
ЗАДУЖБИНА ЈОВАНА ДУЧИЋА
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–)

140

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

140

NOVI SAD
2021

CONTENTS / САДРЖАЈ

ARTICLES AND TREATISES / ЧЛАНЦИ И РАСПРАВЕ

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 9—19, 2021

UDC 633.63:632.26
UDC 633.63: 665.52/.54
https://doi.org/10.2298/ZMSPN2140009S
ORIGINAL ARTICLE

Mira S. STAROVIĆ[1*], *Danijela T. RISTIĆ*[1], *Snežana Đ. PAVLOVIĆ*[2], *Mehmet M. MÖZCAN*[3], *Dragana Lj. JOŠIĆ*[4]

[1] Institute for Plant Protection and Environment,
Teodora Drajzera 9, Belgrade 11000, Serbia
[2] Institute for Medicinal Plant Research "Dr. Josif Pančić",
Tadeuša Košćuška 1, Belgrade 11000, Serbia
[3] Selçuk University, Faculty of Agriculture, Department of Food Engineering,
Alaaddin Keykubat Kampüsü No. 371, Konya 42079, Turkey
[4] Institute of Soil Science,
Teodora Drajzera 7, Belgrade, Serbia

ANTIFUNGAL ACTIVITY OF PLANT ESSENTIAL OILS AND *Pseudomonas chlororaphis* STRAINS AGAINST *Cercospora beticola* Sacc.

ABSTRACT: Leaf spot disease caused by *Cercospora beticola* Sacc. is the most destructive foliar disease of beet. *Cercospora* leaf spot is controlled primarily by fungicides because the non-chemical alternatives do not provide commercially viable control. One of the ways of reducing chemical application is the use of different essential oils (EOs) or antagonistic plant growth-promoting rhizobacteria (PGPB). This study evaluates several EOs and PGPB belonging to *Pseudomonas chlororaphis* as possible control agents of this pathogen. Antifungal properties were determined by *in vitro* microdilution method against five *C. beticola* monosporial isolates originated from the locality Brus, Serbia (53°53' N, 21°04'E and 429 m above sea level) using EOs from medicinal plants: Turkish pickling herb (*Echinophora tenuifolia*), oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), and myrtle (*Myrtus communis*) obtained by a hydro-distillation method. All tested oils displayed some antifungal activity against the fungal isolates. *Origanum vulgare* EO demonstrated the strongest antifungal activity (MIC – 0.0055±0.0051mg/mL), *Ocimum basilicum* slightly lower (MIC – 0.075±0.045mg/mL), followed by *Myrtus communis* (MIC – 0.775±0.045 mg/mL) and *Echinophora tenuifolia* (MIC – 7.75±4.5 mg/mL). Five tested *P. chlororaphis* strains exhibited some antagonistic effect against *C. beticola*. Overnight culture (ONC) of *P. chlororaphis* strain E65 induced the highest percentage of inhibition (75.8%), followed by N3 (72.0%). A cell-free supernatant (CFS) and the CFS treated with EDTA (CFS-EDTA) of these strains showed similar inhibition of 60.2 and 56.0%, and both strains suppressed *C. beticola* growth. *P. chlororaphis* strains M1 and K113 also reduced the fungal growth by

[*] Corresponding author. E-mail: miragavranstarovic@gmail.com

67–70% using ONC and between 48–57% using different CFS fractions. The strains L1 and B25 caused inhibition of 60% using ONC and 50% by CFS. The lowest inhibition (~40%) by CFS-EDTA and heat-treated cell-free supernatant (HT-CFS) was recorded for B25, which was used as a reference strain. The tested isolates of *C. beticola* were susceptible to all selected essential oils and *P. chlororaphis* strains E25, N3, M1, and K113 *in vitro*, making them a promising non-chemical control agent. It is recommended that these findings should be tested in field conditions.
KEYWORDS: antagonism, CLS, essential oils, MIC, *Pseudomonas* sp.

INTRODUCTION

Cercospora leaf spot (CLS) caused by the fungus *Cercospora beticola* Sacc. is the most important foliar disease of sugar beet (*Beta vulgaris*) worldwide (Holtschulte, 2000; Jacobsen and Franc, 2009; Skaracis et al., 2010). Yield reduction due to CLS can be over 50% (Kaiser and Varrelmann, 2008). CLS is known to cause economic damage in Serbia up to 50% (Trkulja et al., 2017). It is usually controlled by single-site fungicides, which can lead to a quite intensive application of pesticides resulting in the development of fungal resistance and environmental pollution. *C. beticola* quickly develops resistance to the fungicides, which makes protection difficult (Georgopoulos and Dovas, 1973). Consequently, an environmentally friendly alternative is required that can also ensure acceptable quality and quantity of agricultural yield (Ma and Michailides, 2005). Biological control agents and essential oils are an attractive and promising alternative for disease control due to their low environmental impact and their ability to slow down fungicide resistance in pathogen populations (Jochum et al., 2006). Biopesticides can be divided into three different types according to their active substance: microorganisms (bacteria, fungi, oomycetes, viruses, and protozoa), biochemicals (secondary metabolites), and semiochemicals (a chemical signal produced by one organism that causes a behavioural change in an individual of the same or a different species) (Chandler et al., 2011). A biopesticide can be defined as a mass-produced agent manufactured from a living microorganism or a natural product and sold for the purpose of controlling plant pests and diseases. This definition encompasses most entities classed as biopesticides within the Organisation for Economic Cooperation and Development countries (OECD, 2009).

Medicinal plants are a rich source of biologically active compounds. Different medicinal plant species have been used in traditional medicine because of their well-known antimicrobial properties. Essential oils (EOs) have been used for their bactericidal, virucidal, and fungicidal properties (Bakkali et al., 2008). There are numerous data on the high inhibitory effect of EOs from different plant species against many phytopathogenic fungal species (Özcan and Erkmen, 2010; Stevic et al., 2014; Combrinck et al., 2011), but there are very few studies on the control of *C. beticola* (Fatoung et al., 2011) using plant extracts.

Genus *Pseudomonas* comprises a number of plant growth-promoting (PGP) species that express characteristics that can lead to the improvement of plant growth and health. *P. putida* WCS358 was involved in inducing systemic

resistance in plants (Meziane et al., 2005). In the biological control of cotton seedling, damping-off disease caused by *R. solani*, *P. aureofaciens* (*chlororaphis*) and *P. fluorescens* were found to produce phenazine, siderophore, and volatile and non-volatile metabolites (Samavat et al., 2014).

Antagonistic activities of Serbian indigenous *Pseudomonas* spp. strains against different phytopathogenic fungi were reported by Djuric et al. (2011), and Jošić et al. (2015). *P. chlororaphis* strains harbouring multiple PGP traits, including antibiotics phenazine-1-carboxylic acid (PCA) and 2-hydroxy-phenazine-1-carboxylic acid (2-OH-PCA), successfully inhibited *Alternaria alternata* growth and disease incidence on cardoon (Jošić et al., 2012), *P. theicola*, and *F. oxysporum* (Starović et al., 2017; Poštić et al., 2019).

In this study, we have assessed the possibility of using Eos, as well as different fractions of bacterial culture of *P. chlororaphis* strains as antifungal agents in preventing the development of one of the most dangerous fungal beet diseases.

MATERIAL AND METHODS

Antifungal activity of EOs

In this study, Turkish pickling herb or Tarhana herb (*Echinophora tenuifolia*), oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), and myrtle (*Myrtus communis*) were obtained from Mersin (Turkey) by hydro-distillation in a Clevenger-type apparatus, as previously reported (Özcan and Erkmen, 2001; Starović et al., 2016). The essential oils obtained this way were stored in sealed glass bottles, protected from the light by aluminium foil wrapping and stored at -18 ^{o}C.

Fungi. The antifungal activity was tested using fungal species identified as *Cercospora beticola* from the collection of the Institute for Plant Protection and Environment, Belgrade, Serbia, and sub-cultured on potato dextrose agar (PDA) at 28 °C. The suspension of aerial mycelia was prepared from a 20-day-old mycelium suspended in 0.9% (w/v) NaCl with 0.1% (v/v) Tween 20 by mixing thoroughly using a vortex mixer.

Antifungal effect *in vitro*. To investigate the antifungal activity of EOs, a micro-dilution method was used (Rodriquez-Tudela et al., 2008). The final volume of microtiter plate wells was 100 µl (90 µl/well potato dextrose medium with appropriate dilutions of EO and 10 µl/well mycelia suspension of *C. beticola*). The experiment was performed in four repetitions. Microtiter plates were incubated for 5 days at 28 °C.

The minimal inhibitory concentration (MIC) was defined as the lowest concentration of essential oils (EO) that completely inhibited the visible fungal growth (Figure 1). Fluconazole was used as a positive control.

The obtained values of MIC were processed by Duncan's multiple range tests. Analysis of the variance was performed on MIC data of four EOs on

C. beticola. The significance was evaluated at $p<0.05$ for all tests. Statistical analyses were done by procedures of STATISTICA v.7 (StatSoft, Inc.) and IBM SPSS Statistics v.20 (SPSS, Inc.).

Antifungal activity of *P. chlororaphis* strains

Five *P. chlororaphis* strains from the rhizosphere of different plants were tested for the antifungal effect on *C. beticola*. The *P. chlororaphis* B25 strain was included in this experiment as a reference strain, whose complete genome sequence has been deposited in DDBJ/503 EMBL/GenBank under the accession number CP027753 (Biessy et al., 2019).

The antagonistic activity of *P. chlororaphis* strains against *C. beticola* was tested on Waksman agar medium by dual culture method (Wolf et al., 2002). A bacterial overnight culture (ONC), extracellular metabolites in cell-free supernatant (CFS), CFS treated with EDTA (ethylenediaminetetraacetic acid disodium salt dehydrate), known as CFS-EDTA, and a heat-treated cell-free supernatant (HT-CFS) were tested for the inhibition of *C. beticola* growth. To obtain the supernatant fraction, an optimized ON culture (10^6 CFU mL^{-1}) was centrifuged twice at 13,000 rpm for 5 min, with or without filtration (filter tubes with microporous membrane 0.22 µm, Merck Millipore Ltd.). To test two additional fractions, CFS was treated with 1mM EDTA or heated at 70 ºC for 30 min.

P. chlororaphis culture and its fraction (10 µL) were placed on the edge of Petri dishes, 2.5 cm distance from fungal mycelia placed in the centre as 1 cm plug, while the control variant contained only mycelia of *C. beticola* on WA and WA with 1mM EDTA added instead of bacterial culture/fraction. Four replicates per variant were used and the test was performed twice. After incubation of the cultures at 25 °C for 7 days, the growth of fungus was measured. The percentage of the fungal growth inhibition was calculated using the following formula: 100*(1-R2/R1), where R1 was the radial distance growth of the fungus in a control plate and R2 was the radial distance growth of the fungus in the bacterial treatment. The results of the fungal growth inhibition by *P. chlororaphis* strains were compared using a statistically significant difference ($p\leq0.01$) from Duncan's test (STATISTICA v.7; StatSoft, Inc.).

RESULTS

Antifungal activity of EOs

All tested oils showed some antifungal activity against the tested fungal pathogen (Figure 1). *Origanum vulgare* EO showed the strongest antifungal activity (MIC – 5.5±0.0051µg/mL), with a slightly lower *Myrtus communis* (MIC – 325.5±0.045µg/mL), followed by *Ocimum basilicum* (MIC – 775.5±0.045µg/mL), and *Echinophora tenuifolia* (MIC – 3250.0±4.5 µg/mL) (Table 1).

Table 1. Antifungal activity of the essential oils expressed as the minimal inhibitory concentrations (µg/mL) against *Cercospora beticola*.

Essential oils	MIC (µg/mL)*
Echinophora tenuifolia	3250.0c
Origanum vulgare	5.5a
Ocimum basilicum	775.5b
Myrtus communis	325.0b

*Different letters in MIC column indicate a significant difference between means ($p<0.05$)

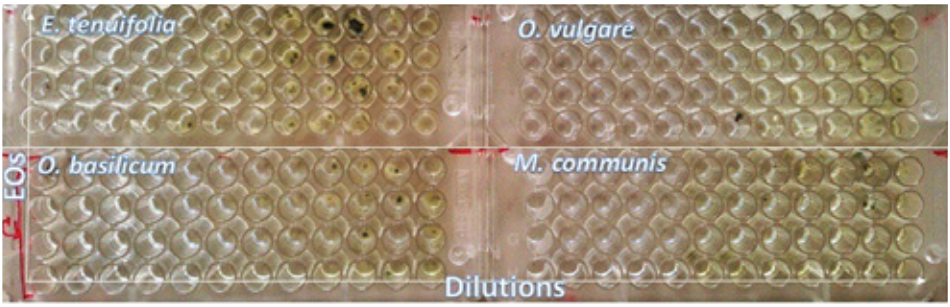


Figure 1. Micro-dilution method: antifungal effect of investigated essential oils.

Antifungal activity of *P. chlororaphis* strains

Our results have demonstrated that all *P. chlororaphis* strains showed an antagonistic effect on *C. beticola* (Table 2). The percentage of fungal growth inhibition by overnight culture of the tested strains ranged from 60.6 to 75.8%, and from 48.8 to 60.2% by cell-free supernatants. The fraction CFS-EDTA of the strain B25 induced the lowest inhibition rate of 39.4% (Figure 2). EDTA did not influence fungal growth at all, showing no statistically different values compared to the control.

Table 2. Growth of *C. beticola* (mm) affected by *P. chlororaphis* strains.

Fungus *C. beticola*	Bacterial strain	Bacterial culture* and fraction			
		ONC	CFS	CFS-EDTA	HT-CFS
C	B25	13.88±0.83	17.38±1.5	21.88±096	20.13±0.82
36.00±1.69	L1	14.25±1.16	18.5±1.07	19.13±1.64	18.63±1.3
C-EDTA	M1	11.88±0.64	15.5±1.26	16.13±0.5	16.88±0.82
36.25±1.28	N3	10.13±0.64	14.38±1.06	15.25±0.89	14.88±0.83
mean	E65	8.75±0.52	14.38±1.06	16.13±0.83	15.38±1.3
36.13±1.45	K113	10.75±0.76	16.63±0.74	18.75±0.89	17.38±1.06

*bacterial culture 10^6 CFU mL^{-1} and its fractions; C – Control; C-EDTA – Control supplemented with 1mM EDTA; ONC – overnight culture; CFS – cell-free supernatant; CFS-EDTA – CFS treated with 1mM EDTA; HT-CFS – heat-treated CFS. Values indicate mean values (±SD).

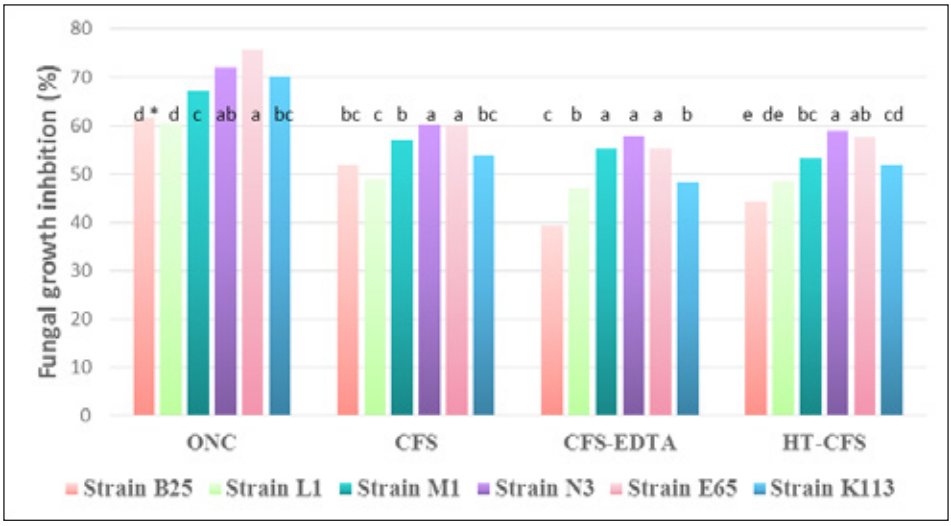


Figure 2. Growth inhibition of *C. beticola* affected by *P. chlororaphis* strains.
* The bars with the same letters are not significantly different at P < 0.01 using Duncan's test.

DISCUSSION

The selected essential oils, especially oregano, exhibited significant antifungal activity against *C. beticola*. These results are encouraging considering how resistance of *C. beticola* to the fungicides is widely established. Another advantage of using EOs is that they can be applied in organically grown crops. The results of this investigation support our earlier study of antifungal activity against *Phomopsis theicola*, as the most potent oil was oregano (MIC-5.5µg/mL) (Starović et al., 2017).

Numerous studies from other researchers have shown significant antifungal activity of oregano's essential oils, such as Kocić-Tanackov et al. (2012) which have demonstrated that the concentration of 25 µg/mL inhibits the growth of *Fusarium oxysporum*, *F. proliferatum*, *F. subglutinans*, and *F. verticilioides* by 81–88%. Bedoya-Serna et al. (2018) obtained the MIC of oregano essential oil of 0.20 µg/mL, 0.26 µg/m L, and 0.30 µg/mL against *Fusarium* sp., *Cladosporium* sp., and *Penicillium* sp. respectively. In another study, the MICs of oregano essential oil against *Botrytis cinerea* and *Colletrotrichum gleosporioides* were 0.8 and 1.0 mg/mL respectively (Cid-Pérrez et al., 2016). In addition, the MIC of myrtle essential oil against *Fusarium* spp. was 0.3–3.25 mg/mL (Starović et al., 2016). Citral, methyl anthranilate, and nerol (citrate EOs) in a concentration of 5.0 mL/L reduced *C. beticola* by 78–80% in field condition and manifested a very high antagonistic effect against *C. beticola in vitro* conditions (Fatouh et al., 2011).

Dual culture assay *in vitro* and inhibition zone area was taken as a measure of the antagonistic potential, which led to the selection of bacterial strains with a better inhibitory potential on the growth of *C. beticola*. ONC of *P. chlororaphis* strain E65 induced the highest percentage of inhibition (75.8%), followed by N3 (72.0%), showing a significant difference at $P<0.01$ using Duncan's test. The reverse significance was calculated for their HT-CFS fraction. No significant difference was observed in fungal inhibition of CFS and CFS-EDTA of these strains. Both strains were highly effective in antagonistic action toward *C. beticola* growth. The lowest percentage of inhibition – around 60% for ONC and 50% for CFS was caused by *P. chlororaphis* strains L1 and B25. B25 showed the lowest inhibition for CFS-EDTA and HT-CFS. *P. chlororaphis* strains M1 and K113 also reduced the fungal growth at a high percentage (67–70%) by ONC and between 48–57% by different CFS. No significant difference between strains E65, N3, and M1 in the inhibition of fungus comparing CFS-EDTA fractions was exhibited.

This finding is in agreement with the results obtained by other researchers. Arzanlou et al. (2016) reported that *C. beticola*, a causal agent of Cercospora leaf spot disease in sugar beet, was inhibited by strains from different genera isolated from the rhizosphere of sugar beet – *Bacillus*, *Pseudomonas*, and *Paenibacillus*. These strains significantly decreased the disease severity in laboratory and greenhouse assays. In their study, *Pseudomonas* strain inhibited two *C. beticola* strains by 53.13 and 64.84% in dual culture assay, which is in concordance with our results. Poornima et al. (2011) tested *Bacillus subtilis*, *P. fluorescens*, *Trichoderma harzianum*, *T. koningii,* and *T. viride* as bioagents, under *in vitro* and *in vivo* conditions, against *C. beticola* that caused CLS of palak (*Beta vulgaris* var. bengalensis Hort). Although *T. harzianum* was significantly superior under *in vitro* condition, *P. fluorescens* was the most effective under *in vivo* conditions showing the lower percent of disease severity.

To assess the effectiveness of biological control of another phytopathogen from genera *Cercospora*, the causal agent of frogeye leaf spot of soya bean – *C. sojina*, Simonetti et al. (2012) used one *P. fluorescens* and two *Bacillus amyloliquefaciens* indigenous strains. The fungal growth was inhibited by 52–53% using the culture of *Bacillus* strains and 32–34% with the *P. fluorescens* strain. Besides two different species of pathogen and an antagonist from the same genera, we noticed that *P. fluorescens* caused a smaller reduction in *C. sojina* growth than culture and three fractions of all *P. chlororaphis* strains of *C. beticola* in our study.

In an attempt to find alternatives to fungicides against *C. beticola*, Derbalah et al. (2013) tested several different formulations, including the biological agent containing *Bacillus subtilis*, *B. pumilus*, *P. fluorescens*, *Epicoccum nigrum*, and found that biological formulations were the most effective treatments against sugar beet leaf spot, followed by nanosilica and nanozinc oxide.

CONCLUSIONS

Our study provides sound scientific evidence of the practical use of traditional medicinal plants in biological control of fungal diseases and encourages selection and testing of other plant products as potential alternatives to pesticides. Based on our promising results on the effectiveness of the EOs from Turkish pickling herb, oregano, basil, and myrtle in *C. beticola* control, further studies could lead to the selection of essential oils and their concentrations for *in vivo* trials of products with fungicidal properties. Further studies should focus on the investigation of the most effective antagonistic *P. chlororaphis* strains E65, N3, and M1 in the control of CLS disease under greenhouse and field conditions.

ACKNOWLEDGMENTS

The authors are thankful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for financial support during this study through the Projects grant TR31018 and III46007. A special thanks to Mrs. S. Bajic-Raymond, a Director of Raymond Educational Consultancy Ltd, and Professor G. Raymond, University of Bristol, UK, for proof-reading the paper and linguistic corrections.

REFERENCES

Arzanlou M, Mousavi S, Bakhshi M, Khakvar R, Bandehagh A (2016): Inhibitory effects of antagonistic bacteria inhabiting the rhizosphere of the sugarbeet plants, on Cercospora beticola Sacc., the causal agent of Cercospora leaf spot disease on sugarbeet. *J. Plant Prot. Res*. 56: 6–14.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008): Biological effects of essential oils – review. *Food Chem. Toxicol*. 46: 446–475.

Bedoya-Serna C, Dacanal G, Fernandes A (2018): Antifungal activity of nanoemulsions encapsulating oregano (*Origanum vulgare*) essential oil: *in vitro* study and application in Minas Padräo cheese. *Braz. J. Microbiol*. 49: 929–935.

Biessy A, Novinscak, A, Blom J, Léger G, Thomashow LS, Cazorla FM, Josic D, Filion M (2019): Diversity of phytobeneficial traits revealed by whole–genome analysis of worldwide–isolated phenazine–producing *Pseudomonas* spp. *Environ Microbiol*. 21: 437–455.

Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP (2011): The development, regulation and use of biopesticides for integrated pest management. *Phil. Trans. R. Soc. B* 366: 1987–1998.

Cid-Pérrez T, Torre-Muňoz J, Nevárez-Moorillón G, Palou E, López-Malo A (2016): Chemical characterization and antifungal activity of *Poliomintha longiflora* Mexican oregano. *J. Essent. Oil Res*. 28: 157–165.

Combrinck S, Regnier T, Kamatou GPP (2011): In vitro activity of eighteen essential oils and some major components against common postharvest fungal pathogens of fruit. *Ind. Crop. Prod.* 33: 344–349.

Derbalah AS, El-Moghazy SM, Godah MI (2013): Alternative Control Methods of Sugar-beet Leaf Spot Disease Caused by the Fungus *Cercospora beticola* (Sacc). *Egypt J. Biol. Pest. Contr.* 23: 247–254.

Djuric S, Pavic A, Jarak M, Pavlovic S, Starovic M, Pivic R, Josic D (2011): Selection of indigenous fluorescent pseudomonad isoletes from maize rhizospheric soil in Vojvodina as possible PGPR. *Rom. Biotech. Lett.* 16: 6580–6590.

Fatouh YO, El-Kareem F, Abd-El-Latif F, El-Mohammedy R (2011): Effects of citrus essential oil compounds on management leaf spot disease on sugar beet plant under field condition. *J. Agr. Sci. Tech.* 7: 869–877.

Georgopoulos SG, Dovas C (1973): Occurrence of *Cercospora beticola* strains resistant to benzimidazole fungicides in northern Greece. *Plant Dis.* 62: 321–324.

Holtschulte B (2000): *Cercospora beticola* – worldwide distribution and incidence. In: MJC Asher, B. Holtschulte, MR Molard, F. Rosso, G. Steinrücken, R. Beckers (eds), *Advances in Sugar Beet Research Cercospora beticola Sacc. Biology, agronomic influence and control measures in sugar beet*, Vol. 2, International Institute for Beet Research, Brussels.

Jacobsen BJ, Franc GD (2009): Cercospora leaf spot. In: RM Harveson, LE Hanson, GL Hein (eds.), *Compendium of Beet Diseases and Pests*, American Phytopathological Society, St. Paul, MN.

Jochum CC, Osborne LE, Yuen GY (2006): *Fusarium* head blight biological control with *Lysobacter enzymogenes* strain C3. *Biol. Con.* 39: 336–344.

Jošić D, Protolipac K, Starović M, Stojanović, S, Pavlović S, Miladinović M, Radović S (2012): Phenazine producing *Pseudomonas* isolates decrease *Alternaria tenuissima* growth, pathogenicity and disease incidence on cardoon. *Arch. Biol. Sci.* 64: 1495–1503.

Jošić D, Ćirić A, Soković M, Stanojković-Sebić A, Pivić R, Lepšanović Z, Glamočlija J (2015): Antifungal Activities of Indigenous Plant Growth Promoting *Pseudomonas* spp. from Alfalfa and Clover Rhizosphere. *Front. Life Sci.* 8: 131–138.

Kaiser U, Varrelmann M (2009): Development of a field biotest using artificial inoculation to evaluate resistance and yield effects in sugar beet cultivars against *Cercospora beticola*. *Eur. J. Plant. Pathol.* 124: 269–281.

Kocić-Tanackov S, Dimić G, Tanackov I, Pejin D, Mojović L, Pejin J (2012): Antifungal activity of Oregano (*Origanum vulgare* L.) extract on the growth of *Fusarium* and *Penicillium* species isolated from food. *Hem. Ind.* 66: 33–41.

Ma Z, Michailides TJ (2005): Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Prot.* 24: 853–863.

Meziane H, Van der Sluis I, Van Loon LC, Höfte M, Bakker PAH (2005): Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. *Mol. Plant Pathol.* 6: 177–185.

Organisation for Economic Co-operation and Development (2009): Series on pesticides no. 448. Report of Workshop on the Regulation of Biopesticides: Registration and Communication Issues. Available to: http://www.oecd.org/dataoecd/3/Collego55/43056580.pdf. Accessed: 7 October 2010.

Özcan M, Erkmen O (2001): Antimicrobial activity of the essential oils of Turkish plant spices. *Eur. Food Res. Technol*. 212: 658–660.

Poornima Y, Hegde R, Jagdish J (2011): Biological Management of Leaf Spot of Palak Caused by *Cercospora beticola* Sacc. *Res. J. Agr. Sci*. 2: 655–657.

Poštić D, Jošić D, Lepšanović Z, Aleksić G, Latković D, Starović M (2019): The effect of *Pseudomonas chlororaphis* subsp. *aurantiaca* strain Q16 able to inhibit *Fusarium oxysporum* growth on potato yield. *Ratar. Povrt*. 56: 41–48.

Rodriquez-Tudela JL, Donnelly JP, Arendrup MC, Arikan S, Barchiesi F, Bille J (2008): Euacast technical note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds. *Clin Mirobiol. Infect*. 14: 982-984.

Samavat S, Heydari A, Zamanizadeh HR, Rezaee S, Aliabadi AA (2014): A comparison between *Pseudomonas aureofaciens* (chlororaphis) and *P. fluorescens* in biological control of cotton seedling damping-off disease. *J. Plant Protect. Res.* 54: 115–121.

Simonetti E, Carmona MA, Scandiani MM, García AF, Luque AG, Correa OS, Balestrasse KB **(**2012): Evaluation of indigenous bacterial strains for biocontrol of the frogeye leaf spot of soya bean caused by *Cercospora sojina*. *Lett. Appl. Microbiol.* 55: 170–173.

Skaracis GN, Pavili OI, Biancardi E (2010): Cercospora leaf spot disease of sugar beet. *Sugar Tech*. 12: 220–228.

