88–90%, while this value was higher under irrigation conditions and ranged from 92 to 98%.

CONCLUSION

The comparison of differences between the accelerated ageing test and the standard germination test of soybean seeds showed the statistically significant decrease in the first count (26.75%) and germination (29.25%), and the increase of the percentage of non-germinated seeds (61.75%) after the application of the accelerated ageing test. The following seed traits were statistically significantly reduced after the application of the accelerated ageing test in comparison to the standard germination test: germination, root length (106.75 mm), root fresh weight (1.39 g) and root dry weight (0.11 g), while the percentage of the off-type seedlings (10.75%) was increased. From the aspect of differences in the standard germination tests between organically and conventionally produced soybean seeds, statistically significantly higher were germination (73.00%), root length (176.63 mm) and the root fresh weight (2.24 g) in seedlings of conventionally produced seeds, while percentage of non-germinated seeds (38.75%) was higher in organically produced seeds. By monitoring the differences in the accelerated ageing test between organically and conventionally produced soybean seeds, it was determined that the first count (60%), germination (63.25%) and the root length (106.75 mm) were statistically significantly higher in conventionally produced seeds, while higher percentage of non-germinated seeds (61.75%) and the length of seedling above-ground parts (121.63 mm) were recorded in organically produced seeds.

REFERENCES

Bailly C (2004): Active oxygen species and antioxidants in seed biology. *Seed Sci. Res.*, 14: 93–107.

Balešević-Tubić S, Tatić M, Đorđević V, Nikolić Z, Subić J, Đukić V (2011): Changes in soybean seeds as affected by accelerated and natural aging. *Rom. Biotechnol. Lett.* 16: 6740–6747.

Ciabotti S, Silva ACBB, Juhasz ACP, Mendonça CD, Tavano OL, Mandarino JMG, Gonçalves CAA (2016): Chemical composition, protein profile, and isoflavones content in soybean genotypes with different seed coat colors. *Int. Food Res. J.* 23: 621–629.

Hoffman JR, Falvo MJ (2004): Protein – Which is Best? *J. Sports Sci. Med.* 3: 118–130.

ISTA (2014): International Rules for Seed Testing. International Seed Testing Association, Switzerland.

ISTA Rules (2016): International Rules for Seed Testing 2016 Edition. International Seed Testing Association, ISTA Bassersdorf, Switzerland.

Kalentić M, Stefanović E, Simić I, Maerz U (2014): Organska poljoprivreda u Srbiji. Nacionalno udruženje za razvoj organske proizvodnje Serbia organica, Beograd. Available to: http://www.kombeg.org.rs/Slike/CeTranIRazvojTehnologija/2015/jun/Organska%20poljoprivreda%20u%20Srbiji%202014.pdf

Khaje-Hoseini M, Powell AA, Bingham IJ (2003): The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Sci. Technol.* 31: 715–725.

Lekić S (2003): *Životna sposobnost semena*. Beograd: Društvo selekcionera i semenara.

Maksimović L, Pejić B, Milić S, Đukić V, Balešević-Tubić S, Vujaković M (2004): Uticaj navodnjavanja na prinos, kvalitet i evapotranspiraciju semenske soje. *Vodoprivreda* 36: 421–426.

Nkang A, Umoh EO (1997): Six month storability of five soybean cultivars as influenced by stage of harvest, storage temperature and relative humidity. *Seed Sci. Technol.* 25: 93–99.

Official Gazette of RS (1987): issue 47/87.

Prijić LJ, Jovanović M (1989): Hypocotyl growth of different soybean genotypes. *Semenarstvo* 6: 279–286.

Rastegar Z, Sedghi M, Khomari S (2011): Efects of Accelerated Aging on Soybean Seed Germination Indexes at Laboratory Conditions. *Not. Sci. Biol.* 3: 126–129.

Srebrić M, Đukanović L, Jovanović Ž (2010): Životna sposobnost semena soje pri različitim uslovima testiranja. *Selekcija i smenarstvo* 16: 31–37.

Tatić M (2007): *Uticaj endogenih i egzogenih činilaca na proces starenja i životnu sposobnost semena soje Glycine max (L) Merr.* Doktorska disertacija, Univerzitet u Novom Sadu, Poljoprivredni fakultet.

TeKrony DM (2005): Accelerated Aging Test: Principles and Procedures. *Seed Technol.* 27: 135–146.

Ugrenović V, Filipović V (2012): Organska proizvodnja i biodiverzitet. II Otvoreni dani biodiverziteta, 26. Jun, Pančevo.

Vujaković M, Milošević M, Nikolić Z, Taški-Ajduković K (2006): Uticaj navodnjavanja na klijavost i životnu sposobnost semena soje (*Glycine max*. (L.) MERR.). *Treći simpozijum*

selekcije za oplemenjianje organizama Društva genetičara i Četvrti naučnostručni simpozijum iz selekcije i semenarstva Društva selekcionera i semenara Srbije, Zlatibor, 16–20. maja 2006.
Živanović LJ, Popović V (2016): Proizvodnja soje (*Glycine max.)* u svetu i kod nas. *21. Savetovanje o biotehnologiji sa međunarodnim učešćem*, Čačak, 11.–12. 03. 2016. Zbornik radova 21: 129–135.

ОРИГИНАЛНИ НАУЧНИ РАД

УТИЦАЈ НАЧИНА ПРОИЗВОДЊЕ НА ЖИВОТНУ СПОСОБНОСТ СЕМЕНА СОЈЕ

Јелена М. ГОЛИЈАН[1], Душица Д. ЈОВИЧИЋ[2], Славољуб С. ЛЕКИЋ[1], Миле Д. СЕЧАНСКИ[3]

[1] Универзитет у Београду, Пољопривредни факултет
Немањина 6, Београд 11080, Србија
[2] Институт за ратарство и повртарство
Максима Горког 30, Нови Сад 21000, Србија
[3] Институт за кукуруз „Земун Поље”
Слободана Бајића 1, Београд 11085, Србија

РЕЗИМЕ: Тест убрзаног старења семена један је од најважнијих тестова за испитивање животне способности семена, који омогућава утврђивање степена очувања клијавости и одређивање дужине периода чувања семена у складишту. Циљ овог рада био је да се испита утицај два различита начина производње – органског и конвенционалног – на животну способност семена соје сорте *Каћа*, применом теста убрзаног старења. Семе је излагано стресним условима температуре од 45 ºC и влажности ваздуха (100%) у трајању од 72 часа. Након примене теста дошло је до повећања броја неклијалог семена органске соје, чиме је смањен проценат клијавости. У односу на стандарни лабораторијски метод, након теста убрзаног старења дужина надземног дела клијанца (121,63 mm), маса свежег надземног дела клијанца (8,9 g) и маса осушеног надземног дела клијанца (1,05 g) били су већи, док су дужина (100,25 mm), свежа (1,26 g) и сува маса (0,1 g) корена били већи код стандардног лабораторијског метода. Након теста убрзаног старења забележен је већи проценат атипичних клијанаца (10,75%) и неклијалог семена (26%) код конвенционалне соје, док је с друге стране дошло до смањења дужине надземног дела клијанца (100,63 mm), дужине корена (106,75 mm) и свеже масе корена (1,39 g).

КЉУЧНЕ РЕЧИ: тест убрзаног старења, конвенционална производња, органска производња, соја

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 141, 69—80, 2021

UDC 633.1:663/664]:547.94:632.51
https://doi.org/10.2298/ZMSPN2141069S
SHORT REPORT

Tijana N. STOJANOVIĆ[1], *Gorica LJ. VUKOVIĆ*[2*], *Aleksandra P. PETROVIĆ*[1], *Bojan B. KONSTANTINOVIĆ*[1], *Nikola M. PUVAČA*[3], *Dušan S. MARINKOVIĆ*[1], *Sonja M. GVOZDENAC4*, *Vojislava P. BURSIĆ*[1]

[1] University of Novi Sad, Faculty of Agriculture
Department of Plant and Environmental Protection
Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia
[2] University of Belgrade, Faculty of Agriculture
Nemanjina 6, Belgrade–Zemun 11080, Serbia
[3] University Business Academy in Novi Sad
Faculty of Economics and Engineering Management in Novi Sad
Department of Engineering Management in Biotechnology
Cvećarska 2, Novi Sad 21000, Serbia
[4] Institute of Field and Vegetable Crops, Department of Agronomy
Maksima Gorkog 30, Novi Sad 21000, Serbia

DETERMINATION OF TROPANE ALKALOIDS IN CORN PUFFS BY THE LC-MS/MS

ABSTRACT: The interest in tropane alkaloids (TA) as food contaminants is increasing. A sensitive and selective LC-MS/MS method was applied for the analysis of corn puff samples from the Serbian market. Only atropine was quantified in 22% of the samples. In case of scopolamine, although not quantified, it was detected in 22% of the samples. Whether the acute reference dose (ARfD) could be exceeded was checked on a case-by-case basis for the individual products under assessment. Due to their low body weight and relatively high snack consumption, preschool children were at the highest risk of TA exposure. Assuming that the average consumption is 50 g of corn puffs per day, the sample with the highest concentration of TAs (2.05 μg/kg, 1.58 μg/kg of atropine) could contribute with 32.0% to the ARfD, of which 24.7% owing to atropine. If the same amount of corn puffs is consumed by older age classes, corresponding exposure contributions to the ARfD would progressively decline, down to 8.4% for adult population. The study revealed no health risk from TAs exposure through the consumption of the corn puffs in Serbian population.

KEYWORDS: atropine, corn, food analysis, risk assessment, scopolamine

[*] Corresponding author. E-mail: goricavukovic@yahoo.com

INTRODUCTION

It is well known that certain plants, animals and microorganisms produce natural toxins which are not toxic to them, but can be toxic to humans when ingested through food. Those natural toxins have received significant attention nowadays as a potential health hazard to humans (Puvača et al., 2020). Bearing that in mind, the World Health Organization accentuates the importance of monitoring the most relevant natural toxins in food (Casado et al., 2020).

The tropane alkaloids (TAs) are a group of over 200 secondary metabolites, found in all parts of the tropane alkaloids producing plants (Mulder et al., 2016). Besides the *Solanaceae* family, tropane alkaloids are also found in the following families: *Convolvulaceae*, *Euphorbiaceae*, *Proteaceae*, *Brassicaceae* and *Erythroxylaceae* (Gutiérrez-Grijalva et al., 2020). The tropane alkaloids may be divided into: tropane alkaloids produced by the family *Solanaceae* (atropine (AT), scopolamine (SC), hyoscyamine) (Figure 1), coca alkaloids (cocaine) and a newly discovered group of tropane alkaloids – calystegines (Kohnen-Johannsen and Kayser, 2019). The toxic effects of the tropane alkaloids in humans are related to the inhibition of muscarinic acetylcholine receptors in the central and the autonomic nervous systems (EFSA, 2013).

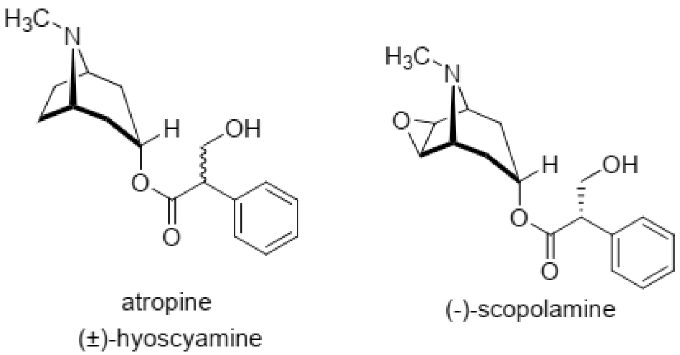

Figure 1. Chemical structure of atropine (racemic mixture of (±)-hyoscyamine) and scopolamine

The food contamination could be the consequence of the raw food material in which the TAs are naturally present. On the other hand, the contamination could occur through the co-harvesting plants, i.e. weeds containing tropane alkaloids, with the species of family *Solanaceae* being the most prominent, such as *Datura stramonium*. The parts of this plant have been found as the accidental impurities in the most important agricultural crops – maize, buckwheat, sunflower, soybean, millet and other (Gonçalves et al., 2020). In order to obtain more occurrence data on the presence of TAs in food, the EU Commission adopted Recommendation 2015/976/EU1 on the monitoring of the presence of TAs in food (EC, 2015). However, the monitoring is limited due to the limited availability of reliable analytical methods combined with the appropriate sensitivity. The maximum level was established only in cereal-based foods for infants and young children, containing millet, sorghum, buckwheat, or their derived products (Regulation 2016/239), limiting atropine and scopolamine concentration to 1 µg/kg for each alkaloid (EC, 2016). The discussions are continuing to define the maximum levels on corn, buckwheat, millet and sorghum (grains and milling products). The herbal infusions are also under consideration.

Taking into account the growing interest in plant secondary metabolites, the aim of the present study was to investigate the presence of tropane alkaloids in corn puffs, popular extruded snacks made out of cornmeal, i.e. corn, by the liquid chromatography with tandem mass spectrometry (LC-MS/MS) and estimate the level of the exposure of Serbian population.

MATERIALS AND METHODS

Chemicals and reagents

Atropine and scopolamine reference standards were obtained from the Sigma-Aldrich. The standard solutions of atropine and scopolamine were prepared at 1 mg/mL in methanol, each. The working standard solution mixtures were prepared at the concentration of 10 µg/mL and 1 µg/mL in methanol and stored in the dark at -20 °C. Acetonitrile and methanol were purchased from J. T. Baker. Both organic solvents were HPLC Ultra Gradient HPLC grade. The formic acid was analytical grade (Fisher Scientific UK). The QuEChERS extraction (Cat. No. 5982-5650) and QuEChERS dispersive kit 15 mL (Cat. No. 5982-5156) were obtained from Agilent Technologies, USA.

Instrumentation

HPLC Agilent 1290 Infinity II chromatograph equipped with a quaternary pump, multisampler and column compartment thermostat was used for the detection of atropine and scopolamine. The HPLC system was coupled to an Agilent 6495 LC/TQ triple quadrupole mass spectrometer with AJS ESI (Jet Stream Technology Ion Source). The Zorbax Eclipse Plus C18 column Rapid Resolution HD (50x2.1mm, 1.8 µm particle size) was used for the chromatographic separation. The column temperature was held at 35 ºC and the injection volume for the LC system was 2 µL. The chromatographic separation of AT and SC was carried out with mobile phase consisting of water (A) and methanol (B), both containing formic acid (0.1%, v/v), in a gradient mode and flow rate of 0.25 mL/min. A gradient elution started at 5% of B and held 1 min. This composition was increased to 40% B at 7 min, 90% B at 8 min and held for 2 min. The composition of the mobile phase returned to the initial conditions in one min and the system was equilibrated during two min. The total running time was 11 min. The ESI source was used with the following settings: drying gas (nitrogen) temperature 200 °C, drying gas flow rate 16 L/min, nebulizer pressure 30 psi, sheath gas temperature of 300 ºC, sheath gas flow 12 L/min and capillary voltage 3,000 V. The detection was performed using the dynamic multiple reactions monitoring mode (dMRM). The Agilent MassHunter software (version B.10.0 SR1 Agilent Tehnologies, 2006–2019) was used for the optimization and quantification.

Sample collection and preparation

Eighteen corn puffs samples were collected from the local shops and supermarkets in Novi Sad, Serbia. The sampling was performed in accordance with the EU directive 2002/63/EC. The samples were dry ground into powder prior to the analysis (particle size of less than 1 mm and sieved to obtain a homogenous sample particle size).

Atropine and scopolamine were extracted from ground corn puff samples using the QuEChERS method described on Figure 2.

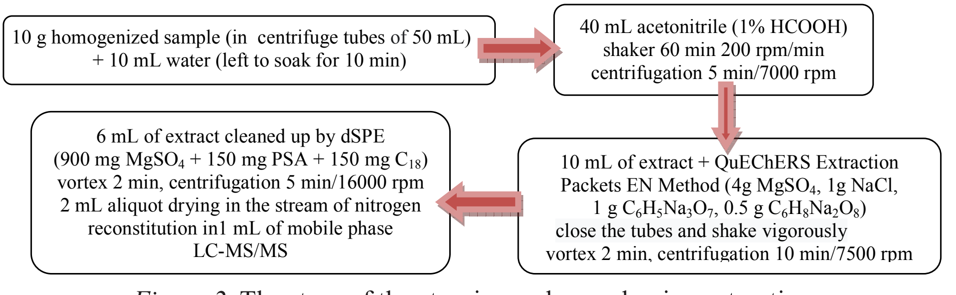

Figure 2. The steps of the atropine and scopolamine extraction

Acquisition parameters

Atropine and scopolamine were analyzed using ESI+ (electrospray positive ionization) by dynamic multiple reactions monitoring mode. The fragmentation of the protonated atropine and scopolamine ions yielded 3 product ions, respectively (Table 1). The most intense MRM transitions for atropine m/z 290.2 > 124.2 and scopolamine 304.2 > 138.2 were monitored for the quantification and the second most intense (other two) transitions were used for the confirmation (Vuković et al., 2018).

Table 1. LC-ESI-MS/MS parameters for the analysis of AT and SC in MRM mode

TA	Molecular formula	Molecular weight (g/mol)	Retention time (min)	Precursor ion [M+H*] (m/z)	Product ion (m/z)	Fragmentation voltage (V)	Collision energy (V)
AT	$C_{17}H_{23}NO_3$	289.2	9.63	290.2	124.2* 93.2 77.1	150 150 150	24 36 68
SC	$C_{17}H_{21}NO_4$	303.2	8.42	304.2	156 138.2* 103.2	150 150 150	12 24 44

* Quantification product ion

RESULTS AND DISCUSSION

The previous studies (Vuković et al., 2018; Vuković et al., 2020) pointed out that the addition of the formic acid to the mobile phase resulted in more efficient ionization and gave the finer peak of the studied tropane alkaloids. The MRM chromatograms and mass spectra of atropine and scopolamine transitions are shown in Figure 3.

The limit of detection (LOD) was determined as the lowest concentration giving a response of three times the average baseline. The ratio signal/noise in the obtained chromatograms for the LOD was calculated by MassHunter Qualitative Software and was estimated to be 0.1 mg/kg for both tested compounds. The limit of quantification (LOQ) (1 mg/kg) was calculated as 3.3*LOD and was in accordance with the Commission Recommendation (EU) 2015/976 related to the LOQ: "preferably below 5 mg/kg and not higher than 10 mg/kg for agricultural commodities, ingredients, food supplements and herbal teas and lower than 2 mg/kg for finished foods and 1 mg/kg for cereal-based foods for infants and young children".

The quantification was carried out by "recovery calibration" method (a known amount of analyte is spiked into the sample before extraction begins) by adding the tropane alkaloids standards into each sample to five calibration levels of 1, 2, 5, 10 and 20 µg/kg. The obtained calibration curves (both atropine and scopolamine) were used for the calculation of the atropine and scopolamine concentrations in the samples. The calibration curves of the studied atropine and scopolamine in the range of 1–20 µg/kg are shown in Figure 4.

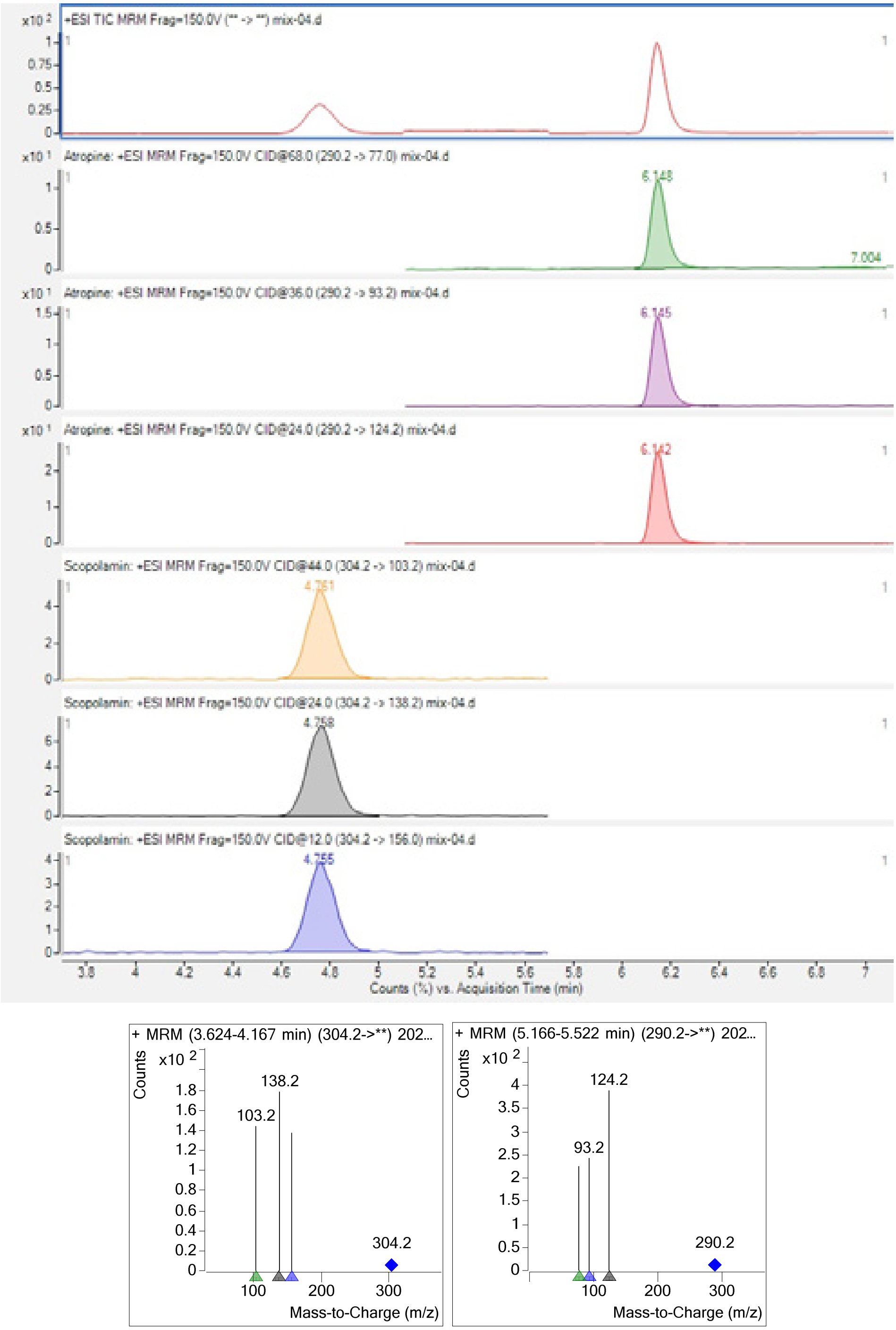

Figure 3. MRM chromatograms and mass spectra of AT and SC

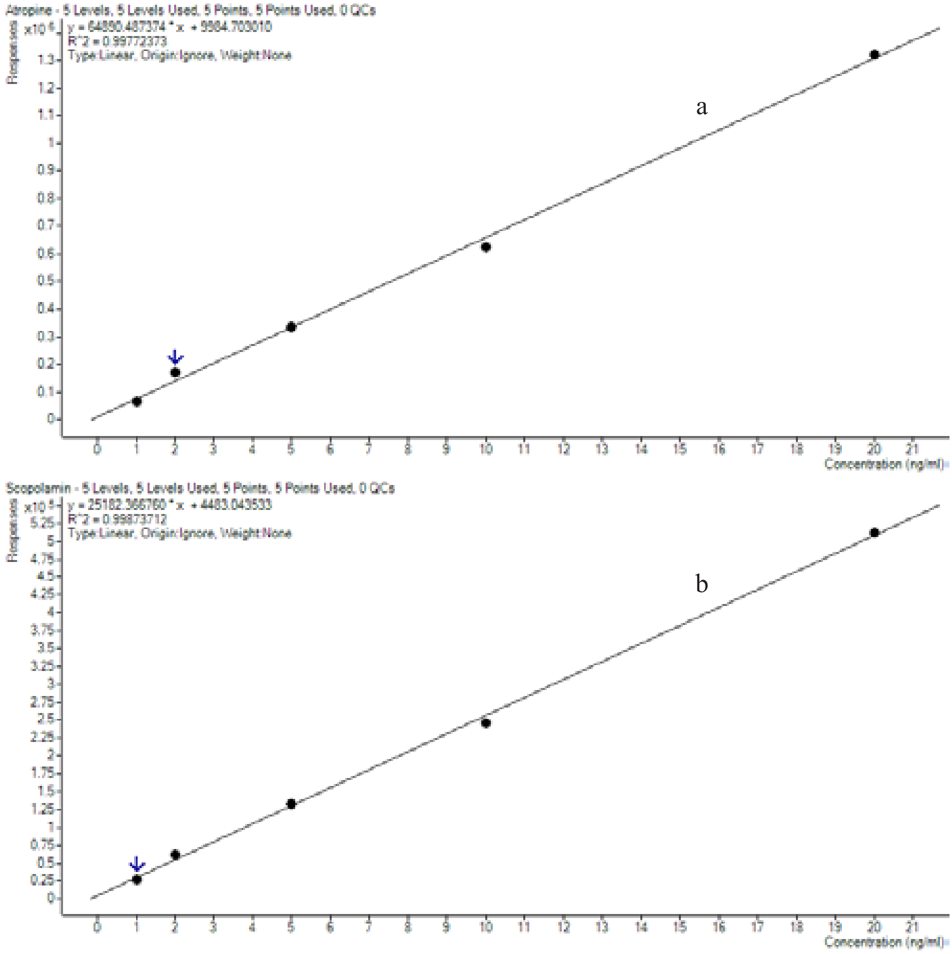

Figure 4. Calibration curves of atropine (a) and scopolamine (b) in matrix

Determination of tropane alkaloids in corn puffs

A total of 18 corn puffs samples were analysed for the presence of atropine and scopolamine. As shown in Table 2, only atropine results were quantified (22%, all the remaining 78% left-censored results were reported as below the LOD). With regard to scopolamine, 22% of the results were reported as numerical values, but below the LOQ.