Starović M, Ristić D, Pavlović S, Ristić M, Stevanović M, AlJuhaimi F, Naydun S, Özcan MM (2016): Antifungal activities of different essential oils against anise seeds mycopopulations. *J. Food Saf. Food Qual*. 67: 72–78.

Starović M, Ristić D, Aleksić G, Pavlović S, Özcan MM, Knežević M, Jošić D (2017): Antifungal activity of plant essential oils and selected *Pseudomonas* strains to the *Phomopsis theicola*. *Pestic. Fitomed.* 32: 121–127.

Stevic T, Beric T, Savikin K, Sokovic M, Godjevic D, Dimkic I, Stankovic S (2014): Antifungal activity of selected essential oils against fungi isolated from medicinal plant. *Ind. Crop Prod*. 55: 116–122.

Trkulja N, Milosavljević A, Mitrović M, Jović J, Toševski I, Khan MFR, Secor GA (2017): Molecular and experimental evidence of multi-resistance of *Cercospora beticola* field populations to MBC, DMI and QoI fungicides. *Eur. J. Plant Pathol*. 149: 895–910.

Wolf A, Fritze A, Hagemann M, Berg G (2002): *Stenotrophomonas rhizophila* sp. nov., a novel plant-associated bacterium with antifungal properties. *Int. J. Syst. Evol. Micr*. 52: 1937–1944.

АНТИФУНГАЛНА АКТИВНОСТ БИЉНИХ ЕТАРСКИХ УЉА И СОЈЕВА *Pseudomonas chlororaphis* НА *Cercospora beticola* Sacc.

Мира С. СТАРОВИЋ[1], Данијела Т. РИСТИЋ[1], Снежана Ђ. ПАВЛОВИЋ[2], Мехмет М. МОЗКАН[3], Драгана Љ. ЈОШИЋ[4]

[1] Институт за заштиту биља и животну средину,
Теодора Драјзера 9, Београд 11000, Србија
[2] Институт за проучавање лековитог биља „Др Јосиф Панчић”,
Тадеуша Кошћушка 1, Београд 11000, Србија
[3] Универзитет у Селџуку, Пољопривредни факултет,
Катедра за прехрамбено инжењерство, Конија 42079, Турска
[4] Институт за земљиште,
Теодора Драјзера 7, Београд 11000, Србија

РЕЗИМЕ: Пегавост лишћа шећерне репе чији је проузроковач *Cercospora beticola* Sacc. је врло деструктивно обољење ове биљне врсте. Хемијска средства се уобичајено користе за контролу ове болести, јер за сада алтернативне мере заштите нису комерцијализоване. У овом раду испитиван је *in vitro* антифунгални утицај неких етарских уља (ЕУ) и *Pseudomonas chlororaphis* сојева – ризобактерија стимулатора раста биљака, као могућих агенаса за контролу овог патогена. Микродилуционом методом су одређене минималне концентрације инхибиције (MIC) етарских уља. Сва примењена етарска уља су испољила задовољавајући степен инхибиције. Етарско уље оригана испољило је најјачи антифунгални ефекат (MIC – 0,0055±0,0051mg/mL), нешто нижи босиљка (MIC – 0,075±0,045 mg/mL), мирте (MIC – 0,775±0,045 mg/mL) и најслабији турске киселе биљке (MIC – 7,75±4,5 mg/mL). Ангонистички ефекат на пораст мицелије *C. beticola* детектован је код 5 сојева *P. chlororaphis*, а сој B25 коришћен је као референтни. Прекоћна култура *P. chlororaphis* соја E65 испољила је највиши степен инхибиције (75,8%), затим соја N3 (72,0), док су фракције супернатаната са и без EDTA изазвале инхибицију 60,2 и 56,0%. Прекоћне културе сојева M1 и K113 инхибирале су 67–70% пораст мицелије, а 48–57% различите фракције супернатаната. Инхибицију раста гљиве 60% условиле су прекоћне културе сојева L1 и B25, а 50% њихови супернатанти, док су супернатант обогаћен EDTA и термички третирани супернатант соја B25 остварили најнижи степен инхибиције (~40%). Сва примењена етарска уља и *P. chlororaphis* сојеви E25, N3, M1 и K113 испољили су значајан степен инибиције пораста мицелије изолата *C. beticola* пореклом са шећерне репе, што их чини потенцијално перспективним нехемијским агенсима, чији ефекат треба проверити и у пољским условима.

КЉУЧНЕ РЕЧИ: антагонизам, етарска уља, MIC, CLS, *Pseudomonas* sp.

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 21—27, 2021

UDC 631.576.3: 632.25
https://doi.org/10.2298/ZMSPN2140021I
ORIGINAL ARTICLE

Maja V. IGNJATOV[*1], *Dragana N. MILOŠEVIĆ*[1], *Gordana D. TAMINDŽIĆ*[1], *Žarko S. IVANOVIĆ*[1]

[1] Institute of Field and Vegetable Crops,
National Institute of the Republic of Serbia
Maksima Gorkog 30, Novi Sad 21000, Serbia
[2] Institute for Plant Protection and Environment
Teodora Drajzera 9, Belgrade 11000, Serbia

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *Fusarium graminearum* Schwabe AS A CAUSAL AGENT OF *Hyssopus officinalis* L. SEED ROT

ABSTRACT: Symptoms of seed rot of *Hyssopus officinalis* L. were noticed during seed health testing in 2018. According to morphological and cultural characteristics, isolates belong to *Fusarium* spp. and *Alternaria* spp.. Based on morphological and pathogenic properties, as well as sequence analysis, isolate designated as 4003/3 was identified as *Fusarium graminearum* deposited in NCBI gene bank under Acc. Number MK061542. To our knowledge *F. graminearum* as the causal agent of *Hyssopus officinalis* L. seed rot in Serbia was noticed for the first time. This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-9/2021-14/200032.
KEYWORDS: Hyssop, *Fusarium graminearum*, seed rot

INTRODUCTION

Hyssop (*Hyssopus officinalis* L.) is a perennial polymorphous plant species of the Lamiaceae family. It is grown in Southern Europe with essential oil accumulated in the flowers and leaves and often used as condiment and spices in food industries (Fathiazad and Hamedeyazdan, 2011; Ogunwande et al., 2011; Zawiślak, 2013).

Fusarium graminearum Schwabe belongs to a group of important soil- and seed-borne pathogens infecting numerous crop plants and occurring worldwide

* Corresponding author. E-mail: maja.ignjatov@ifvcns.ns.ac.rs

in various climatic zones producing diverse families of secondary metabolites such as zearalenone, deoxynivalenol, and trichothecene (Leslie and Summerell, 2006; Nesic et al., 2014).

During routine quality control of hyssop seeds in 2018, fungal infection followed by seed rot was noticed and confirmed by microscopic examination. Multilocus sequence typing scheme analysis has been applied to members of the *Fusarium* genus and DNA sequence-based identification of some unknown isolates can be achieved using the translation-elongation factor *1-α* TEF gene region. Thus the TEF gene has become the marker of choice as a single-locus identification tool in *Fusarium* (Geiser et al., 2004).

The aim of this research was the identification of the causal agent of hyssop seed rot, based on morphological properties of isolates and molecular features using partial sequences of the translation elongation factor gene (*1-α* TEF).

MATERIAL AND METHODS

Pathogen isolation

Symptoms of infected hyssop seeds appear to be soft, covered with white, light pink, or reddish fungal mycelium, followed with violet pigmentation under the seeds (Figure 1a). In order to perform isolation of the pathogen, infected hyssop seeds were transferred onto a Potato Dextrose Agar (PDA) medium with streptomycin sulfate amended (300 mg/l) (w/v). Plates were incubated for seven days at 25 °C under ultraviolet light ("black light") with a 12^h photoperiod (Mathur and Kongsdall, 2003).

Pathogenicity test

Pathogenicity test was performed *in vitro* using a modified agar slant method in the test tube with PDA amended (Porter et al., 2015). At the bottom of the tube, there was placed a piece of mycelium of each isolate grown on PDA and dried hyssop seed was carefully placed 2 cm above. As a positive control, there was used an identified isolate of *Fusarium graminearum* from the collection of the Institute of Field and Vegetable Crops (Laboratory for Seed Testing). Hyssop seed placed on agar without mycelia was used as a negative control. For each isolate, set of five tubes in four repetitions were inoculated. All test tubes were placed in aseptic sealed plastic boxes and incubated for two weeks at 25 °C. All isolates were re-isolated and sub-cultured on Potato Dextrose Agar (PDA) and Carnation Leaf Agar (CLA) using a hyphal tip transfer technique (Leslie and Summerell, 2006), fulfilling Koch's postulates. Identification of *Fusarium* species and morphological characterization was performed according to Gerlach and Nirenberg (1982) and Leslie and Summerell (2006).

DNA extraction and molecular species identification

Fusarium isolates were grown on PDA plates for 7 days, and mycelia were harvested and ground in liquid nitrogen. DNA was extracted using a DNeasy Mini Kit (QIAGEN Inc., Hilden, Germany), according to the manufacturer's instructions. The translation elongation factor *1-α* TEF gene region was amplified by PCR with the primers EF1 (ATGGGTAAGGAGGACAAGAC) and EF2 (GGAAGTACCAGTGATCATGTT) (O'Donnell et al., 1998; Geiser et al., 2004). The polymerase chain reaction (PCR) was done in S-thermal cycler (Eppendorf, Germany) with a total volume of 25 µl consisting of 12.5 µl of 2x Eppendorf Master Mix (Fermentas, Lithuania); 1.25 µM of each primer (100 pmol/µl), 1µl of fungal DNA, and 9 µl of RNase-free water. The reaction was performed in a thermal cycler (Eppendorf, Germany) under the following programs: initial denaturation of 2 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 53 °C, and 2 min at 72 °C, with a final extension of 10 min at 72 °C. PCR products were separated using electrophoresis on 1.5% agarose gel containing ethidium bromide (0.5 g/mL) and visualized using a UV light with Bio-print cx4 (VilberLourmat, Germany).

The amplified product from isolate JBL4003/3 was purified with a QIAquick PCR Purification Kit (Qiagen) and sequenced in both directions with an automated sequencer (Macrogen, Korea). The obtained sequence was compared with the previously reported isolates available in the GenBank (http://www.ncbi.nlm.nih.gov/BLAST/), using the ClustalW program (Thompson et al., 1994) and MEGA6 software (Tamura et al., 2013).

The phylogenetic tree was generated using Maximum likelihood implemented in MEGA 6 software (Tamura et al., 2013). The edited *1-α* TEF sequence was compared with other available *Fusarium* species sequences in the GenBank. The reliability of the obtained tree was evaluated using the bootstrap analysis based on 1000 replicates, and bootstrap values <50% were omitted. Kimura 2-parameter model was chosen as the best-fitting model of nucleotide substitution.

RESULTS AND DISCUSSION

Twelve isolates distinguished based on colonies appearances and morphological characteristics were chosen for further investigation (JBL4003/1-4003/12). The presence of *Fusarium* spp. and *Alternaria* spp. (JBL4003/5) was confirmed with microscopic observation. Pigmentation of the most *Fusarium* spp. isolates was variably changing from whitish to pinkish, dark purple to vinaceous with a dash of brown, with macro or microconidia formed which indicated presence of *Fusarium* spp. Isolate JBL4003/3 was distinguished based on cultural and morphological characteristics, and according to the description given by Gerlach and Nirenberg (1982), it belongs to *F. graminearum*. Regarding the daily mycelial growth rate, isolate JBL4003/3 was fast growing causing the

hyssop seed rot after the fifth day. No fungi recovered from the negative control. All isolates were re-isolated from symptomatic tissue thus fulfilling Koch's postulates, and stored by freezing at -70 °C. A previous study showed that hyssop seed is the host of different Fusarium species (Ignjatov et al., 2020). Since 2018, an increase in cases of fusariosis has been observed in Serbia, present on various seeds of many different species (hyssop, garlic, onion, "evening stock" etc.) (Ignjatov et al., 2017, 2020). *Fusarium graminearum* is a major pathogen worldwide and the main causal agent of fusarium head blight, a disease complex of wheat and other small grains. In addition to causing considerable crop yield decrease, it is of particular concern because of the ability of Fusarium species to produce mycotoxins in the grain that are harmful to human and animal consumers (Petru et al., 2016).

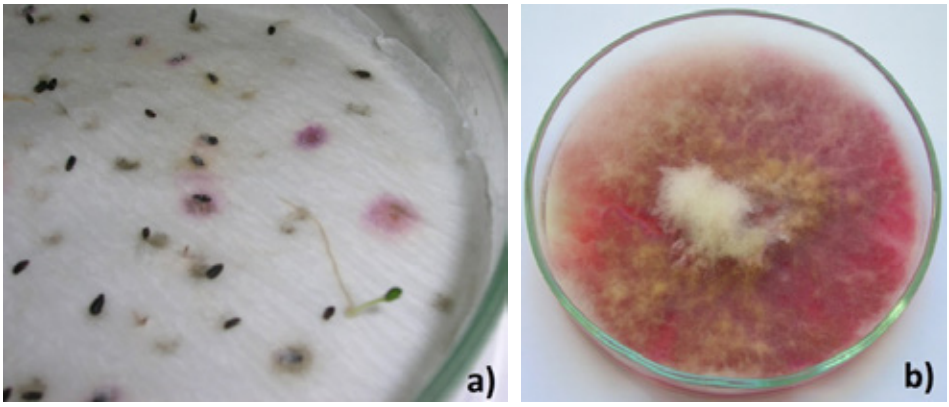


Figure 1. Fusarium graminearum Schwabe on hyssop seed (*Hyssopus officinalis* L.): a) Symptoms of the primary infection of *Hyssopus officinalis* (*Fusarium* spp., *Alternaria* spp.; b) Colony of *F. graminearum* grown on PDA – isolate JBL4003/3

Polymerase chain reaction (PCR) with primers designated as EF1 and EF2 was created as choice of a single locus identification tool in *Fusarium* genus (Geiser et al., 2004). The amplified and purified DNA fragment of representative JBL4003/3 isolate was sequenced in both directions and deposited in the GeneBank under Accession Number MK061542.1. Genetic analysis of the translation elongation factor *1-α* TEF sequence confirmed that Serbian isolate originating from hyssop belongs to *F. graminearum* Schwabe species showing 100% homology with strains from GenBank (Acc. Nos. KM052642 and JX118875). A Maximum likelihood tree (Figure 2), reconstructed based on the *1-α* TEF sequences of different *Fusarium* species selected from GenBank show that JBL4003/3 isolate used in this study was grouped with the isolates previously characterized as *F. graminearum* Schwabe (Acc. Nos. JF270173, JF278592 and HQ702569). Certain groups are well covered in *FUSARIUM*-ID (NCBI) database, particularly *Gibberella fujikuroi* species complex. *Fusarium graminearum* is a common pathogen infecting multiple crops from various climatic zones.

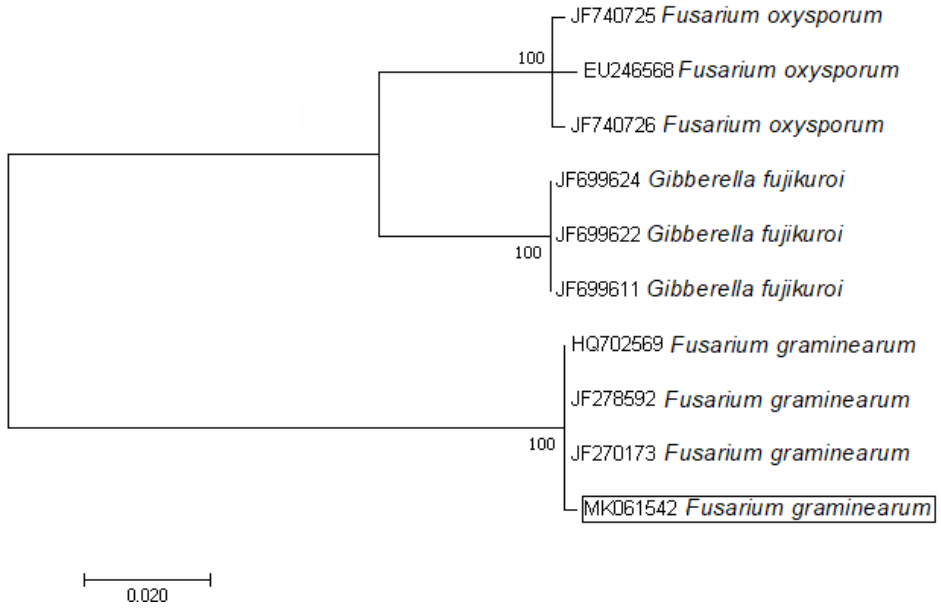


Figure 2. Phylogenetic tree: Phylogram was generated with MEGA6 using bootstrap analysis with 1000 replicates while the bootstrap values (>50%) are shown next to relevant branches. *F. graminearum* Schwabe species showing 100% homology with *F. graminearum* strains from GenBank JF270173, JF278592 and HQ702569

Molecular detection based on the TEF gene of *Fusarium* species could be a powerful tool in identification of the pathogenic species, giving results in a shorter period of time compared to the morphological identification (Pavlović et al., 2016). Generally, a management of rot disease, caused by *Fusarium* species, is usually based on the crop rotations to reduce the inoculum levels in soil (Davis et al., 2006) as well as using healthy seeds or genetically resistant cultivars.

CONCLUSION

Based on morphological and pathogenic properties, as well as sequence analysis, to our knowledge, this is the first case of *F. graminearum* as the causal agent of *Hyssopus officinalis* L. seed rot in Serbia.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-9/2021-14/200032.

REFERENCES

Davis RM, Colyer PD, Rothrock CS, Kochman JK (2006): Fusarium wilt of cotton: population diversity and implications for management. *Plant Dis.* 90: 692–703.

Fathiazad F, Hamedeyazdan S (2011): A review on *Hyssopus officinalis* L.: Composition and biological activities. *Afr. J. Pharm. Pharmaco.* 5: 1959–1966.

Geiser DM, Del Mar Jiménez-Gasco M, Kang S, Makalowska I, Narayanan, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O'Donnell K (2004): FUSARIUM-ID v. 1.0: A DNA Sequence Database for Identifying Fusarium. *Eur. J. Plant Pathol.* 110: 473–479.

Gerlach W, Nirenberg H (1982): Mitteilungen aus der Biologischen Bundesanstalt Für Land- und Forstwirtschaft (Berlin – Dahlem), *The genus Fusarium – A pictorial atlas,* 309: 1–405.

Ignjatov M, Milošević D, Nikolić Z, Gvozdanović-Varga J, Tatić M, Popović T, Ivanović Ž (2017): First Report of *Fusarium tricinctum* causing rot of stored garlic bulbs in Serbia. *Plant Dis.* 101: 382.

Ignjatov M, Milošević D, Nikolić Z, Tamindžić G, Stojanović M, Popović V, Ivanović Ž (2020): First report of *Fusarium proliferatum* as the causal agent of seed rot of *Hyssopus officinalis* in Serbia. *Plant Dis.* 104: 1863.

Leslie JF, Summerell BA (2006): The *Fusarium* laboratory manual, Oxford, UK: Wiley-Blackwell Publishing, 388–390.

Lević J, Stanković S, Krnjaja V, Bočarov-Stančić A (2009): *Fusarium* species: The occurrence and the importance in agricultural of Serbia. *Matica Srpska Proc. Nat. Sci.* 116: 33–48.

Mathur SB, Kongsdal O (2003): *Common laboratory seed health testing methods for detecting fungi*. ISTA, Switzerland.

Nesic K, Ivanovic S, Nesic V (2014). Fusarial toxins: secondary metabolites of Fusarium fungi. *Rev. Environ. Contam. Toxicol.* 228: 101–120.

O'Donnell K, Cigelnik E, Nirenberg HI (1998): Molecular systematic and phytogeography of the *Gibberella fujikuroi* species complex. *Mycologia* 90: 465–493.

Ogunwande IA, Flamini G, Alese OO, Cioni PL, Ogundajo AL, Setzer WN (2011): A new chemical form of essential oil of *Hyssopus officinalis* L. (*Lamiaceae*) from Nigeria. *Int. J. Biol. Chem. Sci.* 5: 46–55.

Pavlović S, Ristić D, Vučurović, I, Stevanović M, Stojanović S, Kuzmanović S, Starović M (2016): Morphology, pathogenicity and molecular identification of *Fusarium spp.* associated with anise seeds in Serbia. *Not. Bot. Horti. Agrobot.* 44: 411– 417.

Bozac P, Popescu S, Botau D, Boldura OM, Pirvulescu P (2016): Molecular Characterization for some new *Fusarium* Isolates Collected from the West Part of Romania. *Rom. Biotechnol. Lett.* 21: 11560–11568.

Porter LD, Pasche JS, Chen W, Harveson RM (2015): Isolation, identification, storage, pathogenicity tests, hosts, and geographic range of *Fusarium solani* f. sp. *pisi* causing *Fusarium* root rot of pea. *Plant Health Prog.* [DOI: 10.1094/PHP-DG-15-0013]

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013): MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30: 2725–2729.

Thompson JD, Higgins DG, Gibson TJ (1994): CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position–specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673–4680.

Zawiślak G (2013): Morphological characters of *Hyssopus officinalis* L. and chemical composition of its essential oil. *Mod. Phytomorphol.* 4: 93–95.

МОРФОЛОШКА И МОЛЕКУЛАРНА КАРАКТЕРИЗАЦИЈА *Fusarium graminearum* Schwabe КАО ПРОУЗРОКОВАЧА ТРУЛЕЖИ СЕМЕНА *Hyssopus officinalis* L.

Маја В. ИГЊАТОВ[1], Драгана Н. МИЛОШЕВИЋ[1], Гордана Д. ТАМИНЏИЋ[1], Жарко С. ИВАНОВИЋ[2]

[1] Институт за ратарство и повртарство,
Институт од националног значаја за Републику Србију
Максима Горког 30, Нови Сад 21000, Србија
[2] Институт за заштиту биља и животну средину,
Теодора Драјзера 9, Београд 11000, Србија

РЕЗИМЕ: Симптоми трулежи семена *Hyssopus officinalis* L. примећени су током испитивања здравственог стања семена 2018. године. Према морфолошким и одгајивачким карактеристикама изолати припадају врстама *Fusarium* spp. и *Alternatia* spp.. Идентификација *Fusarium* spp. потврђена је применом ланчане реакције полимеразе са паром прајмера EF1 и EF2, при чему је амплификација и секвенционирање гена TEF-1α извршена за изолат JBL4003/3 (MK061542.1), чиме је потврђено да је изоп нови домаћин врсте *Fusarium graminearum* Schwabe.

КЉУЧНЕ РЕЧИ: изоп, *Fusarium graminearum*, трулеж семена

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 29—44, 2021

UDC 634.322:632.25(497.11)
https://doi.org/10.2298/ZMSPN2140029S
ORIGINAL ARTICLE

Stefan S. STOŠIĆ[*], *Danijela T. RISTIĆ*, *Svetlana T. ŽIVKOVIĆ*

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

POSTHARVEST DECAY OF MANDARIN FRUIT IN SERBIA CAUSED BY *Penicillium expansum*

ABSTRACT: Mandarin fruits are one of the most popular among the *Citrus* genus. They are consumed because of their nutritional and health benefits, as well as pleasant taste and smell. This paper describes the identification and characterization of *Penicillium expansum*, isolated from molded mandarin fruits. The obtained isolates were cultivated on five media [Czapek Yeast Autolysate agar (CYA), MEA (Malt extract agar), Creatine sucrose agar (CREA), Yeast extract sucrose agar (YES), and Oatmeal agar (OA)] and at five different incubation temperatures (5, 15, 25, 30, and 37 °C). Isolates were sequenced for two molecular loci: internal transcribed spacer and beta-tubulin. Based on the results from morphological, physiological, molecular, and phylogenetic analyses, the recovered isolates were identified as *P. expansum*. The isolated species was confirmed as pathogenic to mandarin fruits in a pathogenicity test. To the best of our knowledge, this is the first report of *P. expansum* as a postharvest pathogen of mandarin fruit in Serbia.

KEYWORDS: *Citrus reticulata*, identification, morphological analysis, molecular characterization, pathogenicity

INTRODUCTION

Citrus fruits (oranges, lemons, limes, grapefruit, tangerines, and their varieties) are among the most important crops worldwide. They belong to the Rutaceae plant family and they are cultivated in more than 140 countries across the globe (Liu et al., 2012; Wu et al., 2018). Their nutritional values make them an important part of the healthy human diet.

One of the most popular citrus fruits is mandarin (*Citrus reticulata* Blanco). It encompasses many varieties, hybrids, and mutants that have distinct names, but several terms are used in colloquial speech (mandarins, clementines, tangerines, etc.). Together with citron (*C. medica*) and pummelo (*C. maxima*), mandarins are considered ancestral *Citrus* species (Wu et al., 2018). The tasty

[*] Corresponding author. E-mail: stefan.stosic@izbis.bg.ac.rs

flavour, alimentary importance, seedlessness in some sorts, the ease of peeling, and the small amount of the residual oils on hands during peeling are some of the reasons for being often the first choice among the consumers of citrus fruits (El-Otmani et al., 2011; Wang et al., 2018). Mandarin fruits are consumed in fresh form, used for mandarin juice production, or applied in the manufacturing of jellies, essential oils, confectionery, and sweets. Mandarin juice is also used for adulteration of other citrus juices or for making fermented beverages (Putnik et al., 2017). Nutritional benefits of mandarin fruit consumption include vitamins C, E, and A, carotenoids (phytoene, β-cryptoxanthin, and violaxanthin), phenolic compounds, sugars, organic acids, potassium, phosphorus, magnesium, amino acids (asparagine, arginine, aspartic acid, proline, and glutamine). These compounds have various antioxidant, anti-inflammatory, anticarcinogenic, and cardioprotective effects on human health (Codoñer-Franch and Valls-Bellés, 2010; Putnik et al., 2017).

Production of mandarin fruit and its varieties in 2018 was 34,393,430 tons with a steadily increase in the last three decades (FAOSTAT, 2020). Mandarins and belonging hybrids are ranked the second of all the citrus fruits produced in the world (Palou et al., 2015). In Serbia, the annual import of mandarin/clementine/tangerine fruits in 2019 was 28,568.4 tons. Mandarin with its varieties was the second of all the citrus fruits, and the fourth of all the fruits imported in Serbia in 2019 (*Statistical Office of the Republic of Serbia*, 2020).

Many fungi can attack citrus fruits (including mandarins) and cause diseases, in the field or after harvest. Pathogens that infect the plants in the orchard often can remain latent and the disease can develop later in the packinghouse. Some of the most important postharvest fungal pathogens from this group are *Lasiodiplodia theobromae*, *Phomopsis citri*, *Alternaria citri*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, and *Phytophthora citrophthora*. Pathogenic fungi from the second group infect the mandarin fruit through injuries or micro-wounds. These are known as wound pathogens and some of the characteristic species that cause postharvest diseases are *Penicillium digitatum*, *P. italicum*, and *Geotrichum candidum* (Barkai-Golan, 2001; Palou et al., 2015). Two listed *Penicillium* species are considered maybe the most important postharvest diseases of citrus fruits, contributing up to 90% of the total loss (Yang et al., 2019). Besides mentioned, other species of *Penicillium* spp. are reported as pathogens of mandarin fruit and its varieties/hybrids: *P. crustosum* (Garcha and Singh, 1976), *P. ulaiense* (Tashiro et al., 2012; Park et al., 2018), and *P. expansum* (Moosa et al., 2019). *P. expansum* was also isolated from mandarin fruit (*Citrus unshiu* (Swingle) Marcov.) in a study by Liu et al. (2009), but the pathogenicity test was not performed.

To the best of our knowledge, the only reported postharvest pathogen of mandarin fruit in Serbia is *C. gloeosporioides* (Živković et al., 2012). Considering that there are no literature data about postharvest diseases caused by *Penicillium* species on stored mandarin fruit in Serbia, the aim of this study was to elucidate the etiology of the *Penicillium*-like decay, to identify the causal agent(s) using the polyphasic approach – combining morphological, physiological, and molecular methods – and to test the pathogenicity of the obtained isolates.