It is important to notice full co-occurrence, as well as the ratio of atropine to scopolamine in the range from 3.4 to 3.9, coherent with a potential contamination with *Datura stramonium*. It would be valuable to know whether and how much of tropane alkaloids was lost during the extrusion – the food processing technique used in the corn puffs production.

Table 2. Summary of the presence of atropine and scopolamine in corn puffs

Parameter	Atropine	Scopolamine	Sum of atropine and scopolamine
N	18	18	18
N pos (%)	4 (22.2)	4 (22.2)	4 (22.2)
min c (μg/kg)	1.03	0.29	1.32
max c (μg/kg)	1.58	0.47	2.05

N – number of the samples; pos – positive samples; c – concentration.
LOD = 0.1 μg/kg and LOQ = 1.0 μg/kg for atropine and scopolamine.

The surveys performed in the European countries reported different levels of contamination, both in terms of the fraction of positive samples and of total TA content, in a broad range of foods (flours, infant formulas, botanicals or honey). It was confirmed that atropine and scopolamine were the most frequently found TAs in cereal-based foods (Mulder et al., 2016; EFSA, 2018). Over the last five years, Rapid Alert System for Food and Feed (RASFF, https://ec.europa.eu/food/safety/rasff_en) reported 24 notifications related to TAs in food, out of which 14 related to cereals and bakery products (made from corn, millet, buckwheat and soy), nine to tea and herbal preparations and one to infant food. The highest measured concentrations were 180 and 36 mg/kg of atropine and scopolamine in baking mix, respectively, as well as 213 and 44.7 mg/kg in herbal infusion. It is interesting to note that the country of origin of two products was Serbia (corn grits and peppermint). A peppermint sample from Serbia contained even 200.5 and 488.7 mg/kg of atropine and scopolamine, respectively.

The most recent research considering TAs has been published by Vuković et al. (2021). The study included 71 food product samples, such as corn puffs, popcorn, corn and corn grits. The TAs detections above the LOQ (2 μg/kg, which is in accordance with the Commission Recommendation (EU) 2015/976), appeared in 29.57% of the analyzed samples. According to the literature data, these concentrations can have serious negative effects on human and animal health. The highest mean concentrations of atropine and scopolamine were detected in corn grits samples, followed by popcorn and corn.

Health risk assessment

The acute reference dose (ARfD), i.e. the amount of substance, expressed on a body weight basis, that could be ingested via food over a day without a risk for the consumer health, was established by the European Food Safety Authority (EFSA) at 16 ng/kg bw per day for the sum of atropine and scopolamine (EFSA, 2013). However, a tolerable daily intake (TDI) for chronic exposure was not established, since the TAs “are not bio accumulative, or genotoxic, and do not exhibit chronic toxicity” (EFSA, 2013). Whether the ARfD could be exceeded was estimated on a case-by-case basis for the individual products under assessment, based on the measured atropine and scopolamine concentrations and the estimated corn puffs consumption. The acute exposure to atropine and scopolamine through the consumption of corn puffs, estimated on a per day basis across age classes, is presented in Tab. 3, as well as the exposure to the sum of atropine and scopolamine, calculated as the sum of both alkaloids in the same sample.

Table 3. Exposure assessment to atropine and scopolamine through consumption of corn puffs, across age classes.

	Exposure (ng/kg bw)		% of group ARfD		> ARfD
Age class	Min	Max	Min	Max	%
			Atropine		
Preschool children	2.6	4.0	16.1	24.7	0
Children 7–10 y.	1.6	2.4	9.8	15.0	0
Adolescents 11–14 y.	1.0	1.5	6.2	9.5	0
Adults 15+ y.	0.7	1.0	4.2	6.5	0
			Scopolamine		
Preschool children	0.7	1.2	4.53	7.3	0
Children 7–10 y.	0.4	0.7	2.75	4.5	0
Adolescents 11–14 y.	0.3	0.5	1.74	2.8	0
Adults 15+ y.	0.2	0.3	1.19	1.9	0
			Sum of atropine and scopolamine		
Preschool children	3.3	5.1	20.6	32.0	0
Children 7–10 y.	2.0	3.1	12.5	19.4	0
Adolescents 11–14 y.	1.3	2.0	7.9	12.3	0
Adults 15+ y.	0.9	1.3	5.4	8.4	0

ARfD – group acute reference dose for the sum of atropine and scopolamine (16 ng/kg bw per day) (EFSA, 2013). y – years of age. Consumed amount of corn puffs: 50 g (one pack). Body weight: preschool children 20 kg, children (7–10 y.) 33 kg, adolescents (11–14 y.) 52 kg, adults (15+ y.) 76 kg.

The moderate differences were observed between the minimum and maximum exposure estimates for the age class. Due to their low body weight and relatively high snack consumption, preschool children were at the highest risk to the TA exposure. For a preschool child of around 20 kg bw, the ARfD would correspond to an intake of 320 ng TAs per day. Assuming the consumption of 50 g of corn puffs (one pack) per day, the sample with the highest TA concentration (2.05 μg/kg of total TAs, 1.58 mg/kg of atropine) could contribute with 102.5 ng of TAs (32.0% of the ARfD, of which 79.0 ng (24.7%) as atropine contribution). The ARfD would be exceeded when the product contained more than 6.4 μg TAs per kg. If the same amount of corn puffs is consumed by older age classes, the corresponding exposure contribution to the ARfD would progressively decline with the increase of age and average body weight, down to 8.4% (6.5% owing to atropine) for the adult population. As shown in Table 3, the fraction of the products under assessment that would cause exceeding 10% of the ARfD was 22% for the preschool children and the children, 6% for the adolescents, while for the adults that level was not reached by any of the products. The study revealed no health risk from tropane alkaloids exposure through corn puffs for Serbian population.

The snacks are especially popular with the children for which the highest mean acute exposure for the sum of atropine and scopolamine has been observed in the assessment conducted by the EFSA (children 0.97–18.91 ng/kg bw/day, toddlers 1.82–18.65 ng/kg bw/day and other children 1.13–18.13 ng/kg bw/day) (EFSA, 2018). From a toxicological point of view, if ARfD is exceeded, adverse health effects can no longer be ruled out.

CONCLUSION

The study results do not indicate a serious health concern related to the exposure to the tropane alkaloids through the consumption of corn puffs commercialized on the Serbian market. The younger age groups (preschool children and children) are acutely exposed to the higher levels of the tropane alkaloids compared to the older age groups.

The study included only one type of food with a limited number of samples. Bearing in mind that the consumption of snacks is increasing, especially among the children and adolescents, obtained insight into the actual contamination levels is considered important. Furthermore, the study results highlight the importance of monitoring the tropane alkaloids in other food categories that could potentially be contaminated with the tropane alkaloids.

ACKNOWLEDGEMENT

The authors would like to acknowledge the generous support in writing the study by the prof. dr Ljilja Torović, Faculty of Medicine, University of Novi Sad.

REFERENCES

Casado N, Gañán J, Morante-Zarcero S, Sierra I (2020): New advanced materials and sorbent-based microextraction techniques as strategies in sample preparation to improve the determination of natural toxins in food samples. *Molecules* 25: 1–2.

EC (European Commission) (2015): Commission recommendation 2015/976 of 19 June 2015 on the monitoring of the presence of tropane alkaloids in food. *Off. J. Eur. Union* 157: 97–98.

EFSA (2013): Scientific Opinion on Tropane alkaloids in food and feed. *EFSA J.* 11: 3386.

EFSA (2018): Human acute exposure assessment to tropane alkaloids. *EFSA J.* 16: 22.

EU (European Commission) (2016): Commission regulation (EU) 2016/239 amending regulation (EC) No 1881/2006 as regards maximum levels of tropane alkaloids in certain cereal-based foods for infant and young children. *Off. J. Eur. Union* L45: 3–5.

Gonçalves C, Dernovics M, Moreno-Gonzalez D, Bouten K, Garcia-Reyes JF, Stroka J (2020): Occurrence and Determination of Tropane Alkaloids in Food and Feed. *Encyclopedia of Analytical Chemistry*, 2.

Gutiérrez-Grijalva EP, López-Martínez LX, Contreras-Angulo LA, Elizalde-Romero CA, Heredia JB (2020): *Plant Alkaloids: Structures and Bioactive Properties*. Singapore: Springer.

Kohnen-Johannsen KL, Kayser O (2019): Tropane Alkaloids: Chemistry, Pharmacology, Biosynthesis and Production. *Molecules* 24: 796.

Mulder PPJ, de Nijs M, Castellari M, Hortos M, MacDonald S, Crews C, Hajslova J, Stranska M (2016): Occurrence of tropane alkaloids in food. *EFSA* Supporting Publications 13: 8.

Puvača N, Bursić V, Vuković G, Budakov D, Petrović A, Merkuri J, Avantaggiato G, Cara M (2020): Ascomycete fungi (*Alternaria* spp.) characterization as major feed grains pathogens. *J. Agron. Technol. Eng. Manag.* 3: 499.

Vuković G, Bursić V, Stojanović T, Petrović A, Gvozdenac S, Starović M, Kuzmanović S, Aleksić G (2018): LC-MS/MS determination of tropane alkaloids in maize crop. *Contemporary Agric*. 67: 222.

Vuković G, Bursić V, Stojanović T, Petrović A, Konstantinović B, Puvača N, Marinković D (2020): Determination of atropine and scopolamine in popcorn by the LC-MS/MS. *26th International Symposium on Analytical and Environmental Problems*, 23–24. November 2020, Szeged, Hungary, Proceedings, 356.

Vuković G, Stojanović T, Konstantinović B, Petrović A, Špirović Trifunović B, Marinković D, Bursić V (2021): Skrivena opasnost prisustva korovskih vrsta: Tropanski alkaloidi u hrani i žitaricama. *Biljni lekar (Plant Doctor)* 49: 657–667.

КРАТКО САОПШТЕЊЕ

ОДРЕЂИВАЊЕ ТРОПАНСКИХ АЛКАЛОИДА У КУКУРУЗНОМ ФЛИПСУ ПРИМЕНОМ LC-MS/MS

Тијана Н. СТОЈАНОВИЋ[1], Горица Љ. ВУКОВИЋ[2], Александра П. ПЕТРОВИЋ[1], Бојан Б. КОНСТАНТИНОВИЋ[1], Никола М. ПУВАЧА[3], Душан С. МАРИНКОВИЋ[1], Соња М. ГВОЗДЕНАЦ[4], Војислава П. БУРСИЋ[1]

[1] Универзитет у Новом Саду, Пољопривредни факултет
Департман за фитомедицину и заштиту животне средине
Трг Доситеја Обрадовића 8, Нови Сад 21000, Србија
[2] Универзитет у Београду, Пољопривредни факултет,
Немањина 6, Београд–Земун 11080, Србија
[3] Универзитет Привредна академија у Новом Саду
Факултет за економију и инжењерски менаџмент у Новом Саду
Департман за агрономију, технологију и инжењерски менаџмент
Цвећарска 2, Нови Сад 21000, Србија
[4] Институт за ратарство и повртарство
Департман за агрономију
Максима Горког 30, Нови Сад 21000, Србија

РЕЗИМЕ: Интересовање за тропанске алкалоиде као контаминанте хране је у порасту. За анализу кукурузног флипса са тржишта Србије примењена је осетљива и селективна LC-MS/MS метода. Атропин је квантификован у 22% узорака. У случају скополамина, који није квантификован, детектовано је у 22% узорака. Да ли може доћи до прекорачења акутне референтне дозе проверено је за сваки појединачни производ који је уврштен у процену. Мала телесна маса и релативно велике конзумиране количине снек-производа истичу предшколску децу као групу у највећем ризику од изложености тропанским алкалоидима. Под претпоставком о конзумирању 50 грама кукурузног флипса дневно, узорак с највећом концентрацијом тропанских алкалоида (2,05 μg/kg, 1,58 mg/kg атропина) може допринети са 32,0% од акутне референтне дозе, од чега 24,7% одговара атропину. Уколико исту количину кукурузног флипса конзумирају старије узрасне групе, допринос следствене изложености тропанским алкалоидима би прогресивно опадао до 8,4% за одраслу популацију. Студија није указала на здравствени ризик услед изложености тропанским алкалоидима путем конзумирања кукурузног флипса за популацију у Србији.

КЉУЧНЕ РЕЧИ: анализа хране, атропин, кукуруз, процена ризика, скополамин

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 141, 81—93, 2021

UDC 631.812: 546.74(497.11)
https://doi.org/10.2298/ZMSPN2141081B
ORIGINAL ARTICLE

Dušana D. BANJAC[1*], *Jordana M. NINKOV*[1], *Stanko B. MILIĆ*[1], *Snežana P. JAKŠIĆ*[1], *Milorad S. ŽIVANOV*[1], *Biljana I. RADOVIĆ*[1], *Marko V. MALIĆANIN*[2]

[1] Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia
Maksima Gorkog 30, Novi Sad 21000, Serbia
[2] University of Niš, Faculty of Agriculture in Kruševac
Kosančićeva 4, Kruševac 37000, Serbia

NICKEL CONTENT IN FIELD CROP SEEDS AND AGRICULTURAL SOIL IN CENTRAL SERBIA

ABSTRACT: Nickel (Ni) is an essential nutrient for animals and it has an important role in many physiological and biochemical processes in higher plants. At the same time, it belongs to the group of potentially toxic elements (PTEs). The aim of this study is to determine Ni concentrations in the soil-plant relationships between the main crops and agricultural land in Central Serbia. A total of 71 bulked soil samples are taken from the topsoil at the depth of 0–30 cm in an area belonging to 6 statistical districts of Central Serbia. A total of 71 seed samples are collected during harvest as an average sample of seed from each observed plot, of which 26 are corn, 19 sunflower, 17 wheat, and 9 soybean samples. Analysis of the collected samples includs the main soil parameters and Ni total and available concentrations in soil, as well as Ni total concentration in seeds. The median value of total Ni concentration in soil is 44.8 mg kg^{-1}, close to MAC. The median nickel concentration in wheat and corn seeds is 0.5 mg kg^{-1}, while soybean and sunflower seeds have higher median Ni content of 8.40 and 10.26 mg kg^{-1}, respectively. Bioaccumulation factors in seeds (BAF) in the present study ranges from 0.013 (corn) to 0.256 (soybean). According to statistically significant differences, all crops have equal total Ni_T concentration in soil, while the available Ni_A concentration differs in soils under corn and sunflower cultivars. Based on Ni concentration in seed and BAF, two groups are distinguished − the group of soybeans and sunflowers with higher Ni content and the group of wheat and corn with lower Ni content in seed. The obtained differences confirm that plant species have a significant role in the bioaccumulation of Ni. The determined BAF parameter is in a statistically significant negative correlation with the total Ni content in soil in all observed crops except maize. However, the BAF parameter for maize alone is in a statistically significant negative correlation with the readily available Ni concentration in the soil. The obtained correlations indicate that higher Ni concentration in soil causes lower Ni concentration in seeds, which might be due to the activation of plant defense mechanism to preserve the reproductive organs – seeds – from

[*] Corresponding author. E-mail: dusana.banjac@nsseme.com

harmful effects. Based on the obtained results, Ni concentration in seeds of the main field crops in Central Serbia is safe for feed and food usage. Increased content of Ni found in agricultural soils in Central Serbia requires constant monitoring for maintaining sustainable agriculture production.
KEYWORDS: nickel, field crops, soil, seed

INTRODUCTION

The content of hazardous and harmful substances in soils requires constant monitoring due to the negative impact of such substances on crops, as well as their presence in the food chain.

Monitoring potentially toxic elements (PTE) is conducted to assess soil contamination by agrochemicals. Also, soil conservation is crucial to the establishment of sustainable agriculture. Numerous studies examining possible soil contamination by PTE indicate a high content of nickel (Ni) in Serbian soils as a result of soil geochemical origin (Mrvić, 2009; Albanese et al., 2015).

Ni is an essential nutrient for animals and a beneficial element for higher plants. As a cofactor for urease and hydrogenase, Ni plays a significant role in the enzyme-catalyzed metabolic processes of higher plants. According to Drzewiecka et al. (2012), Ni is an important metal in plant metabolism and a cofactor of numerous metalloenzymes. On the other hand, nickel causes toxic effects on plants in relatively small doses. Although the increased content of nickel in Serbian soils results from soil geochemical origin, a detailed investigation of Ni content should be conducted, including its correlation with the main soil properties and different plant species.

Ni is relatively stable in an aqueous solution. In soil solution, Ni may exist as aqueous Ni^{2+}, complexed with inorganic and organic ligands, and/or associated with suspended mineral colloids, where the organic complexes may be dominant in soil solution (Adriano, 2001). Compared to other metals, nickel shows exceptional mobility from soil to surface plant parts, which can directly affect plant photosynthetic activity (Huillier et al., 1996).

The establishment of a critical level of Ni in soil and plant, from the aspect of both deficiency and toxicity, is still the subject of discussion by many authors, due to the unexplained essentiality of nickel for plants and animals, and Ni is required only in ultra-trace amounts. In addition, Ni is comparatively abundant in most soils and its deficiency is a very rare condition, even where bioavailability is low due to high carbonate, sesquioxide, and organic matter content (Adriano, 2001). According to Kabata-Pendias and Pendias (2001), the toxic effects of nickel on plant growth and development ranged from 10 to 100 mg kg^{-1} dry weight of soil, indicating the need for a more detailed examination of the effect of nickel on economically important plant species. Considering that Serbian plant production is mainly focused on maize, wheat, soybean, and sunflower, it must be determined how the readily available nickel is adopted by these plant species, as well as the extent to which nickel is toxic to plants.

This study aims to determine the concentrations of Ni in the soil-plant relationships between the main crops and agricultural land in Central Serbia.

MATERIALS AND METHODS

Sample collection and processing

Field trials were carried out in the second half of 2018. The locations of collected soil samples are shown in Figure 1 and belong to 6 statistical districts of Central Serbia: East, Bor, South, West, Belgrade, and Central District. A total of 71 bulked soil samples are taken from topsoil at the depth of 0–30 cm, using a probe drill. One composite soil sample represented 15–25 subsamples from production plots (up to 5 ha area).

Field crops are sampled during harvest as an average sample of seed from each observed plot. A total of 71 seed samples are collected, of which 26 samples are corn, 19 sunflower, 17 wheat, and 9 soybeans.

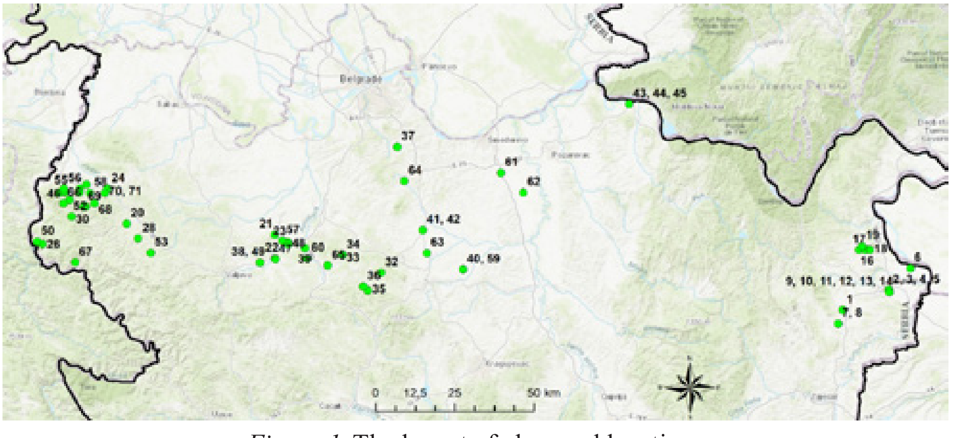

Figure 1. The layout of observed locations

Laboratory analyses

The soil samples are air-dried at room temperature, milled, and sieved to <2 mm particle size, following ISO 11464:2006. Particle size distribution in the <2 mm fraction is determined by the pipette method (Van Reeuwijk, 2002). The size fractions are defined as clay (<2 µm), silt (2–20 µm), fine sand (20–200 µm), and coarse sand (200–2000 µm). The applied methods are as follows: the pH value (ISO 10390:2005), the free $CaCO_3$ content (ISO 10693:1995), the organic matter content (OM) (ISO 14235:1998), the total N (ISO 13878:1998), and the total organic C (TOC) (ISO 10694:1995). Readily available phosphorus

(P_2O_5) and available potassium (K_2O) are extracted by the AL method (Egner and Riehm, 1955) and measured by the means of spectrophotometry and flame photometry, respectively.

The samples are analyzed for the total content of Ni after the soil microwave digestion in concentrated acid solution (5:1 HNO_3/H_2O_2, and 1:12 solid/solution ratio). In parallel, the bioavailable nickel concentration in soil is determined using buffered EDTA extraction solutions according to extraction protocols for IRMM BCR reference materials CRM-484 (5 g soil/50 ml EDTA concentration 0.05 $molL^{-1}$ pH=7.00).

The plant samples are air-dried and ground in a mill to extract the plant material. As moisture content is determined gravimetrically, the reported results refer to dry mass. Plant materials are analyzed for total Ni content after the microwave digestion of the samples in a concentrated acid solution (0.5 g of sample material in a digestion solution comprised of 10 ml HNO_3 + 2 ml H_2O_2, Vt = 50 ml).

The concentration of nickel in prepared soil and plant samples is determined by ICP-OES (Vista Pro-Axial, Varian) following the US EPA method 200.7:2001.

Quality assurance and quality control (QA/QC) assessments are conducted using certified reference materials IRMM ERM-CC141 (loam soil), IRMM BCR-484 (sewage sludge amended terra rossa soil), and SRM1515 (apple leaves); for total, available Ni concentration in soil and plant matrix, respectively. The percentage of recovery, defined as the ratio of measured concentrations and certified values of two reference materials, ranged from 87% to 111%, which provided adequate analytical accuracy and precision.

The results of the plant material and soil analysis are calculated on dry matter (DM).

Calculation of bioaccumulation factor

To estimate the nickel uptake rates by studied crop seeds from the soil, the bioaccumulation factors (BAF) are used, defined as flowing equation:

$$BAF = \frac{\text{Ni concentration in seed } [mg \cdot kg^{-1}]}{\text{Total Ni concentration in soil } [mg \cdot kg^{-1}]}$$

Statistical analysis

Data are statistically processed by analyzing the main descriptive parameters for each element. Correlations between all the examined parameters are determined, while the statistical differences between the tested Ni contents in plants and soil are determined by Duncan's multiple range test.

All statistical analyses are performed using STATISTICA for Windows version 13 (TIBCO, 2018). Statistical parameters are shown in tables.

RESULTS AND DISCUSSION

Soil characterization and nickel concentration in soil

In the examined samples, the distribution of soil mechanical fractions differs among districts, as well as within each district. Texture classes range from light sandy to heavy clay, but soils with a medium mechanical composition still dominate, with a slight dominance of the silt fraction (Table 1). The soil pH values vary from plot to plot, both within one district and between districts. The potential pH values (in 1M KCl solution) range from acidic (pH 4.93) to slightly alkaline (pH 8.20), while the median value is 6.63 that belongs to a neutral class (Table 1). Since the pH reaction of the soil is highly correlated with the $CaCO_3$ content, the obtained results are very similar to the pH distribution. According to the results of the research, weak calcareous and non-calcareous soils are the most common, while a strong calcareous class is determined in a small number of plots (Table 1). The contents of organic matter (OM) and total organic carbon in soil (TOC) are also highly correlated, so the obtained results show similar distribution between the soil classes. The content of OM ranges from 0.81 to 6.57%, but the prevailing OM content does not exceed 3%, which is characteristic of low humus soils (Table 1). Based on the median of all results obtained for total nitrogen, the examined soils are moderately supplied with total nitrogen (Table 1). The content of readily available phosphorus ranges from very low (ameliorative class) to extremely high (toxic) content. Most of the samples are classified as poor in phosphorus. The content of readily available potassium ranges from low through very high to harmful class. Most of the tested samples are classified in the optimal class (Table 1). A determined wide range of phosphorus and potassium concentration in soil, as well as high statistical variation between soil samples (Table 1) confirm that P_2O_5 and K_2O content is strongly affected by inadequate fertilization practices.

Table 1. Descriptive statistics of the observed soil parameters

Parameter	Aver. value	Median value	MIN	MAX	ST DEV	CV [%]	SE
Coarse sand[%]	6.10	2.46	0.70	37.03	8.028	131.63	0.953
Fine sand [%]	33.55	31.76	11.11	65.30	11.541	34.40	1.370
Silt [%]	31.97	35.04	6.88	47.12	8.670	27.12	1.029
Clay [%]	28.39	28.84	6.76	62.08	11.355	40.00	1.348
pH in H_2O	5.61	5.28	3.79	7.62	1.203	21.45	0.143
pH in 1MKCl	6.63	6.32	4.93	8.20	1.039	15.67	0.123
$CaCO_3$[%]	1.21	0.25	0.00	12.27	2.578	213.74	0.306
OM [%]	2.45	2.22	0.81	6.57	1.198	48.95	0.142
TOC [%]	1.71	1.41	0.09	5.93	1.188	69.33	0.141
Total N [%]	0.192	0.164	0.061	0.574	0.106	55.23	0.013
P_2O_5[mg 100g^{-1}]	11.56	7.30	0.90	182.00	21.793	188.49	2.586
K_2O [mg 100g^{-1}]	20.57	20.19	5.56	72.74	10.027	48.75	1.190
Ni_T[mg kg^{-1}]	50.34	44.82	19.23	113.40	23.028	45.74	2.733
Ni_A[mg kg^{-1}]	5.71	5.13	0.84	16.33	3.802	66.61	0.451

Values for total nickel content Ni_T in soil obtained in the present study ranges from 19.2 (location 17) to 113.4 mg kg^{-1} (location 69) (Figure 1, Table 1). The maximum allowable concentration – MAC (OG 23/1994) is 50 mg kg^{-1}, and 30 soil samples out of 71 observed in the present study have a higher concentration than the MAC threshold. The median value obtained in the study is 44.8 mg kg^{-1} (Table 1). The median exceeds the listed background concentration for European soils (<35 mg kg^{-1}) (Houskova and Montanarella, 2006). Based on the GEMAS project, the median for Europe is 14.7 mg kg^{-1} (Albanese et al., 2015), which is much lower than the value obtained in this study.

The available Ni content in EDTA Ni_A detected in all samples ranges from 0.8 (location 21) to 16.3 mg kg^{-1} (location 57), with the median value of 5.1 mg kg^{-1} (Figure 1, Table 1). The percentage of accessible content in the total content is a good indicator of soil pollution and it ranges from 1.4 to 22.7%. Out of eight observed samples with a share higher than 20%, seven have a total content above MAC. According to the observed statistics, all studied regions have a median in the range of 40–50 mg kg^{-1}, except for the southern region where the median is 34.5 mg kg^{-1}. It has been pointed out that the soils in Serbia have increased nickel content due to the geochemical origin of soils formed from the parent substrate rich in nickel (Dozet et al., 2011). The present study is therefore in line with the previously conducted research.

According to the established statistically significant correlation (data not shown), the total concentration of Ni is positively correlated with clay content but negatively correlated with fine sand content. In addition, total Ni is positively correlated with organic matter content. Contrary to the expectations of this study, a lower pH reaction has not been proven to increase the content of Ni, according to the established positive correlation between Ni_T and pH values, which is attributed to the predominantly acidic reaction of the observed samples.

Nickel concentration in seed

The concentration of nickel in most natural vegetation is about 0.5 mg kg^{-1}, which is the median value for wheat and corn in this study (Table 2). Soybean and sunflower seeds have a much higher Ni content of 8.40 and 10.26 mg kg^{-1} median value, respectively. Average contents of Ni in cereal grains vary from 0.34 to 14.6 mg kg^{-1} (Kabata-Pendias and Mukherjee, 2007) in different countries, which is in accordance with the results of the present study. The highest value of 19.4 mg kg^{-1} (Table 2) is found in soybeans at location 40 (Figure 1), originating from soils where nickel is below the MAC (Ni_T=36.4 and Ni_A=5.2 mg kg^{-1}). The most variable results between the analyzed samples are found in corn, while the other crops have similar variations in results (Table 2). According to Adriano (2001), the critical level of Ni deficiency in plants is <0.1 mg kg^{-1}, while sufficient levels in plants vary in a wide range from 0.01 to 10 mg kg^{-1}. Therefore, all tested crops in this study have sufficient nickel content. The phytotoxic Ni concentrations vary widely among plant species and cultivars, reported to range from 40 to 246 mg kg^{-1} (Kabata-Pendias and Pendias 2001). According to Kastori (1997), the critical toxic concentrations of Ni in plants amount to 20 and 30 mg kg^{-1}, respectively. Therefore, no toxic effects of Ni on plants are expected in the present study.

According to the rulebook on the quality of feed (OG RS 39/2016), there are no special restrictions for the nickel content. According to the rulebook on the quality of food (OG RS 90/2018), there is a limit of 0.5 mg kg^{-1} for Ni content in oils, fats, margarine, and related products. Based on all the obtained results, Ni concentration in the seed of the main field crops in Central Serbia is safe for feed and food usage.

Ni is required in a nutrient solution to prevent the accumulation of toxic concentrations of urea in not only urea-fed but also mineral N-fed (nitrate, ammonium) or N-fixing soybean (Kutman et al., 2013). In this study, soybean seeds are produced by growing plants in nutrient solutions containing different Ni levels, and their urease activities are measured. According to the obtained results, seeds with Ni concentrations vary between 0.04–8.32 mg kg^{-1}. Depending on the Ni concentration, a significant difference is observed between seed urease activities and the increased rates of nickel supply increases seed yield by up to 25%.

The results obtained for BAFs range from 0.004 (corn) to 0.866 (sunflower) (Table 2). According to BAF results, the observed plant species have no hyperaccumulation characteristics (BAF>1). The median value of the determined bioaccumulation factors (BAF) in the present study coincides with Ni concentrations in seed in the following order (Table 2):

BAF corn < BAF wheat < BAF sunflower < BAF soybean.

The obtained results point that sunflower and soybean have tenfold higher potential for Ni accumulation in seeds, compared to corn and wheat (Table 2).

Table 2. Descriptive statistics of Ni concentration in plant seeds and of bioaccumulation factors

Parameter	Aver. value	Median value	MIN	MAX	ST DEV	CV [%]	SE
Ni wheat [mg kg^{-1}]	0.813	0.500	0.500	1.658	0.449	55.17	0.109
BAF wheat	0.020	0.019	0.005	0.052	0.014	68.73	0.003
Ni corn [mg kg^{-1}]	0.608	0.500	0.500	3.300	0.549	90.36	0.108
BAF corn	0.013	0.012	0.004	0.036	0.007	50.85	0.001
Ni soybean[mg kg^{-1}]	9.981	8.405	4.690	19.420	4.896	49.06	1.632
BAF soybean	0.256	0.157	0.082	0.563	0.191	74.52	0.064
Ni sunflower[mg kg^{-1}]	8.937	10.260	0.500	17.860	5.109	57.17	1.172
BAF sunflower	0.255	0.126	0.006	0.866	0.244	95.81	0.056

Aver. – Average value; MIN – Minimum value; MAX – Maximum value; ST DEV – Standard deviation; CV – Coefficient of variation; SE – Standard error of the arithmetic mean

The present study investigates the statistically significant differences between Ni concentration in soil, plant, and BAF parameters in the observed plant species (Table 3). The first important observation is that all crops have equal total Ni_T concentration in soil. According to available Ni_A concentrations in soil, mutual differences are found between soils under maize and sunflower. According to Ni concentration in seed and BAF, two groups are clearly distinguished – the group of soybeans and sunflowers with higher Ni content and the group of wheat and corn with lower Ni content in seed (Table 3). The obtained differences confirm that plant species have a dominant role in the bioaccumulation of Ni, as documented in previous research (Sheoran et al., 2016).

Table 3. The average value and statistical difference of Ni concentration in soil and plant for observed crops species

Crops species	Ni_T [mg kg^{-1}] in soil	Ni_A[mg kg^{-1}] in soil	Ni [mg kg^{-1}] in seed	BAF
Wheat	48.1^{a}	5.2^{ab}	0.813^{b}	0.020^{b}
Corn	50.8^{a}	7.3^{a}	0.608^{b}	0.013^{b}
Soybean	50.2^{a}	5.0^{ab}	9.981^{a}	0.256^{a}
Sunflower	51.9^{a}	4.4^{b}	8.937^{a}	0.255^{a}

Values marked with the same letter do not differ in statistical significance (according to Duncan's multiple range test. $p \leq 0.05$)

Table 4. Correlations between BAF, Ni concentration in plant and soil for observed crops species

	Parameter	Ni in seed	Ni_T in soil	Ni_A in soil	BAF
Wheat	Ni in seed	1.000	-0.114^{ns}	-0.066^{ns}	0.834**
	Ni_T in soil		1.000	0.585*	-0.565*
	Ni_A in soil			1.000	-0.359^{ns}
	BAF				1.000
Maize	Ni in seed	1.000	0.358^{ns}	-0.034^{ns}	0.694**
	Ni_T in soil		1.000	0.630**	-0.367^{ns}
	Ni_A in soil			1.000	-0.579**
	BAF				1.000
Soybean	Ni in seed	1.000	-0.543^{ns}	-0.230^{ns}	0.944**
	Ni_T in soil		1.000	0.597*	-0.754*
	Ni_A in soil			1.000	-0.388^{ns}
	BAF				1.000
Sunflower	Ni in seed	1.000	-0.443^{ns}	-0.182^{ns}	0.821*
	Ni_T in soil		1.000	0.782**	-0.730*
	Ni_A in soil			1.000	-0.424^{ns}
	BAF				1.000

** $p \leq 0.01$ significantly correlated; * $p \leq 0.05$ significantly correlated; ns – no statistical signification

Concerning soil-plant nickel distribution relationships, the determined correlations are shown in Table 4. A significant positive correlation is found between soil total and available Ni concentration, as well as between Ni concentration in seed and BAF, which is expected. Ni concentration in seeds is not significantly correlated with either total or available Ni concentration in soil in any of the observed crop species, highlighting the importance of the bioaccumulation factor (BAF).

Determined parameter BAF is in statistically significant negative correlation with total Ni content in soil for all observed crops, except maize, where BAF is negatively correlated with available Ni concentration in soil (Table 4). The obtained correlations indicate that higher Ni concentration in the soil causes lower Ni concentration in the seed. This might be due to activation of the plant defense mechanisms for preservation of its reproductive organs – seeds, in this case – from the harmful effects. In future research, these relationships need to be examined in detail. The Ni uptake by plants from the serpentine soils, which geochemically contain enormously high Ni concentrations, have been widely investigated and based on these studies. The uptake and translocation of Ni primarily depend on the plant species, where some serpentine endemic species have adapted and act as Ni hyperaccumulators with the concentration of even 1,000 mg kg^{-1} in plant tissue (Milić et al., 2021). However, non-endemic serpentine plant species generally uptake low Ni content compared to the uptake of other PTEs, although they grow in soil with such high Ni content (Freitas et al., 2004; Bani et al., 2010; Vicić et al., 2014; Tomović et al., 2017; Milić et al., 2021). The reason for the lower accumulation of Ni in plants, despite the high content of Ni in the soil medium, can be the activation of defense mechanisms for the accumulation of nickel in plant tissue.

CONCLUSION

The median value of total Ni concentration in soil under main field crops in Central Serbia is 44.8 mg kg^{-1}, which is close to MAC. The median concentration of Ni in wheat and corn seeds is 0.5 mg kg^{-1}, while soybean and sunflower seeds have much higher Ni content of 8.40 and 10.26 mg kg^{-1}median value, respectively. The median value of determined bioaccumulation factors (BAF) in the present study coincides with Ni concentration in seed and ranges from 0.013 (corn) to 0.256 (soybean). Therefore, the observed plant species have no hyperaccumulation characteristics. The obtained results indicate a tenfold higher Ni accumulation potential in sunflower and soybean seed compared to corn and wheat. According to statistically significant differences, all crops have equal total Ni_T concentration in soil, while the available Ni_A concentration in soil under corn and sunflower is different. According to Ni concentration in seed and BAF, two groups are clearly distinguished − the group of soybeans and sunflowers with higher Ni content and the group of wheat and corn with lower Ni content in seed. The obtained differences confirm that plant species have a dominant role in the bioaccumulation of Ni. Ni concentration in seeds is not significantly correlated with either total or available Ni concentration in the soil in any of the observed crops. The determined BAF parameter is in a statistically significant negative correlation with total Ni content in the soil in all observed crops except maize, where BAF is negatively correlated with available Ni concentration in soil. The obtained correlations indicate that higher Ni concentration in soil caused lower Ni concentration in seed, due to the activation of plant defense mechanisms for preservation of its reproductive organs, i.e. seeds, from harmful effects. In future research, these relationships need to be examined in detail.

Based on the obtained results, the main field crops in Central Serbia have safe Ni concentration levels in seed intended for feed and food usage. Increased content of Ni in agriculture soils of Central Serbia requires permanent monitoring aiming at sustainable agriculture production.

ACKNOWLEDGMENT

This study was conducted as a part of the Project: "Global assessment of the tolerance of field crops on hazardous and harmful substance in agricultural soils and irrigation water", supported by the Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia in 2018. This research was also supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-9/2021-14/200032.

REFERENCES

Adriano D (2001): *Trace Elements in Terrestrial Environments, Biogeochemistry, Bioavailability and Risks of Metals.* Second Edition. New York: Springer.

Albanese S, Sadeghi M, Lima A, Cicchella D, Dinelli E, Valera P, Falconi M, Demetriades A, De Vivo B (2015): GEMAS: Cobalt, Cr, Cu and Ni distribution in agricultural and grazing land soil of Europe. *J. Geochem. Explor.* 154: 81–93. [DOI: 10.1016/j.gexplo.2015.01.004]

Bani A, Pavlova D, Echevarria E, Mullaj A, Reeves R, Morel JL, Sulçe S (2010): Nickel hyperaccumulation by the species of Alyssum and Thlaspi (Brassicaceae) fromthe ultramafic soils of the Balkans. *Bot. Serb.* 34: 3–14.

Dozet D, Nešić Lj, Belić M, Bogdanović D, Ninkov J, Zeremski T, Dozet D, Banjac B (2011): Poreklo i sadržaj nikla u aluvijalno-deluvijalnim zemljištima Srema [Origin and Content of Nickel in Alluvial-Diluvial Soils of Srem, Serbia]. *Ratar. Povrt*. 48: 369–374.

Drzewiecka K, Mleczek M, Gąsecka M, Magdziak Z, Goliński P (2012): Changes in Salixviminalis L. cv. 'Cannabina' morphology and physiology in response to nickel ionshydroponic investigations. *J. Hazard. Mater.* 217–218: 429–438. [DOI: 10.1016/j. jhazmat.2012.03.056].

Egner H, Riehm H (1955): *Die Untersuchung von Böden*. In: R. Thun, R. Herrmann, E. Knickmann (Eds.), Berlin, Germany: Neumann Verlag Radebeul, 110–125.

Freitas H, Prasad MNV, Pratas J (2004): Analysis of serpentinophytes from north-east of Portugal for trace metal accumulation-relevance to the management of mine environment. *Chemosphere* 54: 1625–1642. [DOI: 10.1016/j.chemosphere.2003.09.045].

Houskova B, Montanarella L (2006): *Parent material as a source of natural background values in soils*. The European Soil Datebase, version V2.0, EUR 19945 EN, Available from: https://esdac.jrc.ec.europa.eu/ESDB_Archive/eusoils_docs/Poster/ParentMaterial.pdf

Huillier LL, d'Auzac J, Durand M, Michaud-Ferrièr N (1996): Nickel effects on two maize (*Zea mays*) cultivars: growth, structure, Ni concentration, and localiza-tion, *Canad. J. Bot.* 74: 1547–1554.

ISO 10390, 2010. *Soil Quality – Determination of pH*. International Organization for Standardization, Genève, Switzerland.

ISO 10693, 1995. *Soil Quality – Determination of Carbonate Content – Volumetric Method*. International Organization for Standardization, Genève, Switzerland.

ISO 10694, 1995. *Soil quality – Determination of organic and total carbon after dry combustion (elementary analysis)*. International Organization for Standardization, Genève, Switzerland.

ISO 11464, 2006. *Soil Quality – Pretreatment of Samples for Physico-chemical Analysis*. International Organization for Standardization, Genève, Switzerland.

ISO 13878, 1998. *Soil quality – Determination of total nitrogen content by dry combustion ("elemental analysis")*. International Organization for Standardization, Genève, Switzerland.

ISO 14235, 1998. *Soil Quality – Determination of Organic Carbon by Sulfochromic Oxidation*. International Organization for Standardization, Genève, Switzerland.

Kabata-Pendias A, Mukherjee AB (2007): *Trace Elements from Soil to Human*. New York: Springer.

Kabata-Pendias A, Pendias H (2001): *Trace Elements in Soils and Plants*. 3rd edition, Boca Raton, FL: CRC Press Inc.

Kastori R (ed.) (1997): *Teški metali u životnoj sredini*. Novi Sad: Naučni institut za ratarstvo i povrtarstvo [*Heavy metals in environment*. Research Institute of Field and Vegetable Crops].

Kutman BY, Kutman UB, Cakmak I (2013): Nickel-enriched seed and externally supplied nickel improve growth and alleviate foliar urea damage in soybean. *Plant Soil* 363: 61–75. [DOI: 10.1007/s11104-012-1284-6]

Milić D, Bubanja N, Ninkov J, Milić S, Vasin J, Luković J (2021): Phytoremediation potential of the naturally occurring wetland species in protected Long Beach in Ulcinj, Montenegro. *Sci. Total Environ.* 797: 148995. [DOI: 10.1016/j.scitotenv.2021.148995]

Mrvić V, Antonović G, Martinović Lj (Eds.) (2009): *Plodnost i sadržaj opasnih i štetnih materija u zemljištima centralne Srbije* [Fertility and Content of Harmful and Dangerous Substances in the Soils of Central Serbia]. Beograd: Institut za zemljište.

OG 23/1994 *Serbian Official Gazette*. Regulation on permitted amounts of hazardous and noxious substances in agricultural soil and irrigation water and methods of their testing.

OG 39/2016 *Serbian Official Gazette*. Regulation on the quality of animal feed.

OG 90/2018 *Serbian Official Gazette*. Regulation on the maximum permitted amounts of plant protection matter residues in food and animal foods and about food and animal foods for which the maximum allowable residues they are to be determine.

Sheoran V, Sheoran AS, Poonia P (2016): Factors affecting phytoextraction: a review. *Pedosphere* 26: 148–166. [DOI: 10.1016/S1002-0160(15)60032-7]

Tibco Software Inc (2018): Statistica (data analysis software system), version 13. http://tibco.com.
Tomović G, Buzurović U, Đurović S, Vicić D, Mihailović N, Jakovljević K (2017): Strategies of heavy metal uptake by three Armeria species growing on different geological substrates in Serbia. *Environ. Sci. Pollut. Res. Int.* 25: 507–522. [DOI: 10.1007/s11356-017-0445-9]
US EPA, 2001. Method 200.7: Trace Elements in Water, Solids, and Biosolids by Inductively Coupled Plasma – Atomic Emission Spectrometry, Rev. 5, EPA-821-R-01- 0102001, 57.
Van Reeuwijk LP (2002): *Procedures for soil analysis*. Sixth edition, ISRIC FAO Technical Paper vol. 9. International Soil Reference and Information Centre Wageningen, 12–16.
Vicić D, Stoiljković M, Bojat N, Sabovljević S, Stevanović B (2014): Physiological tolerance mechanisms of serpentine tolerant plants from Serbia. *Annu. Rev. Ecol. Evol. Syst.* 69: 185–195.

ОРИГИНАЛНИ НАУЧНИ РАД

САДРЖАЈ НИКЛА У СЕМЕНУ РАТАРСКИХ УСЕВА И ПОЉОПРИВРЕДНОМ ЗЕМЉИШТУ ЦЕНТРАЛНЕ СРБИЈЕ

Душана Д. БАЊАЦ[1], Јордана М. НИНКОВ[1], Станко Б. МИЛИЋ[1], Снежана П. ЈАКШИЋ[1], Милорад С. ЖИВАНОВ[1], Биљана И. РАДОВИЋ[1], Марко В. МАЛИЋАНИН[2]

[1] Институт за ратарство и повртарство
Институт од националног значаја за Републику Србију
Максима Горког 30, Нови Сад 21000, Србија
[2] Универзитет у Нишу, Пољопривредни факултет Крушевац
Косанчићева 4, Крушевац 37000, Србија

РЕЗИМЕ: Никал је есенцијални хранљиви елемент за животиње и има важну улогу у бројним физиолошким и биохемијским процесима виших биљака, али истовремено припада групи потенцијално токсичних елемената (ПТЕ). Циљ овог истраживања био је да се утврде концентрације Ni у релацији земљиште–биљка између главних ратарских биљних врста и пољопривредног земљишта за централну Србију. Укупно је узето 71 узорак земљишта из горњег слоја земљишта 0–30 cm, с подручја шест статистичких округа централне Србије. Прикупљено је укупно 71 узорак семена током жетве као просечан узорак семена са сваке посматране парцеле, од чега је 26 узорака било семе кукуруза, 19 сунцокрета, 17 пшенице и девет соје. Прикупљени узорци анализирани су на основне параметра земљишта и укупну и приступачну концентрацију никла у земљишту, као и укупну његову концентрацију у семену. Вредност медијане за укупну концентрацију никла у земљишту била је 44,8 mg kg^{-1}, што је близу вредности МДК. Средња концентрација никла у семену пшенице и кукуруза била је 0,5 mg kg^{-1}, док је семе соје и сунцокрета имало знатно већи садржај Ni од 8,40 односно 10,26 mg kg^{-1}. Фактори биоакумулације у семену (BAF) у овој студији кретали су се од 0,013 (кукуруз) до 0,256 (соја). Према статистички значајним разликама, све ратарске биљне врсте имале су једнаку укупну концентрацију Ni_T у земљишту, док су се према приступачној концентрацији Ni_A у земљишту, међусобно разликовала земљишта под кукурузом и сунцокретом. На основу концентрације Ni у семену и BAF-а, јасно се издвајају две групе: у једној групи су соја и сунцокрет са већим садржајем Ni, док су у другој групи пшеница и кукуруз са нижим садржајем Ni у семену.

Добијене разлике потврђују да биљне врсте имају значајну улогу у биоакумулацији Ni. Утврђени параметар BAF био је у статистички значајној, негативној корелацији са укупним садржајем Ni у земљишту за све посматране усеве, осим кукуруза. Међутим, вредност BAF-a је само за кукуруз била у статистички значајној, негативној корелацији са приступачном концентрацијом Ni у земљишту. Добијене корелације указују да је већа концентрација никла у земљишту узроковала његову мању концентрацију у семену, вероватно услед активирања одбрамбених механизама биљака за очување репродуктивних органа – семена, од штетних утицаја. На основу свих добијених резултата, главне ратарске биљне врсте у централној Србији имале су безбедну концентрацију никла у семену за сточну и људску храну. Повећан садржај никла у пољопривредном земљишту централне Србије захтева стално праћење у циљу одрживе пољопривредне производње.
КЉУЧНЕ РЕЧИ: никал, ратарске културе, земљиште, семе

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 141, 95—105, 2021

UDC 543.645.7:636.045
https://doi.org/10.2298/ZMSPN2141095T
ORIGINAL SCIENTIFIC ARTICLE

Dragana Z. TOMANIĆ[*], *Zorana R. KOVAČEVIĆ*, *Dragica M. STOJANOVIĆ*, *Branislava M. BELIĆ*, *Nenad S. STOJANAC*, *Ivan B. STANČIĆ*, *Ivan M. GALIĆ*

University of Novi Sad, Faculty of Agriculture, Department of Veterinary medicine
Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia

METRONIDAZOLE IN THE PROPHYLAXIS AND TREATMENT OF DOGS AND CATS

ABSTRACT**:** Ever since their discovery, antimicrobials have helped in controlling and treating infections in both humans and animals. The control of infectious diseases is endangered by the rise of microorganisms that are resistant to this group of drugs. Limited availability of authorized veterinary drugs leads to prescription of human approved drugs. The aim of our study was to describe metronidazole use patterns and its accordance with scientific literature in Serbia. Results have shown that majority of prescriptions were written to dogs, while 27.1% prescriptions were for cats. Most common general conditions were dental and digestive disorders. Our study shows that metronidazole is available in oral and injectable form, while cats were only treated with injectable formulation. Even though prescription of human approved drugs for companion animals is allowed by Law, there is no official record of data on the extent or nature of off-label use in Serbia. Such information is essential for guiding antimicrobial use policy in small animal veterinary practice as well as for assessing the risk of transmission of antimicrobial resistance to humans. Recognizing the importance of antimicrobial resistance and ensuring more judicious use of antibiotics are key elements to any strategy for maintaining the usefulness of antimicrobial drugs in animals and humans.

KEYWORDS: antimicrobials, antimicrobial resistance, cats, dogs, metronidazole

INTRODUCTION

Antimicrobial drugs are used frequently for the therapy and prevention of infectious diseases in companion animals (Pomba et al., 2017; Morley et al., 2005; Marshall et. al. 2011). These drugs are among the most important treatment options available to modern medicine (Morley et al., 2005). Antibiotic use in companion animals is likely more liberal than in animal food because

[*] Corresponding author. E-mail: tomanicd@live.com

compared to agricultural use, antibiotic use in companion animals is not strictly regulated and is driven by people's emotional attachment to their pets' needs (Shea et al., 2011). Availability of effective antimicrobial therapy has had a profound impact on human and animal health, improved welfare and on safe and economical production of food (Weese et al., 2015). However, their efficacy has been compromised by antimicrobial resistance which has resulted in tremendous impacts on human and animal health, and the economics of both medicine and agriculture (Morley et al., 2005; Hur et al., 2020).