MATERIALS AND METHODS

Sample collection and fungal isolation

Mandarin fruits with blue mold symptoms were collected from the markets in Serbia during 2017–2019. Symptoms included pale, watery spots or the presence of the blue sporulation on the fruit surface. Forty-eight samples have been collected in total and the isolations were carried out immediately upon bringing them to the laboratory. Small pieces on the margin of the diseased and healthy tissue were removed with a sterilized scalpel and surface-sterilized for 3 min in 1% aqueous home bleach solution. After that, pieces were rinsed three times in sterile distilled water, placed and incubated on malt extract agar (MEA) for 7 days at 25 °C. Mycelial fragments were taken from the growing colony margin and transferred to new MEA plates. Ten monosporial isolates were produced and preserved on potato dextrose agar (PDA) slants in the refrigerator at 4 °C.

Morphological and physiological characterization

The morphology of these ten isolates was examined on five media: Czapek Yeast Autolysate agar (CYA), MEA, Creatine sucrose agar (CREA), Yeast extract sucrose agar (YES), and Oatmeal agar (OA). Cultures were three-point inoculated with 1 µl of conidial suspension and incubated for 7 days at 25 °C (Visagie et al., 2014). After the incubation period, colony growth and texture, degree of sporulation, the colour of spores, the presence and colours of soluble pigments and exudates, colony reverse colours, and degree of growth and acid production (on CREA) were recorded.

The isolates were also inoculated on CYA plates and incubated for 7 days in the dark at five different temperatures (5, 15, 25, 30, and 37 °C) to examine the growth and the appearance of the cultures and to detect limiting temperatures for fungal development.

Microscopic observation

Microscopic slides were prepared from 10-day old MEA cultures with 60% lactic acid used as a mounting fluid (Visagie et al., 2014). Conidiophores' branching patterns, shape and texture of phialides, the shape of conidia and ornamentation of the conidial cell wall were recorded for all tested isolates. Observations were performed using an Olympus microscope (model BX51, Olympus Corporation, Japan) and slides were photographed with Olympus camera (model E620, Olympus Corporation, Japan) attached to the microscope. Measurements of conidia (two diameters – length and width) were done in the Quick Photo Camera software program (PROMICRA, s.r.o., Czech Republic).

DNA extraction and PCR

Genomic DNA was extracted from cultures grown on MEA for 7 days at 25 °C. A sterile blade was used to scrape roughly 100 mg of mycelium from the plate surface and to transfer it to a 2 ml centrifuge microtube. Harvested mycelium was frozen with liquid nitrogen and ground using a sterile micropestle. Fungal DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The obtained DNA was preserved at -20 °C until further use.

To confirm morphological identification, partial sequences of the internal transcribed spacer (ITS) region of the rDNA and β-tubulin gene (*BenA*) were amplified in a polymerase chain reaction (PCR), using the fungal specific primers V9G/LS266 and Bt2a/Bt2b, respectively (Glass and Donaldson, 1995; Masclaux et al., 1995; de Hoog and van den Ende, 1998) and following the instructions from Visagie et al. (2014). Amplification conditions were as follows: an initial denaturing step of 5 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 55 °C, 1 min at 72 °C, and a final denaturing step of 7 min at 72 °C. The compounds and volumes for PCR reactions were prepared as described in our previous work (Stošić et al., 2020).

Sequencing and phylogenetic analyses

The same primers used for PCR amplifications were also used for the sequencing of the obtained PCR products. Purification and sequencing in both directions of the recovered PCR amplicons were done by Macrogen's Europe commercial sequencing service (Macrogen Europe B.V., Amsterdam, the Netherlands). The sequences' quality was examined using FinchTV software (version 1.4.0, www.geospiza.com/finchtv) and the ClustalW algorithm (Thompson et al., 1994) integrated into MEGA7 software (Kumar et al., 2016) used to compute consensus sequences. Nucleotide BLAST search algorithm was applied to compare the similarity of the sequences from this study with the fungal sequences from the NCBI GenBank database. After the similarity search and definitive identification, all generated sequences were deposited in the NCBI GenBank database (Table 1, accession numbers MT556009 and MT556010 (ITS), MT563326, and MT563327 (*BenA*)).

The phylogeny was assessed using MEGA software (version 7.0.26, Kumar et al., 2016). The maximum likelihood (ML) tree was constructed for a combined dataset of ITS and *BenA* sequences (Table 1). The gamma-distributed Tamura-Nei model (G+I) with invariant sites was employed as the best fitting model of nucleotide substitution (5 discrete gamma categories, complete deletion of gaps/missing data treatment, nearest neighbour interchange as ML heuristic method). The reliability of the obtained trees was evaluated with 1,000 bootstrap replications for branch stability. Bootstrap confidence values less than 70% were omitted and the sequences of *Aspergillus niger* (isolate NRRL

326) were designated as an outgroup in all analyses. Phylogenetic trees were edited and prepared for publication in Adobe Illustrator CS6 (Adobe, U.S.A.).

Table 1. Accession numbers of *Penicillium* spp. isolates with collection details used in the phylogenetic analysis; isolates from this study are bolded.

Species	Strain/isolate	Substrate and origin	GenBank accessions	
			ITS	*BenA*
A. niger	NRRL 326 = CBS 554.65	Tannin-gallic acid fermentation, Connecticut, U.S.A.	EF661186	EF661089
P. allii	IBT 3056=CBS 188.88	Food item, U.K.	AJ005484	AY674333
P. crustosum	FRR 1669 = CBS 115503 = IMI 091917	Lemon fruit, Aberdeen, Scotland, UK	AY373907	AY674353
P. crustosum	SFC20140101-M781 = 5501	Unknown	KJ527442	KJ527407
P. crustosum	CV1267 = DTO182I3	*Protea repens* infructescence, Riverlands (Malmesbury), South Africa	JX091401	JX091537
P. crustosum	CV1529 = DTO183C4	*Protea repens* infructescence, Riverlands (Malmesbury), South Africa	JX091402	JX091538
P. crustosum	CV0241 = DTO181D2	*Protea repens* infructescence, Stellenbosch, South Africa	JX091403	JX091536
P. crustosum	CV0251 = DTO181D6	Mite from *Protea repens* infructescence, Stellenbosch, South Africa	JX091404	JX091530
P. crustosum	CNU 6043	Apple fruit, Yesan, Korea	HQ225711	HQ225724
P. digitatum	CBS 112082	Lemon, Italy	KJ834506	KJ834447
P. expansum	CBS 325.48 = ATCC 7861	Apple fruit, U.S.A.	AY373912	AY674400
P. expansum	SFC20140101-M737 = 5537	Unknown	KJ527444	KJ527409
P. expansum	F758	Sugar beet root, Idaho, U.S.A.	MG714838	MG714864
P. expansum	CV2860 = DTO180F6 = CV 407	Soil, South Africa	FJ230989	JX091539
P. expansum	CV2861 = DTO180F7 = CV 432	Soil, South Africa	FJ230990	JX091540
P. expansum	CNU 7003	Apple fruit, Daejoen, Korea	HQ225715	HQ225727
P. expansum	**MP/4**	**Mandarin fruit, Serbia**	**MT556009**	**MT563326**
P. expansum	**MP/5**	**Mandarin fruit, Serbia**	**MT556010**	**MT563327**
P. italicum	CBS 339.48	Citrus fruit, Riverside, CA, U.S.A.	KJ834509	AY674398
P. italicum	SFC20140101-M724 = 5340	Unknown	KJ527447	KJ527412
P. italicum	CNU 6089	Apple fruit, Yesan, Korea	HQ225716	HQ225728
P. polonicum	CBS 222.28 = NRRL 995	Soil, Poland	AF033475	AY674305
P. polonicum	F775	Sugar beet root, Idaho, U.S.A.	MG714841	MG714868
P. solitum	CBS 424.89 = FRR 937	Unknown, Germany	AY373932	AY674354
P. solitum	CNU 4096	Apple fruit, Daegu, Korea	HQ213935	HQ225721
P. viridicatum	CBS 390.48 = DTO 005-C9 = FRR 963	Air, Washington DC, U.S.A.	AY373939	AY674295

Pathogenicity assay

A pathogenicity test was conducted on intact, symptomless mandarin fruits for all obtained isolates. Mandarins were surface-sterilized with 70% ethyl alcohol, allowed to air dry, and then wounded with a sterile needle. Fifty microliters of spore suspension were injected into the wound using an automated micropipette and the corresponding micropipette tip. The conidial suspension was prepared by diluting the spores collected from the 14-day-old MEA cultures in 1 ml of sterile distilled water. Spore concentration was determined and adjusted to a concentration of 1×10^6 spores/ml using a Neubauer hemocytometer. Fruits inoculated with 50 µl of sterile distilled water represented negative control. Three replicates per isolate were used. All tested fruits were placed in plastic boxes and incubated at 25 °C and 95% relative air humidity. Seven days after the inoculation, the developed symptoms were observed and the horizontal and vertical (stem-calyx axis) diameters of the lesions were measured. Reisolations from the inoculated fruits were performed on MEA using the same method as previously described. The symptoms expressed on the artificially wounded fruits, colony appearance and growth, and conidiophore and spore morphology were inspected to check the fulfillment of Koch's postulates.

Data analysis

All experiments were performed in three replicates per isolate. Basic descriptive values (average and standard deviation) were computed for each colony, spore, and lesion diameters. Calculations were done in Microsoft Excel 2007 (Microsoft Corporation, U.S.A.).

RESULTS AND DISCUSSION

Blue mold symptoms were observed on collected mandarin fruits. They included discolored or sometimes brown tissue on the fruit surface, concave, circular, and watery spots. Decay was present in different stages – from small lesions with no evident sporulation to some samples with white mycelia and blue-green sporulation. Ten isolates were recovered from symptomatic fruits (MP/1-MP/10), and two isolates (MP/4 and MP/5) were chosen for sequencing and phylogenetic analysis.

After 7 days of incubation at 25 °C, all isolates had radially sulcate colonies on CYA and plane to moderately radially sulcate on MEA. Sulcation was also present on YES, varying from slight radial to the radial and concentric combined. Cultures manifested different textures on tested media – velvety to loosely floccose on CYA and YES, fasciculate on CREA, plane and weak

fasciculate on MEA and OAT. Reverse, colonies had light brown colour with a brighter margin (CYA), pale yellow with a hint of greenish (MEA), greyish yellow (OAT), purple with a yellow margin (CREA), and cream-yellow with brown center (YES). Strong acid production (change of the medium colour from purple to yellow) followed by base production was noticed on CREA. Intensive conidiogenesis was present on all five media, with blue-green spores on CYA, MEA, and YES, green on CREA, and dull green on OAT. Isolates had white mycelia on all inoculated media, present as the margin of the culture, broad on CYA (6–7 mm width), moderate on MEA, YES, and OAT (2–3 mm) and narrow on CREA (up to 1.5 mm). Clear exudate droplets were noticed on CREA, YES, and OAT, while there was no exudate formation on CYA and MEA.

All tested isolates manifested intensive growth across all five tested media, with YES being the most stimulative medium (58.63 mm). The mycelial development was the least intensive on CREA (37 mm), and the average growth diameters on the remaining three media (CYA, OAT, and MEA) were 41.88, 40.38, and 40.25 mm, respectively (Figure 1). The growth diameters and culture appearance agree with the data from the relevant literature sources (Pitt, 1979; Frisvad and Samson, 2004; Pitt and Hocking, 2009; Visagie, 2012; Vico et al., 2014).

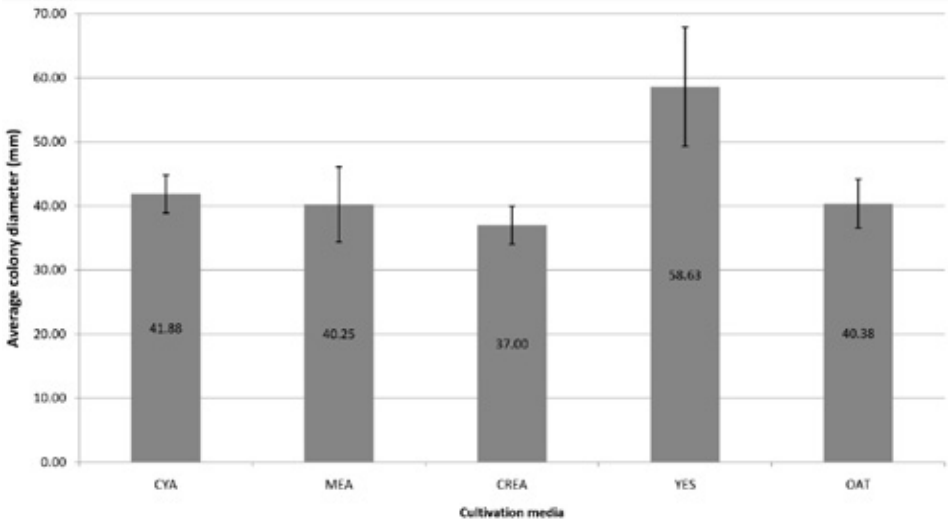


Figure 1. The average colony growth of *P. expansum* isolates on five different media after 7 days of incubation at 25°C. Vertical error bars indicate the standard deviation (SD).

Isolates' cultivation at different temperatures on CYA showed that the optimal (and in the same time maximal) temperature for their development was 25 °C (41.88 mm, Figure 2). Of all tested temperatures, the smallest average

diameter was measured at 5 °C (18.46 mm). Fungal growth was not recorded at 37 °C (Figure 2).

The most restrictive temperature for fungal development was 37 °C and the most optimal was 25°C which is in agreement with previous data (Pitt, 1979; Frisvad and Samson, 2004; Pitt and Hocking, 2009; Visagie, 2012; Vico et al., 2014). Growth was still possible at the lowest tested temperature (5 °C), but our values were closer to those reported by Vico et al. (2014) (18.46 and 14.3–17.6 mm, respectively) than to those noted in Frisvad and Samson (2004), Pitt and Hocking (2009), and Visagie (2012) (up to 5 mm, collectively). The average growth diameter at 30 °C was in concordance with the data in the work by Visagie (2012).

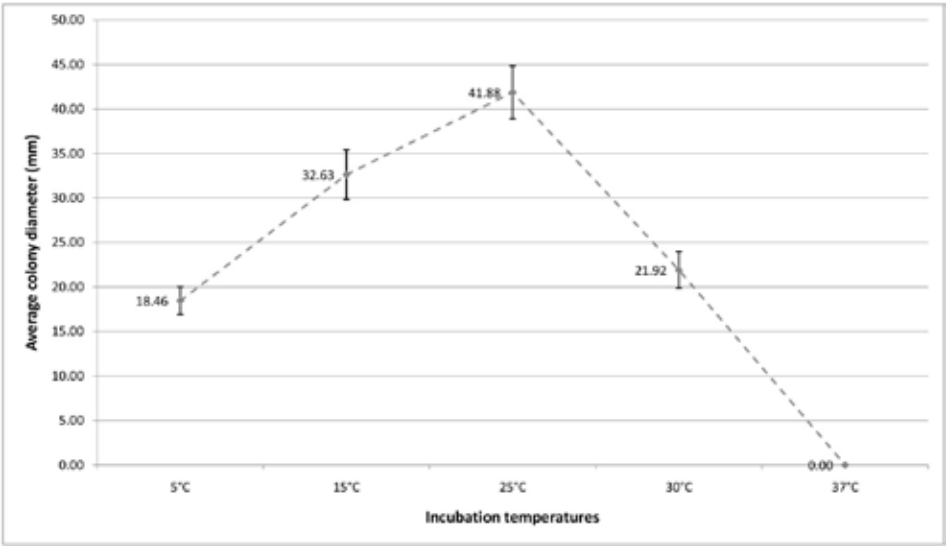


Figure 2. The average colony growth of *P. expansum* isolates on CYA at five different incubation temperatures after 7 days. Vertical error bars indicate the standard deviation (SD).

Microscopic observations revealed that all analyzed isolates formed hyaline, mostly terverticillate conidiophores, with usually smooth stipes and cylindrical phialides. Phialides had a short but distinctive neck. Conidia were subglobose or ellipsoidal, green and with smooth walls, 2.5–3.8–5.25 µm × 2.5–3.52–5.00 µm (minimum-average-maximum values). Ascomata were not formed, as expected (Samson et al., 2010). The listed micromorphological characteristics were in agreement with previous findings (Pitt, 1979; Frisvad and Samson, 2004; Pitt and Hocking, 2009; Samson et al., 2010; Vico et al., 2014).

In the molecular analysis, ITS sequences of the selected Serbian isolates of *P. expansum* from mandarin fruit were 100% identical to each other. BLAST analysis showed 100% similarity with *P. expansum* isolates NRRL 6069,

NRRL 35231, and NRRL 2304 (GenBank Accession numbers DQ339562, DQ339558, and DQ339556, respectively). The sequences of *BenA* of our *P. expansum* isolates shared a 99.75% similarity between each other (1 base pair difference). Nucleotide identity of Serbian *P. expansum* and GenBank *BenA* sequences was in the range of 99.75–100%. The most similar *BenA* sequences from GenBank to ours were the following isolates of *P. expansum*: YC-IK11 sampled from pear (*Pyrus* × *bretschneideri*) in China (Acc. No. MK862430), isolate CMV017H9 from apple in South Africa (MN031410), and isolate DTO 216-G4 from foliar tissue of *Psedotsuga menziesii* in USA (MF990777).

Inferring phylogenetic relationships started with multiple sequence alignments of ITS and *BenA* sequences which were 452 nucleotides (nt) and 387 nt long, respectively. ML phylogenetic tree was constructed using the aligned combined dataset (ITS+*BenA*) which had a total length of 839 nt and it included 26 sequences from 9 taxa (representative isolates of 8 *Penicillium* species and *A. niger* as an outgroup). In a multilocus phylogeny, our isolates of *P. expansum* (MP/4 and MP/5) clustered together with the other *P. expansum* isolates with maximum bootstrap support (100%, Figure 3). Subclades of *P. expansum* and *P. italicum* formed a joint clade with a high confidence level (94%).

Sequences of the ITS region have been used before for resolving *Penicillium* phylogenies (Berbee et al., 1995; Skouboe et al., 1999) and although this region is accepted as the universal genetic barcode for all fungi (Schoch et al., 2012), its resolution is limited at the species level in *Penicillium* (Visagie et al., 2014). With all its disadvantages in mind, *BenA* is proposed as the secondary molecular marker, especially in routine identification procedures (Visagie et al., 2014). We applied ITS and *BenA* sequences in this research and representative isolates were identified as *P. expansum* for both sequenced loci. In a subsequent multilocus analysis, our sequences of the *P. expansum* were clustered in the same clade with other isolates of this species, thereby validating the results of morpho-physiological and molecular characterization.

In a pathogenicity test, isolates of *P. expansum* recovered in this study induced decay on healthy mandarin fruits after 7 days of incubation (Figure 4, A-D). The symptoms on wounded fruits were quite similar to those observed on naturally infected mandarin fruits. The average lesion diameter (± standard deviation, SD) ranged from 30±0 mm to 33.5±2.12 mm. Control fruits remained symptomless. Fungi reisolated from decayed fruits in the pathogenicity test expressed the same morphological characteristics as the original isolates, thus confirming Koch's postulates.

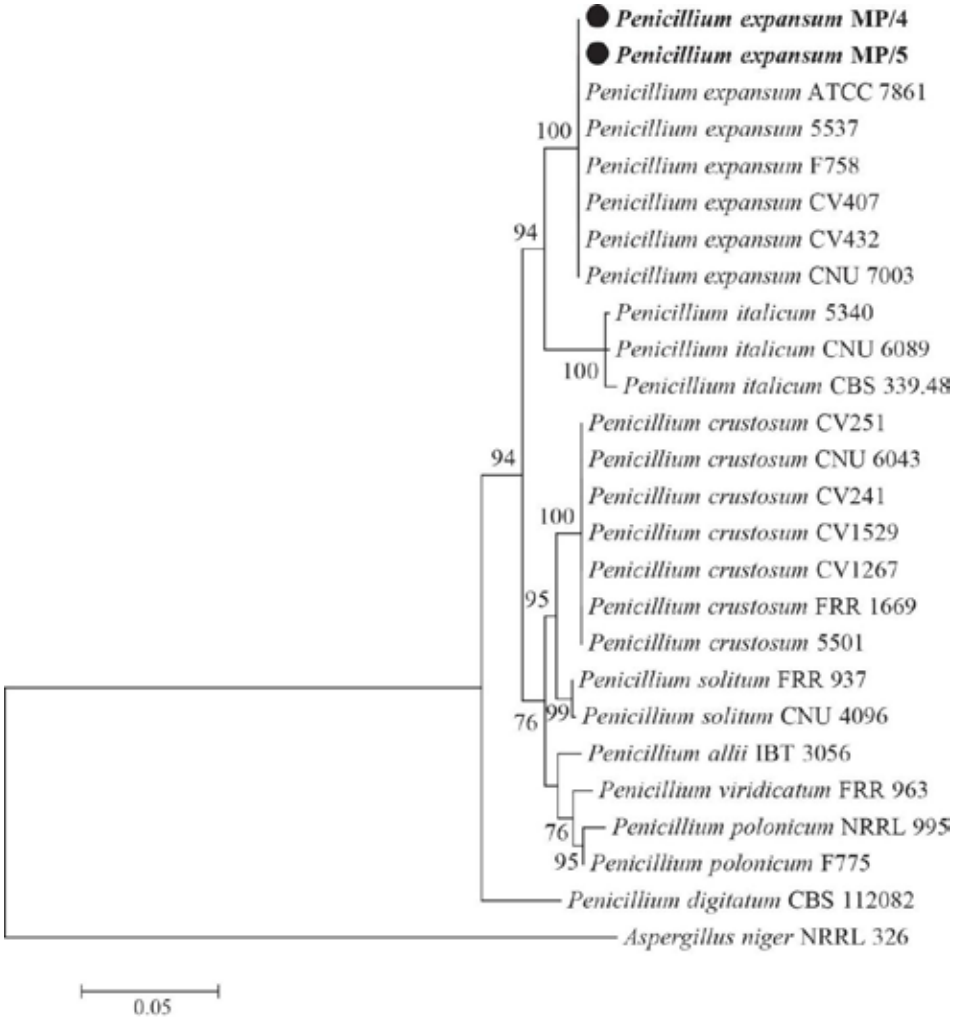


Figure 3. Phylogenetic tree based on combined ITS and *BenA* sequences using the maximum likelihood (ML) method for selected *Penicillium* species. The isolate of *A. niger* NRRL 326 was designated as an outgroup. The bootstrap analysis included 1,000 replicates and only bootstrap values >70% are displayed near the corresponding nodes. The scale represents the number of substitutions per site. Isolates in bold preceded with black dots are from this study.

Figure 4. A) Pathogenicity of *P. expansum* in mandarin fruit; B) Cross-sections of the inoculated fruits (left A and B – fruit inoculated with the pathogen suspension, right A and B – control fruit); C) Close view of the lesion. D) Close view of the cross-section of the diseased fruit.

P. digitatum and *P. italicum* are considered among the most important *Penicillium* postharvest pathogens of citrus fruits, including mandarins (Jhalegar et al., 2015; Louw and Korsten, 2015; Saito and Xiao, 2017; Yang et al., 2019; Cheng et al., 2020). Other *Penicillium* species important for the citrus industry as the decay agents are *P. ulaiense*, *P. crustosum*, *P. expansum*, and *P. dierckxii* Biourge 1923 (=*P. fellunatum*), but the data on *P. dierckxii* is not confirmed (Louw and Korsten, 2015).

In this study, *P. expansum* was isolated from molded mandarin fruits. It was identified at the morphological and molecular level and confirmed as the postharvest pathogen in stored fruits of mandarin. Findings from this research report for the first time *P. expansum* as the cause of the mandarin fruit rot in Serbia.

There are only two studies where *P. expansum* was isolated from mandarin fruit. In research by Liu et al. (2009), *P. expansum* originating from decayed mandarin fruit (cv. *Citrus unshiu* Marcov) was used to test the impact of the quorum-sensing molecule, farnesol, on inducing morphogenetic changes in this fungal pathogen. The isolate was identified using morphological and

molecular methods (sequencing of the ITS region). However, the pathogenicity of the acquired isolate was not tested since that was not the aim of the study. Last year, Moosa and co-authors (2019) isolated and confirmed *P. expansum* as the pathogen of the stored mandarin fruit (*Citrus reticulata,* cv. 'Kinnow') in Pakistan. Using morphological methods and ITS sequencing (primers ITS1/ITS4), they identified the isolated fungus as *P. expansum*.

This species was recorded as capable of producing decay in other members of citrus fruits. The pathogenic potential of *P. expansum* was detected in grapefruit, lemon, and orange fruit in the work by Macarasin et al. (2007). The lesion formation occurred, but it was artificially stimulated by the addition of citric, ascorbic, and oxalic acids and enzyme catalase before the inoculation of *P. expansum*. Vilanova et al. (2012) studied compatible (*P. digitatum*) and incompatible (*P. expansum*) orange fruit–pathogen interactions. The results of their study showed that *P. expansum* can be pathogenic in two varieties of orange fruits (*Citrus sinensis*). The rot development caused by *P. expansum* was dependent on the fruit maturity, storage temperature, and the inoculum concentration. Decay was possible in mature and over-matured fruit, higher at the lower storage temperature (4 °C) and with inoculum concentration of 10^6 and 10^7 spores/ml. In the research of Louw and Korsten (2015), *P. expansum* was able to produce decay in two mandarin cultivars (Nules Clementine and Owari Satsuma). The pathogenic isolate was not sampled from citrus fruit, but it originated from a citrus export chain, more specifically from a wall of distributer/repack facility. It is important to point out that this isolate was reintroduced into apples and isolated from the produced lesions.

P. expansum is usually regarded in the literature as the spoilage agent of pomaceous fruits (apples, pears, quinces) (Pitt, 1979; Snowdon, 1990; Frisvad and Samson, 2004; Pianzzola et al., 2004; Amiri and Bompeix, 2005; Pitt and Hocking, 2009; Louw et al., 2014). Worldwide distribution, the occurrence on a very wide variety of living plant tissues, and the ability to cause severe decay in various fruits and vegetables indicate that *P. expansum* is a broad-spectrum pathogen (Pitt, 1979; Pitt and Hocking, 2009; Neri et al., 2010; Samson et al., 2010; Vilanova et al., 2012). The results from our study and other studies with citrus hosts are congruent with the last statement.

Furthermore, beside causing decay on mandarin and other fruits, it is important to note that *P. expansum* is a consistent producer of mycotoxin patulin (Andersen et al., 2004). Consumption of patulin contaminated products can lead to immunological, neurological, and gastrointestinal health disorders (Puel et al., 2010). Therefore, the maximal allowed concentration of patulin in apples and apple products is limited to 0.4 mg/kg of body weight per day by the FAO-WHO Expert Committee (Bennett and Klich, 2003). Besides patulin, *P. expansum* is designated as the possible producer of other secondary metabolites which also can compromise human health: citrinin, chaetoglobosins, communesins, roquefortine C, and expansolides A and B (Andersen et al., 2004).

CONCLUSION

Several *Penicillium* spp. species can cause decay on mandarin fruits. In this research, isolates of *P. expansum* from mandarin fruits were morphologically identified and the results were confirmed using PCR, sequencing, and phylogenetic analysis of the two loci: ITS and *BenA*. The obtained isolates caused rot in the artificially inoculated mandarin fruits in pathogenicity assay. To the best of our knowledge, this is the first report of *P. expansum* as the decay agent of stored mandarin fruits in Serbia, and one of the few in the rest of the world. The findings of this study could provide a basis for future efficient protection measures of the stored mandarin fruits in Serbia.

ACKNOWLEDGEMENTS

This research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number 451-03-68/2020-14/200010.

REFERENCES

Amiri A, Bompeix G (2005): Diversity and population dynamics of *Penicillium* spp. on apples in pre- and postharvest environments: consequences for decay development. *Plant Pathol.* 54: 74–81.

Andersen B, Smedsgaard J, Frisvad JC (2004): *Penicillium expansum*: consistent production of patulin, chaetoglobosins, and other secondary metabolites in culture and their natural occurrence in fruit products. *J. Agric. Food Chem*. 52: 2421–2428.

Barkai-Golan R (2001): *Postharvest diseases of fruits and vegetables: Development and control*, 1st ed. Elsevier Science B. V., Amsterdam.

Bennett JW, Klich, M (2003): Mycotoxins. *Clin. Microbiol. Rev.* 16: 497–516.

Berbee ML, Yoshimura A, Sugiyama J, Taylor JW (1995): Is *Penicillium* monophyletic? An evaluation of phylogeny in the family Trichocomaceae from 18S, 5.8S and ITS ribosomal DNA sequence data. *Mycologia* 87: 210–222.

Cheng Y, Lin Y, Cao H, Li Z (2020): Citrus postharvest green mold: recent advances in fungal pathogenicity and fruit resistance. *Microorganisms* 8: 449.

Codoñer-Franch P, Valls-Bellés V (2010): Citrus as functional foods. *Curr. Top. Nutraceutical Res*. 8: 173–184.

De Hoog GS, Van den Ende, AHGG (1998): Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* 41: 183–189.

El-Otmani M, Ait-Oubahou A, Zacarías L (2011): *Citrus* spp.: orange, mandarin, tangerine, clementine, grapefruit, pomelo, lemon and lime. In: E. M. Yahia (Ed.), *Postharvest biology and technology of tropical and subtropical fruits*, vol. 2: *Açai to Citrus*. Woodhead Publishing Limited, Cambridge, UK, 437–516.

Food and Agriculture Organization of the United Nations (2020): FAOSTAT Statistical Database, 20.04.2020. Available at: http://www.fao.org/faostat/en/#home.

Frisvad JC, Samson RA (2004): Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*: A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. *Stud. Mycol*. 49: 1–173.

Garcha HS, Singh V (1976): *Penicillium crustosum*, a new pathogen of *Citrus reticulata* (mandarin) from India. *Plant Dis. Report*. 60: 252–254.

Glass NL, Donaldson GC (1995): Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol*. 61: 1323–1330.

Jhalegar MJ, Sharma RR, Singh D (2015): In vitro and in vivo activity of essential oils against major postharvest pathogens of Kinnow (*Citrus nobilis* × *C. deliciosa*) mandarin. *J. Food Sci. Technol*. 52: 2229–2237.

Kumar S, Stecher G, Tamura K (2016): MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Mol. Biol. Evol*. 33: 1870–1874.

Liu P, Deng B, Long C-A, Min X (2009): Effect of farnesol on morphogenesis in the fungal pathogen *Penicillium expansum*. *Ann. Microbiol*. 59: 33–38.

Liu Y, Heying E, Tanumihardjo SA (2012): History, global distribution, and nutritional importance of citrus fruits. *Compr. Rev. Food Sci. Food Saf*. 11: 530–545.

Louw JP, Korsten L (2014): Pathogenic *Penicillium* spp. on apple and pear. *Plant Dis*. 98: 590–598.

Louw JP, Korsten L (2015): Pathogenicity and host susceptibility of *Penicillium* spp. on citrus. *Plant Dis*. 99: 21–30.

Macarisin D, Cohen L, Eick A, Rafael G, Belausov E, Wisniewski M, Droby S (2007): *Penicillium digitatum* suppresses production of hydrogen peroxide in host tissue during infection of citrus fruit. *Phytopathology* 97: 1491–1500.

Masclaux F, Guého E, de Hoog GS, Christen R (1995): Phylogenetic relationships of human-pathogenic *Cladosporium* (*Xylohypha*) species inferred from partial LS rRNA sequences. *J. Med. Vet. Mycol*. 33: 327–338.

Moosa A, Farzand A, Sahi ST, Gleason ML, Khan SA, Zhang X (2019): First report of postharvest fruit rot of *Citrus reticulata* 'Kinnow' caused by *Penicillium expansum* in Pakistan. *Plant Dis*. 103: 155.

Neri F, Donati I, Veronesi F, Mazzoni D, Mari M (2010): Evaluation of *Penicillium expansum* isolates for aggressiveness, growth and patulin accumulation in usual and less common fruit hosts. *Int. J. Food Microbiol*. 143: 109–117.

Palou L, Valencia-Chamorro S, Pérez-Gago M (2015): Antifungal edible coatings for fresh citrus fruit: a review. *Coatings* 5: 962–986.

Park JH, Hyun J-W, Park MJ, Choi YJ (2018): First report of whisker mold as a postharvest disease caused by *Penicillium ulaiense* on citrus (*Citrus unshiu*) in Korea. *Plant Dis*. 102: 2643.

Pianzzola MJ, Moscatelli M, Vero S (2004): Characterization of *Penicillium* isolates associated with blue mold on apple in Uruguay. *Plant Dis*. 88: 23–28.

Pitt JI (1979): *The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces*. London: Academic Press.

Pitt JI, Hocking A (2009): *Fungi and food spoilage*, 3rd ed. New York: Springer-Verlag, New York Inc.

Puel O, Galtier P, Oswald IP (2010): Biosynthesis and toxicological effects of patulin. *Toxins* (Basel) 2: 613–631.

Putnik P, Barba FJ, Lorenzo JM, Gabrić D, Shpigelman A, Cravotto G, Bursać Kovačević D (2017): An integrated approach to mandarin processing: food safety and nutritional quality, consumer preference, and nutrient bioaccessibility. *Compr. Rev. Food Sci. Food Saf.* 16: 1345–1358.

Saito S, Xiao CL (2017): Prevalence of postharvest diseases of mandarin fruit in California. *Plant Heal. Prog.* 18: 204–210.

Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B (2010): *Food and indoor fungi*, 1st ed., CBS Laboratory Manual Series. Utrecht: CBS-KNAW Fungal Biodiversity Centre.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W (2012): Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. U.S.A.* 109: 6241–6246.

Skouboe P, Frisvad JC, Taylor JW, Lauritsen D, Boysen M, Rossen L (1999): Phylogenetic analysis of nucleotide sequences from the ITS region of terverticillate *Penicillium* species. *Mycol. Res.* 103: 873–881.

Snowdon AL (1990): *Color atlas of post-harvest diseases and disorders of fruits and vegetables*, Vol. 1: General introduction and fruits. Boca Raton: CRC Press.

Statistical Office of the Republic of Serbia (2020): Database of the Statistical Office of the Republic of Serbia, 30.06.2020. Available from: https://data.stat.gov.rs/?caller=SDDB.

Stošić S, Ristić D, Gašić K, Starović M, Grbić MLj, Vukojević J, Živković S (2020): *Talaromyces minioluteus*: new postharvest fungal pathogen in Serbia. *Plant Dis.* 104: 656–667.

Tashiro N, Manabe K, Ide Y (2012): First report of whisker mold, a postharvest disease on citrus caused by *Penicillium ulaiense* (in Japan). *J. Gen. Plant Pathol.* 78: 140–144.

Thompson JD, Higgins DG, Gibson TJ (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673–4680.

Vico I, Duduk N, Vasić M, Nikolić M (2014): Identification of *Penicillium expansum* causing postharvest blue mold decay of apple fruit. *Pestic. Fitomed.* 29: 257–266.

Vilanova L, Viñas I, Torres R, Usall J, Jauset AM, Teixidó N (2012): Infection capacities in the orange-pathogen relationship: compatible (*Penicillium digitatum*) and incompatible (*Penicillium expansum*) interactions. *Food Microbiol.* 29: 56–66.

Visagie CM (2012): *The polyphasic taxonomy of Penicillium and Talaromyces spp. isolated from the diverse Fynbos biome*. Doctoral dissertation. Stellenbosch University, Stellenbosch.

Visagie CM, Houbraken J, Frisvad JC, Hong S-B, Klaassen CHW, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA (2014): Identification and nomenclature of the genus *Penicillium* *Stud. Mycol.* 78: 343–371.

Wang L, He F, Huang Y, He J, Yang S, Zeng J, Deng C, Jiang X, Fang Y, Wen S, Xu R, Yu H, Yang X, Zhong G, Chen C, Yan X, Zhou C, Zhang H, Xie Z, Larkin RM, Deng X, Xu Q (2018): Genome of wild mandarin and domestication history of mandarin. *Mol. Plant* 11: 1024–1037.

Wu GA, Terol J, Ibanez V, López-García A, Pérez-Román E, Borredá C, Domingo C, Tadeo FR, Carbonell-Caballero J, Alonso R, Curk F, Du D, Ollitrault P, Roose ML, Dopazo J, Gmitter FG, Rokhsar DS, Talon M (2018): Genomics of the origin and evolution of *Citrus*. *Nature* 554: 311–316.

Yang Q, Qian X, Dhanasekaran S, Boateng NAS, Yan X., Zhu H, He F, Zhang H (2019): Study on the infection mechanism of *Penicillium digitatum* on postharvest citrus (*Citrus reticulata* Blanco) based on transcriptomics. *Microorganisms* 7: 672.

Živković S, Trkulja N, Popović T, Oro V, Ivanović Ž (2012): Morphological and molecular identification of *Colletotrichum gloeosporioides* from *Citrus reticulata*. Proceedings, International Symposium: Current Trends in Plant Protection. Institute for Plant Protection and Environment, Belgrade (Serbia), Belgrade, Serbia, 293–298.

ТРУЛЕЖ ПЛОДОВА МАНДАРИНЕ СКЛАДИШТЕНЕ У СРБИЈИ ПРОУЗРОКОВАНА ВРСТОМ *Penicillium expansum*

Стефан С. СТОШИЋ, Данијела Т. РИСТИЋ, Светлана Т. ЖИВКОВИЋ

Институт за заштиту биља и животну средину,
Теодора Драјзера 9, Београд 11000, Србија

РЕЗИМЕ: Мандарина је једна од најтраженијих воћних врста из рода *Citrus*. Конзумира се због својих нутритивних и здравствених добробити, као и због пријатног мириса и укуса. У овом раду описана је идентификација и карактеризација врсте *Penicillium expansum*, изоловане са плесњивих плодова мандарине у Србији. Прикупљени изолати су гајени на пет микробиолошких подлога [Чапекова аутолизатна подлога са додатком квасца (CYA), сладни агар (MEA), креатин сахарозна подлога (CREA), агар са квашчевим екстрактом и сахарозом (YES) и подлога од овсеног брашна (OA)], као и на пет различитих температура (5, 15, 25, 30, 37 °C). Добијени изолати су секвенцирани на два молекуларна маркера (интерни транскрибовани регион и бета-тубулин). Резултати морфолошких, физиолошких, молекуларних и филогенетичких анализа указују да добијени изолати припадају врсти *P. expansum*. Изолована врста је потврђена као патоген плодова мандарине. Према нашим сазнањима, ово је први налаз *P. expansum* као складишног патогена на плодовима мандарине у Србији.

КЉУЧНЕ РЕЧИ: идентификација, морфолошка анализа, молекуларна карактеризација, патогеност, *Citrus reticulata*

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 45—57, 2021

UDC 582.303/.305(285.3)(497.11)
https://doi.org/10.2298/ZMSPN2140045C
ORIGINAL ARTICLE

Miloš ĆIRIĆ[1], *Jelena KRIZMANIĆ*[2], *Biljana P. DOJČINOVIĆ*[1], *Bojan GAVRILOVIĆ*[3*], *Dalibor M. MARINKOVIĆ*[1]

[1] University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Njegoševa 12, Belgrade 11000, Serbia
[2] University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", Takovska 43, Belgrade 11000, Serbia
[3] Serbian Academy of Sciences and Arts, Geographical Institute "Jovan Cvijić", Department of Physical Geography, Đure Jakšića 9, Belgrade 11000, Serbia

OCCURRENCE OF *Botryococcus terribilis* Komárek & Marvan IN A SMALL SANDPIT LAKE – THE FIRST REPORT FROM SERBIA

ABSTRACT: Alkaline saline ponds and lakes are habitats with unique biodiversity. The green alga of the genus *Botryococcus* was accidentally discovered during a micro-algal survey of these habitats in Serbia. Species *B. terribilis* was found and identified for the first time in Serbia in the small sandpit lake of the Rusanda Nature Park. The first sampling was incomplete due to the unknown origin of the orange-red surface scum. After the identity of the species had been confirmed, three additional samplings were conducted aiming to collect phytoplankton and water samples for physical and chemical analyses. Major anions and cations were analysed in order to characterize the chemical type of sandpit lake, as well as nutrient content. Sandpit lake is an alkaline water body that belongs to sodium bicarbonate chemical type. Morphometric analysis of *B. terribilis* was performed using light and transmission electron microscopy. *B. terribilis* was found to be numerous in the phytoplankton community in late autumn and winter. Species from the genus *Botryococcus* are known as a rich source of different lipids and this discovery can be the first step in the further biotechnological application of this species in sustainable biofuel production.

KEYWORDS: Peskara, *Botryococcus terribilis*, phytoplankton

* Corresponding author. E-mail: b.gavrilovic@gi.sanu.ac.rs

INTRODUCTION

Algae from the genus *Botryococcus* (Trebouxiophyceae, Chlorophyta) are an interesting group of organisms in terms of their ecology, physiology and their application in biotechnology. Beside cyanobacteria, there are not so many photosynthetic microorganisms which can dominate or bloom in hypertrophic lakes and *Botryococcus* species are one of them (Jeppesen et al., 2007). Moreover, cells of their colonies are surrounded by mucilage containing numerous oil globules (John et al., 2011). Due to the high content of different hydrocarbons some species of the genus have the potential for use in biotechnology (Rai et al., 2007) and sustainable biofuel production.

Classification of *Botryococcus* taxa into several species or sub-species is still under debate (Metzger and Largeau, 2005). Komárek and Marvan (1992) analysed morphological variability of 47 populations, defined five new species (*B. comperei*, *B. australis*, *B. fernandoi*, *B. terribilis* and *B. pila*), and proposed a key for determination of 13 morphological types with the status of species. On the other hand, some authors in their studies have continued to refer to a single species, *B. braunii* Kützing, but there is molecular evidence based on the nuclear 18S rDNA that different *B. braunii* strains probably belong to more than one species (Senousy et al., 2004). In addition, there were some doubts about the taxonomic position of *Botryococcus* taxa, but nowadays it is generally accepted that *B. braunii* belongs to class Trebouxiophyceae, Chlorophyta (Senousy et al., 2004).

Botryococcus species occur in various aquatic habitats from ponds, oligotrophic and mesotrophic lakes to reservoirs with higher electrolyte content. They are generally euryhaline, tolerating salinities ranging from fresh, brackish to saline water (Hammer, 1986; John et al., 2011). In addition, there are strains that are eurythermal, such as two *B. braunii* strains (BOD-NG17 and BOD-GJ2) that can survive temperatures between -20 °C and 40 °C, and tolerate desiccation for over six months (Demura et al., 2014). Experiments with species *Botryococcus protuberans* West & G. S. West revealed that this organism can tolerate pH levels ranging from 7.0 to 9.5, with an optimum at about 8.5 (Rai et al., 2007). In spite of its slow growth rate, some *Botryococcus* species can form a dense bloom in subtropical and tropical regions (Shimamura et al., 2012; Janse van Vuuren and Levanets, 2019) or form benthic mats in shallow salt lakes (Hammer, 1986). The red and green blooms of *B. braunii* are reported in Australian freshwater lakes (Wake and Hillen, 1981). In some water bodies, such as Darwin River Reservoir in Australia Townsend (2001) recorded persistent dominance of *B. braunii* as a result of stable physical conditions. During the massive development, it is not rare to see colonies of *Botryococcus* floating on the surface and forming coloured scum. The higher density of this planktic microalga is usually associated with lower diversity of phytoplankton, zooplankton and even fish community. It is possible that some *Botryococcus* taxa, such as strains of *B. braunii*, produce different extracellular fatty acids with allelopathic effects on other organisms (Chiang et al., 2004).

The *Botryococcus* taxa are known as a group of algae that have an ability to produce and accumulate different hydrocarbon molecules and even some of those molecules, such as botryococcenes, are named after them (Metzger and Largeau, 2005). Moreover, within the same species, there can be several chemical races. For instance, different *B. braunii* strains can be divided into three chemical races, called A, B and L (Metzger and Largeau, 2005). Since some *Botryococcus* cells can have a very high percentage of oil-related molecules (up to 75% of the dry weight), these microalgae can be used as a potent feedstock for renewable liquid fuel production (Demura et al., 2014). Interestingly, species *B. sudeticus* Lemmermann is able to produce and accumulate lipids with oil composition that is similar to the content of olive oil (Senousy et al., 2004).

Distribution of colony-forming planktic algae of the genus *Botryococcus* is most likely cosmopolitan (Komárek and Marvan, 1992; John et al., 2011). For instance, *B. terribilis* Komárek & Marvan is observed in slightly alkaline waters in temperate and tropical regions. The species was found in southern Sweden, Czechoslovakia, Austria, southern Spain, Chad and Cuba (Komárek and Marvan, 1992; Fanés Treviño et al., 2009). In Serbia, *Botryococcus* species have been found in the northern part of the country, in the Vojvodina province (Milovanović and Živković, 1953; Szemes, 1967; Guelmino, 1973; Seleši, 1981, 1982; Obušković, 1982; Đukić et al., 1991a, 1991b; Maletin et al., 1994; Pujin et al., 1996; Pujin, 1998; Trbojević, 2018). Most findings are related to species *B. braunii* that was recorded in lakes and reservoirs (Sava Lake, Lake Palić, Lake Ludaš, and Borkovac reservoir), in rivers and channels (Danube River, Tisa River, Jegrička, Mostonga, and Bajski channel), and different ponds that are a part of Ečka fishpond system. It is interesting that Obušković (1982) reported summer maxima of *B. braunii* in July and August in Sava Lake. Finally, in Tisa River besides *B. braunii* Guelmino (1973) observed one more species of this genus, *B. micromorus* W. West & G. S. West.

The main aims of this study were: (1) to describe the green alga that was found and identified for the first time in Serbia in the small sandpit lake and (2) to analyse the relationship between the chemical composition of the water and the occurrence of the recorded *Botryococcus* species. The morphological and ecological characterisation of this micro-organism should be the first step in the realisation of its biotechnological potential in our country.

MATERIAL AND METHODS

Description of the study site

Water and plankton sampling as well as *in situ* measurements of physical and physico-chemical parameters were conducted on the shore of the sandpit lake known as Peskara (N45°31’06” E20°17’54”). This waterbody is located in the protected zone of the Rusanda Nature Park, between two lakes – Lake Velika

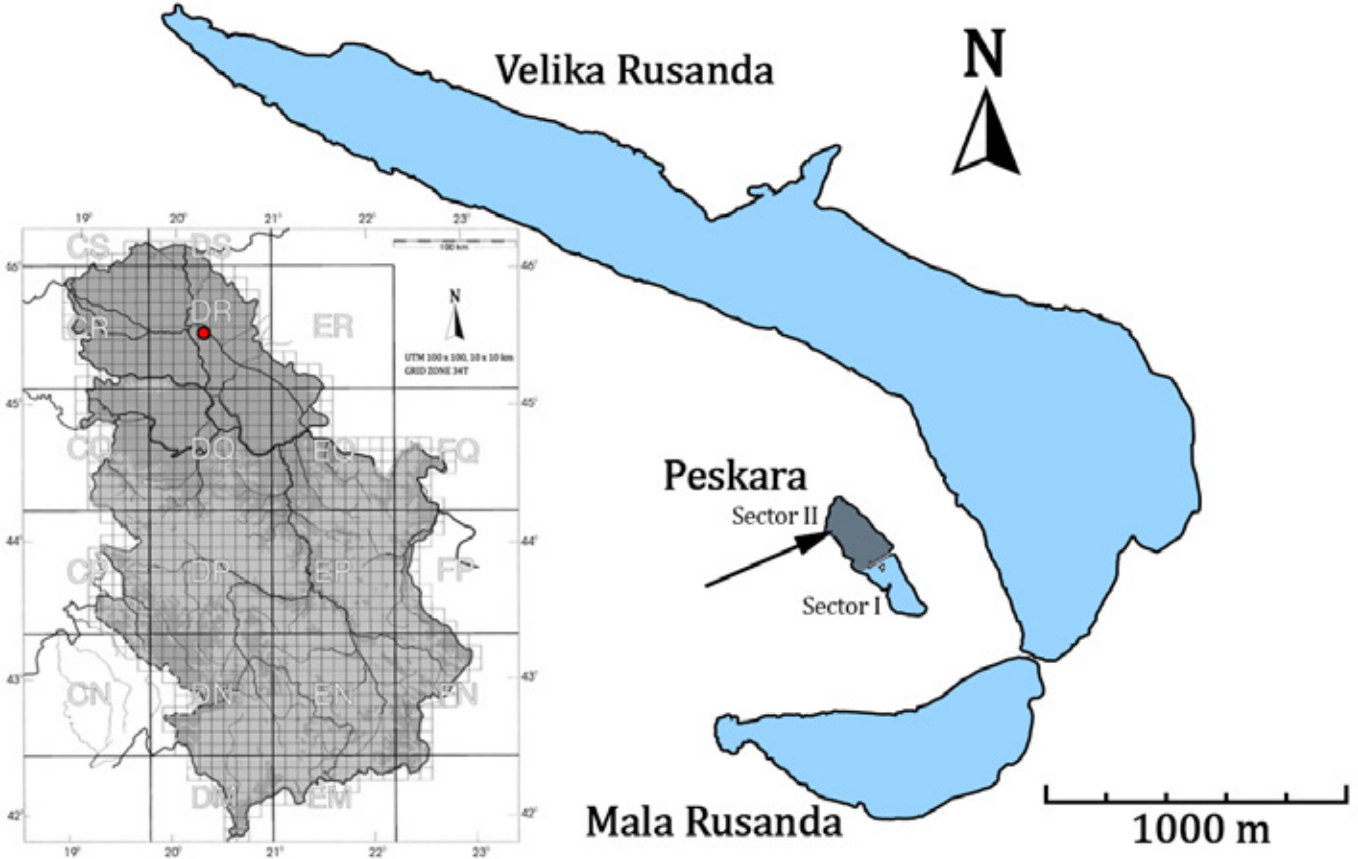


Figure 1. Location of the sampling site. Black dot denotes position of the study site on UTM map. Black arrow indicates the deeper part of the sandpit lake "Peskara" where the sampling was conducted.

Rusanda and Lake Mala Rusanda (Figure 1). Description of the sandpit lake origin is given in the document published by the Institute for Nature Conservation of the Vojvodina Province (2011). Since the data on the sandpit lake morphometry were not available, we conducted measurements of depth at several sites along two axes inside the lake (Figure 1). The deeper part of the lake (Sector II) is 205 m long, approximately 107 m wide, with the maximum and average depth of 6.6 m and 4.4 m respectively.

Physical and physico-chemical parameters

Several water quality parameters were measured *in situ*: water temperature (T), pH, conductivity (COND), and dissolved oxygen (DO). T, pH, and COND were monitored using Water Multimeter 18.52.01 (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) and DO was measured with DO meter HI9147 (Hanna Instruments, Woonsocket, USA). Samples for chemical analyses of different cations and anions were collected with two 0.5-L plastic bottles. The concentrations of different metals (Na^+, K^+, Ca^{2+} and Mg^{2+}) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, UK) according to the procedure described in Vidaković et al. (2019). The water NH_4^+ content was measured using 930 Compact IC Flex ion chromatograph (Metrohm, Herisau, Switzerland) following SRPS EN ISO 14911:2009. The determination of NO_2^-, NO_3^- and PO_4^{3-} was done using the same ion chromatograph according to U.S. EPA (1997). The total phosphorus (TP) concentration was measured with UV/Vis spectrophotometer Specord 50 (Analytic Jena, Jena, Germany) following SRPS EN ISO 6878:2008. The calculation of CO_3^{2-} and HCO_3^- was based on p– and m–alkalinity that was determined by the titration method (APHA, AWWA & WPCF 1995a). Cl^- content was determined by the argentometric method following APHA, AWWA & WPCF (1995b). Finally, the determination of SO_4^{2-} concentration was done using the gravimetric method with the ignition of residue according to APHA, AWWA & WPCF (1995c).

Sampling and identification of *Botryococcus terribilis*

For the purpose of alga identification, a 10-L water sample was taken and filtered through a plankton net (mesh size of 25 μm, HYDRO-BIOS Apparatebau GmbH, Altenholz, Germany). Identification of *B. terribilis* was done using Leica DM750 microscope (Leica Microsystems) with objective HI PLAN 40/0.65 (at 40× magnification) according to the following literature: John et al. (2011) and Komárek and Marvan (1992). Microphotographs of *B. terribilis* were made using Zeiss Axioimager.M1 microscope with AxioVision 4.9 software at 400× magnification.

Counting of *B. terribilis* colonies was performed according to Utermöhl (1958). Firstly, 10 mL subsample was taken from 0.5-L unfiltered water sample and left in the HYDRO-BIOS sedimentation chamber for 48 hours. Calculation of *B. terribilis* abundance was done after counting its colonies using the inverted microscope INVE 500T (COLO Lab Experts, Novo Mesto, Slovenia) with objectives 20× and 40×. The abundance of *B. terribilis* was expressed as a number of colonies per ml.

Algal cells preparation for transmission electron microscopy

The liquid algal sample was fixed in 5% glutaraldehyde, rinsed toughly with phosphate buffer, embedded in agar and postfixed in osmium tetroxide in the same buffer for 2h. Afterwards, samples were dehydrated through ethanol (30–100%) and routinely embedded in Araldite. Ultrathin sections (100 nm thickness) were obtained using a Leica UC6 ultramicrotome (Leica Microsystems, Germany), mounted on copper grids, contrasted in uranyl acetate and lead citrate using Leica EM STAIN (Leica Microsystems), and examined on a Philips CM12 transmission electron microscope (Philips/FEI, Eindhoven, The Netherlands) equipped with the digital camera SIS MegaView III (Olympus Soft Imaging Solutions, Münster, Germany).

RESULTS

Physical and chemical analysis of water

The sandpit lake "Peskara" is subsaline (according to classification given by Hammer, 1986) and alkaline pond filled with water that can be classified into sodium bicarbonate chemical type. The first sampling, when only a few water quality parameters were determined, was conducted in October 2019 when the temperature was higher (17 °C) (Table 1). A more detailed water analysis conducted in December 2019, and May and July 2020 revealed that among cations and anions sodium and bicarbonate were dominant, respectively. In all samples, pH values were high indicating alkaline conditions. When it comes to the nutrient content, all nitrogen compounds (NH_4^+, NO_2^-, NO_3^-) and PO_4^{3-} were under the detection limit. TP level indicated mesotrophic conditions according to OECD (1982).

Table 1. Physical and chemical characteristics of water in the small sand pit lake "Peskara" during the sampling in 2019 and 2020 (TRANS Transmission; T Water temperature; COND Conductivity; DO Dissolved oxygen; TP Total phosphorus)

Parameter	Unit	October 2019	December 2019	May 2020	July 2020
TRANS	m	–	–	0.85	0.89
T	°C	17.0	6.7	20.9	25.1
pH		8.95	8.99	8.97	9.06
COND	µS/cm	1860	1895	1947	1952
DO	mg/L	12.2	16.8	9.1	10.5
Na^+	mg/L	–	173.30	428.3	–
K^+	mg/L	–	3.76	8.60	–
Ca^{2+}	mg/L	–	5.58	13.75	–
Mg^{2+}	mg/L	–	17.09	32.52	–
NH_4^+	mg/L	–	<0.05	<0.05	–
NO_2^-	mg/L	–	<0.02	<0.02	–
NO_3^-	mg/L	–	<0.5	<0.5	–
PO_4^{3-}	mg P/L	–	<0.02	<0.02	–
TP	mg P/L	–	0.027	0.024	–
CO_3^{2-}	mg/L	–	118.2	174.0	–
HCO_3^-	mg/L	–	723.5	750.3	–
Cl^-	mg/L	–	99.1	102.0	–
SO_4^{2-}	mg/L	–	60.0	70.6	–

Morphometric analysis and abundance of *B. terribilis*

The young colonies were more or less spherical. Older ones, with an irregularly ovoid shape, were 17.5–115.0 µm long and 17.5–77.5 µm wide (n=30), and composed of several sub-colonies that are joined with short mucilaginous connections. Cells were completely embedded within the wrinkled mucilage with numerous oil droplets that helped a colony to float (Figure 2. A, B).

The mucilage was indistinctly layered, yellow or orange, with short and simple gelatinous processes on its margin. Cells had obovoid shape and were usually radially oriented (Figure 2. C). Cells were 5.5–8.0 µm long and 3.5–5.0 µm wide (n=15). The abundance of *B. terribilis* in December 2019, May 2020 and July 2020 was 132, 45, and 20 colonies per ml, respectively.