Due to the inadequate use of antibiotics described in both human and veterinary medicine, bacteria have developed resistance to many classes of antimicrobial and this resistance has been disseminated among different bacterial populations, resulting in treatment failures in both human and veterinary medicine (Hughes et al., 2012; Miranda et al., 2021). Antimicrobial resistance (AMR) showed a dramatic increase over the last decade and is currently considered a major world health problem (Hur et al., 2020). Although the true magnitude of antimicrobial resistance is not fully known, growing population of dogs and cats can contribute to the spreading of antimicrobial resistance. Close contact with humans, but also the increasing attention to animal welfare results in the extensive use of antimicrobial agents in these animals (Miranda et al., 2021; Guardabassi et al., 2004) particularly in canine medicine (Guardabassi et al., 2004).

Lately, the risks posed by the use of antimicrobials in the treatment of infections in pets have been assumed to be quite low. However, recent publications indicate that antimicrobial resistance is increasing among organisms causing infections in pets (Lloyd, 2007). While there is considerable data on antimicrobial drug use and drug resistance in bacteria of animal food origin, there is little useful data about the same issue in companion animals, based on a review of the current literature (Prescott et al., 2002; Guardabassi et al., 2004; Lloyd, 2007; Miranda et al., 2021; Shea et al., 2011). Several case studies have documented the presence of antibiotic-resistant bacterial strains in small animal veterinary medicine (Damborg et al., 2004; Damborg et al., 2016; Nienhoff et al., 2009; Duijkeren et al., 2011) because there is a large overlap in antibiotics used in human and in small animal veterinary medicine, and because of the close contact many pet owners have with their dogs and cats (Shea et al., 2011). Companion animals are able to acquire and exchange multidrug resistant pathogens with humans (Hur et al., 2020; Lloyd, 2007; Miranda et al., 2021) and thus, the benefits of their use in animals must be weighed against the risk to public health (Chambers et al., 2018). Knowledge of antimicrobial usage patterns is critical in the implementation and monitoring of antimicrobial stewardship programs (Hur et al., 2020).

Some antimicrobial products authorized for human use are also used in companion animals, in application of the 'cascade' (Pomba et al., 2017; EMA/CVMP/AWP/237294/2017; Guardabassi et al., 2004). This consumption is regulated by EU directive 2001/82/EEC and it allows veterinarians to use human drug only if a suitable veterinary drug for another animal species does not exist (Hölsö et al., 2005); Law on medicines and medical devices of Republic of Serbia, 2012). When prescribing under the cascade, veterinarians should take into account the importance of the antimicrobial to human medicine and the risk for transmission of AMR from treated animals to humans (EMA/CVMP/AWP/237294/2017). According to some authors, reasons why human drugs are so widely used in companion animal practice can be sometimes associated with lower price (Escher et al., 2011; Gomez et al., 2018), but also with more suitable drug formula, the strength, package size or non-availability of a comparable veterinary product (Hölsö et al., 2005).

Metronidazole belongs to the group of nitroimidazoles (ALIMS, 2015; Bergvall et al., 2009) and it is not authorized for use in animals in Serbia (ALIMS, 2015). It has bactericidal effect against a wide range of microorganisms (EMEA/MRL/173/96-FINAL, 1997; ALIMS, 2015). The exact mechanism of action is not fully known, but metronidazole diffuses into the microorganism cell and causes loss of helical structure, rupture of strands in bacterial and protozoal DNA, and inhibits nucleic acid synthesis, resulting in cell death (Boothe, 2015; Tauro et al., 2018). The microbiological properties of metronidazole are known from its use in human medicine (EMEA/MRL/173/96-FINAL, 1997). It is used in humans for treatment and prophylaxis of anaerobic bacterial and protozoal infections (ALIMS, 2015; Boothe, 2015; EMEA/MRL/173/96-FINAL, 1997; Ceruelos et al., 2017). In Serbia, the use of metronidazole is off label, since it is not in accordance with its summary of the product characteristics (ALIMS, 2015), but it is commonly prescribed by veterinarians. In companion animals it is mostly used in the treatment of gastrointestinal infections, in the treatment of gingivitis, hepatitis and osteomyelitis (Seol et al., 2010). For treatment of these infections metronidazole is used in doses of 10–15 mg/kg b.w./12 h, or 25–50 mg/kg b.w./h (Cupic et al., 2019). According to the Food and Drug Administration Agency (FDA) and the European Medicines Agency (EMA), this drug is banned in animal food (Payne et al., 1999).

The goal of the present study was to describe metronidazole use patterns in dogs and cats in Serbia and to determine if that consumption is in accordance with prudent use guidelines and scientific literature.

MATERIALS AND METHODS

Sampling design

The study was conducted at private veterinary ambulance in Novi Sad, Serbia, between January and May of 2020. Fifty nine medical records that included dogs and cats were used. All prescriptions are written in electronic database along with clinical history of animals and diagnostic work-up, which allows a computer search and a transfer of information to the spreadsheets (Excel version 9.0). A computer search was made to identify all animals where metronidazole was used in treatment during survey period. Following information were gathered: animal species, age, drug brand name, active substance, pharmaceutical form, dosage, duration of the treatment and treated condition, as well as route of administration.

Data analysis

The data were analyzed first at species level (dogs and cats), secondly by indication and thirdly by route of application. Each prescription was then classified as either therapeutic or prophylactic use by reviewing the medical charts. When animal received pharmaceutical for a condition, this was classified as a therapeutic treatment. When animal was treated with a pharmaceutical as a part of a surgical procedure (administration prior, during or after the surgical procedure), the administration was classified as prophylactic. Therapeutic treatments were grouped according to the main indication (dental and digestive) and prophylactic treatments according to similar criteria (dental, digestive and orthopedic). The route of application (per oral and intravenous) was also distinguished. The mean treatment period for each indication was calculated. For each case, it was determined whether diagnostic procedures (bacteriological culture, antimicrobial susceptibility test, cytology and rapid SNAP test) were performed and if so, which procedures were those.

RESULTS AND DISCUSSION

During the survey, 59 medical records containing prescriptions of metronidazole were analyzed. The majority of prescriptions were written to dogs (43 or 72.9%) while 16 or 27.1% prescriptions were written for cats. Their age ranged from two months to 15 years for cats, while for dogs this range was from two months to 13 years (Figure 1).

Conditions (Treatment Indication)

For dogs, therapeutic treatment was used in 65.1% cases (28/43), and the respective percentage for cats was 68.8% (11/16). Conditions for therapeutic use of antimicrobials were dental and digestive disorders (Table 1 and Table 2). Prophylactic (perioperative) treatment was administered in 34.9% (15/43), while in cats 31.2% (5/16) of administrations were prophylactic. Surgical procedures which included interventions like dental, digestive and orthopedic are shown in Table 3. The specific diseases included periodontitis and gingivitis; gastroenteritis and enteritis; in orthopedic, fractures and amputation. When route of administration was considered, 53.5% of dogs and 100% of cats were prescribed injectable metronidazole, and 46.5% of dogs were prescribed an oral drug, while no oral formulations were prescribed to cats.

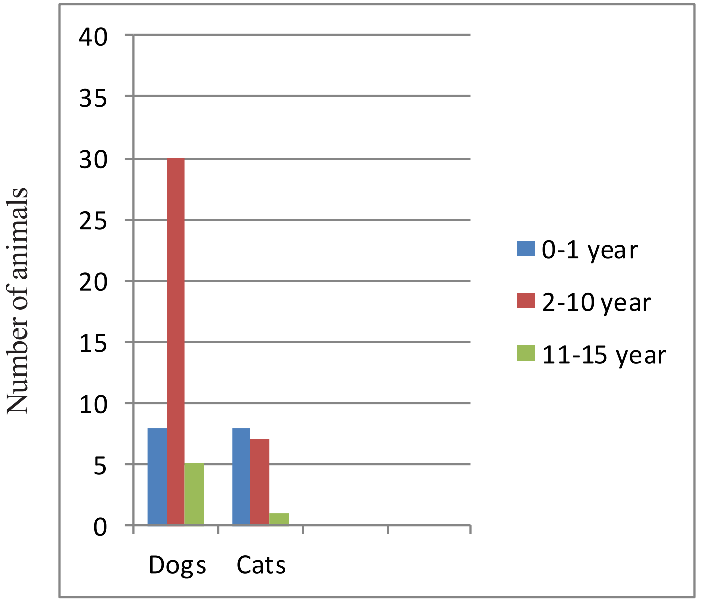

Figure 1. Age range of animals

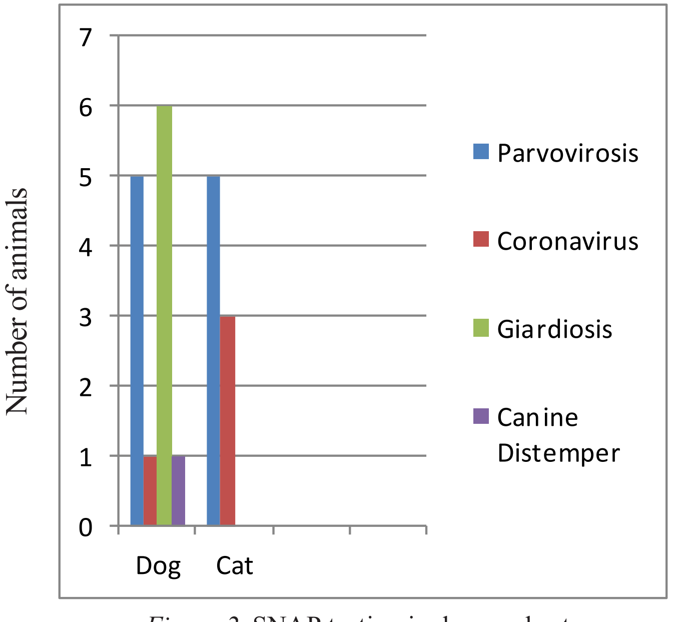

Figure 2. SNAP testing in dogs and cats

In dogs, the mean duration of the treatment period for oral formulations varied from three to ten days, while for injectable metronidazole that period was 1–5 days. The mean duration of the treatment in cats varied between 1–5 days.

Diagnostic procedures

Antimicrobial susceptibility and bacteriological testing in dogs had been performed in 9.3% and 6.7% cases. However, no information about this testing in cats was recorded. SNAP testing was used every time it was suspected on infectious diseases like Parvo virus, Corona virus, Giardiasis and Canine distemper (Figure 2).

Table 1. Use of metronidazole in therapy of 43 dogs according to the indication and route of administration

Indication	Route of administration		N	%
	Per oral	Parenteral		
Dental				
Gingivitis	3		3	7.0
Periodontitis	2		2	4.7
Digestive				
Gastroenteritis	5	5	10	23.2
Enteritis	6	7	13	30.2
Surgery	4	11	15	34.9
Total			43	100

Table 2. Use of metronidazole in therapy of 16 cats according to the indication and route of administration

Indication	Route of administration	N	%
	Parenteral		
Digestive			
Gastroenteritis	3	3	18.8
Enteritis	8	8	50.0
Surgery	5	5	31.2
Total		16	100

Table 3. Use of metronidazole in prophylaxis of dogs and cats according to the indication in this study

Indication	Dental		Digestive	Orthopedic	
	Gingivitis	Periodontitis	Enterotomia	Fractura osis	Amputation
Dogs	8	2	2	3	
Cats	2	1	1		1

In Serbia, there are no surveillance reports on usage of antimicrobials approved for human use. As in Serbia, these drugs are frequently prescribed for companion animals all over the world. Furthermore, the availability of similar studies in the literature is very limited. Metronidazole is not approved for veterinary purposes despite its common place usage in companion animal medicine. The percentage of metronidazole prescriptions in our study was significantly higher for dogs (72.9%) than cats (27.1%). The most common conditions for metronidazole use were dental and digestive disorders, what is in agreement with Danish guideline for antibiotic use in companion animals (Jessen et al., 2018).

Metronidazole is commonly used by small animal's practitioners, as it is believed to reduce the duration of clinical signs and the severity of diarrhea, although evidence is lacking (Robbing et al., 2020; Lutz et al., 2020; Johnston et al., 2000). Regarding systematic use in our study, metronidazole was the most used for digestive disorders what is in agreement with the European data (Gómez et al., 2018; Lutz et al., 2020). Acute diarrhea, including acute hemorrhagic diarrhea syndrome is frequently encountered in dogs. This condition is commonly treated with metronidazole, but several recent publications suggest that this disease is typically self-limiting and that probiotic administration can result in as rapid a resolution of clinical signs as antimicrobial therapy (Robbins et al., 2020; Rantala et al., 2004; Lehner et al., 2020). While nitroimidazoles are not classified as critically important antimicrobials in Serbia, their use to treat acute diarrhea should nevertheless be restricted. Current guidelines only recommend the use of antimicrobials in cases of acute diarrhea if clinical or laboratory signs consistent with sepsis are present (Lutz et al., 2020; Jessen et al., 2018). Another indication for metronidazole use was gingivitis and periodontitis. The results of study conducted by Heijl et al. (1980) demonstrated that administration of metronidazole can reduce the rate of plaque formation, change the composition of the developing subgingival microbiota and prevent the onset of gingivitis. Holloway et al. (2013) pointed out that metronidazole and clindamycin are preferred because they reach more effective levels within the biofilm in the vicinity of the periodontal space.

Bergvall et al. (2009) revealed that perioperative use of antibiotics is referred to the administration before, during or after surgical procedure. Our results have shown that perioperative use of metronidazole was in cases of dental, digestive and orthopedic disorders (Table 3). A prophylactic use of is always indicated prior to operations with a high risk of anaerobic infections (ALIMS, 2015). Guidelines for the prudent use of antimicrobials in veterinary medicine (2015/C 299/04) recommend that perioperative use of antimicrobials should be minimized by using aseptic techniques also suggested by Lehner et al. (2020). Besides, Swedish guideline emphasize that they never should be used as a substitute for good asepsis while Danish guidelines emphasize the dog's status and expected surgery as the main criteria for perioperative use of antimicrobials (Jessen et al., 2018; Bergvall et al., 2006).

When route of administration was considered, there is a small difference between oral (46.5%) and parenteral administration (53.5%) in dogs whereas cats were treated only with metronidazole parenterally (100%). This may reflect the challenge of giving oral medication to cats when compared to injectable antimicrobial agents (Burke et al., 2016), but also the fact that although tablets have been broken or crushed for oral administration to cats, they find these unpalatable (Macintire et al., 2012).

Prescribing under the cascade should be supported by a full diagnostic investigation including bacterial culture and antimicrobial susceptibility testing, when possible (EMA/CVMP/AWP/237294/2017). Our results revealed very low rate of diagnostic procedures, which can be associated with excessive use of antimicrobials, representing a critical issue not only for a public health, but also for animal welfare.

CONCLUSION

Although the Law allows the cascade use of drugs, there is no official record of data on the extent or nature of off-label use in Serbia. Without this information it is impossible to estimate how recommendations of antimicrobial use are followed which may lead to drug incompliance and inadequate treatment of infectious diseases, which can, in turn, lead to development of antimicrobial resistance. National recommendations, as well as surveillance system, are mandatory in order to support responsible use of antibiotics, particularly of human approved drugs, like metronidazole. Antimicrobial drugs are valuable, versatile, safe and have a specific role in controlling bacterial disease in animals, but their usefulness can be only maintained if being used in accordance with good and professional veterinary practice.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Science and Technological Development, Republic of Serbia [Grant Number 451-03-68/2020-14/200117].

REFERENCES

Agencija za lekove i medicinska sredstva Srbije (ALIMS) (2015): Sazetak karakteristika leka – Metronidazol. Available to: www.alims.gov.rs

Bergvall K, Greko C, Ingman J, Larsson CI, Mannerfelt T, Odensvik K, Pelander L, Sjöström L, Holst BS, Weidman A, Wellander M (2009): Guidelines for the clinical use of antibiotics in the treatment of dogs and cats, Swedish Veterinary Association. Available to: https://www.svf.se/media/jzqbjer5/policy-ab-english-dogs-and-cats.pdf

Boothe DM (2015): Nitroimidazoles. *Merck Manual, Veterinary Manual*. Available to: https://www.merckvetmanual.com/pharmacology/antibacterial-agents/nitroimidazoles

Burke S, Black V, Sánchez-Vizcaíno F, Radford A, Hibbert A, Tasker S (2016): Use of cefovecin in a UK population of cats attending first-opinion practices as recorded in electronic health records. *J. Feline Med. Surg.* 19: 687–692.

Ceruelos AH, Romero-Quezada LC, Ledezma JCR, Contreras LL (2017): Therapeutic uses of metronidazole and its side effects: an update. *Eur. Rev. Med. Pharmacol. Sci*. 23: 397–401.

Chambers P, Gibson I, Manson K, Millar A, Scott D (2018): Guidelines for the clinical use of antimicrobial agents in the treatment of dogs and cats. New Zealand Veterinary Association. Available to: www.amrvetcollective.com/assets/guidelines/guide_comp.pdf

Ćupić V, Muminović M, Kobal S, Velev R (2019): *Pharmacology for students of veterinary medicine*. Belgrade–Sarajevo–Ljubljana–Skoplje.

Damborg P, Broens EM, Chomel BB, Guenther S, Pasmans F, Wagenaar JA, Weese JS, Wieler JH, Windahl U, Vanrompay D, Guardabassi L (2016): Bacterial Zoonoses Transmitted by Household Pets: State-of-the-Art and Future Perspective for Targeted Research and Policy Actions. *J. Comp. Path.* 155: S27–S40.

Damborg P, Olsen KEP, Nielsen EM, Guardabassi (2004): Occurrence of Campylobacter jejuni in Pets living with human patients infected with C. jejuni. *J. Clin. Microbiol.* 42: 1363–1364.

De Briyne N, Atkinson J, Pokludová L, Borriello SP (2014): Antibiotics used most commonly to treat animals in Europe. *Vet. Rec.* 175: 325.

Duijkeren E, Kamphuis M, Mije IC, Laarhoven LM, Duim B, Wagenaar JA, Houwers DJ (2011): Transmission of methicillin-resistant Staphylococcus pseudintermedius between infected dogs and cats and contact pets, humans and the environment in households and veterinary clinics. *Vet. Microbiol.* 150: 338–343.

EMEA/CVMP (2017): Reflection paper on off-label use of antimicrobials in veterinary medicine in The European Union. Available to: https://www.ema.europa.eu/en

EMEA/MRL/173/96-FINAL (1997): Metronidazole summary report. Committee for veterinary medical products. Available to: www.ema.europa.eu

Escher M, Vanni M, Intorre L, Caprioli A, Tognetti R, Scavia G (2011): Use of antimicrobials in companion animal practice: a retrospective study in a veterinary teaching hospital in Italy. *J. Antimicrob. Chemother.* 66: 920–927.

Gómez-Poveda B. and Moreno MA (2018): Antimicrobial Prescriptions for Dogs in the Capital Of Spain. *Front. Vet. Sci.* 5: 309.

Guardabassi L, Schwarz S, Lloyd DH (2004): Pet animals as reservoirs of antimicrobial-resistant bacteria. *J. Antimicrob. Chemother.* 54: 321–332.

Heijl L, Lindhe J (1980): Effect of selective antimicrobial therapy on plaque and gingivitis in the dog. *J. Veterinary Periodontol.* 7: 463–478.

Holloway S, Trott D, Shipstone M, Barrs V, Malik R, Burrows M (2013): Antibiotic prescribing detailed guidelines. Australian Infectious Diseases Advisory Panel. Available to: http://www.ava.com.au/sites/default/files/AVA_website/pdfs/AIDAPguidelines.pdf. Accessed August 12, 2016.

Hölsö K, Rantala M, Lillas A, Eerikäinen S, Huovinen P, Kaartinen L (2005): Prescribing Antimicrobal Agents for Dogs and Cats via University Pharmacies in Finland – Patterns and Quality of Information. *Acta. Vet. Scand.* 46: 87–93.

Hughes LA, Williams N, Clegg P, Callaby R, Nuttall T, Coyne K, Pinchbeck G, Dawson S (2012): Cross-sectional survey of antimicrobial prescribing patterns in UK small animal veterinary practice. *Prev. Vet. Med.* 104: 309–316.

Hur BA, Hardefeldt LY, Verspoor KM, Baldwin T, Gilkerson JR (2020): Describing the antimicrobial usage patterns of companion animal veterinary practices; free text analysis of more than 4.4 million consultation records. *PLoS One* 15: e0230049.

Jessen LR, Damborg P, Spohr A, Goericke-Pesch S, Langhorn R, Houser G, Willesen J, Schjærff M, Eriksen T, Sørensen TM, Jensen VF, Obling F, Guardabassi L (2018): Antibiotic use

guidelines for companion animal practice (2nd edition). Companion Animal Group, Danish Veterinary Association.
Johnston KL, Lamport AI, Ballèvre O, Batt R (2000): Effects of oral administration of metronidazole on small intestinal bacteria and nutrients of cats. *Am. J. Vet. Res.* 61: 1106–1112.
Law on medicines and medical devices Republic of Serbia ("Official Gazette of RS", No. 30/2010, 107/2012 – other law and 113/2017 – other law). Available to: https://www.alims.gov.rs/eng/regulations/law-on-medicines-and-medical-devices/
Lloyd DH (2007): Reservoirs of antimicrobial resistance in pet animals. *Clin. Infect. Dis.* 45: 148–152.
Lutz B, Lehner C, Schmitt K, Willi B, Schüpbach G, Mevissen M, Peter R, Müntener C, Hanspeter Naegeli H, Schuller S (2020): Antimicrobial prescriptions and adherence to prudent use guidelines for selected canine diseases in Switzerland in 2016. *Vet. Rec. Open*. 7: e000370.
Macintire DK, Drobatz KJ, Haskins SC, Saxon WD (2012): Manual of Small Animal Emergency and Critical Care Medicine, 2nd Edition. John Wiley & Sons, Inc.
Marshall BM, Levy SB (2011): Food Animals and Antimicrobials: Impacts on Human Health. *Clin. Microbiol. Rev.* 24: 718–733.
Miranda C, Silva V, Igrejas G, Poeta P (2021): Impact of European pet antibiotic use on enterococci and staphylococci antimicrobial resistance and human health. *Future Microbiol.* 16: 185–201.
Morley PS, Apley MD, Besser TE, Burney DP, Fedorka-Cray PJ, Papich MG, Traub-Dargatz JL, Weese JS (2005): Antimicrobial drug use in veterinary medicine. *J. Vet. Intern. Med.* 19: 617–629.
Nienhoff U, Kadlec K, Chaberny IF, Verspohl J, Gerlach GF, Schwarz S, Simon D, Nolte I (2009): Transmission of methicillin-resistant Staphylococcus aureus strains between humans and dogs: two case reports. *J. Antimicrob. Chemother.* 64: 660−662.
Official Journal of the European Union (2015): Guidelines for the prudent use of antimicrobials in veterinary medicine (2015/C 299/04). Available to: https://ec.europa.eu/health/sites/health/files/antimicrobial_resistance/docs/2015_prudent_use_guidelines_en.pdf
Payne, MA, Baynes RE, Sundlof SE et al. (1999): Drugs prohibited from extralabel use in food animals. *J. Am. Vet. Med. Assoc.* 215: 28–32.
Pomba C, Rantala M, Greko C, Baptiste KE, Catry B, Duijkeren E, Mateus A, Moreno MA, Pyörälä S, Ružauskas M, Sanders P, Teale C, Threlfall EJ, Kunsagi Z, Edo JT, Jukes H, Törneke K (2017): Public health risk of antimicrobial resistance transfer from companion animals. *J. Antimicrob. Chemother.* 72: 957–968.
Prescott JF, Hanna WJB, Reid-Smith R, Drost K (2002): Antimicrobial drug use and resistance in dogs. *Can. Vet. J.* 43: 107–116.
Rantala M, Hölsö K, Lillas A, Huovinen P, Kaartinen L (2004): Survey of condition-based prescribing of antimicrobial drugs for dogs at a veterinary teaching hospital. *Vet. Rec.* 155: 259–262.
Rollin BE (2002): An ethicist's commentary on extra-label drug use. *Can. Vet. J.* 43: 749–750.
Shea A, McCarthy R, Lindenmayer J (2011): Therapeutic Antibiotic Use Patterns in Dogs: Observations from a Veterinary Teaching Hospital. *J. Small Anim. Pract.* 52: 310–318.
Šeol B, Matanović K, Terzić S. (2010): *Antimikrobna terapija u veterinarskoj medicini*. Zagreb: Medicinska naklada.
Tauro A, Beltran E, Cherubini GB, Coelho AT, Wessmann A, Driver CJ, Rusbridge CJ (2018): Metronidazole-induced neurotoxicity in 26 dogs. *Aust. Vet. J.* 96: 495–501.

Weese JS, Giguère S, Guardabassi L, Morley PS, Papich M, Ricciuto DR, Sykes JE (2015): ACVIM Consensus Statement on Therapeutic Antimicrobial Use in Animals and Antimicrobial Resistance. *J. Vet. Intern. Med*. 29: 487–498.