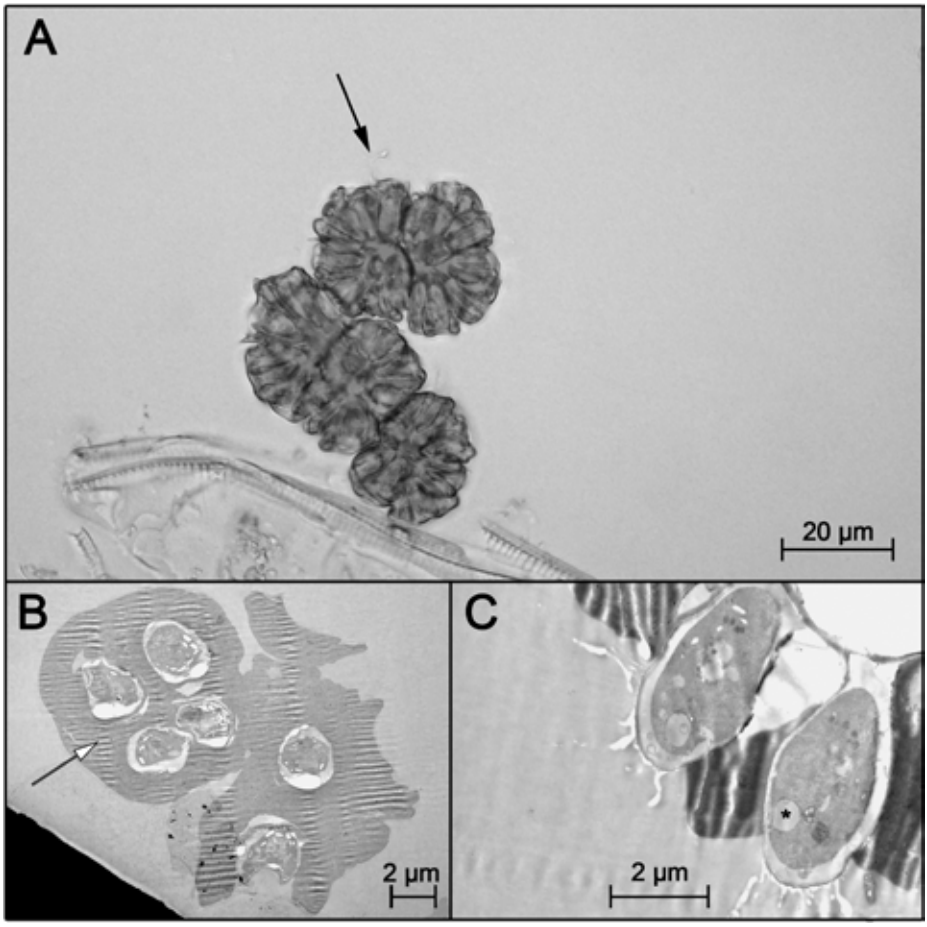


Figure 2. Microphotographs of *B. terribilis*: **A** Light microscopy of colony collected in July 2020, branched gelatinous processes at the periphery (black arrow); **B**, **C** Transmission electron microscopy (TEM) of colonies collected in July 2020. **B** showing colony in cross section with cells completely embedded within the wrinkled mucilage (white arrow); **C** cells in longitudinal section with lipid bodies (asterisk)

DISCUSSION

The species that we found during our long-term algal survey of salt alkaline ponds in the Vojvodina Province, had different morphological and ecological features than *B. braunii*. The colonies found in the sandpit lake Peskara appear to belong to species *B. terribilis* since the cup-shaped, mucilaginous sheath around cells, a characteristic of *B. braunii*, was absent and cells were completely hidden within extracellular matrix. In their study of eight *B. terribilis* strains isolated from freshwaters of Romania, Hegedűs et al. (2015) also noticed colonies

composed of tight subcolonies with cells completely or partially embedded in the hydrocarbon matrix. Moreover, in our study short, branched mucilaginous processes were observed on the colony margin. *B. neglectus* is another species that forms similar gelatinous processes at the periphery, but compared to *B. terribilis* it has smaller colonies and cells (Komárek and Marvan, 1992; De Queiroz Mendes et al., 2012). Finally, chemical analysis of water in Peskara revealed the existence of alkaline conditions that are favourable for the development of *B. terribilis.* It is possible that the species of the genus *Botryococcus* found earlier in some alkaline lakes in Serbia and designated as *B. braunii* was, in fact, *B. terribilis*. Therefore, only a comparative analysis based on molecular and ultrastructural evidence of strains isolated from different lakes in Serbia can give an unambiguous answer to this question.

In the course of our study, we regularly reported the presence of *B. terribilis* in the sandpit lake Peskara. In October 2019, *B. terribilis* formed an orange-red surface scum, but later, especially in summer 2020, the number of colonies decreased. It is known that buoyant colonies of *Botryococcus* may stay in the water column long after the maximum growth period and even be abundant under conditions which are no longer optimal for them (Tyson, 1995). Thus, it is possible that *Botryococcus* bloom was an accidental event, not so frequent in this lake.

A recent study focused on hydrocarbon biosynthesis in *B. terribilis* strain collected in adjacent Romania revealed that this taxon has similar properties as the most extensively studied *B. braunii* (Szöke-Nagy et al., 2020). Since our finding of *B. terribilis* is the first report of this alga in Serbia, further work should be focused on the isolation of *B. terribilis* strain from the sandpit lake "Peskara" and characterisation of its lipid molecules.

CONCLUSION

The green alga *B. terribilis* was discovered and identified for the first time in Serbia in the small sandpit lake in the Rusanda Nature Park. Its ecology and potential application in biotechnology is still insufficiently known and should be further investigated.

ACKNOWLEDGEMENTS

This study was financially supported by the Serbian Ministry of Education, Science, and Technological Development (Grant No. OI172001, Grants No. 451-03-68/2020-14/200026 and 451-03-68/2020-14/200178). Transmission electron microscope analysis was done in the Center for electron microscopy at the University of Belgrade – Faculty of Biology. Finally, we would like to thank professor Zlatko Levkov for his valuable advice, and professor Lothar Krienitz for his help in confirming the identity of the species.

REFERENCES

APHA, AWWA, WPCF (1995a): Method 2320 B. In: Eation AD, Clesceri LS & Greenberg AE. (eds.), *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, USA, 2–26.

APHA, AWWA, WPCF (1995b): Method 4500-Cl^- B. In: AD Eation, LS Clesceri & AE Greenberg (eds.), *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, USA, 4–49.

APHA, AWWA, WPCF (1995c): Method 4500-SO_4^{2-} C. In: AD Eation, LS Clesceri & AE Greenberg (eds.), *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, USA, 4–135.

Chiang IZ, Huang WY, Wu JT (2004): Allelochemicals of *Botryococcus braunii* (Chlorophyceae). *J. Phycol.* 40: 474–480.

De Queiroz Mendes MC, González AAC, Moreno MLV, Figueira CP, De Castro Nunes JM (2012): Morphological and ultrastructural features of a strain of *Botryococcus terribilis* (Trebouxiophyceae) from Brazil. *J. Phycology* 48: 1099–1106. [DOI: 10.1111/j.1529-8817.2012.01181.x]

Demura M, Ioki M, Kawachi M, Nakajima N, Watanabe MM (2014): Desiccation tolerance of *Botryococcus braunii* (Trebouxiophyceae, Chlorophyta) and extreme temperature tolerance of dehydrated cells. *J. Appl. Phycol.* 26: 49–53.

Đukić N, Pujin V, Maletin S, Gajin S, Gantar M, Petrović O, Ratajac R, Seleši Đ, Matavulj M (1991a): Eutrofizacija stajaćih voda Vojvodine – "Borkovac" (Lentic waters eutrophication in Vojvodina – "Borkovac"). *Godišnjak Vodoprivrede Vojvodine* I: 4–6.

Đukić N, Pujin V, Maletin S, Gajin S, Gantar M, Petrović O, Ratajac R, Seleši Đ, Matavulj M (1991b): Eutrofizacija stajaćih voda Vojvodine – "Palić" (Lentic waters eutrophication in Vojvodina – "Palić"). *Godišnjak Vodoprivrede Vojvodine* I, 52–57.

Fanés Treviño I, Sánchez Castillo P, Comas González A (2009): Contribution to the taxonomic study of the family Botryococcaceae (Trebouxiophyceae, Chlorophyta) in southern Spain. *Cryptogam. Algol.* 30: 17–30.

Guelmino J (1973): Zenta és környékének növényei II. Virágtalanok (Plants of Senta and its surroundings II. Non-flowering plants). *Građa za monografiju Sente* 13: 39–102.

Hammer UT (1986): *Saline lake ecosystems of the world*. Springer Science & Business Media.

Hegedűs A, Mocan A, Barbu-Tudoran L, Comon C, Drugă B, Sicora C, Dragos N (2015): Morphological, biochemical, and phylogenetic assessments of eight *Botryococcus terribilis* strains collected from freshwaters of Transylvania. *J. Appl. Phycol.* 27: 865–878. [DOI: 10.1007/s10811-014-0387-2]

Institute for Nature Conservation of Vojvodina Province (2011): *Studija zaštite: Park Prirode "Rusanda" – Predlog za stavljanje pod zaštitu kao zaštićenog područja II kategorije*. Novi Sad, Srbija.

Janse van Vuuren S, Levanets A (2019): First record of *Botryococcus braunii* Kützing from Namibia. *Bothalia* 49: 1–5.

Jeppesen E, Meerhoff M, Jacobsen BA, Hansen RS, Søndergaard M, Jensen JP, Lauridsen TL, Mazzeo N, Branco CWC (2007): Restoration of shallow lakes by nutrient control and

biomanipulation–the successful strategy varies with lake size and climate. *Hydrobiologia* 581: 269–285. [DOI: 10.1007/978-1-4020-6158-5_28]

John DM, Whitton BA, Brook AJ (2011): *The freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae*. Cambridge: Cambridge University Press.

Komárek J, Marvan P (1992): Morphological differences in natural populations of the genus *Botryococcus* (Chlorophyceae). *Arch. Protistenkunde* 141: 65–100. [10.1016/S0003-9365(11)80049-7]

Maletin S, Pujin V, Kostić D (1994): The diet, growth and fecundity of the allochtonous planktivorous fish in the Danube. In: DV Janković, MŽ Jovičić (eds.), *The Danube in Yugoslavia – contamination, protection and exploitation*. Belgrade: Institute for Biological Research "Siniša Stanković", Institute for Development of Water Resources "Jaroslav Černi", 174–184.

Metzger P, Largeau C (2005): *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Appl. Microbiol. Biotechnol.* 66: 486–496. [DOI: 10.1007/s00253-004-1779-z]

Milovanović D, Živković A (1953): Ispitivanja planktonske produkcije u ribnjacima Ečke, prilog regionalnoj limnologiji stajaćih voda Panonske nizije [Plankton production investigations in Ečka fishponds, contribution to regional limnology of stagnat waters in the Pannonian plain]. *Zbornik radova* XXIX, Institut za ekologiju i biogeografiju SAN, 3: 197–264.

Obušković Lj (1982): Dinamika fitoplanktona i nekih ekoloških faktora kao odraz eutrofizacije u Savskom jezeru kod Beograda [Dynamics of phytoplankton and some ecological factors as an eutrophication effect in the Sava Lake near Beograd]. *Vodoprivreda* 14: 123–128.

OECD (Organization for Economic Cooperation and Development) (1982): Eutrophication of waters. Monitoring, assessment and control. Paris, France: OECD.

Pujin V, Đukić N, Maletin S, Miljanović B, Ivanc A (1996): Hidrobionti kao pokazatelji kvaliteta vode Jegričke [Hydrobionts as indicators of water quality of Jegrička water]. In: L. Lazić (ed.), *Jegrička*, Edicija Tija Voda I, PČESA, Novi Sad, Serbia, 69–78.

Pujin V (1998): Sastav planktona u nekim kanalima hidrosistema DTD [Plankton composition of some channels in DTD hydrosystem]. In: L. Lazić (ed.), *Mostonga i vode Zapadne* Bačke, Edicija Tija Voda II, PČESA, Novi Sad, Serbia, 54–57.

Rai UN, Dwivedi S, Baghel VS, Tripathi RD, Shukla OP, Shukla MK (2007): Morphology and cultural behavior of *Botryococcus protuberans* with notes on the genus. *J. Environ. Biol.* 28: 181–184.

Seleši Đ (1981): Limnološka istraživanja Ludoškog jezera [Limnological investigations of Lake Ludoško]. *Vode Vojvodine* 9: 333–352.

Seleši Đ (1982): Limnološka istraživanja Palićkog jezera [Limnological studies on Lake Palić]. *Vode Vojvodine* 10: 345–368.

Senousy HH, Beakes GW, Hack E (2004): Phylogenetic placement of *Botryococcus braunii* (Trebouxiophyceae) and *Botryococcus sudeticus* isolate UTEX 2629 (Chlorophyceae). *J. Phycol.* 40: 412–423. [10.1046/j.1529-8817.2004.03173.x]

Shimamura R, Watanabe S, Sakakura Y, Shiho M, Kaya K, Watanabe MM (2012): Development of *Botryococcus* seed culture system for future mass culture. *Procedia Environ. Sci.* 15: 80–89. [DOI: 10.1016/j.proenv.2012.05.013]

SRPS EN ISO 6878:2008: *Water Quality – Determination of phosphorus – Ammonium molybdate spectrometric method (ISO 6878:2004)*. Institute for Standardization of Serbia, Belgrade.

SRPS EN ISO 14911:2009: *Water Quality – Determination of dissolved Li^+, Na^+, NH_4^+, K^+, Mn^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} using ion chromatography – Method for water and waste water.* Institute for Standardization of Serbia, Belgrade.

Szemes G (1967): Systematisches Verzeichnis der Pflanzenwelt der Donau mit einer zusammenfassenden Erläuterung. In: R. Liepolt (ed.), *Limnologle der Donau*, Stuttgart, Germany: E. Schweizerbart'sche Verlagsbuchhandlung, 70–131.

Szöke-Nagy T, Porav SA, Dragoș N (2020): In silico modeling and analysis of squalene synthase-like 1 (SSL-1) enzyme from green microalga *Botryococcus terribilis* AICB 872. *Studia UBB Biologia* 65: 5–18.

Townsend SA (2001): Perennial domination of phytoplankton by *Botryococcus* and *Peridinium* in a discontinuously polymictic reservoir (tropical Australia). *Arch. Hydrobiol.* 151: 529–548. [DOI: 10.1127/archiv-hydrobiol/151/2001/529]

Trbojević I (2018): *Analiza perifitona sa veštačkih podloga iz Savskog jezera i jezera Vrutci* [Analysis of periphyton developed on artificial substrates in the Sava lake and the Vrutci lake]. PhD thesis. Faculty of Biology, University of Belgrade, Serbia.

Tyson RV (1995): Distribution of the Palynomorph Group: Phytoplankton Subgroup, Chlorococcale Algae. In: RV Tyson (ed.), *Sedimentary Organic Matter*, Springer, Dordrecht, 309–317.

U.S. EPA 1997: *Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography*. Revision 1.0, Cincinnati, OH.

Utermöhl H (1958): Zur vervollkommnung der quantitativen phytoplankton-methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel [Methods of collecting plankton for various purposes are discussed]. *Mitt. – Int. Ver. Theor. Angew. Limnol.* 9: 1–38. [DOI: 10.1080/05384680.1958.11904091]

Vidaković D, Krizmanić J, Dojčinović B, Pantelić A, Gavrilović B, Živanović M, Novaković B, Ćirić M (2019): Alkaline soda Lake Velika Rusanda (Serbia): the first insight into diatom diversity of this extreme saline lake. *Extremophiles* 23: 347–357. [DOI: 10.1007/s00792-019-01088-6]

Wake LV, Hillen LW (1981): Nature and hydrocarbon content of blooms of the alga *Botryococcuc braunii* occuring in Australian freshwater lakes. *Aust. J. Mar. Freshw. Res.* 32: 353–367.

ПОЈАВА АЛГЕ *Botryococcus terribilis* Komárek & Marvan У ПЕСКАРИ – ПРВИ НАЛАЗ ЗА СРБИЈУ

Милош ЋИРИЋ[1], Јелена КРИЗМАНИЋ[2], Биљана П. ДОЈЧИНОВИЋ[1], Бојан ГАВРИЛОВИЋ[3], Далибор М. МАРИНКОВИЋ[1]

[1] Универзитет у Београду, Институт за хемију, технологију и металургију, Институт од националног значаја за Републику Србију, Његошева 12, Београд 11000, Србија
[2] Универзитет у Београду, Биолошки факултет, Институт за ботанику и ботаничка башта „Јевремовац", Таковска 43, Београд 11000, Србија
[3] Српска академија наука и уметности, Географски институт „Јован Цвијић", Департман за физичку географију, Ђуре Јакшића 9, Београд 11000, Србија

РЕЗИМЕ: Алкалне слане баре и језера су станишта са јединственим биодиверзитетом. Зелена алга из рода *Botryococcus* је случајно откривена током алголошких истраживања ових станишта у Србији. Врста *B. terribilis* је пронађена и по први пут идентификована у Србији у Пескари у Парку природе „Русанда". Прво узорковање током кога је на површини воде забележена наранџасто-црвена скрама непознатог порекла било је непотпуно. Након што је врста идентификована спроведена су још три додатна узорковања с циљем сакупљања фитопланктона и воде за физичко-хемијске анализе. Анализирани су доминантни анјони и катјони како би се утврдио хемијски тип воде у Пескари, али и садржај нутријената. Утврђено је да је вода у Пескари алкална и да припада натријум-бикарбонатном хемијском типу. Морфометријска анализа *B. terribilis* је изведена светлосном и трансмисионо-електронском микроскопијом. *B. terribilis* је био нарочито бројан у фитопланктонској заједници током касне јесени и и току зиме. Врсте рода *Botryococcus* су познате као богати извори различитих липида, па ово откриће може бити први корак у примени ове врсте у одрживој производњи биогорива.

КЉУЧНЕ РЕЧИ: Пескара, *Botryococcus terribilis*, фитопланктон

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 59—70, 2021

UDC 633.33
https://doi.org/10.2298/ZMSPN2140059P
ORIGINAL ARTICLE

Gordana R. PETROVIĆ[1], Tomislav T. ŽIVANOVIĆ[2], Radmila I. STIKIĆ[2], Zorica T. NIKOLIĆ[1], Dušica D. JOVIČIĆ[1], Gordana D. TAMINDŽIĆ[1], Dragana N. MILOŠEVIĆ[1]*

[1] Institute of Field and Vegetable Crops, Seed Testing Laboratory, Maksima Gorkog 30, Novi Sad 21000, Serbia
[2] University of Belgrade, Faculty of Agriculture, Department for Agrochemistry and Plant Physiology, Nemanjina 6, Belgrade-Zemun 11080, Serbia

EFFECTS OF DROUGHT STRESS ON GERMINATION AND SEEDLING GROWTH OF DIFFERENT FIELD PEA VARIETIES

ABSTRACT: Germination and seedling growth of seven genotypes of field pea (*Pisum sativum* L.) were studied in PEG-6000 solution having osmotic potentials -0.1 and -0.2 MPa. A study was undertaken to evaluate the influence of different osmotic potentials (MPa) on seed germination percentage (GP) and mean germination time (MGT). Results show that the percentage of germination decreased with a decrease in osmotic potential, while mean germination time increased. Variety Javor is much more sensitive than the other varieties at all levels of osmotic stress. By contrast, the lowest sensitivity of germination and MGT was found in varieties Mraz and Trezor. Seed germination tests at -0.1 to -0.2 MPa have the potential to be used as tests in field pea. Osmotic stress exposure and its duration significantly affected the growth of seedlings (shoot and root) and the accumulation of biomass, while its effect was more prominent on the growth of the shoot than on root growth, which was also confirmed by the root tolerance index.

KEYWORDS: *Pisum sativum*, seed, shoot, varieties, tolerance index

INTRODUCTION

Pea (*Pisum sativum*) is the second most important food legume worldwide after common beans. The increasing demand for protein-rich raw materials for animal feed or intermediary products for human nutrition has led to a greater interest in this crop as a protein source. Pea seeds are rich in protein (23–25%),

* Corresponding author. E-mail: gordana.petrovic@ifvcns.ns.ac.rs

slowly digestible starch (50%), soluble sugars (5%), fibre, minerals, and vitamins (Bastianelli et al., 1998; Červenski et al., 2017).

Nowadays, legumes provide one-third of the entire amount of protein for human consumption, representing an important source of fodder and forage for animals, as well as in the production of edible and industrial oils (Smýkal et al., 2012). Due to their high protein content, they can partially replace soybean in the meal of ruminants and non-ruminants (Gružauskas et al., 2016). One of the most important attributes of legumes is their capacity for symbiotic nitrogen fixation, underscoring their importance as sources of nitrogen in both natural and agricultural ecosystems (Heldt and Piechulla, 2011; Smýkal et al., 2012).

During their growth, crop plants are usually exposed to different environmental stresses that can limit their growth and productivity (Fleury et al., 2010; Vujaković et al., 2011). Salinity and drought are major environmental factors limiting plant growth and productivity, causing great economic losses (Jovičić et al., 2014; Petrović et al., 2016). Field pea, like other pulses, is moderately sensitive to a number of abiotic stress factors, particularly involving water deficit, soil nutrition such as salinity and alkaline-induced boron toxicity, reproductive frost damage, and heat stress (Dita et al., 2006; Petrović et al., 2016).

Seed germination is the first critical and the most sensitive stage in the life cycle of plants and it can compromise the seedlings' establishment (Kolb and Barsch, 2010; Karagić et al., 2010). Seeds exposed to unfavorable environmental conditions, like water stress, germinate with difficulties (Jovičić et al., 2014). Water stress limits plant survival and early seedling growth by delaying its beginning or decreasing the final germinability (Okçu et al., 2005; Kaydan and Yagmur, 2008; Silva et al., 2010). Considering that pea, like other legumes, requires a relatively large amount of water during the germination process, it is suggested that this is the most critical phase of development stages.

Drought tolerance testing in the initial stages of plant development is of vital importance because the seed with more rapid germination under water deficit conditions can be expected to achieve a rapid seedling establishment, resulting in higher yields (Jovičić et al., 2014). Polyethylene glycol (PEG 6000) is commonly used to simulate water-stress conditions in seed germination studies. PEG 6000 is an inert osmotic agent whose molecules are too large to penetrate the seed, thus preventing any toxic effects. Because PEG 6000 does not enter the apoplast, water is withdrawn not only from the cell but also from the cell wall (Verslues et al., 2006). PEG-based in vitro screening for drought tolerance has proven to be an effective screening method for large sets of germplasm (Okçu et al., 2005; Generozova et al., 2009).

Measurement of germination can provide valuable information about the start, rate, uniformity, and final percentage of germination. However, two seed lots can have the same germination percentage but differ in speed or uniformity. Therefore, a total germination percentage after a specific period does not give a full explanation of the dynamics of germination (Joosen et al., 2010). It is also useful to calculate MGT. MGT is defined as a measure of the rate and time-spread of germination (Soltani et al., 2016).

Since researchers have paid little attention to improve the locally cultivated pea in this area, the present study contributes to understanding the responses under osmotic and drought stress conditions and the further improvement of our field pea cultivars.

The aim of this study was to determine whether the pea seed germination and seedling growth of different field pea cultivars were inhibited by the osmotic effect of water deficit, as well as to establish a simple and fast screening method for drought tolerance in pea genotypes.

MATERIALS AND METHODS

The study was conducted using seven selected genotypes of the genus *Pisum* from the collection of the Institute of Field and Vegetable Crops in Novi Sad. Seeds were sterilized with 0.5% sodium hypochlorite solution for 1 min and after that washed with distilled water. Pea seeds were germinated in 15 x 24 cm plastic boxes filled with sand. For each cultivar, 4 replicates of 100 seeds were sown for each treatment.

Drought stress was stimulated by two different osmotic potential levels (-0.1 MPa and -0.2 MPa) using PEG 6000. Solutions of PEG 6000 were prepared according to Michel and Kaufmann (1973). Distilled water served as a control.

Plastic boxes were placed in a germination chamber at 20 °C under conditions of a 12h light/dark cycle.The sand was moistened daily with distilled water for control and the solution of PEG for treatments. Seed germination was recorded every 24h for 10 days by evaluating normal seedlings. A seed was considered to have germinated when the radicle was 2 mm long (Kaya et al., 2006; Kim et al., 2006).

The germination percentage was determined by counting the number of germinated seeds as follows: (GP) = Number of germinated seed / Total Number of seed tested x 100.

Mean germination time (MGT) was determined according to Kandil et al. (2012): $MGT = \sum dn / \sum n$, where (n) is the number of seeds that were germinated on the day (d), and (dn) is the number of days counted from the beginning of germination.

The radicle and hypocotyl length (mm) and the seedling fresh and dry weights (g) were measured on the 10th day after sowing. Dry weights of hypocotyl and radicle were measured after drying samples in the oven at 80 °C for 24h, to an accuracy of 0.1 mg using an analytical balance, until a constant weight was achieved. The results were expressed as the total mass of ten shoots (g).

The shoot/root ratio of dry weight was calculated from the obtained results. Tolerance index (TI) was calculated for the shoot and the root according to Maiti et al. (1994):

Tolerance index (TI) = Dry weight of plant material in the control group (g) / Dry weight of plant material in the treated group (g).

Data given in percentages were subjected to arcsine transformation before statistical analysis. For all investigated parameters, the analysis of variance was performed using the Statistical software (Sigmaplot 10.0., Systat Software Inc., San Jose, CA, U.S.A.). Significant differences among the mean values were compared by Student's t-test (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$). Figures were drawn using Sigmaplot.

RESULTS AND DISCUSSION

Germination of seeds is a complex physiological process triggered by the imbibition of water after possible dormancy mechanisms have been released by appropriate triggers. Under favorable conditions, a rapid expansion growth of the embryo culminates in rupture of the covering layers and emergence of the radicle. Radicle emergence is considered the completion of germination. Water stress can affect germination by decreasing the percentage of germination.

The results of this study show that different concentrations of PEG in germination media significantly affect the seed germination of field pea. An increase in osmotic stress significantly decreased germination percentage (GP) in all the tested varieties. Increased Mean Germination Time (MGT) and decreased total germination time occurred already at the lowest level of stress (-0.1 MPa).

Germination in controls ranged between 96.25% (Partner) and 99.75% (Mraz), except for variety Javor where it amounted to 83.75%. In all varieties, a decrease in germination depending on the level of stress was observed, while this reduction differs among varieties (Figure 1a).

Variety Mraz showed the least reduction of GP (control 100%; -0.1 MPa 99%; -0.2 MPa 97%), while the largest decrease was in Javor variety (control 84%; -0.1 MPa 64; -0.2 MPa 58%). GP decreased with the increased PEG 6000 concentration, which is due to the reduction in water potential gradient between the seed and surrounding media (Okçu et al., 2005). Obtained results are in accordance with the results of other similar studies (Almansouri et al., 2001; Kaydan and Yagmur, 2008; Jovičić et al., 2013; Petrović et al., 2016).

Mavi et al. (2010) suggest that differences in MGT or the rate of germination between seed lots are crucial for determining the emergence performance both in terms of the rate of emergence, final count, and seedling size and variation. It is useful to calculate MGT because, according to Demir et al. (2008), slower germinating lots, as indicated by MGT, with a greater spread of germination over time, produced smaller and more variable seedlings in the laboratory.

MGT increased progressively as osmotic potential decreased (Figure 1B). Okçu et al. (2005) also found that mean germination time was delayed by decreasing water potential. MGT in controls ranged from 5.04 (Mraz) and 5.58 (Javor) days, at -0.1 MPa between 5.14 (Pionir) and 6.91 (Javor) days, while in the solution of -0.2 MPa PEG ranged between 5.17 (Trezor) and 7.24 (Javor) days.

The trend of increasing MGT, or slowing germination, with increasing levels of stress was observed in all varieties, except for varieties Mraz and Trezor in the solution of -0.2 MPa when they shorten the germination compared

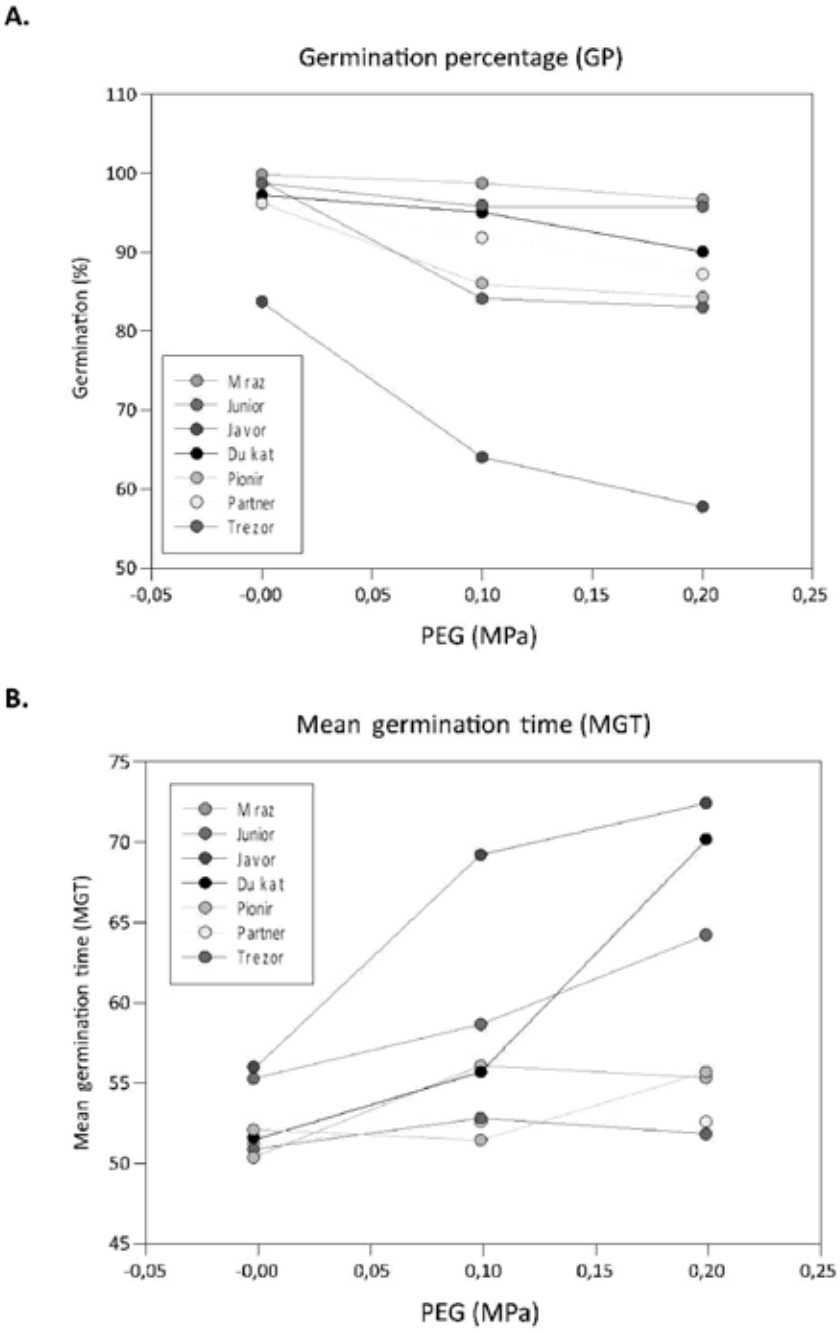


Figure 1. A. The effect of different concentrations of osmoticum PEG 6000 on seed germination in field pea genotypes; B. The effect of different concentrations of osmoticum PEG 6000 on mean germination time of field pea genotypes.

to -0.1 MPa. The increase of MGT in conditions of osmotic stress was particularly noticeable in variety Javor in which germination occurred later compared to other varieties.

Taking into account the results of measurements of the germination percentage and mean germination time, it is observed that variety Javor is much more sensitive than the other varieties at all levels of osmotic stress. On the contrary, the lowest sensitivity of germination and MGT was found in varieties Mraz and Trezor.

In order to examine the influence of different levels of osmotic stress on morphological changes of seedlings, growth parameters were determined.

An increase in osmotic stress resulted in decreased shoot and root length, but there were differences between the examined varieties. The Student's t-test showed that the decrease in shoot length compared to the control was very significant ($p < 0.001$), already at the lowest level of osmotic stress (-0.1 MPa) in all tested varieties. The lowest rate of reduction in the shoot length, relative to the control, was in the variety Dukat (73.4%, from 149.52 mm in control to 39.75 mm). On the contrary, the highest reduction (83.7%) was measured in Junior (from 215 mm in control to 35 mm in -0.1 MPa PEG solution, respectively) (Table 1).

A significant decrease in root length compared to the control was also observed. The smallest reduction in the root length relative to the control was in the variety Trezor (43.9%, from 200.48 mm to 112.48), while the highest reduction (70.7%) was in the variety Javor (211.50 mm to 112.48 mm) at the lowest level of osmotic stress (Table 1). The analysis of results showed differences between the examined varieties.

Water stress depressed the shoot growth of the cultivars rather than their root growth. The roots of the seedlings were longer indicating that the PEG significantly influenced the reduction of cell division and cell elongation. The same conclusion was made by Okçu et al. (2005) examining the effects of salt and drought stresses at the water potentials of -2, -4, -6, and -8 bars, induced by NaCl and PEG 6000 each, on germination and early seedling growth for three pea cultivars. PEG-induced osmotic stress caused more growth inhibition as compared to NaCl-induced osmotic stress (Petrović et al., 2016). The obtained results are in accordance with the results of Dobranszki et al. (2006) who analyzed the effect of PEG on 8 pea genotypes and found that osmotic stress caused by the solution of PEG 6000 affected the development of shoots more than the roots of the seedlings and that these changes were statistically significant at a solution of 5%, which would correspond to a solution of the osmotic potential of -0.1 MPa.

The average dry mass of the shoot in control ranged from 0.24 g (Pionir) to 0.47 g (Dukat). Varieties Junior, Pionir, and Trezor had the smallest dry mass of the shoots in the PEG solution of -0.1 MPa (0.07 g), while Dukat had a maximum of 0.12 g. At -0.2 MPa, Javor and Pionir had the lowest dry mass (0.05 g), while Dukat had 0.09 g. It is noticeable that the Dukat variety had the highest value of the dry mass of the shoot in all the examined groups (Table 1). The decrease of the shoot dry mass in the control was the largest in the Mraz variety

Table 1. Seedlings growth at a different level of osmotic stress

Variety	Treatment (PEG)	Shoot length (mm)	Root length (mm)	Shoot dry weight (g)	Root dry weight (g)	Shoot dry weight/ Root dry weight
Mraz	Control	119.38±1.23	212.38±1.48	0.290±0.000	0.168±0.000	1.72±0.004
	-0.1MPa	31.38***±1.16	105.62***±1.16	0.100***±0.001	0.080***±0.000	1.26***±0.02
	-0.2MPa	20.50***±0.79	83.00***±0.54	0.700***±0.000	0.080***±0.002	0.88***±0.03
Junior	Control	215.00±0.04	165.12±0.43	0.394±0.001	0.160±0.002	2.18±0.04
	-0.1MPa	35.00***±0.16	107.00***±0.12	0.069***±0.001	0.078***±0.002	0.88***±0.00
	-0.2MPa	29.05***±0.06	64.00***±0.04	0.070***±0.001	0.060***±0.001	1.16***±0.04
Javor	Control	126.50±0.18	211.50±0.74	0.350±0.002	0.208±0.002	1.69±0.02
	-0.1MPa	27.50***±0.08	62.00***±0.20	0.080***±0.000	0.080*±0.001	0.10***±0.01
	-0.2MPa	11.00***±0.04	53.50***±0.31	0.050±0.001	0.070**±0.001	0.71***±0.01
Dukat	Control	149.52±0.70	210.05±1.03	0.470±0.001	0.310±0.001	1.52±0.00
	-0.1MPa	39.75***±0.53	88.50***±0.11	0.120***±0.004	0.160***±0.000	0.75***±0.00
	-0.2MPa	23.05***±0.18	77.50±0.04	0.091***±0.001	0.131***±0.000	0.69***±0.01
Pionir	Control	210.00±0.63	161.5±1.39	0.240±0.001	0.070±0.000	3.44±0.00
	-0.1MPa	52.50***±0.28	77.02***±0.16	0.070***±0.001	0.040***±0.000	1.60*±0.01
	-0.2MPa	29.50***±0.04	63.00±0.63	0.050***±0.001	0.040***±0.001	1.26**±0.01
Partner	Control	163.50±0.18	183.50±0.12	0.370±0.001	0.330±0.001	1.12±0.00
	-0.1MPa	38.52***±0.02	85.00***±0.07	0.100***±0.001	0.180***±0.001	0.56***±0.00
	-0.2MPa	29.95***±0.73	58.000±0.04	0.080***±0.001	0.010***±0.000	0.81***±0.11
Trezor	Control	153.52±0.06	200.48±0.20	0.360±0.001	0.230±0.000	1.56±0.00
	-0.1MPa	24.92***±0.05	112.48***±0.06	0.070***±0.000	0.130***±0.000	0.54***±0.00
	-0.2MPa	22.98***±0.19	103.48***±0.19	0.070***0.000	0.120***±0.000	0.56***±0.00

*** – $p<0.001$, **- $p<0.01$, * $p<0.05$

(65.1%), and the smallest in the Dukat variety (35.8%). Results of the stress of -0.2 MPa showed that the largest decrease in the shoot dry mass was in the Javor variety (81.7%), and the smallest in the Dukat variety (44.3%).

The average value of the root dry mass in control was from 0.07 g (Pionir) to 0.33 g (Partner), in -0.1 MPa solution from 0.04 g (Pioneer) to 0.18 g (Partner), and in -0.2 MPa solution from 0.04 g (Pionir) to 0.13 g (Dukat). The decrease in control was the largest in the Javor variety (61.6%) and the smallest in the Trevor variety (43.5%). At the stress of -0.2 MPa, the largest decrease in relation to control was in the Partner (69.8%), while the smallest reduction was in the Pionir variety (57%). Comparing to other varieties, the duration of osmotic stress had no large effect on the change in dry weight in the Pionir variety.

In the Dukat variety, only the ratio of dry matter of the shoot and root dropped below 1.00 already at -0.1 MPa, which indicates that this variety, in the first stages of plant exposure to stress, assimilated referring to the root.

Jaleel et al. (2009) pointed out that dry mass accumulation is a desirable characteristic in the case of water deficiency, while Sakthivelu et al. (2008) claim that the dry mass is in correlation with tolerance to drought.

Table 2. Tolerance index (TI) of the shoot and root of the seedlings of field pea at different levels of osmotic stress.

Variety	Treatment (PEG)	Shoot TI	Root TI
Mraz	Control	1.00	1.00
	-0.1 MPa	0.35*	0.47*
	-0.2 MPa	0.24*	0.47*
Junior	Control	1.00	1.00
	-0.1 MPa	0.20*	0.49*
	-0.2 MPa	0.20*	0.38*
Javor	Control	1.00	1.00
	-0.1 MPa	0.23*	0.38*
	-0.2 MPa	0.14*	0.33*
Dukat	Control	1.00	1.00
	-0.1 MPa	0.26*	0.52*
	-0.2 MPa	0.19*	0.42*
Pionir	Control	1.00	1.00
	-0.1 MPa	0.29*	0.57*
	-0.2 MPa	0.21*	0.57*
Partner	Control	1.00	1.00
	-0.1 MPa	0.27*	0.41*
	-0.2 MPa	0.22*	0.26*
Trezor	Control	1.00	1.00
	-0.1 MPa	0.19*	0.43*
	-0.2 MPa	0.19*	0.32*

* $p<0.05$

Based on dry mass data of shoot and root of the seedlings in the control and treated group, values of tolerance index on drought, as well as other stress factors, could be calculated (Maiti et al., 1994). The tolerance index (TI) in this study is calculated for shoot and root separately, for each level of stress (Table 2).

There was a statistically significant downward trend in the value of TI of the shoot, which points to the reduced tolerance of the tested varieties to enhanced and prolonged osmotic stress (Table 2). TI at the lowest level of osmotic stress was from 0.56 (Trezor) to 0.47 (Mraz). At -0.2 MPa TI was between 0.14 (Javor) and 0.24 (Mraz). The largest decrease in TI of the shoot at osmotic stress of -0.1 MPa was in the Mraz variety (65%) and the smallest in the Dukat variety (35.5%). In stress conditions of -0.2 MPa, the largest decrease was found in the Javor variety (82%) and the smallest in the Dukat variety (44%).

The values of TI of the seedling roots (Table 2) also showed a downward trend like in the shoot. In the -0.1 MP solution, the TI was between 0.38 (Javor) and 0.56 (Trezor), while in osmotic stress of -0.2 MPa it was between 0.30 (Partner) and 0.57 (Pionir). The largest decrease in the TI at the osmotic stress of -0.1 MPa was in the Javor variety (62%) and the smallest in the Trezor variety (43.8%). In the case of the stress of -0.2 MPa, the largest decrease was observed in the varieties Pionir and Partner (70%) and the smallest in the Trezor variety (47.8%).

Maiti et al. (1994) concluded that the stress conditions have a greater impact on the IT of the shoot of the plant than on the roots. The decrease in tolerance index, under the influence of osmotic stress caused by NaCl, was determined by Dang et al. (2011).

Comparison of the values of the tolerance index of the shoot and root indicates that the values of the root tolerance index are significantly higher than the values for the shoot, at all levels of stress. The values obtained for the root under the influence of stress are closer to the control values compared to the values for the shoot, which indicates a lesser impact of osmotic stress on the dry mass accumulation in the root comparing to the shoot. Taking into account the obtained values of the index of tolerance of the shoot and root at all levels of stress and mutual comparison of varieties, it can be said that the Mraz variety is in the group of tolerant varieties, along with the Dukat and Pionir varieties. Trezor and Partner belong to a group of medium tolerant varieties, while Junior and Javor are the least tolerant varieties.

CONCLUSION

The obtained results show that the reaction to osmotic stress at low osmotic potential can be tested in different pea plants, based on simple methods such as germination percentage and seedling growth rate. Seed germination at -0.1 to -0.2 MPa appears to be a good selection criterion for field pea varieties to be planted in arid or semi-arid areas.

ACKNOWLEDGEMENT

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-68/2020-14/200032.

REFERENCES

Almansouri M, Kinet J, Lutts S (2001): Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant Soil* 231: 243–254.

Bastianelli D, Grosjean F, Peyronnet C, Duparque M, Régnier JM (1998): Feeding value of pea (*Pisum sativum*, L.) 1. Chemical composition of different categories of pea. *Anim. Sci.* 67: 609–619. [DOI: 10.1017/s1357729800033051]

Červenski J, Danojević D, Savić A (2017): Chemical composition of selected winter green pea (*Pisum sativum* L.) genotypes. *J. Serbian Chem. Soc.* 82: 1237–1246. [10.2298/JSC170323094C]

Dang HQ, Tran NQ, Gill S, Tuteja R (2011): A single subunit MCM6 from pea promotes salinity stress tolerance without affecting yield. *Plant Molec. Biol.* 76: 19–34.

Dita MA, Rispail N, Prats E, Rubiales D, Singh KB (2006): Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. *Euphytica* 147: 1–24.

Demir I, Ermis S, Mavi K, Matthews S (2008): Mean germination time of pepper seed lots (*Capsicum annuum* L.) predicts size and uniformity of seedlings in germination tests and transplant modules. *Seed Sci. Technol.* 36: 21–30.

Dobranszki J, Iszaly-Toth J, Magyar-Tabori K (2006): Inhibition recovery of germination and growing ability of seedlings under and after osmotic stress induced by polyethylene glycol in 8 pea genotypes. *Int. J. Hortic. Sci.* 12: 53–59.

Fleury D, Jefferies S, Kuchel H, Langridge P (2010): Genetic and genomic tools to improve drought tolerance in wheat. *J. Exp. Bot.* 61: 3211–3222.

Generozova IP, Maevskaya SN, Shugaev AG (2009): The inhibition of mitochondrial metabolic activity in etiolated pea seedlings under water stress. *Russ. J. Plant Physiol.* 56: 38–44.

Gružauskas R, Kudlinskienė I, Stanytė G, Alijošius S, Stankevičius R, Šašytė V, Bliznikas S, Kliševičiūtė V, Racevičiūtė-Stupelienė A (2016): The Potential of Native Raw Materials in the Animal Nutrition and its effects on the Production Quality: A review. *Vet. Zoot.* 73: 42–50.

Heldt H, Piechulla B (2011): Nitrogen fixation enables plants to use the nitrogen of the air for growth. *Plant Biochemistry*, 307–322. [DOI: 10.1016/B978-0-12-384986-1.00011-9]

Jaleel CA, Manivannan P, Wahid A, Farooq M, Somasundaram R, Panneerselvam R (2009): Drought stress in plants:a review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.* 11: 100–105.

Jovičić D, Nikolić Z, Zorić M, Marjanović-Jeromela A, Petrović G, Milošević D, Ignjatov M (2014): Viability of oilseed rape (*Brasicca napus* L.) seed under salt stress. *Genetika* 46: 137–148. [DOI: 10.2298/GENSR1401137J]

Jovičić D, Zdjelar G, Nikolić Z, Ignjatov M, Milošević D, Karagić Đ, Milošević B (2013): Effect of drought and salinity stress on pea (*Pisum sativum* L.) germination and seedlings. *1st International Conference on Plant Biology, 20th Symposium of the Serbian Plant Physiology*

Society, Beograd: Serbian Plant Physiology Society, Institute for Biological Research "Siniša Stanković", June 4–7, Subotica, Serbia, 127.

Joosen RVL, Kodde J, Willems LAJ, Ligterink W, Van der Plas LHW, Hilhorst HVM (2010): GERMINATOR: a software package for high-throughput scoring and curve fitting of *Arabidopsis* seed germination. *Plant J.* 62: 148–159.

Kandil AA, Sharief AE, Ahmed SRH (2012): Germination and Seedling Growth of Some Chickpea Cultivars (*Cicer arietinum* L.) under Salinity Stress. *J. Basic Appl. Sci.* 8: 561–571.

Karagić Đ, Katić S, Mikić A, Vujaković M, Milić D, Vasiljević S, Milošević B (2010): Effects of genotype and mechanical damage during harvest on field pea (*Pisum sativum* L.) seed quality. *Genetika* 42: 425–434.

Kaya MD, Okçu G, Atak M, Cikili Y, Kolsarici O (2006): Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *Eur. J. Agron.* 24: 291–295.

Kaydan D, Yagmur M (2008): Germination, seedling growth and relative water content of shoot in different seed sizes of triticale under osmotic stress of water and NaCl. *Afr. J. Biotechnol.* 7: 2862–2868. [DOI: 10.5897/AJB08.512]

Kim HJ, Feng H, Kushad MM, Fan X (2006): Effects of Ultrasound, Irradiation, and Acidic Electrolyzed Water on Germination of Alfalfa and Broccoli Seeds and *Escherichia Coli* O157:H7. *J. Food Sci.* 71: M168–M173. [DOI: 10.1111/j.1750-3841.2006.00064.x]

Kolb A, Barsch K (2010): Environmental factors and seed abundance influence seedling emergence of a perennial forest herb. *Acta Oecol.* 36: 507–513. [10.1016/j.actao.2010.07.003]

Maiti RK, De La Rosa-Ibarra M, Sandoval ND (1994): Genotypic Variability in Glossy Sorghum Lines for Resistance to Drought, Salinity and Temperature Stress at the Seedling Stage. *J. Plant Physiol.* 143: 241–244. [DOI: 10.1016/S0176-1617(11)81694-9]

Mavi K, Demir I, Matthews S (2010): Mean germination time estimates the relative emergence of seed lots of three cucurbit crops under stress conditions. *Seed Sci. Technol.* 38: 14–25. [DOI: 10.15258/sst.2010.38.1.02]

Michel BE, Kaufmann MR (1973): The Osmotic Potential of Polyethylene Glycol 6000. *Plant Physiol.* 51: 914–916. [DOI: 10.1104/pp.51.5.914]

Okçu G, Kaya MD, Atak M (2005): Effects of Salt and Drought Stresses on Germination and Seedling Growth of Pea (*Pisum sativum* L.). *Turk. J. Agric. For.* 29: 237–242.

Petrović G, Jovičić D, Nikolić Z, Tamindžić G, Ignjatov M, Milošević D, Milošević B (2016): Comparative study of drought and salt stress effects on germination and seedling growth of pea. *Genetika* 48: 373–381. [DOI: 10.2298/GENSR1601373P]

Sakthivelu G, Devi MKA, Giridhar P, Rajasekaran T, Ravishankar GA, Nedev T, Kosturkova G (2008): Drought-induced alterations in growth, osmotic potential and in vitro regeneration of soybean cultivars. *Gen. Appl. Plant Physiol.* 34 (Special Issue): 103–112.

Silva EN, Ribeiro RV, Ferreira-Silva SL, Viégas RA, Silveira JAG (2010): Comparative effects of salinity and water stress on photosynthesis, water relations and growth of *Jatropha curcas* plants. *J. Arid Environ.* 74: 1130–1137. [10.1016/j.jaridenv.2010.05.036]

Smýkal P, Aubert G, Burstin J, Coyne CJ, Ellis NTH, Flavell AJ, Ford R, Hýbl M, Macas J, Neumann P, McPhee KE, Redden RJ, Rubiales D, Weller JL, Warkentin TD (2012): Pea (*Pisum sativum* L.) in the genomic era. *Agron.* 2: 74–115. [10.3390/agronomy2020074]

Soltani E, Ghaderi-Far F, Baskin CC, Baskin JM (2016): Problems with using mean germination time to calculate rate of seed germination. *Aust. J. Bot.* 63: 631–635. [DOI: 10.1071/BT15133]

Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu JK (2006): Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant J.* 45: 523–539. [10.1111/j.1365-313X.2005.02593.x]

Vujaković M, Balešević-Tubić S, Jovičić D, Taški-Ajduković K, Petrović D, Nikolić Z, Đorđević V (2011): Viability of soybean seed produced under different agro-meteorological conditions in Vojvodina. *Genetika* 43: 625–638. [DOI: 10.2298/GENSR1103625V]

УТИЦАЈ СТРЕСА СУШЕ НА КЛИЈАЊЕ И ПОРАСТ ПОНИКА РАЗЛИЧИТИХ ГЕНОТИПОВА СТОЧНОГ ГРАШКА

Гордана Р. ПЕТРОВИЋ[1], Томислав Т. ЖИВАНОВИЋ[2],
Радмила И. СТИКИЋ[2], Зорица Т. НИКОЛИЋ[1], Душица Д. ЈОВИЧИЋ[1],
Гордана Д. ТАМИНЏИЋ[1], Драгана Н. МИЛОШЕВИЋ[1]

[1] Институт за ратарство и повртарство, Лабораторија за испитивање семена,
Максима Горког 30, Нови Сад 21000, Србија
[2] Универзитет у Београду, Пољопривредни факултет,
Катедра за агрохемију и физиологију биљака,
Немањина 6, Београд-Земун 11080, Србија

РЕЗИМЕ: У раду је проучавано клијање и пораст поника седам генотипова сточног грашка (*Pisum sativum* L.) у раствору PEG-6000 са вредностима осмотских потенцијала од -0,1 и -0,2 MPa. Испитивање је извршено како би се утврдио утицај различитих осмотских потенцијала (MPa) на проценат клијавости семена (GP) и просечно време клијања (MGT). Резултати показују да се проценат клијавости смањивао упоредо са смањењем осмотског потенцијала, док се просечно време клијања повећавало. Сорта „Јавор" много је осетљивија од осталих сорти при свим нивоима осмотског стреса. Супротно томе, најмањи утицај на клијање и MGT утврђен је код сорти „Мраз" и „Трезор". Одређивање клијавости семена у растворима PEG од -0,1 до -0,2 MPa може се користити за тестирање сточног грашка. Изложеност осмотском стресу и његово трајање значајно су утицали на раст поника (изданак и корен) и акумулацију биомасе, при чему је његов утицај био израженији на раст изданка него на раст корена, што је потврдио и индекс толерантности корена.

КЉУЧНЕ РЕЧИ: *Pisum sativum*, семе, клијанац, сорте, индекс толерантности

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 71—84, 2021

UDC 579.81(497.11 Petnička pećina)
https://doi.org/10.2298/ZMSPN2140071P
ORIGINAL ARTICLE

Slađana S. POPOVIĆ[1,*], *Kristina M. PETROVIĆ*[2], *Dušica S. TRNAVAC-BOGDANOVIĆ*[3], *Dragana L. MILOŠEVIĆ*[1] *Ana D. GRAOVAC*[2], *Ivana S. TRBOJEVIĆ*[2], *Gordana V. SUBAKOV-SIMIĆ*[2]

[1] University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Center for Ecology and Technoeconomics, Njegoševa 12, Belgrade 11000, Serbia
[2] University of Belgrade, Faculty of Biology, Studentski trg 16, Belgrade 11000, Serbia
[3] University of Belgrade, Faculty of Geography, Studentski trg 3, Belgrade 11000, Serbia

CYANOBACTERIA AND ALGAE FROM BIOFILM AT THE ENTRANCE ZONE OF PETNICA CAVE

ABSTRACT: The importance of biofilms in caves, the diversity of microorganisms in them, their mutual relationship and relationship with the substratum are among the advancing research topics in microbial biospeleology. This research is making contribution to the knowledge about biofilms at cave entrances and phototrophic communities in them. In that manner, biofilms from the entrance zone of the Petnica Cave were examined. Light microscopy showed that cyanobacteria were exclusively dominant phototrophs (34 taxa out of 39 total taxa recorded) with coccoid forms prevailing (28 taxa); simple trichal forms were present to a lesser extent, while heterocytous ones were completely absent. Genera *Gloeocapsa, Chroococcus*,*Gloeothece* and *Leptolyngbya* were the most diverse. Four green algal genera characteristic for aerophytic habitats (*Apatococcus*, *Desmococcus*, *Haematococcus* and *Trentepohlia*) were also recorded, while Bacillariophyta were observed sporadically. Three groups of sampling sites were distinguished based on recorded taxa, their richness and similarity, using non-metric multidimensional scaling (NMDS). Quantitative biofilm characteristics were also assessed – the content of chlorophyll *a* (Chl *a*) was determined, as well as the contents of water, organic and inorganic matter. Chl *a* had a significant positive correlation with the content of organic matter (r=0.904, P=0.013).

KEYWORDS: aerophytic phototrophs, algae, biofilm, cyanobacteria, Petnica Cave

[*] Corresponding author. E-mail: spopovic.bio@gmail.com

INTRODUCTION

Numerous different cavities are present widely underneath the Earth's surface, with many of them being solutional – karst caves which usually occur in limestone. Those are so far the most frequently studied types. Even though many caves are explored worldwide so far, it still is a small part of the present underground passages (Klimchouk, 2004). Cave entrance habitats are a part of caves characterized by the presence of daylight and may be considered as ecotones between the surface and dark cave habitats. The depth to which light penetrates, considering cave morphology, depends on the shape, position or width of the cave opening (Sket, 2004). For caves with large entrances, the light intensity reduces 1% or less per meter toward the interior, while in caves characterized with extremely small entrances (<1 m), almost complete darkness can ensue only after 1 m from the entrance (Pentecost, 2004). Here, at cave entrances, due to the presence of light, phototrophs are present (Sket, 2004). Different photosynthetic species may differently tolerate lack of light, while cyanobacteria and algae may have some adaptations to low light intensities and some can grow on rock walls, even if the only source of water is condensation (Pentecost, 2004).

Cyanobacteria and algae in caves are often found forming biofilms with other microorganisms/organisms. The variety of biofilms in caves can be quite high, and it seems that they are "unusually diverse compared to those found on the surface" (Boston et al., 2001 cited in Boston, 2004). Since they help in the survival and growth of microorganisms and their protection, it is even suggested that the primary evolutionary units are not the organisms incorporated in biofilms, but biofilms themselves (Boston, 2004).

Cyanobacteria are quantitatively among the most important organisms on Earth: they had a key role in the oxygenation of the atmosphere in the past and today. Their ability to detect and respond to variations in the environment is of key importance for their success and their presence in a diverse range of habitats (Whitton and Potts, 2012) and among them, in caves (Albertano, 2012). They are more adaptable compared to eukaryotic algae and some of the adaptations to subaerophytic life are a better tolerance of desiccation and water stress (Whitton and Potts, 2012). The cyanobacterial community inside caves, unlike those inhabiting outside habitat, is influenced by the characteristics of this somehow confined environment and it is mostly influenced by quality and quantity of light and air humidity (Albertano, 2012). They are resistant to different conditions in caves, including darkness (Czerwik-Marcinkowska and Massalski, 2018).