ОРИГИНАЛНИ НАУЧНИ РАД

МЕТРОНИДАЗОЛ У ПРОФИЛАКСИ И ТЕРАПИЈИ КОД ПАСА И МАЧАКА

Драгана З. ТОМАНИЋ, Зорана Р. КОВАЧЕВИЋ, Драгица М. СТОЈАНОВИЋ, Бранислава М. БЕЛИЋ, Ненад С. СТОЈАНАЦ, Иван Б. СТАНЧИЋ, Иван М. ГАЛИЋ

Универзитет у Новом Саду, Пољопривредни факултет
Департман ветеринарске медицине
Трг Доситеја Обрадовића 8, Нови Сад 21000, Србија

РЕЗИМЕ: Од свог открића, антибиотици помажу у контроли и лечењу инфекција код људи и животиња. Ефикасност антибиотика је угрожена порастом броја микроорганизама отпорних на ову групу лекова. Ограничена доступност регистрованих ветеринарских лекова доводи до тога да се у пракси користе лекови регистровани за примену код људи. Циљ овог истраживања био је да се анализира употреба метронидазола у приватној ветеринарској амбуланти у Србији, као и да се утврди да ли је та употреба у складу са научном литературом. Резултати су показали да се овај лек углавном примењује у лечењу паса, док је 27,1% метронидазола коришћено у лечењу мачака. Најчешће индикације за употребу метронидазола биле су дентални и дигестивни поремећаји. Ово истраживање показало је да су, иако је метронидазол доступан у оралном и ињекционом облику, мачке лечене само ињекционим формулацијама. Иако је законом дозвољено прописивање хуманих лекова за лечење животиња, у Србији не постоје званични подаци о обиму или природи те употребе. Такве информације су кључне за спровођење смерница о употреби антимикробних средстава у ветеринарској пракси малих животиња, као и за процену ризика од преноса антимикробне резистенције на људе. Препознавање важности антимикробне резистенције као и разумнија употреба антибиотика кључни су елементи сваке стратегије за одржавање ефикасности антимикробних лекова.

КЉУЧНЕ РЕЧИ: антибиотици, антимикробна резистенција, мачке, пси

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 141, 107—117, 2021

UDC 665.52: 582.943.15
https://doi.org/10.2298/ZMSPN2141107B
ORIGINAL SCIENTIFIC ARTICLE

Mirjana A. BOGAVAC[1], *Jovana K. MIŠKOVIĆ*[2], *Nenad J. KRSMANOVIĆ*[2], *Milana S. RAKIĆ*[2], *Maja A. KARAMAN*[2*]

[1] University of Novi Sad, Clinical Centre of Vojvodina, Faculty of Medicine
Department of Obstetrics and Gynaecology
Hajduk Veljkova 1, Novi Sad 21000, Serbia
[2] University of Novi Sad, Faculty of Sciences
Department of Biology and Ecology
Trg Dositeja Obradovića 2, Novi Sad 21000, Serbia

Origanum vulgare L. ESSENTIAL OIL EFFECTS ON MICROBIAL PATHOGENS CAUSING VAGINITIS

ABSTRACT: Multidrug resistance of human isolates of bacteria that cause vaginal infections is one of most recent topics in scientific investigations along with the search for novel antimicrobial drugs originating from nature. Monitoring of antimicrobial activity of traditionally used herbal essential oils may give us basic perspective and directions for future studies design. Antimicrobial activity of commercial essential oil (EO) of *Origanum vulgare* against human clinical isolates of bacteria and *Candida* was determined by microdilution method, obtaining minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) (CLSI protocol). Both investigated strains of *Candida* as well as all bacterial strains, except *P. aeruginosa*, showed susceptibility to investigated EO. The most susceptible strain was Gram positive *S. aureus*[1] strain unlike *S. aureus*[2] which indicates strain specificity. Both Gram negative *E. coli* isolates (*E. coli*[1] and *E. coli*[2]) showed resistance to all tested antibiotics in this study, and susceptibility to oregano EO at 12.5 µl/ml. GC/MS analysis identified a total of 35 components (96.13%) in commercial oregano EO, with the most dominant phenol carvacrol (64.12%). Our data suggest that oregano EO possess potent antimicrobial activity and that human vaginal isolates shows strain specificity in antimicrobial susceptibility.
KEYWORDS: antimicrobial activity, essential oil, *Origanum vulgare*, vaginal infection

INTRODUCTION

In recent years, essential oils (EOs) have been the centres of research in several industrial and scientific fields, mainly in cosmetics and food processing,

[*] Corresponding author. E-mail: maja.karaman@dbe.uns.ac.rs

but with ever increasing potential for biomedical application (Sarikurkcu et al., 2015). According to the traditional medicine knowledge, EOs have been used as analgesics, sedatives, anti-inflammatory drugs, and antimicrobial agents (Gulluce et al., 2012).

One of the most widely used EOs is oregano essential oil (EO). Plants of the *Origanum* genus (*Lamiaceae* family) are found mainly in the Mediterranean region, with 39 distinct species described so far (Sözmen et al., 2012). Ethnopharmacological applications of oregano are diverse and include use of infusions for respiratory and digestive system, to relieve cough, sore throat and indigestion (Barros et al., 2010). Recently, *O. vulgare* EO has been proved as potent antimicrobial agent in a treatment of vaginal infections (Karaman et al., 2017). In fact, vagina infections caused by both bacteria and yeasts and recognized as vaginosis and vaginitis, represent a serious problem, particularly during pregnancy, due to increased susceptibility for infection owing to changes in immune system itself, hormonal oscillations and changes of normal physiological processes (Machado and Cerca, 2015). Furthermore, the most effective antibiotic therapy cannot always be administered during pregnancy (Palmeira-de-Oliveira et al., 2015).

New emerging trends in use of more natural products for medicinal treatment are getting scientific validation in recent years. Finding an effective treatment option, which is safe for women, is of great importance with the need for prophylactic therapy of vaginosis before final decision on type of alternative therapy (Stefanakis et al., 2013). Since routinely antibiogram tests are recommended for every single microbial isolate (strain) causing vaginitis, we have chosen to analyse susceptibility of several strains – six bacterial and two *Candida*, that have been mostly recognized as a transient microorganisms or temporary skin microbiome that normally inhabits the rectum but may sometimes increase in number and cause nonspecific vaginitis making irritation, inflammation, itching together with appearance of vaginal candidiasis caused by an overgrowth of a commensal yeast *Candida albicans* that naturally lives in vagina (Bortz et al., 2013).

Hence, the aim of the study was to investigate *in vitro* antimicrobial activity of commercial *Origanum vulgare* L. EO against clinical isolates of six bacterial and two *Candida* strains.

MATERIALS AND METHODS

Bacterial and yeast strains

Bacterial and *Candida* strains were obtained, by approved protocol of Ethical Board of Clinical Centre of Vojvodina, from women with symptoms of vaginal infection at the Department of Obstetrics and Gynaecology, Clinical Centre of Vojvodina, Faculty of Medicine, University of Novi Sad. Two Gram-positive strains (*S. aureus*^{1} and *S. aureus*^{2}), four Gram-negative strains (*E. coli*^{1}, *E. coli*^{2}, *P. aeruginosa* and *P. mirabilis*) and two *Candida* isolates (*C. albicans*^{1} and *C. albicans*^{2}) were investigated.

Antimicrobial assay

Antimicrobial activity of commercial *O. vulgare* EO (ProBotanic, Belgrade, Serbia HACCP, ISO 9001) was assessed by microdilution method, determining minimum inhibitory and minimum bactericidal/fungicidal concentration (MIC, MBC/MFC). Antimicrobial testing was proceeded according to procedures of Clinical Laboratory Standards Institute (CLSI 2008a; CLSI 2008b). Five double dilutions of initial concentrations of EO were made in a range from 3.12 µl/ml to 50 µl/ml for bacterial and *Candida* strains. Inoculums of strains were prepared from overnight cultures by using McFarland standards to reach the final 1.5×10^8 CFU/ml for bacteria and 1.5×10^6 for yeast. Antibiotics: streptomycin (STR), ampicillin (AMP), tetracycline (TCN), and cefuroxime (CXM) (Sigma, Switzerland) were tested at the final concentration (64–512 µg/ml), while nystatin (Hemofarm, Vršac, Serbia) was used as antimycotic for yeasts (32–256 µg/ml).

Growth curves of *C. albicans*

Change of colony-forming unit (CFU) of *C. albicans* in a period of 72 h was detected in a form of growth curves, as well as IC_{50} values of analysed oregano EO. Spectrophotometrically, method was based on monitoring of medium (malt broth, Torlak, Serbia) with *C. albicans* at λ=560 (Multiskan GO Thermo Scientific, Finland), at every two hours in a period of 72 h. Inhibition (%) of *C. albicans* growth was calculated according to equation:

$$1\text{-}A_{test}/A_{control} \times 100$$

A_{test} and $A_{control}$ stands for absorbance of tested and control reactions (Bogavac et al., 2015, Karaman et al., 2017).

Germination test of *C. albicans*

Oregano EO ability to inhibit the germination of *C. albicans* was tested at the following concentrations from 2.30 to 4.50 µl/ml. Human blood serum (Euro Lab Polyclinic, Novi Sad, Serbia) was inoculated with *C. albicans* and incubated for 3 h at 37 °C. Positive results were considered if no *C. albicans* filaments were detected in ten microscopic visual fields detected microscopically (Olympus BX51 microscope, Olympus, Tokyo, Japan).

Brine shrimp toxicity assay

Toxicity of EO was evaluated by *A. salina* brine shrimp bioassay (Meyer et al., 1982) with the same protocol modifications described previously (Bogavac et al., 2017; Karaman et al., 2017).

Chemical composition analysis of *O. vulgare* EO

Composition of oregano EO was investigated by the gas chromatography with mass spectrometry (GC/MS) at Institute for Public Health of Vojvodina (Novi Sad, Serbia). Agilent capillary gas chromatograph directly coupled to the mass spectrometer (MSD) system GC Agilent 7890A model; MS 5875C (Santa Clara, CA, USA) was used.

Statistical analysis

Means and standard deviations were obtained by Microsoft Excel for Windows, while IC_{50} values were derived from the best fit line obtained by linear regression analysis in software Origin 8.0 (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

Antimicrobial activity and growth curves

Antibacterial and antifungal activity of oregano EO is shown in Table 1. Oregano EO showed bacteriostatic and bactericidal activity against all examined clinical strains, except *P. Aeruginosa,* according to MIC and MBC, respectively. Both investigated strains of *Candida* showed susceptibility to tested EO. The MIC values for EO against bacterial strains ranged from 3.12 to 12.5 μl/ml, MBC values from 3.12 to 25 μl/ml, while both MIC and MFC values against *Candida* strains were detected at higher conc. (12.5–25 μl/ml). Obtained growth curve of *C. albicans*, used as control, was sigmoidal with the clear exhibition of lag, log and stationary phases and approximately 6 hours was necessary for cells to proliferate and enter the log phase (Figure 1). Moreover, the growth curves obtained after treatment were observed to shift to the right due to the extension of the lag phase, which strongly implies that EO has suppressed the cell growth and exhibited fungistatic effect. Exposure of *Candida* species to EO showed dose–response relationship demonstrating reduction of cells from 49% to 80% at 0.31 and 2.5% mass concentration applied, respectively (Figure 1).

The most susceptible bacterial strain was Gram positive *S. aureus*[1] strain, while *S. aureus*[2] showed higher MIC and MBC values indicating strain specificity. In comparison, MBCs values of analysed EO against *S. aureus* were detected at the higher concentrations (22.7–45.4 μl/ml) (Karaman et al., 2017) while in this study their MBC values reached at lower concentration range (3.12–12.5 μl/ml) indicating the impact of EO chemical composition on expressing activities. Furthermore, detected MBCs for *E. coli* isolates ranged from 2.8 to 45.4 μl/ml (Karaman et al., 2017), which is similar with presented results (12.5–25.0 μl/ml). *P. aeruginosa* strain showed to be the most resistant strain against both tested EO and antibiotics, while Man et al. (2019) demonstrated good activity of oregano EO against *P. aeruginosa* (MIC and MBC were 6.3%).

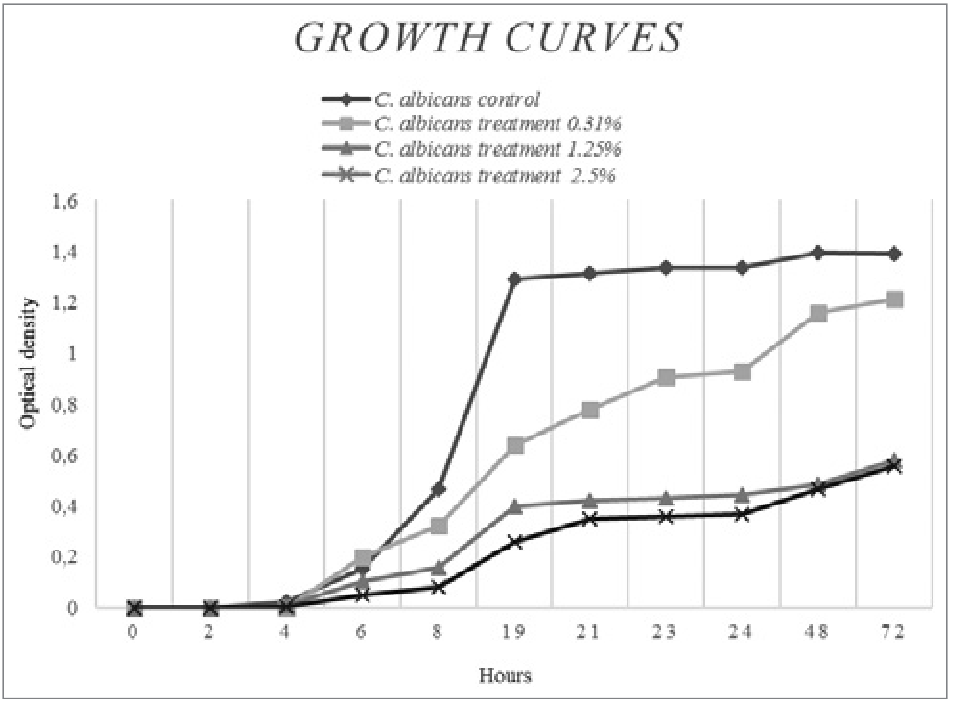

Figure 1. Normal growth curve of *Candida* species cultured in malt broth medium and growth curves of *Candida* treated with different concentration of *O. vulgare* EO.

Table 1. Susceptibility of microbial strains to *O. vulgare* EO (μl/ml) and antibiotics (μg/ml).

Bacterial strain	EO *O. vulgare* L.		Antibiotics (MIC) (μg/ml)				
	MIC (μl/ml)	MBC (μl/ml)	STR	AMP	TCN	CXM	
S. aureus[1]	3.12 [a]	3.12 [a]	512	128**	64**	R	
S. aureus[2]	12.5 [c]	12.5 [c]	512	R	512**	R	
E. coli[1]	12.5 [c]	12.5 [c]	R	R	R	R	
E. coli[2]	12.5 [d]	25.0 [c]	R	R	R	R	
P. mirabilis	6.25 [b]	6.25 [b]	512	256	512**	R	
P. aeruginosa	R	R	R	R	R	R	
Yeast strain	EO *O. vulgare* L.						Antimycotic
	MIC (μl/ml)	MBC (μl/ml)					Nystatin (μg/ml)
C. albicans[1]	12.5 [a]	12.5 [a]					64
C. albicans[2]	25.0 [b]	25.0 [b]					128

MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration; MFC – minimum fungicidal concentration; R- resistant; [a,b,c] – significant differences between antimicrobial activity within strain of bacteria determined by Tukey HSD test at $p<0.01$. In each column different letters mean significant differences. Antibiotics: STR, streptomycin; AMP, ampicillin; TCN, tetracycline; CXM, cefuroxime; **Strains sensitive to antibiotics according to interpretive categories and MIC breakpoints (μg/ml) (Clinical and Laboratory Standards Institute, 2017).

The antibiotic resistance varied among other tested isolates (Table 1). The most efficient antibiotic was AMP against *S. aureus*[1] strain, while the CXM showed the weakest activity since none of investigated isolates were susceptible to CXM. It should be emphasized that both *E. coli* isolates (*E. coli*[1] and *E. coli*[2]) showed resistance to all tested antibiotics, while both strains showed susceptibility to oregano EO. According to standards for antimicrobial susceptibility testing (CLSI, 2017) *P. aeruginosa* ATCC 27853 strain shows MIC breakpoint to TCN at 8 μg/ml, while analysed *P. aeruginosa* showed the resistance to TCN. Similarly, *E. coli* ATCC 25922 strain showed susceptibility to AMP (at 2 μg/ml), TCN (at 4 μg/ml) and CXM (at 8 μg/ml) according to interpretive categories and MIC breakpoints (CLSI, 2017), while in our study both *E. coli* isolates were resistant to all tested antibiotics. According to this, isolated human pathogens need to be routinely antibiogram tested due to possible multidrug resistance, while searching and development of new antibacterial components should be intensified. Regarding yeast isolates, both *C. albicans* strains showed higher susceptibility to nystatin in comparison with EO (Table 1). *C. albicans*[1] isolate showed to be more susceptible to EO and antimycotic, than *C. albicans*[2], pointing out the strain specificity of yeast isolates.

Toxicity and germination

According to brine shrimp toxicity assay oregano EO showed acute toxicity, since all investigated concentration of oregano EO caused absolute mortality of *A. salina* (data not shown). Although recently good correlation between *A. salina* bioassay *in vitro* and *in vivo* toxicity tests ($r = 0.85$, $p < 0.05$) was reported (Parra et al., 2001), some authors obtained different results, without correlation between these assays (Sanchez et al., 1993). In conclusion, additional toxicity testing for oregano EO needs to be included. Oregano EO at concentration of 4.50 μl/ml leads to complete inhibition of germination of *C. albicans* (Figure 2a), in comparison to control, without EO treatment (Figure 2b). Obtained germination inhibition is more than twenty-five times stronger compared to oregano EO purchased in USA, where MIC and MBC values for filament elongation were 125 μl/ml.

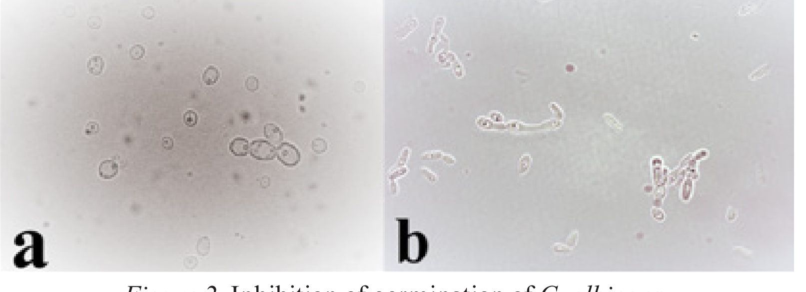

Figure 2. Inhibition of germination of *C. albicans.*
a – Treatment with *O. vulgare* EO (5%); b – Control.

Chemical characterization of EO

GC-MS analysis of commercial oregano EO was conducted in order to detect present bioactive compounds and total of 35 components (96.13%) were identified. The most dominant chemical class were oxidised hydrocarbons (71.15%), with the prevalence of phenol carvacrol (64.12%) (Table 2). Detected antibacterial activity against *S. aureus* and *E. coli* strains may be attributed to this monoterpenoid phenol (Mathela et al., 2010). The mechanism of carvacrol effect on bacterial cell was described earlier and it was shown that this compound causes permeabilization and depolarization of the cytoplasmic membrane (Magi et al., 2015). Specifically, its good antibacterial activity against transient *E. coli* is present thanks to its ability to inhibit microbial toxin production and biofilm formation, together with reducing the fimbriae production (Friedman, 2014; Lee et al., 2017). The dominance of carvacrol (80.5%) in oregano EO was also reported by Man et al. (2019) where oregano EO demonstrated antibacterial activity against *P. aeruginosa*, which may be related to higher carvacrol content since it was shown that carvacrol can impair the cell membrane and decrease the cytoplasmic pH in *P. aeruginosa* (Lambert et al., 2001).

Besides carvacrol, aromatic monoterpenes p-cinnamon (7.00%), bicyclic sesquiter trans-caryophyllene (3.37%) and borneol (3.19%) were detected in significant amounts. The results correspond to previously obtained GC-MS chemical identification of oregano EO with carvacrol as major compound (Karaman et al., 2017). On the other hand, there were some differences in the content of monoterpene hydrocarbons and oxygenated monoterpenes, in regard to previous study, where camphor, thymyl methyl ether, epizonarene, α-humulene, δ-cadinene, isoledene, eremophilene, as well as linalool, terpinene-4-ol and α-terpineol were detected in EO (Karaman et al., 2017). Difference in EO chemical composition is well known characteristic, which can be caused by many factors, especially growth factors (De Falco et al., 2013). Consequently, a further research needs to be more focused on whether a mixture or a pure EO compound is responsible for antimicrobial activity.

Table 2. Chemical composition (%) of the commercial *O. vulgare* EO.

Components	R.I.[a] [min]	*O. vulgaris* (%)
α-Phellandrene	8.091	0.34
α -Pinene	8.274	0.78
Camphene	8.588	0.63
1-Octen-3-ol	8.800	0.28
β-Myrcene	8.972	0.70
2-β-Pinene	9.029	0.15
Phellandrene	9.407	0.16
α-Terpinene	9.567	0.99
p-Cymene	9.704	**7.00**
dl-Limonene	9.784	0.27

Sabinene	9.859	0.19
Eucalyptol	9.899	0.51
γ-Terpinene	10.242	2.73
trans-Sabinene hydrate	10.511	0.73
α-Terpinolene	10.751	0.22
Linalyl alcohol	10.854	1.97
cis-Sabine	11.100	0.36
Borneol	12.617	3.19
Terpinene-4-ol	12.685	1.63
cis-Dihydrocarvone	13.120	0.17
d-Dihydrocarvon	13.303	0.11
Carvacrolmethylether	13.607	0.20
Carvone	14.139	0.19
Thymol	14.602	1.29
Carvacrol	14.905	**64.12**
Carvacrylacetate	16.118	0.11
trans-Caryophyllene	17.595	3.37
Aromadendrene	17.927	0.48
β-Selinene	18.247	0.20
Viridiflorene	18.814	0.27
β-Bisabolene	18.865	1.34
δ-Cadinene	19.157	0.24
Spathulenol	20.336	0.31
Caryophyllene oxide	20.490	0.74
Copaene	21.251	0.16
Chemical classes		
Monoterpene hydrocarbons		4.64
Cyclic terpenes		6.07
Oxidised hydrocarbons		71.15
Terpenoids and sesquiterpenes		5.78
Oxidized sesquiterpenes		0.74
Aromatic monoterpenes		7.00
Total identified compounds		96.13

[a]R.I. – Retention indices measured relative to n-alkanes (C9–C18) on the non-polar NP-5 column and GC MS detection. Bold letters indicate the highest percentage.

CONCLUSION

This study shows that oregano EO possesses antimicrobial activity against multi-drug resistant *E. coli* human isolate. Strain specificity is documented for both bacteria (*S. aureus*[1] and *S. aureus*[2]) and *C. albicans* isolates, which indicates that for every single isolate appropriate antibiogram testing needs to be done. Alternative ways of treatment can be recommended when there are no effective antibiotic treatments, but further toxicology and clinical studies are needed for EO clinical practice approval as antimicrobial agent.

ACKNOWLEDGEMENT

The research was kindly supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant No. OI172058.

REFERENCES

Barros L, Heleno SA, Carvalho AM, Ferreira ICFR (2010): *Lamiaceae* often used in Portuguese folk medicine as a source of powerful antioxidants: Vitamins and phenolics. *LWT – Food Sci. Technol.* 43: 544–550.

Bogavac M, Karaman M, Sudji J, Radovanović B, Janjušević Lj, Ćetković N, Tešanović K (2017): Antimicrobial potential of *Rosmarinus officinalis* commercial essential oil in the treatment of vaginal infections in pregnant women. *Nat. Product Commun.* 12: 127–130.

Bogavac M, Karaman M, Janjušević Lj, Suđi J, Radovanović B, Novaković Z, Simeunović J, Božin B (2015): Alternative treatment of vaginal infections – *in vitro* antimicrobial and toxic effects of *Coriandrum sativum* L. and *Thymus vulgaris* L. essential oils. *J. Appl. Microbiol.* 119: 697–710.

Bortz J, Levinson S, Kirschner M, Cuca RC (2013): *Composition for topical treatment of mixed vaginal infections*. United States Patent Application Publication, Pub. No.: US 2013/0172279 A1.

Clinical and Laboratory Standards Institute (2017): CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100.

Clinical Laboratory Standards Institute (2008a): M07-A9 – Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Ninth Edition; Wayne PA. USA: CLSI document.

Clinical Laboratory Standards Institute (2008b): M27-A3 and Supplement S3Reference method for broth dilution antifungal susceptibility testing of yeasts; approved 0standard. 3d ed. CLSI document.

De Falco E, Mancini E, Roscigno G, Mignola E, Taglialatela-ScafatiO, Senatore F (2013): Chemical composition and biological activity of essential oils of *Origanum vulgare* L. subsp. *vulgare* L. under different growth conditions. *Molecules* 18: 14948–14960.

Friedman M (2014): Chemistry and multibeneficial bioactivities of carvacrol (4-isopropyl-2-methylphenol), a component of essential oils produced by aromatic plants and spices. *J. Agric. Food Chem.* 62: 7652–7670.

Gulluce M, Karadayi M, Guvenalp Z, Ozbek H, Arasoglu T, Baris O (2012): Isolation of some active compounds from *Origanum vulgare* L. ssp. *vulgare* and determination of their genotoxic potentials. *Food Chem.* 130: 248–253.

Karaman M, Bogavac M, Radovanović B, Sudji J, Tešanović K, JanjuševićLj (2017): *Origanum vulgare* essential oil affects pathogens causing vaginal infections. *J. Appl. Microbiol.* 122: 1177–1185.