The aim of this research was to explore the diversity of phototrophic microorganisms from the entrance zone of Petnica Cave.

MATERIALS AND METHODS

Sampling location

Petnica Cave is situated southeast of the town of Valjevo, near Petnica village. The entrances to the cave lie on the northern slope of the Osoj hill in the village of Petnica, at the junction of the hill area and Valjevo and the Kolubara valley. River Banja springs from the Cave and flows into the river Kolubara (Figure 1).

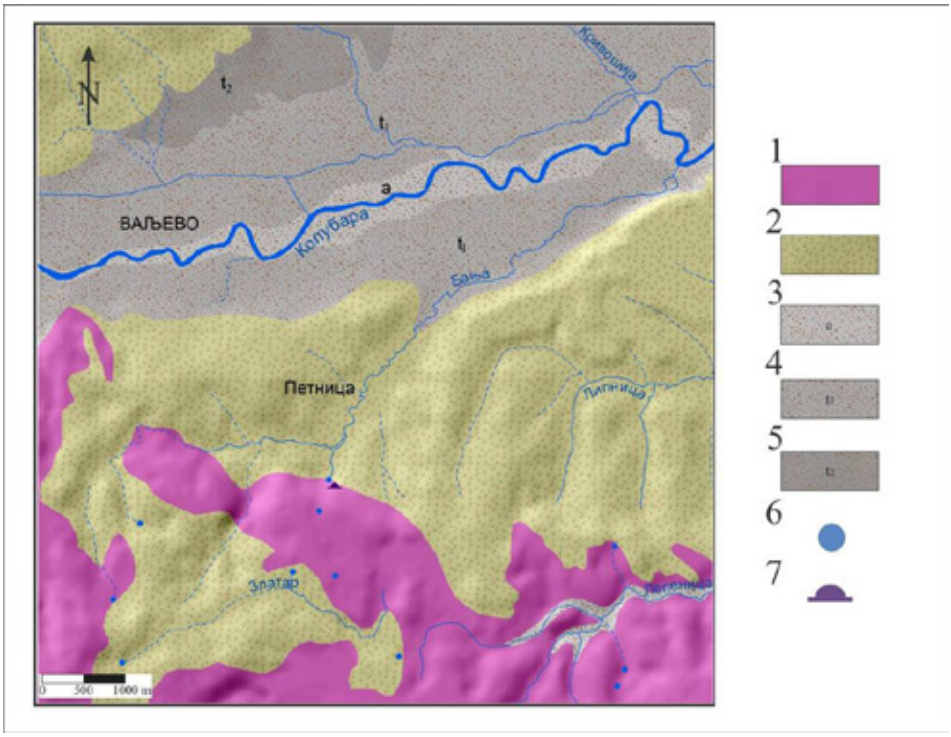


Figure 1. Relief, hydrology and geology of the wider surroundings of Petnica Cave according to geomorphological information system – ArcMap. 1 – carbonate rocks; 2 – Miocene marly-clay sediments; 3 – alluvium; 4,5 – river terraces; 6 – springs; 7 – cave.

Petnica Cave consists of two main parts: Donja pećina (lower cave), which is hydrologically active and Gornja pećina (upper cave), a dry part of the cave characterized with the occasionally present seeping water. They are connected and the total length of canals is 580 m. The entrance of the lower cave has a triangle shape, 14 m wide at the basis and 9 m high, while the entrance of the upper cave is considerably smaller. Due to large openings, external temperature and air humidity influence cave conditions at the entrance and near the

entrance (Đurović, 1998; Lazarević, 1988). The sampling was performed at the entrance of the lower cave.

Petnica Cave is the oldest researched and the most famous cave in the Valjevo karst. It was first protected as an archeological site, and then it was declared a natural monument, as a speleological object and geoheritage site and is among the ten speleological monuments of nature in Western Serbia. A protection zone of 2.8 ha was established around the cave in 1969, while the latest protection study (2012) proposes to protect an area of 8.10 ha (Group of authors, 2012). Also, spring Banja ("Spring Petnica") is an extremely rare hydrological phenomenon, which, according to Simić (2008) belongs to the first groups of hydrological heritage objects of Serbia. The cave itself has so far been infrastructurally arranged and equipped for the needs of the tourist cave three times, but the number of visitors has never been enough for this function to be established. For long it has been the subject of different scientific research and educational activities (Group of authors, 2012).

Sampling sites and sampling procedure

Signs of the presence of phototrophic microorganisms were visible on the walls of the entire entrance zone of the lower level of Petnica Cave. Six sam-

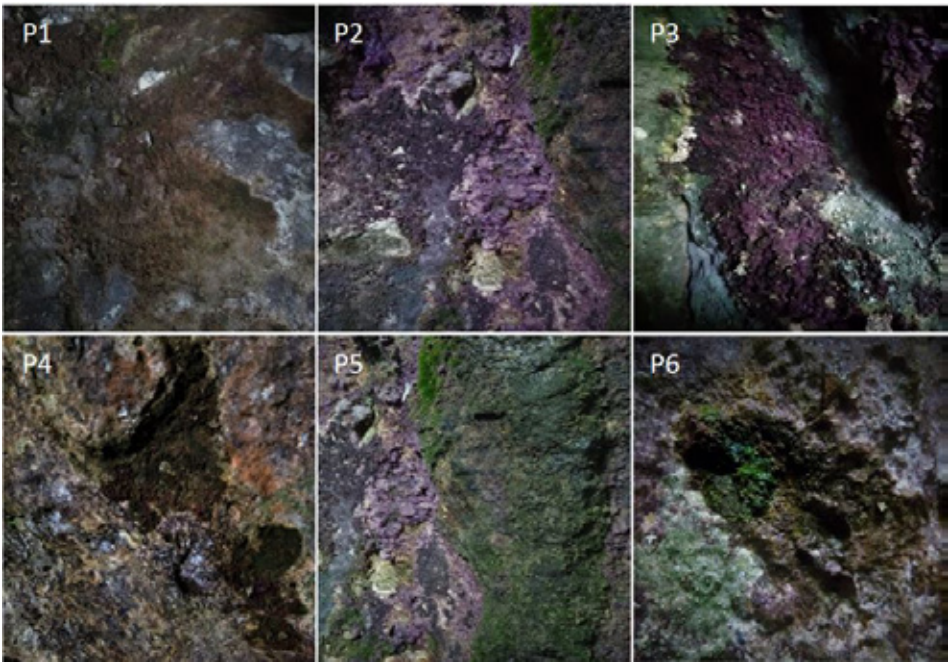


Figure 2. Sampling sites in Petnica Cave: P1) brownish coloured biofilm; P2) light purple gelatinous biofilm; P3) dark purple gelatinous biofilm, P4) brown coloured biofilm; P5) light green coloured biofilm; P6) dark green coloured biofilm.

pling sites (P1–P6) showing variously coloured biofilms, which photographs are shown in Figure 2, are chosen for biofilm sampling. All sampling sites were located, at the distance of approximately 20 m from the entrance of the cave (on the cave wall opposite to the cave entrance), but at different heights from the ground: P1 – 1.4 m, P2 – 1.4 m, P3 – 1.4 m, P4 – 1.65 m, P5 – 1.65 m and P6 – 1.7 m.

Biofilm samples for algological analyses were sampled as described in Popović et al. (2017). A flame sterilized scalpel was used to collect biofilm directly from the rock substratum, by gently scraping the material from the stone surface. The samples of biofilm were stored in labeled sterile polyethylene bags and as such transported to the laboratory for further processing – making of slides for microscopical analysis and identification of cyanobacteria and algae.

Algological analyses

For microscopical analyses of phototrophic microorganisms, two kinds of slides were made: temporarily wet mount slide (a piece of biofilm mixed with water) and semi permanent slide (a piece of biofilm mixed with glycerine). The slides were observed using the light microscope Zeiss Axio-Imager M.1 with software AxioVision 4.8. The identification of cyanobacteria and algae was performed by using the standard literature: John et al. (2003), Komárek and Anagnostidis (1998; 2005) and Starmach (1972).

Determination of chlorophyll *a*, water content, organic and inorganic matter in biofilms

Biofilm was sampled from a certain surface of 3.8 cm^2 using the round matrix as described in Popović et al. (2017), both for the determination of chlorophyll *a* (Chl *a*) content, as well as for biofilm parameters – the content of water, organic and inorganic matter. Biofilm was kept in sterile polyethylene bags until arrival in the laboratory. Chl *a* extraction and calculation were done as described in Popović et al. (2015), after which this parameter was expressed per surface area, as μg Chl *a* cm^{-2}. The content of water, organic and inorganic matter were determined based on the difference between fresh sample weight and weights after drying at 105 °C and ashing at 550 °C (Popović et al., 2017) and were expressed in percentages.

Data analysis

Correlations (Pearson coefficient) between Chl *a* and biofilm parameters were demonstrated using the statistical package XLSTAT (Addinsoft, 2020). Considering sampling sites, non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance was done to see potential similarity/dissimilarity

between them, based on documented cyanobacterial and algal taxa. For this purpose, the Canoco 5 Software Package (Ter Braak and Šmilauer, 2012) was used.

RESULTS AND DISCUSSION

In the aerophytic phototrophic community from the entrance zone of Petnica Cave, a total of 39 cyanobacterial and algal taxa was documented (Table 1). Cyanobacteria prevailed with 34 taxa, but algae belonging to Chlorophyta were also found, though in a lesser extent. Bacillariophyta were spotted too. The dominant Cyanobacteria were coccoid forms with 28 identified representatives (genus or species level) from 12 genera. However, simple trichal forms of Cyanobacteria were recorded too, but heterocytous ones were completely absent at these sampling sites. The most diverse cyanobacterial genera were *Gloeocapsa, Chroococcus* and *Gloeothece* considering coccoid forms and *Leptolyngbya* considering simple trichal forms. *Gloeobacter violasceus* was documented on all sampling sites, while *Chroococcus ercegovicii, Gloeocapsa biformis* and *G.nigrescens* were found on five. The only green algal representatives that were recorded in biofilm samples were members of *Apatococcus*, *Desmococcus*, *Haematococcus* and *Trentepohlia* genera. Few Bacillariophyta were spotted sporadically in biofilm. Some cyanobacterial representatives are shown in Figure 3.

Table 1. List of cyanobacterial and algal taxa from biofilm samples from the entrance of Petnica Cave.

	P1	P2	P3	P4	P5	P6
Cyanobacteria						
Coccoid						
Aphanocapsa fusco-lutea Hansgirg					+	+
Aphanothece saxicola Nägeli				+		
Aphanothece sp. Nägeli					+	
Asterocapsa sp. H.-J.Chu				+	+	+
Chondrocystis dermochroa (Nägeli ex Kützing) Komárek & Anagnostidis			+			
Chroococcidiopsis sp. Geitler						+
Chroococcus cohaerens (Brébisson) Nägeli	+					+
Chroococcus ercegovicii Komárek & Anagnostidis	+	+	+	+	+	
Chroococcus sp. (Kützing) Nägeli	+					
Chroococcus tenax (Kirchner) Hieronymus					+	+
Chroococcus turgidus (Kützing) Nägeli				+	+	+
Gloeobacter violasceus Rippka, J. B. Waterbury & Cohen-Bazire	+	+	+	+	+	+
Gloeocapsa aeruginosa Kützing			+			+
Gloeocapsa atrata Kützing	+			+	+	+

Gloeocapsa biformis Ercegovic	+		+	+	+	+
Gloeocapsa cf. *alpina* Nägeli				+		
Gloeocapsa nigrescens Nägeli	+	+		+	+	+
Gloeocapsa novacekii Komárek & Anagnostidis				+		
Gloeocapsa punctata Nägeli			+			+
Gloeocapsa reicheltii P.G.Richter				+		
Gloeocapsa sp. Kützing		+				
Gloeocapsopsis sp. Geitler ex Komárek					+	+
Gloeothece cf. *palea* (Kützing) Nägeli				+	+	+
Gloeothece confluens Nägeli				+		
Gloeothece rupestris (Lyngbye) Bornet				+	+	+
Gloeothece sp. Nägeli				+		
Pseudocapsa dubia Ercegovic	+				+	
Synechococcus cf. *elongatus* (Nägeli) Nägeli			+			
Unidentified coccoid Cyanobacteria			+	+	+	
Simple trichal						
Leptolyngbya foveolarum (Gomont) Anagnostidis & Komárek						+
Leptolyngbya perforans (Geitler) Anagnostidis & Komárek			+	+	+	
Leptolyngbya spp. Anagnostidis & Komárek	+		+	+	+	
Tapinothrix bornetii Sauvageau						+
Wolskyella cf. *floridana* Maresš & Kaštovský			+			+
Chlorophyta						
Apatococcus lobatus (Chodat) J.B.Petersen					+	
Desmococcus olivaceus (Persoon ex Acharius) J. R. Laundon					+	+
Haematococcus pluvialis Flotow					+	+
Trentepohlia aurea (Linnaeus) C.Martius				+		
Bacillariophyta						
Unidentified Bacillariophyta					+	+

Some data considering phototrophs from cave entrances in Serbia that are published so far indicate similar findings as in Petnica Cave (Popović et al., 2015; 2017; 2019; 2020). Cyanobacteria, more precisely coccoid forms were, for example, dominant in Božana Cave (Popović et al., 2015), Vernjikica and Degurić caves (Popović et al., 2019), Samar and Jezava caves (Popović et al., 2020). In majority, *Gloeocapsa* and *Chroococcus* were recognized as the most diverse genera. Genus *Gloeocapsa* was recognized as the most diverse genera when three caves in western Serbia were explored (Popović et al., 2017). In general genus *Gloeocapsa* is reported from various habitats characterized with variable ecological characteristics, which indicate its tolerance to a wide specter of environmental conditions (Cennamo et al., 2012).

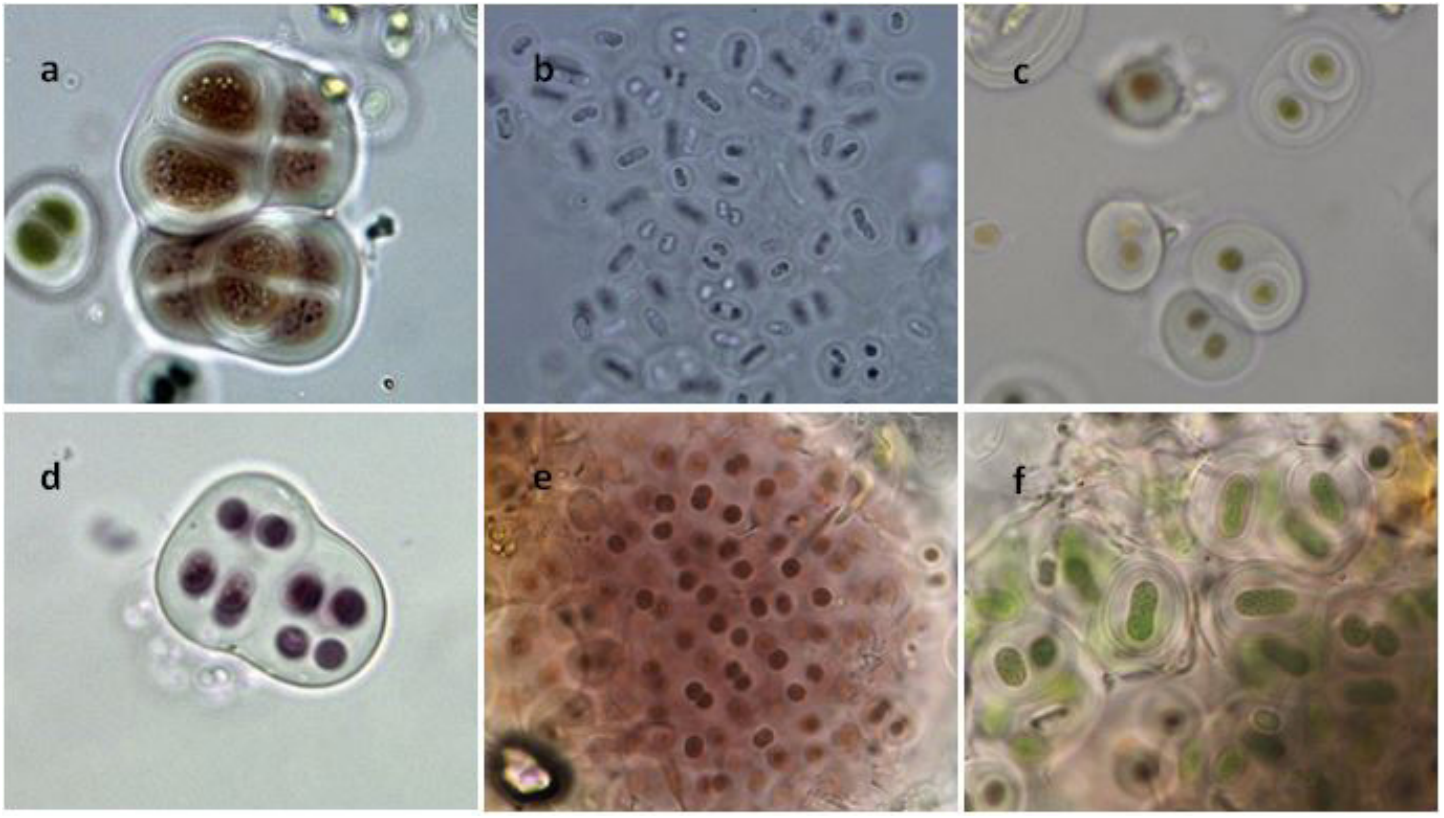


Figure 3. Example of some cyanobacterial representatives recorded in biofilm samples from Petnica Cave. a – *Chroococcus tenax*; b – *Gloeobacter violasceus*; c – *Gloeocapsa atrata*; d – *Gloeocapsa nigrescens*; e – *Gloeocapsa novacekii*; f – *Gloeothece* cf. *palea*

According to Czerwik-Marcinkowska and Massalski (2018), caves represent biodiversity centers for different types of microorganisms, especially for cyanobacteria, which can be very widespread inhabitants of rock surfaces in caves (Albertano, 2012), often making the main part of the phototrophic community in biofilms. Coccoid cyanobacterial representatives are frequent inhabitants of subaerophytic surfaces due to their simplicity and are in general better adapted to lower light conditions since they tolerate low irradiance more easily and thus often can be dominant (Mulec et al., 2008 cited in Popović et al., 2015). The presence of coccoid forms is even seen in fossils where they range from "isolated single cells, not uncommonly enveloped by multilamellar sheaths, to pairs or quartets of sheath-enveloped or sheath-lacking spheroidal cells" or "occur in large aggregates of geometrically ordered or irregularly distributed close-packed colonial cells" (Schopf, 2012) indicating that they were present many time ago and have time to adapt to various kinds of environment.

Considering Chlorophyta, *Desmococcus* was most frequently reported in aerophytic habitats in general (Lopez-Bautista et al. 2007).

Biofilm parameters (the content of water, organic and inorganic matter), together with the concentration of Chl *a*, are demonstrated in Figure 4. Considering the water content in biofilm samples, the highest was determined in the biofilm from sampling site P3. Water content was higher than 40% in all other samples too, except in biofilm from P5. On the other hand, P5 stands out as the sampling site where the highest content of organic matter was determined. The inorganic matter was highest at P1, and lowest at P6. In the time of sampling, it was noticed that biofilms at the selected sites at the cave entrance were well hydrated, which contributed to higher content in water in almost all samples. However, according to Czerwik-Marcinkowska and Massalski (2018), most European caves are moist and their walls at the entrance zone are frequently covered with cyanobacterial dominated biofilms (Pouličkova and Hašler, 2007). Cyanobacterial dominated biofilms are rich in extracellular polymeric substances (EPS) that are is secreted by the microorganisms as the glycocalyx, sheath or envelope and which one of the main role is water retaining (Albertano, 2012).

Considering the determined values of Chl *a*, the lowest was determined in biofilm taken from P1, and the highest in the biofilm from P5. In general, the highest recorded value of Chl *a* coincides with the highest determined value of organic matter. By observing correlation coefficients (Table 2), Chl *a* was significantly positively correlated with the content of organic matter ($r=0.904$, $P=0.013$) in Petnica Cave. As reported by Popović et al. (2017), the highest Chl *a* was also determined on a sampling site where the highest content of organic matter was recorded. However, it happens very often that a positive correlation between these two parameters is not observed, when organic matter in biofilms originate not exclusively from phototrophic organisms, but also from non-photosynthetic ones (bacteria, fungi, different organic particles that are deposited from air, dust, water, etc.).

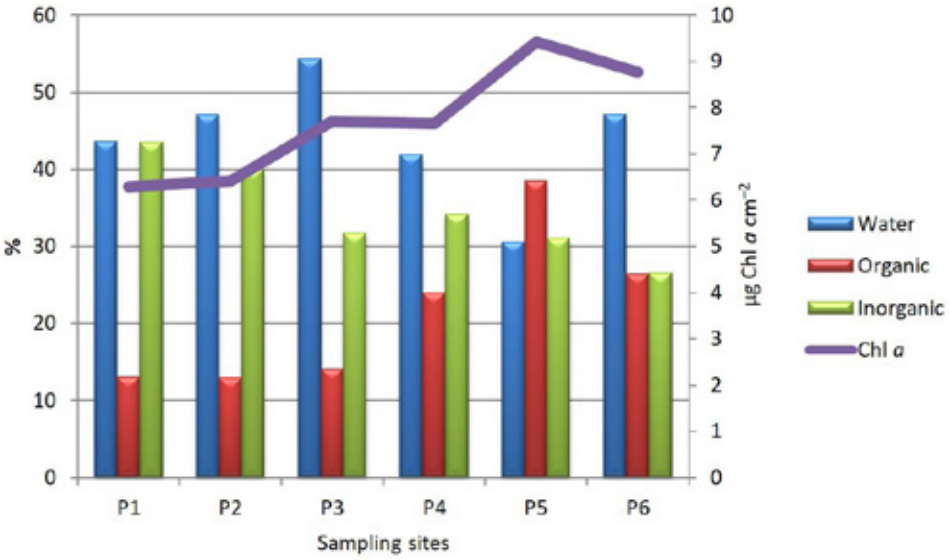


Figure 4. Chl *a* concentration (secondary axis), the content of water, organic and inorganic matter (primary axis) in biofilm samples from the entrance of Petnica Cave (P1–6, sampling sites).

Table 2. Correlation matrix between Chl *a* and biofilm parameters (the content of water, organic and inorganic matter in biofilm) showing correlation coefficients and significance

Correlation matrix (Pearson):				
Variables	Chl *a*	Water (%)	Organic (%)	Inorganic (%)
Chl *a*	1	-0.472	**0.904**	**-0.881**
Water (%)	-0.472	1	-0.791	0.027
Organic (%)	**0.904**	-0.791	1	-0.633
Inorganic (%)	**-0.881**	0.027	-0.633	1
Values in bold are different from 0 with a significance level alpha=0.05				
p-values (Pearson):				
Variables	Chl *a*	Water (%)	Organic (%)	Inorganic (%)
Chl *a*	0	0.344	0.013	0.021
Water (%)	0.344	0	0.061	0.959
Organic (%)	0.013	0.061	0	0.178
Inorganic (%)	0.021	0.959	0.178	0

In this study, we also wanted to represent which sampling sites were the most similar to each other, based on the species composition. Considering NMDS (Figure 5), three groups of sampling sites are distinguished on the ordination diagram. The first group is composed of P1 and P2 sites, and the second group of P4, P5 and P6 sites. The sites within the same group are mutually similar. However, the most distant of all, in other words, the most dissimilar of others

is P3 (upper part of the ordination diagram). P1 and P2 contain a lower number of recorded taxa compared to other sites and the majority of them (almost all) belong to coccoid Cyanobacteria. On the other hand, P4, P5 and P6 are very rich in recorded taxa: at all sites, coccoid, simple trichal Cyanobacteria and Chlorophyta are recorded. P5 and P6 (very close to each other on the ordination diagram) are additionally characterized with the presence of Bacillariophyta. Sampling site P3 is the most distant from others because the number of recorded taxa is somewhere between the previously mentioned two groups and at this site only Cyanobacteria are recorded – coccoid, as well as simple trichal representatives. This sampling site was also characterized with the highest content of water in biofilm (Figure 4).

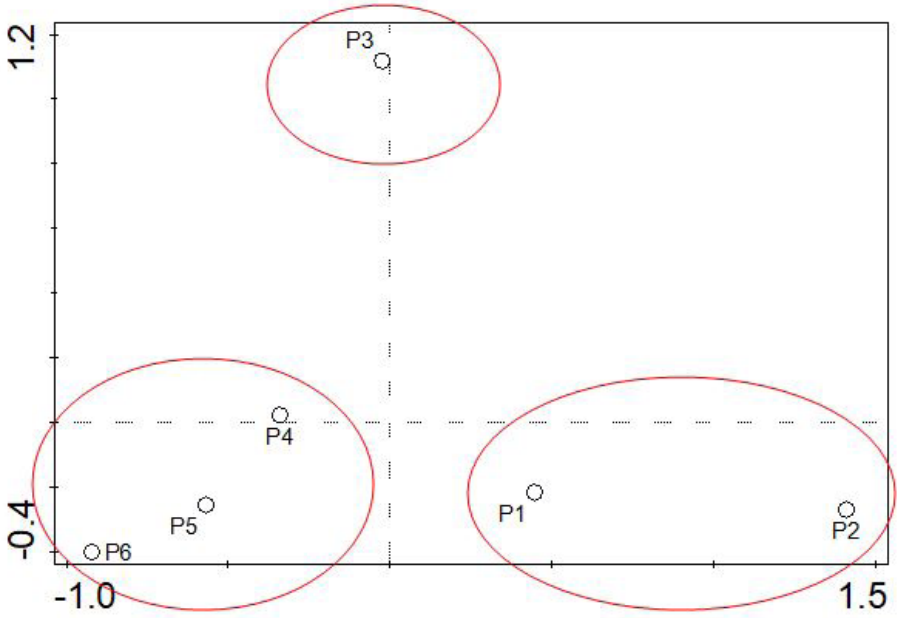


Figure 5. Non-metric multidimensional scaling (NMDS) showing similarity/dissimilarity between the sampling sites P1–6 in Petnica Cave based on recorded cyanobacterial and algal taxa.

CONCLUSIONS

The scientific value of caves due to the development of many different research fields related to them increased during recent decades and many caves are due to their unique characteristics recognized as protected sites. Research of phototrophs can be of greater importance due to their conservation. Phototrophic microorganisms, cyanobacteria and algae, were explored in biofilm samples collected at the entrance zone of Petnica Cave. Cyanobacteria were

exclusively dominant, but Chlorophyta and Bacillariophyta were present too, though to a lesser extent. Coccoid Cyanobacteria prevailed where genera *Gloeocapsa, Chroococcus* and *Gloeothece* were the most diverse. Simple trichal representatives were also present, but heterocytous ones were completely absent from examined samples. By non-metric multidimensional scaling (NMDS) potential similarity/dissimilarity was observed between sampling sites based on recorded taxa. Three groups of sites were distinguished, one with the lower number of recorded taxa (consisting of almost only coccoid Cyanobacteria), second with a high number of recorded taxa (all divisions present) and third, made of only one site, was characterized with the presence of all cyanobacterial groups. Determined Chl *a* was significantly positively correlated with the content of organic matter.

ACKNOWLEDGMENTS

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2021-14/200026 and Grant No. 451-03-9/2021-14/ 200178).

REFERENCES

Addinsoft (2020): XLSTAT statistical and data analysis solution. New York, USA. Available from: https://www.xlstat.com.

Albertano P (2012): Cyanobacterial biofilms in monuments and caves. In: BA Whitton (ed), *Ecology of Cyanobacteria II: their diversity in space and time*, Springer, Netherlands.

Boston PJ, Spilde MN, Northup DE, Melim LA, Soroka DS, Kleina LG, Lavoie KH, Hose LD, Mallory LM, Dahm CN, Crossey LJ, Schelble RT (2001): Cave biosignature suites: Microbes, minerals and Mars. *Astrobiology J.* 1: 25–55.

Boston P (2004): Biofilms. In: J. Gunn (ed), *Encyclopedia of Caves and Karst Sciences*, Fitzroy Dearborn, An imprint of the Taylor and Francis Group, London.