Lambert RJW, Skandamis, PN, Coote PJ, Nychas, GJE (2001): A study of the minimum inhibitoryconcentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.* 91: 453–462.

Lee JH, Kim YG, Lee J (2017): Carvacrol-rich oregano oil and thymol-rich thyme red oil inhibit biofilmformation and the virulence of uropathogenic *Escherichia coli*. *J. Appl. Microbiol.* 123: 1420–1428.

Machado A, Cerca N (2015): Influence of Biofilm Formation by *Gardnerella vaginalis* and other Anaerobes on Bacterial Vaginosis. *J. Infect. Dis.* 212: 1856–1861.

Magi G, Marini E, Facinelli B (2015): Antimicrobial activity of essential oils and carvacrol, and synergy ofcarvacrol and erythromycin, against clinical, erythromycin-resistant Group a Streptococci. *Front. Microbiol.* 6: 165.

Man A, Santacroce L, Iacob R, Mare A, Man L (2019): Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study. *Pathogens* 8: 15–26.

Manohar V, Ingram C, Gray J, Talpur NA, Echard BW, Bagchi D, Preuss HG (2001): Antifungal activities of origanum oil against *Candida albicans*. *Mol. Cell. Biochem.* 228: 111–117.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, Mc Laughlin JL (1982): Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica* 45: 31–34.

Palmeira-de-Oliveira R, Palmeira-de-Oliveira A, Martinez-de-Oliveira J (2015): New strategies for local treatment of vaginal infections. *Adv. Drug Deliv. Rev.* 92: 105–122.

Parra AL, Yhebra RS, Sardinas IG, Buela LI (2001): Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine* 8: 395–400.

Sanchez C, Gupta M, Vasquez M, Noriega Y, Montenegro G (1993): Bioensayo con *Artemia salina* para predecir la actividadantibacteriana y farmacológica. *Revista Médica de Panamá* 18: 62–69.

Sarikurkcu C, Zengin G, Oskay M, Uysal S, Ceylan R, Aktumsek A (2015): Composition, antioxidant, antimicrobial and enzyme inhibition activities of two *Origanum vulgare* subspecies (subsp. vulgare and subsp. hirtum) essential oils. *Ind. Crop. Prod.* 70: 178–184.

Sözmen F, Uysal B, Köse EO, Aktaş Ö, Cinbilgel I, Oksal BS (2012): Extraction of the essential oil from endemic *Origanum bilgeri* P.H.Davis with two different methods: comparison of the oil composition and antibacterial activity. *Chem. Biodivers.* 9: 1356–1363.

Stefanakis MK, Touloupakis E, Anastasopoulos E, Ghanotakis D, Katerinopoulos HE, Makridis P (2013): Antibacterial activity of essential oils from plants of the genus *Origanum*. *Food Control* 34: 539–546.

ОРИГИНАЛНИ НАУЧНИ РАД

ЕФИКАСНО ДЕЈСТВО ЕТАРСКОГ УЉА ВРАНИЛОВЕ ТРАВЕ (*Origanum vulgare* L.) НА МИКРОБНЕ ПАТОГЕНЕ КОЈИ УЗРОКУЈУ ВАГИНИТИС

Мирјана А. БОГАВАЦ[1], Јована К. МИШКОВИЋ[2], Ненад Ј. КРСМАНОВИЋ[2], Милана С. РАКИЋ[2], Маја А. КАРАМАН[2]

[1] Универзитет у Новом Саду, Клинички центар Војводине, Медицински факултет
Катедра за гинекологију и акушерство
Хајдук Вељкова 1, Нови Сад 21000, Србија
[2] Универзитет у Новом Саду, Природно-математички факултет
Департман за биологију и екологију
Трг Доситеја Обрадовића 2, Нови Сад 21000, Србија

РЕЗИМЕ: Мултирезистентност бактерија, изолованих код жена са проблемима вагиналних инфекција, на широк спектар лекова представља једну од најатрактивнијих тема у научном свету заједно са истраживањима нових антимикробних лекова природног порекла. Праћење антимикробне активности традиционално коришћених биљних етарских уља може нам дати основну перспективу и смернице за дизајн будућих студија. Антимикробна активност комерцијалног етарског уља (ЕУ) вранилове траве (*Origano vulgare* L.) против хуманих клиничких изолата бактерија и *Candida* утврђена је микродилуционом методом и добијањем минималне инхибиторне концентрације (МИК) и минималне бактерицидне/фунгицидне концентрације (МБК / МФК) (CLSI протокол). Оба испитивана соја *Candida*, као и сви испитани бактеријски сојеви, осим *P. aeruginosa*, показали су осетљивост на анализирано етарско уље. Најосетљивији сој био је грам позитиван сој *S. aureus*[1], за разлику од *S. aureus*[2] што указује на специфичност соја. Грам негативни изолати *E. coli* (*E. coli*[1] и *E. coli*[2]) показали су резистенцију на све тестиране антибиотике у овој студији и осетљивост на оригано етарско уље на 12.5 μl/ml. ГС/МС анализа идентификовала је укупно 35 компонената (96,13%) у комерцијалном оригано етарском уљу, са најдоминантнијим фенолом карвакролом (64,12%). Наши подаци сугеришу да оригано етарско уље поседује моћну антимикробну активност и да људски изолати показују специфичност соја у осетљивости на антимикробне лекове.

КЉУЧНЕ РЕЧИ: антимикробна активност, етарско уље, *Origanum vulgare*, вагинална инфекција

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 141, 119—137, 2021

UDC 661.471:612.3
https://doi.org/10.2298/ZMSPN2141119K
REVIEW SCIENTIFIC ARTICLE

*Rudolf R. KASTORI, Ivana V. MAKSIMOVIĆ***
Marina I. PUTNIK-DELIĆ

University of Novi Sad, Faculty of Agriculture
Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia

IODINE AND THE HIGHER PLANTS

ABSTRACT: Iodine (I) is widely distributed in the biosphere in small concentrations. Iodide (I^-) and iodate (IO_3^-) are the most important inorganic forms of I that can be found in the biosphere. Iodine is a necessary microelement for some marine algae and higher animals. Approximately 35% of the world's population is insufficiently provided with I, which results in many health problems. Plants are an important source of I for humans, and the knowledge about the uptake, distribution, and accumulation of I in edible plant parts, as well as its effect on life processes and organic production of cultivated species is very important. The results of numerous studies indicate a favorable effect of I application on plant growth, tolerance to stress, and the antioxidant capacity of plants. In addition, its effects on photosynthesis, hydration, carbohydrate metabolism, respiration, and interaction with other elements are analyzed. At the same time, data are indicating that the application of I has no effect, moreover, it may act phytotoxically, especially at higher concentrations. Based on the current understanding of I impact on life processes and the organic production of higher plants, it can be considered a useful element. Plants can complete their life cycle without iodine, but it may have a stimulating effect on their growth under certain circumstances. Considering the importance of I for the health of the human population and higher animals, the application of I to increase its concentration in edible parts of grown plants using cultivation practices (biofortification of crops with I) is an important task for further research.

KEYWORDS**:** accumulation, higher plants, iodine, physiological and stimulating effect, soil

Iodine is not an essential element for higher plants, but plants are an important source of I for both humans and higher animals, for which it is indispensable. Iodine deficiency in human nutrition is widespread in the world, so the knowledge of a relationship between I and higher plants is important from the theoretical but also practical or health point of view. Iodine presence in plants was detected in the mid-19th century by Chatin (1850).

Iodine belongs to group VII B of the periodic table like fluorine, chlorine, and bromine (halogen elements) characterized by giving salts directly with

---

* Corresponding author. E-mail: ivanam@polj.uns.ac.rs

metals. The chemistry of I is very complex with several levels of oxidation: -1, 0, +1, +3, and +5. Iodine is found in nature in inorganic and organic forms and in the form of elemental I (I_2). The most important inorganic I ions that can be found in the biosphere are iodides (I^-) and iodates (IO_3^-). The form in which it is found depends partly on the pH value and the redox state of the medium. Iodine is the only halogen element that occurs in nature in compounds with a positive oxidation state. In addition to inorganic compounds of I, there are many biologically synthesized organic compounds. There is only one stable I isotope, ^{127}I, and more than 20 which are radioactive. Most of them have short or very short half-lives, hence the danger to humans and animals after the release of radionuclides from a nuclear accident is limited to the first weeks after the accident. Iodine reacts easily with numerous biomolecules. Concerning iodine chemistry, it is particularly important that it reacts easily with aromatic compounds, such as phenols or polyphenols and quinones – cyclic compounds having double bonds (Johanson, 2000). The diameter of the I atom is quite large (2.20 Å) and can replace the methyl group in many biomolecules.

Iodine is found in small concentrations in all parts of the biosphere. It is the least widespread of all halogens in nature. The concentration of I in seawater ranges from 45 to 60, in rainwater from 0.5 to 5.0, in river and lake waters from 0.5 to 20 μg L^{-1}, and in the atmosphere from 0.01 to 0.02 ng L^{-1}. It is believed that the most important source of I in the atmosphere is seawater, where its concentration is ten to one hundred times higher than in rainwater (Whitehead, 1984). Significant forms of I in seawater are iodide (I^-) and iodate (IO_3^-) ions and organic I, the concentration of which depends on the geographical region and water depth (Waite et al., 2006; Carrano et al., 2020). Groundwater is characterized by a much higher concentration of I than surface water and ranges within wide limits. For example, Tang et al. (2013) found a concentration of 4,100 μg L^{-1}. Its concentration in igneous rocks ranges from 0.08 to 0.50, in sedimentary rocks from 0.2 to 10.0, and in marine sediments from 3 to 400 mg kg^{-1}.

Studies on the role iodine plays in living organisms were intensified during the mid-19th century when it was found that iodine deficiency is the most important factor in the emergence of endemic hypothyroidism in humans and farmyard animals. Iodine is used by the thyroid gland in the production of hormones that control numerous physiological processes in humans and animals. Insufficient levels of thyroid hormones (thyroxine, triiodothyronine) cause disorders in the growth and development of children, and goiter in adults, as well as low basal metabolism. They enable the proper course of oxidation processes, stimulate the resorption of glucose in the intestines and the breakdown of glycogen, stimulate the mobilization of free fatty acids from adipose tissue, etc. In this regard, iodine is considered a necessary, essential micronutrient both for humans and higher animals. Diseases caused by I deficiency are very important health problems worldwide. Approximately 35% of the world's population is insufficiently provided with I, especially in developing countries, where it causes many economic and social problems (WHO, 2004). Therefore, great attention has been paid lately to the factors affecting the provision of people with a sufficient amount of I.

Studies of I concentration in the soil and its relation to plants began in the early 20th century (Scharer, 1955). In addition to water and air (coastal areas), plants are an important source of I for humans and farmyard animals. Hence, the knowledge on the uptake and influence of external and internal factors on I accumulation in plants, as well as the possibility of increasing its concentration, especially in the edible plant parts, is an important area of research today (Medrano-Macias et al., 2016). The most important source of iodine for terrestrial plants is the soil. Therefore, understanding the state and movement of I in the soil is important for providing plants with I.

Iodine in soils

The concentration of I in the Earth's crust amounts to 0.3 mg kg^{-1}. Sedimentary rocks are characterized by higher concentrations of I than igneous and metamorphic rocks. Oceanic sediments and continental sedimentary rocks are particularly rich in I (Muramatsu et al., 2004). The concentration of I in igneous rock granite is 0.25, basalt 0.22, and in volcanic glass (obsidian) 0.52 mg kg^{-1}. The highest concentration of I in sedimentary rocks is in organic-matter-rich shale 16.7 and limestone 2.7, while the lowest one is in sandstone rock 0.8 mg kg^{-1} (Fuge and Ander, 1998). However, the concentration of I in marine silt is very high – from 10 to 300 mg kg^{-1} (Szabó et al., 1993).

Iodine concentration in the soil ranges in wide limits, from 0.5 to 20 mg kg^{-1}, depending on the soil's physical and chemical characteristics and environmental factors (Whitehead, 1984), while globally, the average concentration is 2.6 mg kg^{-1} (Watts et al., 2010). The concentration of I in the soil represents a balance between its input into the soil and its ability to bind, as opposed to vertical leaching into deeper layers, removal by plant yields, and volatilization. The most important is the iodine input in the soil from the atmosphere. An important factor affecting the transfer of I from the atmosphere to the soil surface is its distribution between gaseous state and particles, as well as the chemical form of gaseous state and particle size (Johanson, 2000). The reduction of a stable iodate ion to a metastable iodide ion in ocean water plays a significant role in the global cycle of I (Fuge and Johnson, 2015). A certain amount of I reaches the soil from dead parts of plants, animals, organic and mineral fertilizers (Chile saltpeter), and applied chemicals (biocides) containing I. Opinions are different regarding the importance of parent substrate decomposition in providing the soil with I. It is considered that it does not have a great role, which is supported by the fact that the concentration of I in the parent substrate is lower than in the soil.

Oceans are the largest reservoir in the global iodine cycle on Earth, where I can be found mostly in ion form I^- and IO_3^-. Reduction of stable iodate ion into metastable iodide in oceans is very important in the global cycle of I. Iodine concentration in the atmosphere is significantly higher above the sea than above the mainland. Its concentration in rainwater is between 1 and 2 μg L^{-1}. About 10 to 50 g I ha^{-1} reaches the soil annually by rainfall, depending on the distance from the sea. In this regard, it was found that the concentration of I in plants depends on the distance from the sea. In red clover, at a distance of 10–50 km, the concentration of I was 272, at a distance of 51–100 km the concentration was 218, at 101–200 km the concentration was 137, while at 201–300 km distance, the concentration was 132 μg of I kg^{-1} dry matter (Szabó et al., 1993). Iodine is found in rainwater in the form of iodide, iodate, and soluble organically bound iodine (Gilfedder et al., 2008). Macro- and microalgae play a key role in the volatilization of I in the atmosphere from the marine environment through biological means (Saiz-Lopez et al., 2012), while the photochemical processes have the same role in non-biological ones (Stemmler et al., 2013). Volatilization of I also occurs from the soil and through microorganisms (Amachi, 2008), as well as through plants (Redeker and Cicerone, 2004). Volatilization of iodine from the soil plays an important role in the global iodine cycle and its transfer to the atmosphere. Both forms I^- and IO_3^- are released from the soil into the atmosphere in the form of HI, $HINO^{3-}$, and HIO_4. Iodine is released from the crop surface into the atmosphere in the form of organic I (CH_3I) with the participation of the enzymes halide ion methyltransferase and halide/thiol methyltransferase. The amount of I emissions into the atmosphere from the soil-plant system per season is 0.28–0.62 kg ha^{-1}, and the average is 0.40 kg ha^{-1} (Redeker et al., 2002). If no I was applied to the soil, its reserves would disappear in nine years. The intensity of I volatilization from the soil depends on its amount in the soil (Keppler et al., 2003), soil microorganisms, and particularly a vegetation cover. By burning fossil coal fuels and petroleum, which can be characterized by fairly high I concentrations, a significant amount can be released into the atmosphere. However, there are significant differences between fossil fuels of different origins (Fuge and Johnson, 2015).

The concentration of I in the soil ranges within wide limits, depending on the soil type, its chemical and physical properties, parent substrate, etc. When parent substrate consists of peat and marine alluvium, I concentration in the soil is the highest. On the other hand, when the parent substrate consists of sand and sandstone rocks, iodine concentration is the lowest. The largest part of I in the soil is bound to organic matter because soil organic matter binds iodine tightly (Dai et al., 2009). I availability for terrestrial plants depends on its solubility in the water while iodine bound to humic substances represents iodine reserve in the soil. Iodine concentration is the highest in the surface soil layer, where the content of organic matter is the highest. In most soil types, the primary container of I is humus. Soil organic matter can bind both iodide and iodate. It is believed that I applied to the soil is transformed from inorganic to organic form rapidly in the liquid and solid state of the soil. Iodine reacts with polyphenols, tyrosine, and thiols of soil organic matter. Many iodine bonds with organic matter are reversible, making desorption possible, which favors its absorption by plants. An important form of binding I in the soil is electrostatic binding. The colloidal fraction of the soil contains clay and organic matter, and a large amount of predominantly negative charges. Based on that, it can be expected that I ions with a negative charge are not particularly tightly bound. Oxides and hydroxides of metals (such as $Fe^{III}(OH)_3$, $Al(OH)_3$,

$Mn^{IV}O_2$) have an important role in the control of I behavior in the soil by adsorption of inorganic I and iodide oxidation (Shetaya et al., 2012). Part of I in the soil is bound to sesquioxides, and part to the organic matter. The peat soils are richer in I than other types of soils. The surface or humus layer of soil characterized by higher organic matter content is richer in I than the sub-arable layer. The distribution of I in the soil profile is affected by the plant root system, as well as the microorganisms' biomass (Bors and Martens, 1992). Sorption of I in the soil also depends on the soil pH. The presence of I in the soil depends on its mechanical composition. Light sandy soils are poorer than clay soils. The coefficient of I distribution between soil particles and soil solution differs largely in certain soil types (Dai et al., 2004). In reductive conditions (e.g. in floodplains), iodine is mostly found in the form of iodide (I^-), while in oxidative conditions it is mostly present in the form of iodate (IO_3^-) (Yuita, 1992).

Iodine is mostly found in the soluble form in the soil. The share of I that is easily leached also depends on the pH value of the soil. Iodine is easier to leach from acidic soils than from neutral ones. Knaelmann (1972) found that there was a high positive correlation (0.81–0.91) between the iodide sorption and the level of organic matter in the soil at pH> 5.5, while the correlation was significantly lower (<0.61) in the soil with a lower pH value. Iodine is leached more intensely from soils with a light mechanical composition than from loamy and clay soils, which is one of the reasons why the former ones are poorer in I. Iodine accumulates primarily in the clay fraction of the soil. The migration of I in the soil may be limited by the carbonate layer. It is believed that soil microorganisms are involved in I fixation in the soil, where bacteria appear to be more important than fungi (Bunzl and Schimmack, 1988).

Iodine uptake, translocation, and accumulation

Plants can take up iodine by root and aboveground parts. Uptake of I depends on numerous factors, internal genetic and ecological, as well as on the concentration and form of I in the zone of the root system. Both forms of I, iodide (I^-) and iodate (IO_3^-), can be present in the soil (Yuita, 1992). Plants accumulate these forms of I with different intensities. In spinach tissues, the concentration of I is significantly higher in plants growing in soil to which I is applied in the form of iodate (KIO_3) than in the form of iodide (KI). The soil-leaf transfer factor in plants grown in the presence of iodate is about ten times higher than in plants where iodide is applied (Dai et al., 2006). Iodine availability for plants in the soil depends on the adsorption and desorption processes that are different for the listed forms of I in certain soil types (Dai et al., 2004). Under the conditions of aquatic cultures in spinach, iodine accumulation in plants increases with an increase of I concentration in the nutrient solution. Thereby, the accumulation of I following the application of iodate is significantly lower than when iodide is applied (Zhu et al., 2003). Pauwels (1961) mentioned that I uptake in turnip rape and oat was higher during the application of KI than of the same dose of KIO_3. It is believed that iodate (IO_3^-) is reduced to iodide (I^-)

at the plant root before the uptake and that its slower uptake is caused by its limited reduction (Böszörményi and Cheh, 1960; Mackowiak and Grossl, 1999). Umaly and Poel (1971) described that with the increasing valence and molecular weight of I form, its uptake decreased, as a result of which the uptake of IO_3^- with valence $^+5$ was not taken up as easily as I^- with valence $^-1$. However, in perennial ryegrass on sandy loam, when I is applied in the form of iodide (KI), elemental I (I_2), and iodate (KIO_3), the accumulation of I is significantly higher from iodate than from the other two applied I forms (Whitehead, 1975). It is believed that KI and KIO_3 have different effects on the cell membrane. Iodide can have an unfavorable effect on the cell membrane, which may lead to reduced root hair growth and an increase in its permeability to iodide, compared to iodate, which has no such effect. The uptake of I in perennial ryegrass grown in the presence of 0.02 to 1 μM I^{-1} is 2–7 times higher than calculated passive uptake, which, according to Whitehead (1975), indicates its active take up.

The iodine uptake can be affected by cultivation practices. According to Whitehead (1975), when applying KI, I_2 and KIO_3, well-decomposed manure reduces the uptake of I by perennial ryegrass more than ten times, whereas the use of chalk reduces the uptake of iodide and increases the uptake of iodate. The application of organic matter (peat) decreases the uptake of ^{125}I by 40 or more percent, depending on the soil type (Moiseyev et al., 1984). Soil calcification reduces the uptake of I by plants. An unfavorable effect of the application of NPK mineral fertilizers on the uptake of I is also noticed.

Plants' ability to take up iodine, as well as other elements through above-ground parts has been studied by several researchers (Wrangel, 1930; Selders and Hugate, 1956). The uptake of I by aerial plant parts is important because the soil, in addition to binding I, also releases it into the atmosphere. Fuge (1996) believed that the release of I from the soil played an important role in the global cycle of iodine and its transfer to the biosphere. Iodine is released into to atmosphere from the soil-plant system in the form of organic I (Muramatsu et al., 1995), while the release intensity depends on the quantity of iodide in the soil (Keppler, 2003). A leaf surface can directly absorb the iodine from the atmosphere, whereby its flow through the stoma opening appears significant (Johanson, 2000). The intensity of I uptake through the above-ground organs depends on weather conditions. If they are favorable for the opening of stomata, I uptake will be more intense than when stomata are closed. Therefore, the share of stomata during the uptake of elemental I may contribute 60% and the other plant parts 40%. The intensity of I uptake through above-ground organs varies among certain plant species, e.g. clovers take up iodine more intensely than grasses (Johanson, 2000). It is believed that the importance of I uptake through root and above-ground plant organs in certain growth and developments stages is different. For *Trifolium repens* and *Typha latifolia*, the uptake through the root is the most important in the early stages of plant development, while the uptake from the air is significant in the final stages of development (Tikhomirov, 1984).

Mobility and the related distribution of iodine in individual plant organs are very important for providing people and animals with this element in edible plant parts. The mobility of I in the phloem is thought to be limited (Herrett et al., 1962). As a result, its accumulation in crop grains is often very low. Translocation of I from vegetative to generative organs requires an active transport mechanism of I in the phloem. Studies of Mackowiak and Grossl (1999) show that iodine taken up by rice is mostly accumulated in the root, stem, and leaves, and its translocation from vegetative organs to the grain is extremely low, which can be explained by the mentioned limited transport of I in the phloem. In leafy vegetables, iodine is notably accumulated in the leaves, which is attributed to its good transport in the xylem. This is indirectly shown by a significantly higher accumulation of I in the leaves than in the root of spinach (Zhu et al., 2003) and radish (Weng et al., 2003). In the same conditions, Hong et al. (2008) recorded a 19.7 times higher accumulation of I in the edible part of Chinese cabbage (in leaves) than in tomato fruit. Distribution of I in vegetables such as Chinese cabbage, lettuce, carrot, and tomato had the following order: root > leaf > stem > fruit. Based on the above, it can be concluded that the application of I can increase its accumulation in vegetative, rather than in generative plant parts, due to its different mobility in the phloem and xylem. This applies to vegetables and cereals which are grown for their grains and fruits, to which I is mostly translocated through the phloem from the vegetative plant part. There is a very low transfer factor of I from soil to wheat grain (0.0016 on average) (Shinonaga et al., 2001). In different soil types, ^{125}I activity in the grain of pea and oat is less than 10 percent of that found in straw (Moieseyev et al., 1984). Sheppard and Evenden (1992) highlighted that maize grown in soil containing 50 mg I kg^{-1} had a concentration of 5.2 mg kg^{-1} of I in leaves, while only 0.6 mg kg^{-1} in grain. In vegetables, such as cabbage, spinach, and radish, the enrichment coefficient of I is higher than 1, which demonstrates the high ability of mentioned plants to take up and accumulate I from the soil (Weng et al., 2003) meaning that these species have higher I use efficiency than the others. This is also indicated by the test results of Hong et al. (2008) who discovered a high value of correlation coefficient (r>0.98 **) between the iodine content in vegetables and the soil. Understanding that I is primarily accumulated in vegetative plant organs, as well as that vegetables are characterized by a great ability to accumulate I, the possibility of providing people with iodine by biofortification is very important.

The effect of iodine on life processes of higher plants

Based on literature data, Kašin (1981) stated that I can favorably affect the metabolism of nitrogen, photosynthesis, and water content in plant tissues. Iodine binds to certain amino acids and proteins and participates as a free ion in the regulation of water content in plant cells. It participates also in redox processes as an electron donor and acceptor.

The application of I affects the antioxidant potential of plants. The effect of I on the plant antioxidant capacity has often been studied in plants that have been exposed to stress, as the formation of reactive oxygen species increases under such conditions. Experimental data show that the effect of I on the plant antioxidant capacity depends on numerous factors: biotic, plant species, and abiotic – concentration, usage and forms of I. A study by Blasco et al. (2011) shows that I effect on oxidative stress depends on I form. Application of iodide (I^-) in lettuce decreases the activity of superoxide dismutase and increases activities of catalase and L-galacto dehydrogenase, as well as the activity of antioxidant compounds ascorbate and glutathione. The application of iodate (IO_3^-) increases the activity of superoxide dismutase, ascorbate peroxidase, catalase, and other enzymes involved in the detoxification of reactive oxygen species. In addition, it increases the concentration of ascorbate and regeneration activities of the Halliwell-Asade cycle. Based on the above, Blasco et al. (2011) conclude that IO_3^- does not have a phytotoxic effect, does not cause lipid peroxidation, and does not reduce biomass. Because of that, its application could improve the plant responses to stress conditions leading to the formation of reactive oxygen species. While applying low concentrations of KIO_3 (< 40 μM) in lettuce, Leyva et al. (2011) discovered the increased activity of superoxide dismutase and ascorbate peroxidase, as well as in non-enzymatic antioxidant substances, such as glutathione and ascorbic acid. The application of 7.88 μM IO_3^- in tomatoes increases the content of ascorbic acid and phenolic compounds (Smolen et al., 2015). Upon applying 20 and 40 μM KIO_3 in lettuce, Blasco et al. (2013) also found the increased antioxidant capacity and increased accumulation of total phenolic compounds. Application of 20 to 80 μM KIO_3 to soybean grown in soil amended by compost increases the activity of superoxide dismutase and ascorbate peroxidase (Gupta et al., 2015). Iodine is a mild reducing agent that chemically provides antioxidant properties. The antioxidant property of I can be quantitatively expressed as a redox potential (Venturi, 2011):

$$\mathbf{I} \rightleftharpoons 1/2\ \mathbf{I}_2 + e^- \text{ (electron)} = -\,0.54 \text{ Volt}$$

When applying KI to tomato in concentrations 1–20 ppm I, Wynd (1934) recorded a significant increase in respiration. Carbon dioxide release increased approximately three times, compared to control. An increase in activities of catalase, peroxidase, and invertase was also recorded, while the application of I did not affect the activity of peptase.

Numerous factors causing stress in plants increase the concentration of reactive oxygen species, which can damage macromolecules necessary for plant growth and development. By increasing the antioxidant capacity of plants, I increases their tolerance to abiotic stress conditions (Leyva et al., 2011; Gupta et al., 2015). However, little is known about the effect of I on plant-pathogen interactions. It is believed that I can affect plant tolerance towards pathogens by activating or modifying the plant defense system via redox signals or by chemical alteration of the cuticle.

Mynett and Wain (1973) found a significant reduction in chlorophyll content and photosynthetic activity, as well as inhibition of Hill-reaction in the leaf discs of beans floated in the solution containing from 0 to $2.5\text{x}10^{-2}$ M iodide and from 0 to $2.3\text{x}10^{-4}$ M iodine. The same authors recorded significantly reduced hydration of bean leaves when iodide was applied in a concentration of 20 μmol.

Iodine also affects nitrogen metabolism. Blasco et al. (2010) studied in detail the effect I had on enzymes of nitrogen metabolism in lettuce and found that application of IO_3^- increased the biomass, stimulated a reduction of NO_3^- and incorporation of NH_4^+ (glutamine synthetase/glutamate synthetase). The application of 40 μM I^- decreases the concentration of NO_3^- in leaves, whereas the production of biomass does not decrease, which is favorable for lettuce quality. A higher concentration of 80 μM I^- has a phytotoxic effect, reduces the biomass and concentration of organic nitrogen in the leaves, as well as the activity of nitrate-reductase, glutamate dehydrogenase, and hydroxypyruvate reductase.

Higher plants can incorporate I into tyrosine, thus synthesizing molecules such as 3-iodotyrosine, 3,5-diiodotyrosine, and 3', 3,5-triiodothyronine that are closely related to thyroxine (Mengel and Kirgby, 2001).

Interaction of I with other elements can directly or indirectly affect the metabolism of plants. Based on current findings, the effect of I on the accumulation of elements primarily depends on I form and ways of its application. It can be synergistic, whereas the uptake, transport, and metabolism of elements are increased, and antagonistic when stated processes are limited or any effect is absent in the presence of I. With the application of KI through the soil and foliar application of KIO_3, Smolen et al. (2011) did not record any significant changes in the mineral composition of lettuce. Later, Smolen et al. (2015) found a negative correlation between I content and a higher number of macro- and microelements in the same plant species. Gracia-Osuna et al. (2014) mentioned an increase of ascorbic acid content, as well as the content of potassium, magnesium, phosphorus, and iron when KIO_3 was applied, but also manganese and copper content using KI in *Opuntia ficus-indica*. When potassium iodide was applied to tomato, a positive correlation was found between I concentration and manganese and copper in the leaves (Hageman et al., 1942). The relationship between I and cobalt was also noticed. In the case of deficiency or excess of cobalt, the uptake of I was reduced (Pais, 1980).

Iodine and productivity of higher plants

The effect of I on plant productivity can be estimated based on knowledge about its impact on plant life processes. Knowledge about this is scarce and often insufficiently grounded. Test results on the effect I has on plant productivity differ greatly, ranging from lack of effect to stimulation, and finally to toxic effects (Table 1). This can be partly explained by the use of different doses and forms of I in the experiments, the method of its application, and supply of nutrient substrate with I, environmental conditions, as well as the reaction of certain plant species to its use. In addition, it is necessary to point out that it is very difficult to perform an experiment where the presence of I is completely excluded since it is also found in the air, and thus accessible to the above-ground plant organs. According to Medrano-Macias et al. (2016), good results are achieved from the angle of biofortification, i.e. plant enrichment with I, when 7.5 kg ha^{-1} KIO_3 is applied to the soil, 10 mg on 1 kg of soil in containers, or 10^{-6}–10^{-5} M in the nutrient solution, and 0.5 kg ha^{-1} KI foliarly.

Table 1. Effect of the application of iodine in different crops

Crops	Effect	References	Crops	Effect	References
Pisum	+	Suzuki (1902)[1]	Crop plants	–	Newton & Toth (1952)[3]
Radish, oat	+	Suzuki & Aso (1903)[1]	Buckwheat	–	Pauwels (1961)
Corn	+	Mazé (1915)[1]	Spinach, wheat, flax, barley, mustard	+	Pauwels (1961)
Sugar beet	+	Stoklas (1924)[1]	Cereals, sugar beet, potato	+	Pais (1980)
Barley, mustard	–	Brenchley (1924)[1]	Rice	–	Mackowiak & Grossl (1999)
Agricultural plants	–	Wrangell (1927)[1]	Spinach	+	Zhu (2003)
Orange trees	–	Haas & Reed (1927)[1]	Chinese cabbage	+	Dai et al. (2004)[4]
Sugar beet, turnips	–	Engles (1928)[1]	Onion	–	Dai et al. (2004)[4]
Sugar beet	+	Stoklas (1929)[1]	Spinach	–	Dai et al. (2006)
Buckwheat	+	Cotton (1930)[1]	Cabbage, lettuce, tomato, carrot	–	Hong et al. (2008)
Citrus	–	Haas (1930)[1]	Soybean	+	Mao et al. (2014)[4]
Wheat	+	Scharrer & Schropp (1931)[1]	Maize	–	Mao et al. (2014)[4]
Tomato	–	Wynd (1934)	Lettuce	–	Smolen et al. (2014)[4]
Tomato	–	Hageman el al. (1942)[3]	Spinach	+	Smolen et al. (2015)[4]

Source: 1 – Wynd (1934), 2 – Scharer (1955), 3 – Pauwels (1961), 4 – Medrano-Macias et al. (2016). Positive effect (+), no effect (-)

Given that iodine affects certain life processes of plants, as well as that it may stimulate their growth, the question arises of whether it can be considered an essential element for higher plants. There is no evidence that higher plants are unable to complete their life in the absence of I in the nutrient substrate. It is interesting to note that the essential elements for plants in the periodic table are grouped around the argon-carbon-argon line. This line is called the "line of nutritive elements" or "line of life" since the majority of essential elements can be found around it but not iodine (Kastori, 1983). Iodine can be considered a useful element based on the current understanding of its role in the life processes of higher plants. Plants can complete their life cycle without it but it can promote plant growth and development under certain conditions.

Mengel and Kirkby (2001) included I in the group of predominantly toxic elements together with some other microelements (F, Al, Ni, Se, etc.). Similar to other elements, high concentrations of I limit the production of plant biomass. Therefore, it seems unreasonable to include I in the group of elements with a predominantly toxic effect on plants. It is believed that 0.5–1.0 μg I g^{-1} of the soil has a toxic effect on plants. In unpolluted agricultural soils, the concentration of soluble I in the soil is significantly lower than the stated value, implying that there is no danger of its toxic effects under normal (usual) circumstances.

There is a significant difference in the sensitivity of some plant species to the increased concentrations of I. Pea and maize are very sensitive, while lettuce and halophytes tolerate the iodine excess well (Pais, 1980). Weng et al. (2003) stated the following order of vegetable sensitivity to the increased presence of I: Chinese cabbage > spinach > radish. Mack and Brasher (1936) mentioned that a concentration of 0.75 μg I g^{-1} of dry matter in pea leaves and 20 μg I g^{-1} in turnip leaves had a toxic effect. When the soil is enriched with 120 mg I kg^{-1}, radish root become rotten, while at a higher dose (180 mg I kg^{-1}) the roots wither. Young seedlings exhibit a greater sensitivity to the presence of higher I concentrations than the older ones (Weng et al., 2003). The toxicity of potassium iodide in pea is higher in the light than in the dark. It is believed that iodide toxicity is a consequence of its intracellular oxidation into iodine (Mynett and Wain, 1973).

Toxic concentrations of I cause visible morphological changes in plants. Iodine has a toxic effect when its concentration in the leaves is higher than 0.5 to 1 mg kg^{-1} of dry matter. With I concentration of 8 mg kg^{-1} of dry matter in leaves, chlorosis occurs. Healthy leaves usually have I concentration of 0.5 mg kg^{-1} of dry matter (Szabó et al., 1993). Signs of I excess are more or less specific to individual plant species. Initial signs of its toxic effect appear on older leaves – they become chlorotic and fall off, while the younger leaves take on a dark green color. Growth is decreased, the leaves bend backward, and necrosis occurs at the apex and marginal part of the leaf. Plant death can also occur (Mengel and Kirkby, 2001). According to Watanabe and Tensho (1970), high concentrations of I cause *Akagare* disease, which is manifested by the occurrence of red spots on the leaves and can lead to plant death. This phenomenon occurs in flooded rice fields in iodine-rich soils. According to Wynd (1934), in conditions of excess I in tomatoes, at first there is a decrease in the intensity of the green color of the leaves. Leaves then become chlorotic, primarily the lower ones. Chlorosis occurs initially between the larger leaf nerves, and later it is covering the entire leaf.

Under hydroponics, plants are more tolerant to high concentrations of iodate than iodide (Zhu et al., 2003). The toxic effect of I occurs due to intracellular oxidation of I^- into I_2, which leads to the inhibition of photosynthesis

(Mynet and Wain, 1973). In lettuce grown on a substrate where I^- concentration is higher than 40 μM, photosynthesis is inhibited (Blasco et al., 2011a). A specific disease is caused in rice by inhibition of photosynthesis at I^-concentration higher than 8 μM (Iwamoto and Shiraiwa, 2012). In contrast to higher plants, inorganic I does not appear to affect photosynthesis in microalgae, as it does not affect Fv/Fm, even at I concentration that inhibits growth. It is believed that FSII in microalgae is protected from the negative effect of iodine present in a cell (Bergeijk et al., 2016). A high level of chlorine can reduce the toxic effect of I, which suggests that competition between these two elements takes place (Mengel and Kirkby, 1978). It is presumed that the release of I into the atmosphere by the plants is one of the defense mechanisms against its excess.

Higher plants as a source of iodine

Plants are a very important source of iodine for the human population, as well as for animals. The daily human requirement for I ranges between 0.1 and 0.2 mg and its content in plants is usually significantly lower than 1.0 mg kg^{-1} of dry matter. The content of I in higher plants ranges widely, from 0.05 to 5 mg kg^{-1} of dry matter (Johanson, 2000). The content of I in plants primarily depends on the presence of available I in the soil and the plant species. Differences in I content in the same plant species depend on the habitat provision with I (Szabó et al., 1993). Sorrel, lettuce, garlic, cucumber, and mangel are cultivated species characterized by a higher content of I. It is believed that about 80% of daily human needs for I is satisfied by vegetable-based food.

Dai et al. (2004a) studied the effect of I application in six vegetable species and found that spinach was efficient in iodine biofortification. Accumulation of I in spinach leaves during application of iodide and iodate differed depending on the applied concentration of I (Zhu et al., 2003). Application of 1 mg L^{-1} of salicylic acid together with I increased the accumulation of I in tomato fruits significantly. The use of salicylic acid along with the application of KIO_3 increased the accumulation of I in the fruits by 157%, and salicylic acid along with KI by 37% (Smolen et al., 2015).

The content of I in particular plant produce differs notably, and in this regard, their share in satisfying the daily human needs for I varies (Table 2). In general, the content of I in plant products is mostly low. By its application, it is possible to increase significantly its content in plants. This knowledge is of great importance, not only theoretically, but also practically, especially in the areas with low concentrations of iodine available to plants in the soil. The presence of I in food is also affected by its preparation. The use of high temperatures during food preparation leads to volatilization of I, and thus to its loss (Zhang et al., 2000).

Table 2. Iodine content in some plant produce and products

Produce	µg I 100 g^{-1} FW	mg I kg^{-1} DW	Produce/product	µg I 100 g^{-1} FW	mg I kg^{-1} DW
Bean, dry	3.0	0.036	Apricot	1.0	0.076
Green peas	4.2	0.168	Pear	1.0	0.064
Mangel	20.1	2.960	Strawberry	1.0	0.098
Tomato	1.7	0.270	White bread	5.8	0.087
Carrot	3.8	0.330	Rye bread	8.5	0.128
Mushrooms	18.1	1.640			

*FW – fresh weight; DW – dry weight

Content of I in seafood is many times higher than in plant produce. Sea fish oil is especially high in iodine, e.g. herring contains 2,480 mg I kg^{-1} of dry matter (Szabó et al., 1993). Drinking water is also a source of iodine for humans. It is estimated that it covers about 10% of the iodine requirement. In the case of an insufficient amount of I in food and water, it is recommended to enrich the salt with 20 mg KI kg^{-1} of salt.

Cereal grains are an important food on a global scale. The accumulation of iodine in cereal grains primarily depends on the soil-grain transfer factors, which depend on plant species, soil characteristics, the chemical form of I, and climate conditions. Similar to wheat, Shinonaga et al. (2001), the transfer factor in the rice grain is 0.002 (Muramatsu et al., 1989). Due to the low transfer factor, cereal grain products are characterized by a relatively low share of I (Table 2).

Marine algae are characterized by a much higher I content than terrestrial plants. Some marine algae accumulate 10 µg I g^{-1} of dry matter (Mengel and Kirkby, 2001), which is 90–2,500 mg I kg^{-1} of dry matter (Johanson, 2000). Red and brown macroalgae are characterized by a large accumulation of I (Phaneuf et al., 1999). An extremely high concentration of I is found in brown algae *Laminaria digitata*, with a maximum value near 5% in dry matter (Argall et al., 2004). Due to the high concentration of I, algae can be used, among other purposes, as iodine fertilizer, and some can even be used for human consumption. Alga *Emiliania huxleyi* accumulates nearly ten times higher concentrations of I than seawater. Because of that, these microalgae can be used for the extraction of I from seawater and iodine-contaminated wastewaters (Iwamoto and Shiraiwa, 2012). According to Hsiao (1969), iodine is an essential element for the growth, morphogenesis, and reproduction of marine brown alga *Patalonia fascia*. Data are suggesting that I accumulates in special cells in red and brown algae (Bollard, 1983). Iodine plays a significant role in the antioxidant metabolism of some aquatic plants.

CONCLUSION

Iodine is found in nature in inorganic and organic forms, including iodide (I^{-1}), iodate (IO_3^-), elemental iodine (I_2), and organic iodine. It is considered an ultra-microelement because of its distribution in plants. Its content in higher plants ranges widely, from 0.05 to 5 mg kg^{-1} of dry matter. In plant tissues, iodine distribution is in the following order: root > leaf > stem > fruit. Higher plants take up I by roots from the soil and by aerial parts from the atmosphere. However, the most important source of I for plants is the soil. Content of I in the soil ranges between 0.5 and 20 mg kg^{-1}, depending on chemical traits, mechanical content, and distance from the sea. Iodine is easily leached from sandy soils, which are poorer in iodine than clay and loamy soils. The most significant input of I into the soil is from the atmosphere. Volatilization is an important aspect of I geochemistry. The quantity of released I into the atmosphere from the soil-plant system during one season amounts to about 0.40 kg ha^{-1}.

The iodine transfer factor from soil to plants is generally low. By introducing I into the nutrient substrate or treating above-ground organs with I solution, it is possible to increase significantly its content in plants, to a greater extent in vegetative than in generative organs. Understanding that I primarily accumulates in the vegetative plant organs, as well as that vegetables are characterized by a greater ability to accumulate I, is very important for providing people with I. Sorrel, mangel, garlic, cucumber, and lettuce, in general, are cultivated species characterized by higher accumulation of I. The precise roles of I in the metabolism of higher plants are not sufficiently known yet. There are records of a favorable effect of the application of I on plant growth, tolerance to stress factors, antioxidant capacity, and differences in I bioaccumulation in cultivated species. In addition, its effect on photosynthesis, carbohydrate metabolism, hydration, respiration, and interaction with other elements is mentioned. Higher concentrations of I (0.5–1.0 µg g^{-1}) in the nutrient substrate have a phytotoxic effect, resulting in chlorosis of young leaves, brown, stunted, and coralloid roots, and inhibition of growth.

Since the biofortification of I through its application is a relatively recent subject of interest, further studies are expected to provide data on the optimal dose, the form of I, and mode of application in the soil-plant system in different soil types and environmental conditions. Iodine is one of the useful elements for higher plants based on the importance it has in the life processes of plants. However, this does not diminish the importance of the problem of I in higher plants, since they are an important source of I in food and feed of humans and higher animals and an essential micronutrient due to health reasons.

REFERENCES

Amachi S (2008): Microbial contribution to global iodine cycling: volatilization, accumulation, reduction, oxidation and sorption of iodine. *Microbes Environ*. 23: 269–276.

Argall E, Kupper FC, Kloareg B (2004): A survey of iodine content in *Laminaria digitata*. *Bot. Mar.* 47: 30–37.

Bergeijk SA, Hernández L, Zubia E, Canavate JP (2016): Iodine balance, growth and biochemical composition of three marine microalgae cultured under various inorganic iodine concentrations. *Mar. Biol.* 163: 1–19.

Blasco B, Leyva R, Romero L, Ruiz JM (2013): Iodine effect on phenolic metabolism in lettuce plants under salt stress. *J. Agric. Food Chem.* 61: 2591–2596.

Blasco B, Rios JJ, Cercilla LM, Sánches-Rodriguez E, Rubio-Wilhelmi MM, Rosales MA, Ruiz MJ, Romero L (2010): Photorespiration and nitrogen metabolism in lettuce plants (*Lactuca sativa* L.): Induced changes in response to iodine biofortification. *J. Plant Growth Regul.* 29: 477–486.

Blasco B, Rios JJ, Leyva R, Cervilla LM, Sánchez-Rodriguez E, Rubio-Wilhelmi MM, Rosales MA, Ruiz JM, Romero L (2011): Does iodine biofortification affects oxidative metabolism in lettuce plants? *Biol. Trace Elem. Res.* 142: 831–842.

Blasco B, Rios JJ, Leyva R, Melgarejo R, Constán-Aguilar C, Sánchez-Rodriguez E, Rubio-Wilhelmi MM, Romero L, Ruiz JM (2011a): Photosynthesis and metabolism of sugars from lettuce plants (*Lactuca sativa* L. var. longifolia) subjected to biofortification with iodine. *Plant Growth Regul.* 65: 137–143.

Bollard EG (1983): Involvement of unusual elements in plant growth and nutrition. In: Lauchli A, Bieleski RL (eds.), *Inorganic plant nutrition,* Encycl. Plant Physiol., New Series, Vol. 15B. Berlin – Heidelberg – New York – Tokyo: Springer Verlag, 695–744.

Bors J, Martens R (1992): The contribution of microbial biomass to the adsorption of radioiodine in soils. *J. Environ. Radioactivity* 15: 35–49.

Böszörményi Z, Cseh E (1960): The uptake and reduction of iodate by wheat roots. *Curr. Sci.* 29: 340–341.

Bunzl K, Schimmack W (1988): Effect of microbial biomass reduction by gamma radiation on the sorption of ^{137}Cs, ^{85}Sr, ^{139}Ce, ^{57}Co, ^{109}Cd, ^{65}Zn, ^{100}Ru and ^{131}I by soils. *Radiat. Environ. Bioph*. 27: 165-176.

Carrano MW, Yarimizu K, Gonzales JL, Cruz-López R, Edwards MS, Tymon TM, Küpper FC, Carrano CJ (2020): The influence of marine algae on iodine speciation in the coastal ocean. *Algae* 35: 167–176.

Chatin A (1850): Existance de l'iode dans les plantes d'eau douce. Conséquences de ce fait pour la géognosie, la physiologie vegetale, la thérapeutique et peut-etre pour l'industrie. *Compt. Rend. Acad. Paris* 30: 352–354.

Dai JL, Zhang M, Hu QH, Haung YZ, Wang RQ, Zhu YG (2009): Adsorption and desorption of iodine by various Chinese soils II. Iodide and iodate. *Geoderma* 153: 130–135.

Dai JL, Zhang M, Zhu YG (2004): Adsorption and desorption of iodine by various Chinese soils. *Environ. Int.* 30: 525–530.

Dai JL, Zhu YG, Huang YZ, Zhang M, Song JL (2006): Availability of iodide and iodate to spinach (*Spinacia oleracea* L.) in relation to total iodine in soil solution. *Plant Soil* 289: 301–308.

Dai JL, Zhu YG, Zhang M, Huang YZ (2004a): Selecting iodine-enriched vegetables and the residual effect of iodate application to soil. *Biol. Trace Elem. Res.* 101: 265–276.

Fuge R (1996): Geochemistry of iodine in relation to iodine deficiency disease. In: Appleton JD, Fuge R, Mccall GJH (eds.), *Environmental Geochemistry and Health*. London: Geological Society Special Publication, 201–211.

Fuge R, Ander EA (1998): Geochemical barriers and the distribution of iodine in the secondary environment: implications for radio-iodine. In: Nicholson K (eds.), *Energy and the Environment: Geochemistry of Fossil, Nuclear and Renewable Resources*. MacGregor Sci Insch, 163–170.

Fuge R, Johnson CC (2015): Iodine and human health, the role of environmental geochemistry and diet, a review. *Appl. Geochem*. 62: 282–302.

Gilfedder BS, Lai SC, Petri M, Biester H, Hoffman T (2008): Iodine speciation in rain, snow and aerosols. *Atmos. Chem. Phys.* 8: 6069–6084.

Gracia-Osuna HT, Benavides-Mendoza A, Rivas-Morales C, Morales-Rubio E, Verde-Star J, Miranda-Ruvalcaba R (2014): Iodine application increased ascorbic acid content and modified the vascular tissue *Opuntia ficus-indica*. *Pak. J. Bot.* 46: 127–134.

Gupta N, Bajpai M, Majumdar R, Mishra P (2015): Response of iodine on antioxidant level of *Glycine max* L. grown under Cd^{2+} stress. *Adv. Biol. Res.* 9: 40–48.

Hageman RH, Hodge ES, McHargue JS (1942): Effect of potassium iodide on the ascorbic acid content and growth of tomato plants. *Plant Physiol.* 17: 465–472.

Herrett RA, Hatfield JHH, Crosby DG, Vlitos AJ (1962): Leaf abscission induced by the iodine ion. *Plant Physiol.* 37: 358–363.

Hong CL, Weng HX, Qin YC, Yan AL, Wie LL (2008): Transfer of iodine from soil to vegetables by applying exogenous iodine. *Agron. Sustain. Dev.* 28: 575–583.

Hsiao SIC (1969): Life history and iodine nutrition of the marine brown algae, *Petalonia fascia* (O.F. Mull.) Kuntze. *Can. J. Bot.* 47: 1611–1616.

Iwamoto K, Shiraiwa Y (2012): Characterization of intracellular iodine accumulation by iodine-tolerant microalgae. *Proced. Environ. Sci.* 15: 34–42.

Johanson KJ (2000): Iodine in soil. *Tehnical Report TR-00-21.* Stockholm: Svensk Kärnbränslehantering AB.

Kašin VK (1981): Značenie joda v metabolizme rastenij. *Agrohemija* 9: 139–153.

Kastori R (1983): *Uloga elemenata u ishrani biljaka*. Novi Sad: Matica srpska.

Kastori R (2006): *Fiziologija biljaka*. Novi Sad: Naučni institut za ratarstvo i povrtarstvo.

Keppler F, Borchers M, Elsner P, Fahimi I, Pracht J, Schöler HF (2003): Formation of volatile iodinated alkanes in soil: results from laboratory studies. *Chemosphere* 52: 477–483.

Knaelmann M (1972): Die Abhaengigkeit der Jodidsorption von verschiedenen Bodenkomponenten. *Atomkernenergie* 19: 329–332.

Leyva R, Sánchez-Rodriguez E, Rios JJ, Rubio-Wilhelmi MM, Romero L, Ruiz JM (2011). Beneficiel effects of exogenous iodine on lettuce plants subjected to salinity stress. *Plant Sci.* 181: 195–202.

Mack WB, Brasher EP (1936): The influence of commercial fertilizers, potassium iodine, and soil acidity on the iodine content of certain vegetables. *J. Agric. Res.* 53: 789–800.

Mackowiak CL, Grossl PR (1999): Iodate and iodide effects on iodine uptake and partitioning in rice (*Oryze sativa* L.) grown in solution culture. *Plant Soil* 212: 135–143.

Medrano-Macias J, Leija-Martinez P, González-Morales S, Juárez-Maldonado A, Benavides-Mendoza B (2016): Use of iodine to biofortify and promote growth and stress tolerance in crops. *Front. Plant Sci.* 7: 1–20.

Mengel K, Kirgby EA (1978): *Principles of Plant Nutrition*. Bern: International Potash Institute.

Mengel K, Kirkby EA (2001): *Principles of Plant Nutrition*. 5th Edition, Dordrecht–Boston–London: Kluwer Academic Publishers.

Moiseyev IT, Tikhomirov FA, Perevezentsev VM, Rerikh LA (1984): Role of soil properties, interspecific plant differences, and other factors affecting the accumulation of radioactive iodine in crops. *Soviet Soil Sci.* 16: 60–66.

Muramatsu Y, Uchida S, Sumiya Y, Ohmomo Y, Obata H (1989): Trace experiments of transfer radio-iodine in the soil-rice plant system. *Water Air Soil Pollut.* 45: 157–171.

Muramatsu Y, Yoshida S, Bannai T (1995): Trace experiments on the behavior of radioiodine in soil-plant-atmosphere system. *J. Radional Nucl. Chem-Articles* 194: 303–310.

Muramatsu Y, Yoshida S, Fehn U, Amachi S, Ohmomo Y (2004): Studies with natural and anthropogenic iodine distribution and cycling in the global environment. *J. Environ. Radioact.* 74: 221–232.

Mynet A, Wain RL (1973): Herbicidal action of iodide: effect on chlorophyll content and photosynthesis in dwarf bean *Phaseolus vulgaris*. *Weed Res.* 13: 101–109.

Pais I (1980): *A mikrotápanyagok Szerepe a Mezőgazgaságban*. Budapest: Mezőgazdasági Kiadó.

Pauwels GWFH (1961): Iodine as a micronutrient for plants. *Plant Soil* 14: 377–392.

Phaneuf D, Cote I, Dumas P, Ferron LA, LeBlanc A (1999): Evaluation of the contamination of marine algae (seaweed) from the St. Lawrence River and likely to be consumed by humans. *Environ. Res.* 80: 175–182.

Redecker KR, Cicerone RJ (2004): Environmental controls over methyl halide emissions from rice paddies. *Glob. Biogeochem. Cycles* 18.

Redeker KR, Andrews J, Fischer F, Sass R, Cicerone RJ (2002): Interfield and intrafield variability of methyl halide emissions from the rice paddies. *Glob. Biogeochem. Cycles* 16: 72–79.

Saiz-Lopez A, Plane JMC, Baker AR, Carpenter LJ, von Glasow R, Gomez Martin JC, McFiggans G, Saunders RW (2012): Atmospheric chemistry of iodine. *Chem. Rev.* 112: 1773–1804.

Scharer K (1955): *Biochemie der Spurenelemente*. Berlin–Hamburg: Paul Parey.

Seldres AA, Hungate FP (1956): The foliar sorption of iodine by plants. U.S. Atomic Energy Commission. *Document* No HW-44890.

Sheppard SC, Evenden WG (1992): Response of some vegetable crops to soil-applied halides. *Can. J. Soil Sci.* 72: 555–567.

Shetaya WH, Young SD, Watts MJ, Ander EL, Bailey EH (2012): Iodine dynamics in soils. *Geochim. Cosmochim. Acta* 77: 457–473.

Shinonaga T, Gerzabek MH, Strebl F, Muramatsu Y (2001): Transfer of iodine from soil to cereal grains in agricultural areas of Austria. *Sci. Total Environ.* 267: 33–40.

Smolen S, Rozek R, Ledwozyw-Smolem I, Strzetelski P (2011): Preliminary evaluation of the influence of soli fertilization and foliar nutrition with iodine on the efficiency of iodine biofortification and chemical composition of lettuce. *J. Elem.* 16: 613–622.

Smolen S, Skoczylas L, Rakoczy R, Ledwozyw-Smolen I, Kopec A, Piatkowska E (2015): Mineral composition of field-grown lettuce (*Lactuca sativa* L.) depending on the diversified fertilization with iodine and selenium compounds. *Acta Sci. Pol. Hortorum Cultus* 14: 97–114.

Smolen S, Wierzbinska J, Sady W, Kolton A, Wiszniewska A, Liszka-Skoczylas M (2015): Iodine biofortification with additional application of salicylic acid affects yield and selected parameters of chemical composition of tomato fruits (*Solanum lycopesicum* L.). *Sci. Hortic.* 188: 89–96.

Stemmler I, Hense I, Qack B, Maier-Reimer E (2013): Methyl iodide production in the open ocean. *Biogeosci.* 10: 17549–17595.

Szabó SA, Györi D, Regiusné Möcsényi Á (1993): *Mikroelemek* a mezőgazdaságban II. (Stimulatív hatású mikroelemek). Budapest: Akadémiai Kiadó és Nyomda.

Tang Q, Xu Q, Zhang F, Huang Y, Liu J, Wang X, Yang Y, Liu X (2013): Geochemistry oh iodine-rich groundwater in the Taiyuan Basin of central Shanxi Province, North China. *J. Geochem. Explor.* 135: 117–123.

Tikhomirov FA (1984): Patterns of iodine migration in the system atmosphere-soil-plants natural waters. *Biol. Bull. Acad. Sci. USSR* 11: 275–261.

Umaly RC, Poel LW (1971): Effects of iodine in various formulations on the growth of barley and pea plants in nutrient solution culture. *Ann. Bot.* 35: 127–131.

Venturi S (2011): Evolutionary significance of iodine. *Curr. Chem. Biol.* 5: 1–8.

Waite TJ, Truesdale VW, Olafsson J (2006): The distribution of dissolved inorganic iodine in the seas around Iceland. *Mar. Chem.* 101: 54–67.

Watanabe I, Tensho K (1970): Further study on iodine toxicity in relation to "reclamation Akagare" disease of lowland rice. *Soil Sci. Plant Nutr.* 16: 192–194.

Watts MJ, O'Reilly J, Maricelli A, Coleman A, Ander EL, Ward NI (2010): A snapshot of environmental iodine and selenium in La Pampa and San Juan provinces in Argentina. *J. Geochem. Explor.* 107: 87–93.

Weng H, Weng J, Yong W, Sun X, Zhong H (2003): Capacity and degree of iodine absorbed and enriched by vegetable from soli. *J. Environ. Sci.* 15: 107–111.

Whitehead DC (1975): Uptake by perennial ryegrass of iodide, elemental iodine and iodate added to soil as influenced by various amendments. *J. Sci. Food Agric.* 26: 361–367.

Whitehead DC (1984): The distribution and transformation of iodine in the environment. *Environ. Int.* 10: 321–339.

WHO (2004): Iodine status worldwide. In: Benoist B. et al. (eds.), Department of Nutrition for Health and Development, Geneva: World Health Organization.

Wrangel M (1930): Das atmosphaerische Jod und die Pflanze. *Die Umschau* 34: 53–65.

Wynd FL (1934): The effects of increasing the iodine content of the tomato plant on respiration and enzymatic activity. *Ann. Missouri Bot. Gard.* 21: 367–432.

Yuita K (1992): Dynamics of iodine, bromine and chlorine in soil: II Chemical forms of iodine in soil solutions. *Soil Sci. Plant Nutr.* 38: 281–287.

Zhang L, Chen Z, Wang J (2000): Iodine loss from iodinised salt during processing, sale and consumption. *Zhejiang Prev. Mad.* 12: 32–34.

Zhu YG, Huang YZ, Hu Y, Liu YX (2003): Iodine uptake by spinach (*Spinacia oleracea* L.) plants grown in solution culture: effects of iodine species and solution concentrations. *Environ. Int.* 29: 33–37.

ПРЕГЛЕДНИ НАУЧНИ РАД

ЈОД И ВИШЕ БИЉКЕ

Рудолф Р. КАСТОРИ, Ивана В. МАКСИМОВИЋ,
Марина И. ПУТНИК-ДЕЛИЋ

Универзитет у Новом Саду, Пољопривредни факултет
Трг Доситеја Обрадовића 8, Нови Сад 21000, Србија

РЕЗИМЕ: Јод (I) је неопходан микроелеменат за човека и више животиње. Његов недостатак у исхрани представља значајан здравствени проблем у многим областима света. Више биљке представљају важан извор јода за човека, стога је од значаја познавање усвајања, биоакумулације, његову расподелу у јестивим деловима гајених врста, као и његово дејство на животне процесе и органску продукцију виших биљака. Биљке јод могу да усвајају кореном из хранљивог супстрата и надземним огранима из атмосфере. Земљиште је за биљке најзначајнији извор јода. Глиновита и иловаста земљишта богатија су јодом од лаких песковитих из којих се лако испира. Волатилизација има важну улогу у геохемији јода. Значајан је његов унос у земљиште из атмосфере. Количина одатог јода у атмосферу из система земљиште–биљке у једном вегетационмом периоду износи у просеку 0,40 kg/ha. Утицај и улога јода у животним процесима виших биљака нису још у довољној мери проучени. Резултати истраживања указују да примена јода може повољно да утиче на раст биљака, на толерантност према стресним условима, на антиоксидантни капацитет биљака, на његову различиту биоакумулацију појединих гајених врста. Поред наведеног, наводи се и утицај јода на фотосинтезу, метаболизам угљених хидрата и азота, дисање, хидратисаност ткива и узајамно дејство с другим елементима. Код сувишка јод делује фитотоксично, ограничава раст биљака и изазива морфолошке промене. Применом јода преко земљишта или третирањем надземних органа његовим раствором могуће је значајно повећати његов садржај у биљкама и то у већој мери у вегетативним него у генеративним органима. Стога лиснато поврће представља значајан извор јода за човека (спанаћ, кисељак, салата, блитва). Досадашњи резултати испитивања у вези примене јода и његовог утицаја на више биљке указују на бројна отворена и недовољно разјашњена питања. Стога, са становишта обезбеђења људи овим микроелементом, намеће се потреба за детаљнијим испитивањем оптималне дозе, облика јода, начина његове примене код појединих биљних врста на различитим типовима земљишта и еколошким условима. На основу досадашњих сазнања о значају јода у животним процесима биљака он се убраја у корисне елементе за више биљке. То, међутим, не умањује значај проблематике јода у вишим биљкама, пошто гајене врсте представљају важан извор овог микроелемента за човека и више животиње за које је он из здравствених разлога веома важан односно неопходан. Ниже биљке, морске алге, јод накупљају у вишеструко већој мери од терестричних, и за неке од њих он је неопходан.

КЉУЧНЕ РЕЧИ: јод (I), више биљке, земљиште, акумулација, физиолошко и стимулативно дејство

EDITORIAL POLICY

The journal MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES is dedicated to publishing original scientific papers presenting new results of fundamental and applied research, review articles as well as brief communications from all scientific fields as referred to in the title of the journal. Review articles are published only when solicited by the editorial board of the journal. Manuscripts that have already been published in extensor or in parts or have been submitted for publication to other journal will not be accepted. The journal is issued twice a year.

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

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

MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES is an Open Access journal.

Contributions to the Journal should be submitted in the English language, with an abstract also in Serbian.

MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES is available in full-text at the web site of Matica Srpska and in the following data bases: Serbian Citation Index, EBSCO Academic Search Complet, abstract level at Agris (FAO), CAB Abstracts, CABI Full-Text, Thomson Reuters Master Journal List and DOAJ (Directory of Open Access Journals).

Editorial responsibilities

The Editor-in-Chief and the Editorial Board are responsible for deciding which articles submitted to MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES will be published. The Editor-in-Chief and the Editorial Board are guided by the Editorial Policy and constrained by legal requirements in force regarding libel, copyright infringement and plagiarism.

The Editor-in-Chief reserves the right to decide not to publish submitted manuscripts in case it is found that they do not meet relevant standards concerning the content and formal aspects. The Editorial Staff will inform the authors whether the manuscript is accepted for publication within two weeks after receiving from authors manuscript revised following reviewers comments.

The Editor-in-Chief and members of Editorial Board must hold no conflict of interest with regard to the articles they consider for publication. If an Editor feels that there is likely to be a perception of a conflict of interest in relation to their handling of a submission, the selection of reviewers and all decisions on the paper shall be made by the Edittor-in-Chief.

The Editor-in-Chief and members of Editorial Board shall evaluate manuscripts for their intellectual content free from any racial, gender, sexual, religious, ethnic, or political bias.

The Editor-in-Chief and the Editorial Staff must not use unpublished materials disclosed in submitted manuscripts without the express written consent of the authors. The information and ideas presented in submitted manuscripts shall be kept confidential and must not be used for personal gain.

The Editor-in-Chief and members of Editorial Board shall take all reasonable measures to ensure that the reviewers remain anonymous to the authors before, during and after the evaluation process.

Author's responsibilities

Authors warrant that their manuscript is their original work, that it has not been published before, and is not under consideration for publication elsewhere. Parallel submission of the same paper to another journal constitutes a misconduct, and eliminates the manuscript from consideration by MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES.

The Authors also warrant that the manuscript is not and will not be published elsewhere (after the publication in MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES) in any language without the consent of the Editorial Board.

In case a submitted manuscript is a result of a research project, or its previous version has been presented at a conference in the form of an oral presentation (under the same or similar title), detailed information about the project, the conference, etc. shall be provided in Acknowledgements. A paper that has already been published in another journal cannot be reprinted in MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES.

It is the responsibility of each author to ensure that papers submitted to MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES are written with ethical standards in mind. Authors affirm that the article contains no unfounded or unlawful statements and does not violate the rights of third parties. The Publisher will not be held legally responsible should there be any claims for compensation.

Reporting standards

A submitted manuscript should contain sufficient detail and references to permit reviewers and, subsequently, readers to verify the claims presented in it. The deliberate presentation of false claims is a violation of ethical standards. Book reviews and technical papers should be accurate and they should present an objective perspective.

Authors are exclusively responsible for the contents of their submissions and must make sure that they have permission from all involved parties to make the data public.

Authors wishing to include figures, tables or other materials that have already been published elsewhere are required to be referred on the reference used, or to have permission from the copyright holder(s). Any material received without such evidence will be assumed to originate from the authors.

Authorship

Authors must make sure that only contributors who have significantly contributed to the submission are listed as authors and, conversely, that all contributors who have significantly contributed to the submission are listed as authors. If persons other than authors were involved in important aspects of the research project and the preparation of the manuscript, their contribution should be acknowledged in a footnote or the Acknowledgments section.

Acknowledgment of Sources

Authors are required to properly cite sources that have significantly influenced their research and their manuscript. Information received in a private conversation or correspondence with third parties, in reviewing project applications, manuscripts and similar materials must not be used without the express written consent of the information source.

Plagiarism

Plagiarism, where someone assumes another's ideas, words, or other creative expression as one's own, is a clear violation of scientific ethics. Plagiarism may also involve a violation of copyright law, punishable by legal action.

Plagiarism includes the following:

• Word for word, or almost word for word copying, or purposely paraphrasing portions of another author's work without clearly indicating the source or marking the copied fragment (for example, using quotation marks);

• Copying equations, figures or tables from someone else's paper without properly citing the source and/or without permission from the original author or the copyright holder.

Please note that all submissions are thoroughly checked for plagiarism.

Any paper which shows obvious signs of plagiarism will be automatically rejected and those authors will not be permitted to submit papers to MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES for three years.

In case plagiarism is discovered in a paper that has already been published by the journal, it will be retracted in accordance with the procedure described below under Retraction policy, and authors will not be permitted to submit papers to MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES for three years.

Conflict of interest

Authors should disclose in their manuscript any financial or other substantive conflict of interest that might have influenced the presented results or their interpretation.

Fundamental errors in published works

In the event that an author discovers a significant error or inaccuracy in his/her own published work, it is the author's obligation to promptly notify the journal Editor or publisher and cooperate with the Editor to retract or correct the paper.

By submitting a manuscript the authors agree to abide by the MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES's Editorial Policies.

Reviewer responsibilities

Reviewers are required to provide written, competent and unbiased feedback in a timely manner on the scholarly merits and the scientific value of the manuscript.

The reviewers assess manuscript for the compliance with the profile of the journal, the relevance of the investigated topic and applied methods, the originality and scientific relevance of information presented in the manuscript, the presentation style and scholarly apparatus.

Reviewers should alert the Editor to any well-founded suspicions or the knowledge of possible violations of ethical standards by the authors. Reviewers should recognize relevant published works that have not been cited by the authors and alert the Editor to substantial similarities between a reviewed manuscript and any manuscript published or under consideration for publication elsewhere, in the event they are aware of such. Reviewers should also alert the Editor to a parallel submission of the same paper to another journal, in the event they are aware of such.

Reviewers must not have conflict of interest with respect to the research, the authors and/or the funding sources for the research. If such conflicts exist, the reviewers must report them to the Editor without delay.

Any selected referee who feels unqualified to review the research reported in a manuscript or knows that its prompt review will be impossible should notify the Editor without delay.

Reviews must be conducted objectively. Personal criticism of the author is inappropriate. Reviewers should express their views clearly with supporting arguments.

Any manuscripts received for review must be treated as confidential documents. Reviewers must not use unpublished materials disclosed in submitted manuscripts without the express written consent of the authors. The information and ideas presented in submitted manuscripts shall be kept confidential and must not be used for personal gain.

Peer review

The submitted manuscripts are subject to a peer review process. The purpose of peer review is to assists the Editor-in-Chief, Editorial Board and Consulting Editors in making editorial decisions and through the editorial communications with the author it may also assist the author in improving the paper. Scientific papers, review papers, short communications are subjected to the review process.

Reviews are anonymous (single-blind procedure), and at least one positive review should be obtained for a positive decision – acceptance of the paper after minor or major revision. In case MAJOR changes are demanded by reviewer(s), the revised version of the paper will be sent again to the reviewer(s) for their FINAL OPINION. Reviewers are not paid for their reviews.

The choice of reviewer(s) is at the discretion of the Editor-in-Chief and members of Editorial Board. The reviewers must be knowledgeable about the subject area of the manuscript; they must not be from the authors' own institution and they should not have recent joint publications with any of the authors. Editors and the Editorial Staff shall take all reasonable measures to ensure that the reviewers remain anonymous to the authors before, during and after the evaluation process.

All of the reviewers of a paper act independently and they are not aware of each other's identities. If the decisions of the two reviewers are not the same (accept/reject), the Editor may assign additional reviewers.

During the review process Editors may require authors to provide additional information (including raw data) if they are necessary for the evaluation of the scholarly merit of the manuscript. These materials shall be kept confidential and must not be used for personal gain.

The Editorial team shall ensure reasonable quality control for the reviews. With respect to reviewers whose reviews are convincingly questioned by authors, special attention will be paid to ensure that the reviews are objective and high in academic standard. When there is any doubt with regard to the objectivity of the reviews or the quality of the review, additional reviewers will be assigned.

Procedures for dealing with unethical behavior

Anyone may inform the editors and/or Editorial Staff at any time of suspected unethical behavior or any type of misconduct by giving the necessary information/evidence to start an investigation.

Investigation

• Editor-in-Chief will consult with the Subject Editors on decisions regarding the initiation of an investigation.

• During an investigation, any evidence should be treated as strictly confidential and only made available to those strictly involved in investigating.

• The accused will always be given the chance to respond to any charges made against them.

• If it is judged at the end of the investigation that misconduct has occurred, then it will be classified as either minor or serious.

Minor misconduct

Minor misconduct will be dealt directly with those involved without involving any other parties, e.g.:

• Communicating to authors/reviewers whenever a minor issue involving misunderstanding or misapplication of academic standards has occurred.

• A warning letter to an author or reviewer regarding fairly minor misconduct.

Major misconduct

The Editor-in-Chief, in consultation with the Subject, and, when appropriate, further consultation with a small group of experts should make any decision regarding the course of action to be taken using the evidence available. The possible outcomes are as follows (these can be used separately or jointly):

• Publication of a formal announcement or editorial describing the misconduct.

• Informing the author's (or reviewer's) head of department or employer of any misconduct by means of a formal letter.

• The formal, announced retraction of publications from the journal in accordance with the Retraction Policy (see below).

• A ban on submissions from an individual for a defined period.

• Referring a case to a professional organization or legal authority for further investigation and action.

When dealing with unethical behavior, the Editorial Staff will rely on the guidelines and recommendations provided by the Committee on Publication Ethics (COPE).

Retraction policy

Legal limitations of the publisher, copyright holder or author(s), infringements of professional ethical codes, such as multiple submissions, bogus claims of authorship, plagiarism, fraudulent use of data or any major misconduct require retraction of an article. Occasionally a retraction can be used to correct errors in submission or publication. The main reason for withdrawal or retraction is to correct the mistake while preserving the integrity of science; it is not to punish the author.

Standards for dealing with retractions have been developed by a number of library and scholarly bodies, and this practice has been adopted for article retraction by MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES: in the electronic version of the retraction note, a link is made to the original article. In the electronic version of the original article, a link is made to the retraction note where it is clearly stated that the article has been retracted. The original article is retained unchanged; save for a watermark on the PDF indicating on each page that it is "retracted."

Open access policy

MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES is an Open Access Journal. All articles can be downloaded free of charge and used in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International (CC BY-NC-ND) licence.

The MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES journal is funded by Matica srpska and does not charge any fees to authorsis free of charge for authors.

Self-archiving Policy

The MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES allows authors to deposit Author's Post-print (accepted version) and Publisher's version/PDF in an institutional repository and non-commercial subject-based repositories, such as arXiv or similar) or to publish it on Author's personal website (including social networking sites, such as ResearchGate, Academia.edu, etc.) and/or departmental website, at any time after publication. Full bibliographic information (authors, article title, journal title, volume, issue, pages) about the original publication must be provided and a link must be made to the article's DOI.

Disclaimer

The views expressed in the published works do not express the views of the Editors and Editorial Staff. The authors take legal and moral responsibility for the ideas expressed in the articles. Publisher shall have no liability in the event of issuance of any claims for damages. The Publisher will not be held legally responsible should there be any claims for compensation.

Articales are available in full-text at the web site of Matica Srpska, Serbian Citation Index, CABI, EBSCO, Web of Science Master Journal List, AGRIS FAO (abstracts)

INSTRUCTION TO AUTHORS

1. General remarks

1.1. Matica Srpska Journal for Natural Sciences (short title: Matica Srpska J. Nat. Sci.) publishes manuscripts and review articles as well as brief communications from all scientific fields as referred to in the title of the journal. Review articles are published only when solicited by the editorial board of the journal. Manuscripts that have already been published in extenso or in parts or have been submitted for publication to other journal will not be accepted. The journal is issued twice a year.

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

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

2. Planning and preparing of the manuscript

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

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

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

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

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

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

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

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

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

3. Structure the Review articles in Abstract, Key Words, Text of the manuscript, Conclusion, and References; submit Summary and Key Words in Serbian language.

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

5. References

5.1. List the References alphabetically. Examples:

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

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

(c) Books: Last name VG, Last name CS (2009): Title of the cited book. Publisher, City

(d) Dissertations: Last name VA (2009): Title of the thesis. Doctoral dissertation, University, City

(e) Unpublished articles: designation "in press" should be used only for papers accepted for publishing. Unpublished articles should be cited in the same way as published articles except that instead of journal volume and page numbers should write "in press" information.

(f) Articles reported at scientific meetings and published in extenso or in a summary form: Last name FR (2011): Proceedings, Name of the meeting, Meeting organizers, Venue, Country, 24–29.

(g) World Wide Web Sites and other electronic sources: Author's last name, Author's initial. (Date of publication or revision). Title, In: source in Italics, Date of access, Available from: <Available URL>. Use n.d. (no date) where no publication date is available. Where no author is available, transfer the organization behind the website or the title to the author space.

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

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

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

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

6. Units, names, abbreviations, and formulas

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

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

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

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

7. Figures

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

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

8. Tables

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

8.2. Number the tables using Arabic numerals.

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

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

9. Electronic copy of the article

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

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

Зборник Матице српске за природне науке издаје Матица српска
Излази двапут годишње

Уредништво и администрација:
Нови Сад, Улица Матице српске 1
Телефон: (021) 6615798

Matica Srpska Journal for Natural Sciences
Published twice a year
Editorial and publishing office:
1 Matica Srpska Street, 21000 Novi Sad, Serbia
Phone: +381 21/6615798

E-mail: vnikolic@maticasrpska.org.rs
zmspn@maticasrpska.org.rs
Website:
https://www.maticasrpska.org.rs/category/katalog-izdanja/naucni-casopisi/zbornik-matice-srpske-za-prirodne-nauke/

The editors of the *Matica Srpska Journal for Natural Sciences*
completed the selection for Issue 141 (2/2021) on November 17, 2021

For Publishers: Prof. Dragan Stanić, PhD
Editorial Staff Secretary: Vladimir M. Nikolić
Managing editor: Prof. Slavka Gajin, PhD
Language Editor: Ljiljana Tubić and Olivera Krivošić
Proof Reader: Vladimir M. Nikolić
Technical design: Vukica Tucakov

Published in December 2021
Computer set: Vladimir Vatić, GRAFIT, Petrovaradin
Printed by: SAJNOS, Novi Sad

Објављивање часописа помогло је
Министарство просвете, науке и технолошког развоја Републике Србије
Publishing a journal 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