Cennamo P, Marzano C, Ciniglia C, Pinto G, Cappelletti P, Caputo P, Pollio A (2012): A survey of the algal flora of anthropogenic caves of Campi Flegrei (Naples, Italy) archeological district. *J. Caves Karst Stud.* 74: 243–250.

Czerwik-Marcinkowska J, Massalski A (2018): Diversity of Cyanobacteria on Limestone Caves, Cyanobacteria, Archana Tiwari, IntechOpen. Available from: https://www.intechopen.com/books/cyanobacteria/diversity-of-cyanobacteria-on-limestone-caves

Đurović P (1998): *Speleološki atlas Srbije*. SANU, Geografski institut "Jovan Cvijić", Zavod za zaštitu prirode Srbije; Univerzitet u Beograd: Geografski fakultet i Biološki fakultet, Beograd.

Group of authors (2012): Studija zaštite: Spomenik prirode Petnička pećina. Zavod za zaštitu prirode Srbije, Beograd.

John DM, Whitton BA, Brook AJ (2003): *The freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae*. Cambridge University Press, UK.

Klimchouk A (2004): Caves. In: J. Gunn (ed), *Encyclopedia of Caves and Karst Sciences*, Fitzroy Dearborn, An imprint of the Taylor and Francis Group, London.

Komárek J, Anagnostidis K (1998): Cyanoprokaryota 1. Teil/1st Part: Chroococcales. In: H. Ettl, G. Gärtner, H. Heynig, D. Mollenhauer (eds), *Süswasserflora von Mitteleuropa* 19/1, Jena-Stuttgart-Lübeck-Ulm: Gustav Fischer.

Komárek J, Anagnostidis K (2005): Cyanoprokaryota 2. Teil: Oscillatoriales. In: H. Ettl, G. Gärtner, H. Heynig, D. Mollenhauer (eds), *Süswasserflora von Mitteleuropa*, 19/2, Berlin: Spektrum Akademischer Verlag.

Lazarević P (1988): *Petnička pećina*. Valjevo: Turistički savez opštine Valjevo.

Lopez-Bautista JM, Rindi F, Casamatta D (2007): The systematic of subaerial algae. In: J. Seckbach (ed), *Algae and Cyanobacteria in Extreme Environments*, Dordrecht: Springer.

Mulec J, Kosi G, Vrhovšek D (2008): Characterization of cave aerophytic algal communities and effects of irradiance levels on production of pigments. *J. Caves Karst Stud.* 70: 3–12.

Pentecost A (2004): Entrance habitats. In: J. Gunn (ed.), *Encyclopedia of Caves and Karst Sciences*, Fitzroy Dearborn, London: An imprint of the Taylor and Francis Group.

Popović S, Subakov Simić G, Stupar M, Unković N, Predojević D, Jovanović J, Ljaljević Grbić M (2015): Cyanobacteria, algae and microfungi present in biofilm from Božana Cave (Serbia). *Int. J. Speleol.* 44: 141–149.

Popović S, Subakov Simić G, Stupar M, Unković N, Krunić O, Savić N, Ljaljević Grbić M (2017): Cave biofilms: characterization of phototrophic cyanobacteria and algae and chemotrophic fungi from three caves in Serbia. *J. Caves Karst Stud.* 79: 10–23.

Popović S, Nikolić N, Jovanović J, Predojević D, Trbojević I, Manić Lj, Subakov Simić G (2019): Cyanobacterial and algal abundance and biomass in cave biofilms and relation to environmental and biofilm parameters. *Int. J. Speleol.* 48: 49–61.

Popović S, Krizmanić J, Vidaković D, Jakovljević O, Trbojević I, Predojević D, Vidović M, Subakov Simić G (2020): Seasonal dynamics of cyanobacteria and algae in biofilm from the entrance of two caves. *Geomicrobiol.* 37: 315–326.

Pouličková A, Hašler P (2007): Aerophytic diatoms from caves in Central Moravia (Czech Republic). *Preslia* 79: 185–204.

Schopf JW (2012): The fossil record of Cyanobacteria. In: BA Whitton (ed), *Ecology of Cyanobacteria II: their diversity in space and time*. Springer, Netherlands.

Simić S (2008): Vode Valjevske Kolubare – integralni deo zaštićenih prirodnih dobara, *Zaštita prirode* 58: 53–70.

Sket B (2004): Subterranean habitats. In: Gunn J (ed), *Encyclopedia of Caves and Karst Sciences*, Fitzroy Dearborn, London: An imprint of the Taylor and Francis Group.

Starmach K (1972): Chlorophyta III. Zielenice nitkowate: Ulotrichales, Ulvales, Prasiolales, Sphaeropleales, Cladophorales, Trentepohliales, Sipholales, Dichotomosiphonales: Warszawa and Krakow, Panstwowe Wyadwnictwo Naukowe, In: *Flora Slodkowodna Polski* (K. Starmach, J. Sieminska, Eds), Tom 10, 750 p.

Ter Braak CJF, Šmilauer P (2012): Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer Power, Ithaca, USA.

Whitton BA, Potts M (2012): Introduction to the Cyanobacteria. In: BA Whitton (ed.), *Ecology of Cyanobacteria II*, Their Diversity in Space and Time. Springer, Netherlands.

ЦИЈАНОБАКТЕРИЈЕ И АЛГЕ ИЗ БИОФИЛМА СА УЛАЗНЕ ЗОНЕ ПЕТНИЧКЕ ПЕЋИНЕ

Слађана С. ПОПОВИЋ[1], Кристина М. ПЕТРОВИЋ[2], Душица С. ТРНАВАЦ-БОГДАНОВИЋ[3], Драгана Л. МИЛОШЕВИЋ[1], Ана Д. ГРАОВАЦ[2], Ивана С. ТРБОЈЕВИЋ[2], Гордана В. СУБАКОВ-СИМИЋ[2]

[1] 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
[2] Универзитет у Београду, Биолошки факултет, Студентски трг 16, Београд 11000, Србија
[3] Универзитет у Београду, Географски факултет, Студентски трг 3, Београд 11000, Србија

РЕЗИМЕ: Значај биофилмова у пећинама, разноврсност микроорганизама у њима, њихов међусобни однос, као и однос са супстратом, спадају у водеће теме истраживања у пољу микробијалне биоспелеологије. Ово истраживање доприноси општем познавању биофилмова на улазима пећина, као и познавању фототрофних заједница које се налазе у њиховом саставу. Имајући то у виду, испитивани су биофилмови са улазне зоне Петничке пећине. Светлосна микроскопија је показала да су цијанобактерије искључиво доминантни фототрофи (забележено је 34 од укупно 39 таксона), са изузетном доминацијом кокоидних форми (28 таксона); трихалне форме (хомоцитни представници) су у биофилму заступљене у мањој мери, док су хетероцитне потпуно одсутне. У родовима *Gloeocapsa*, *Chroococcus*, *Gloeothece* и *Leptolyngbya* документован је највећи број таксона. Четири рода зелених алги која су иначе карактеристична за аерофитска станишта (*Apatococcus*, *Desmococcus*, *Haematococcus* и *Trentepohlia*) су такође забележена, док су представници раздела Bacillariophyta нађени спорадично. Статистичка анализа NMDS је показала да су се према сличности, односно на основу таксона који су идентификовани, одвојиле три групе тачака узорковања. Квантитативне карактеристике биофилма су такође процењене – одређен је садржај хлорофила *a* (Chl *a*), као и садржај воде, органске и неорганске материје. Значајна позитивна корелација ($r=0.904$, $P=0.013$) уочена је између садржаја органске материје и Chl *a*.

КЉУЧНЕ РЕЧИ: аерофитски фототрофи, алге, биофилм, цијанобактерије, Петничка пећина

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 85—101, 2021

UDC 628.161.1(497.113)
https://doi.org/10.2298/ZMSPN2140085M
ORIGINAL ARTICLE

Stanko B. MILIĆ[1], Dušana D. BANjAC[1], Jovica R. VASIN[1], Jordana M. NINKOV[1], Borivoj S. PEJIĆ[2], Ivana B. BAJIĆ[1], Branka Lj. MIJIĆ[1]*

[1] Institute of Field and Vegetable Crops,
National Institute of the Republic of Serbia,
Maksima Gorkog 30, Novi Sad 21000, Serbia
[2] University of Novi Sad, Faculty of Agriculture,
Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia

ASSESSMENT OF IRRIGATION WATER QUALITY AT THE TERRITORY OF VOJVODINA PROVINCE (SERBIA)

ABSTRACT: Intensive crop cultivation systems require continuous monitoring of irrigation water quality as well as the control of physical and chemical soil properties. In view of the ongoing climate change and a dramatic decrease in soil organic matter content, the use of low quality irrigation water and its adverse effects on soil, cultivated plants and irrigation equipment must not be overlooked. The aim of this paper was to evaluate general quality of irrigation water from the different water intake sources in the Vojvodina Province. The paper presents the results of irrigation water quality, collected during 2018 and 2019. The research included 140 irrigation water samples obtained from three different intake structures which collect water from wells, canals or reservoirs. Water quality was assessed using the following parameters: pH value, electrical conductivity (EC), total dissolved solids (TDS), ionic balance, sodium adsorption ratio (SAR) and residual sodium carbonate (RSC) value. Water quality diagram given by the US Salinity Laboratory (USSL) and FAO guidelines for interpretation water quality for irrigation was used. Additionally, the Nejgebauer classification for irrigation water, developed specifically for the area of Vojvodina, was used as a third classification. Based on the results of mineralization of the irrigation water, the following values of the observed parameters were determined: average pH of the analyzed water samples were 7.89, ranged from 7.14 to 9.01, while electrical conductivity values ranged from 0.10 to 3.50 dS/m, with an average of 0.85 dS/m. TDS analysis resulted in a wide range of values, from 112 mg/l to 2,384 mg/l, with an average of 529,22 mg/l. SAR values varied between 0.04–16.52 with a satisfactory average of 1.97. The USSL water classification produced similar results as FAO classification and RSC index <0, indicating that 57% of investigating samples are without concerns for irrigation use, whereas Nejgebauers classification and RSC index 0–1.25 show that over 75% of analyzed samples are suitable and safe for irrigation and soil properties. Since the quality of irrigation water significantly affects plant productivity, as it determines the chemical and physical properties of agricultural land, monitoring of water quality for irrigation is of high importance.

KEYWORDS: irrigation water, irrigation water salinity, water mineralization, SAR, classification of irrigation water

[*] Corresponding author. E-mail: stanko.milic@nsseme.com

INTRODUCTION

The continuous growth of the world's population leads to an increase in the use of drinking and other types of water necessary for undisturbed flow of numerous processes. The fact that this natural resource is endangered by a large number of factors, which accompany modern society, is often forgotten. In agricultural sector, reduction of available water accompanied by its irrational and inadequate use leads to land degradation, deterioration of water quality, as well as its limited use. In addition, crop production in the open field is threatened by global climate change, characterized by increasing air temperatures and decreasing rainfall, i.e. more frequent occurrence of intense drought (Vuković et al., 2018). Higher negative impact of drought can be expected in the near future, while the need for irrigation is expected to increase from 0.7 to 11.6% by the middle of the 21st century in Serbia. In addition, by the end of the 21st century, the water deficit may will have increased by as much as 27 – 35.6% (UNDP, 2019). These reasons impose the need to increase the area under irrigation in our agro-ecological conditions. The Water management master plan – the strategic documents of the Republic of Serbia (*Službeni list RS*, No. 3/2017) stimulate expansion of the irrigation area by 2034 for additional 100,000 to 250,000 hectares. Monitoring water quality is one of the main requirements for stable and sustainable crop production given the fact that water, in addition to soil, is essential for crop growth and development.

Moreover, meeting the needs of cultivated plants as well as expectations of agricultural producers, requires planned irrigation using good quality irrigation water. Defining water quality is a prerequisite for assessing its irrigation suitability. Numerous factors indirectly limit suitability of water for irrigation, ultimately hindering the achievement of high yields and the desired quality of cultivated crops. Generally, mineral composition of water, the crop species and the soil type are the main indicators of suitability of water for irrigation (Čolić et al., 2016).

Soil and climatic conditions in Vojvodina Province can cause salinization of the soil in irrigation systems, especially when irrigation water is mineralized above the permitted level. The accumulation of water-soluble salts in the layer of active rhizosphere of irrigated soils can be expressed to such an extent that it causes serious problems in crop cultivation (Nešić et al., 2003). Research about soil salinization and water quality for irrigation in Vojvodina Province were initiated even before irrigation development in region and continue to be an important issue. With the development of methods and classifications for determining water quality in the world, national experts comparatively worked on this issue (Nejgebauer, 1949; Vučić, 1965; Miljković, 1986a, 1988). The Nejgebauer's water classification was proposed for agro ecological conditions of Vojvodina in 1949 (Vučić, 1976) and provides four main classes. The influence of salts dissolved in water on the infiltration properties of soil, toxicity to plant production as well as the ecological aspect was considered by Miljković (1986b, 1988) with a new classification. Further clarification of the Nejgebauer's classification in order to adapt classes and subclasses to specific conditions was made by Avakumović (1994).

The risk of soil chemical and physical degradation comes from inadequate irrigation and low quality of irrigation water. Vučić (1987) emphasizes that the degradation of soil water status and overall soil physical state can be observed in the surfaces cultivated with vegetable crops for longer periods without crop rotation or use of manure for fertilization. Gajić (1999) states that long-term irrigation of calcareous chernozem and non-calcareous humogley significantly disturb their physical and mechanical properties, compared to the non-irrigated soils, due to a decrease in $CaCO_3$ and humus content. Soil salinization caused by irrigation can occur either directly, by using mineralized water for irrigation, or indirectly, due to a rise in mineralized ground water if the added amount of irrigation water is not controlled (Dragović et al., 1993).

The three-year long research conducted by Dragović et al. (2007) indicated that the use of saline water (class C3-S1) for irrigation causes soil surface salinization, which can limit the yields of crops sensitive to even low soil salinity. Soil salinization particularly occurs in glasshouse or greenhouse vegetable crop production when the quality of irrigation water is not controlled (Hadžić et al., 2004).

Negative effects of mineralized water on soil and plants posed the necessity of determination and evaluation of irrigation water quality (Bošnjak, 1994). The main findings of previous investigations conducted by Belić et al. (2013), concerning the evaluation of quality of irrigation water from several watercourses (for period 1980–2009) in the Vojvodina Province, show a mild decreasing trend in water quality and an increasing mineralization trend in most of the analysed samples of the Danube-Tisza-Danube Hydro System (DTD HS). The waters of the Danube, Tisza and Begej were found suitable for irrigation, except in some cases where it was recommended to monitor the changes in chemical properties due to the potential adverse effects of these waters.

Neutral trend in SAR value on the Bezdan-Danube River profile for 1969–1996 was noticed by Savić et al. (1997). Analyzing water quality for irrigation of Banat watercourses (Karaš, Moravica and Nera) Ilić et al. (2019) concluded that the analyzed watercourses are suitable for irrigation but also require control of the total salt content as well as the SAR ratio of sodium concentration to calcium and magnesium (SAR value), due to the impact on soil and plants. Close control of bicarbonate concentration is required because it has a greater potential to cause various adverse effects. Continuous monitoring of soil and water quality are necessary for proper irrigation management and sustainable agricultural farming. The aim of this study is to estimate and compare overall quality of irrigation water from different water sources in Vojvodina Province in order to provide general insight into the water quality and encourage development of irrigation practice in the region.

MATERIALS AND METHODS

The paper examines the quality of irrigation water on the territory of the Vojvodina Province, obtained from three different sources: wells, canals and

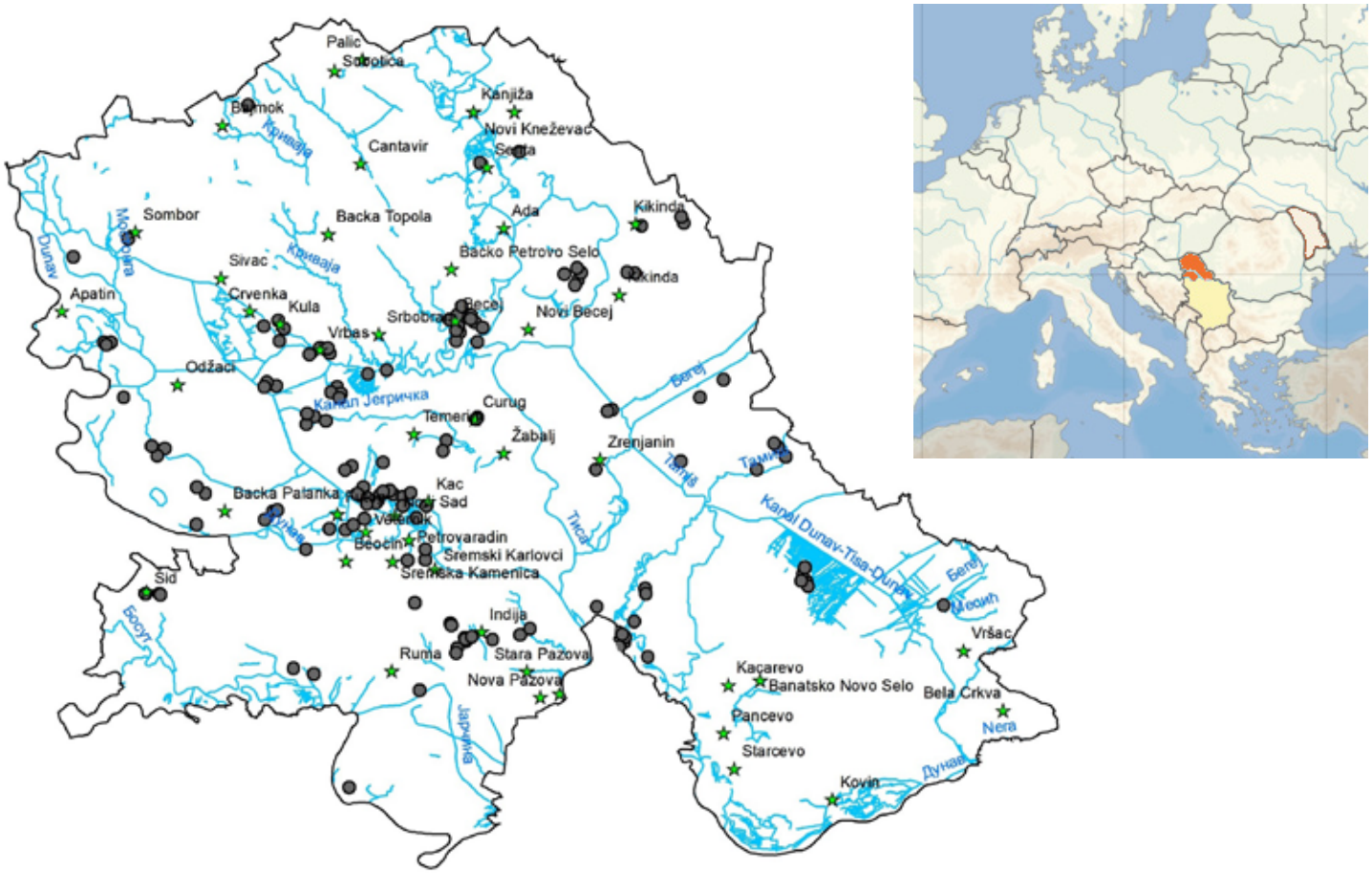


Figure 1. Location of water sampling points in Vojvodina Province

reservoirs. Sampling and laboratory analyses were performed successively during 2018 and 2019. A total of 140 water samples (Figure 1) were analyzed and the following parameters were tested: pH value, electrical conductivity (EC), total dissolved solids (TDS), ionic balance – anions (carbonates, bicarbonates, chlorides, sulfates, nitrates) and cations (calcium, magnesium, potassium and sodium), and sodium adsorption ration (SAR).

Laboratory analyses were conducted at the Institute of Field and Vegetable Crops, The National Institute of the Republic of Serbia – Laboratory for Soil and Agroecology, accredited according to the standard ISO/IEC 17025:2017. The pH value was determined potentiometrically, the electrical conductivity conductometrically at 25 °C, and the TDS by evaporation of water in oven at 105 °C. The content of carbonates, bicarbonates and chlorides according to Mohr was examined by titrimetric methods. Sulfates were determined by gravimetric method with barium chloride, and nitrates by spectrophotometric method. The cation content was determined on a Vista Pro-Varian apparatus, by the induced coupled ICP-OES plasma method. Sodium adsorption ratio (SAR) and residual sodium carbonate (RSC) were calculated.

An assessment of irrigation water quality is given according to: 1) RSC index classification (RSC), (Richards, 1954); 2) The Nejgebauer classification for irrigation water (Nejgebauer, 1949); 3) Water quality diagram given by the US Salinity Laboratory (USSL), US Salinity Laboratory Staff (1954), and 4) FAO guidelines for interpretation water quality for irrigation (FAO), Ayers and Westcot (1985).

Statistical analysis included a total of 140 samples divided into 3 observation groups – wells (58), canals (72) and reservoirs (10). Descriptive statistics and significance of differences were tested by Fisher's least significant difference test (LSD). Statistica for Windows, version 13 was used for all statistical data processing.

RESULTS AND DISCUSSION

The pH values for the tested irrigation water samples range between 7.14 and 9.01 (Table 1). Most of the tested water samples can be classified as neutral, medium alkaline and strongly alkaline. About 90% of the analyzed waters are within the allowed range according to FAO guidelines (pH 6.5–8.4) (Ayers and Westcot, 1985). In addition, significant differences were found between the three types of water sources, where the observed average values exhibited a decreasing trend: reservoirs> canals> wells.

The electrical conductivity (EC) of the observed water samples varied in the range of 0.10 and 3.50 dS m^{-1} (Table 1). The values were in the same range as shown by the previous investigations (Nešić et al., 2003; Vranešević et al., 2016). Only 9% of observed samples had an EC value greater than 1.5 dS m^{-1}. According to the guidelines for Salinity hazard of irrigation water (Follett and Soltanpour, 2002; Bauder et al., 2011) these samples has moderate hazard, water may have adverse effects on many crops what requires careful management practice. This occurs because the plant roots are not able to take up soil water due

to high osmotic potential (Zaman et al., 2018). High EC indicates a high degree of salinity and high index of water solute concentrations (Thompson et al., 2012).

Statistically, the lowest average values of electrical conductivity were determined for water samples from the canal (0.76 dS m^{-1}). Out of the 140 samples observed, only four samples showed significant limitations in terms of their suitability for irrigation, relative to their EC value (>2.25 dS m^{-1}). Assessing the caution of using saline water for irrigation, Hopkins et al. 2007 reported that, at the EC value of 1 dS m^{-1} (salt content about 640 mg/l), over 7.5 t of salt per hectare per year is introduced into the soil at an irrigation rate of 120 l/m^2.

In terms of the content of TDS, the values of the analyzed water samples ranged from 112 to 2,384 mg/l. Statistically, the lowest average value of this parameter (495.68 mg/l) was determined for water samples taken from the canal (Table 2). Salinity is the most important criterion for irrigation water quality evaluation (Ghassemi et al., 1995). High salt concentrations prevent the uptake of water by plants, thus causing crop-yield reductions. This occurs when salts accumulate in the root zone to such an extent that the crop is no longer able to extract sufficient water from the salty soil solution, resulting in water stress for a significant period. The plant symptoms are similar in appearance to those of drought (FAO, 1994). In relation to the total number of samples, 60% of analyzed water samples exhibited the values below 480 mg/l which, according to the classification of total soluble salts in water (Hopkins et al., 2007), has no significant adverse effects on cultivated plants nor causes salt accumulation in soils. According to FAO classification (Ayers and Westcot, 1985), in investigated samples, only one sample (canal) exceeds values shown for severe restrictions (>2,000 mg/l). The majority of analysed samples (53.5%) have no restriction on use regarding concentration of TDS in water. For the achievement of full yield potential, 45.7% of the examined samples require slight to moderate restrictions as well as careful selection of crops and management alternatives. Another crucial issue related to salinity or sodicity management in agriculture is crop selection. Crops vary considerably in their ability to tolerate saline conditions, for example durum wheat, triticale or barley tolerate higher salinity than rice or corn (Mateo-Sagasta and Burke, 2010).

According to the FAO classification of irrigation water (Ayers and Westcot, 1985), chloride content in 85.7% of the tested samples is within the range for safe crop production (<70 ppm or <2 meq/l), while only 7% of water samples can affect hazard in an irrigated farming system, with the possibility of reducing yield of potatoes, alfalfa, wheat, corn, Sudan grass, sorghum, tomatoes squash, etc. Adverse effect of high chlorine concentration is more pronounced with sprinkler irrigation, causing leaf burning (Maral, 2010). Significantly lower values of chloride content in water were found in waters originating from canals (1.26 meq/l) compared to well water and water from reservoirs, where no statistically significant differences were found (Table 2).

The average nitrate content in the analyzed samples was 2.57 mg/l and ranged from 0.01 to 96.76 mg/l (Table 1). The statistically highest average values between the observed water sources were determined in water samples taken from wells (5.92 mg/l), while there was no significant difference between ca-

nal waters and reservoirs (0.61 and 0.54 mg/l) (Table 2). Nitrate content was below 10 mg/l in 96.5% of analyzed water samples, which is the usual value of this parameter according to the FAO water quality assessment (Ayers and Westcot, 1994). The highest concentrations of nitrate in irrigation water were observed in four samples taken from a well (36.4–96.7 mg/l). In the Vojvodina Province chemical fertilizers or manure is commonly applied in order to add nutrients to the soil. A major source of nitrates in well water is deep percolation from fertilizer use on cropland. Nitrate from agriculture is the most common chemical contaminant in the world's groundwater aquifers (WWAP, 2013). In addition, in the areas of intensive agricultural production, one of the basic types of groundwater nitrogen contamination is fertilization, i.e. the application of excessive doses of nitrogen (Suthar et al., 2009; Gao et al., 2012). Other important sources of N in wells include leaching from septic peat, animal manure, land application of municipal or industrial sludge, etc. (Ray and Jain, 1998). Waters with high nitrogen content, besides damaging the environment, can compromise crop quality, excess vegetative growth, e.g. impacts maturity, and/or storability which is of high importance for crop such as potato, sugar beet, grass seed apples (Hopkins et al., 2007).

In view of the content of sulphate in water (SO_4), no differences were found between the observed water sources (Table 2). The average content of these ions was 1.03 meq/l ranging from 0.02 to a maximum of 10.15 meq/l (Table 1). In the largest number of the tested samples (96%) the values did not exceed 3 meq/l (144 ppm). The highest content of sulphate in investigation was 10.2 meq/l (489,5 ppm) sourced from a canal. Waters containing more than 1,000 mg/l sulfates are toxic to plant health, growth and development (Ghoraba et al., 2013). Considering the observed low amount of sulfate, current concentrations of these ions pose no significant threats.

Table 1. Descriptive statistics, average values of the analyzed parameters.

Analyzed parameters	Mean	Min	Max	Percentile 25%	Percentile 75%	St. dev.	Coef. var.
pH value	7.89	7.14	9.01	7.59	8.20	0.41	5.16
EC (dS/m)	0.85	0.10	3.50	0.51	0.99	0.52	61.26
TDS (mg/l)	529.22	112.00	2384.00	316.50	588.50	345.44	65.27
CO_3^{2-} (meq/l)	0.24	0.00	2.08	0.00	0.34	0.42	176.99
HCO_3^- (meq/l)	7.06	1.63	35.24	4.03	8.79	4.30	60.93
Cl^- (meq/l)	1.29	0.00	9.69	0.47	1.53	1.63	126.42
SO_4^{2-} (meq/l)	1.03	0.02	10.15	0.25	1.21	1.39	135.99
NO_3-N (mg/l)	2.57	0.01	96.76	0.06	1.04	11.02	428.39
Ca^{2+} (meq/l)	3.31	0.41	13.80	2.25	3.74	1.92	58.01
Mg^{2+} (meq/l)	3.47	0.17	13.58	1.72	4.52	2.33	67.00
K^+ (meq/l)	0.24	0.01	4.60	0.08	0.20	0.50	207.35
Na^+ (meq/l)	3.39	0.08	16.62	1.31	4.35	3.10	91.53
SAR	1.97	0.04	16.52	0.80	2.42	2.10	106.68
RSC	0.51	-12.82	21.58	-1.12	0.94	3.80	739.57

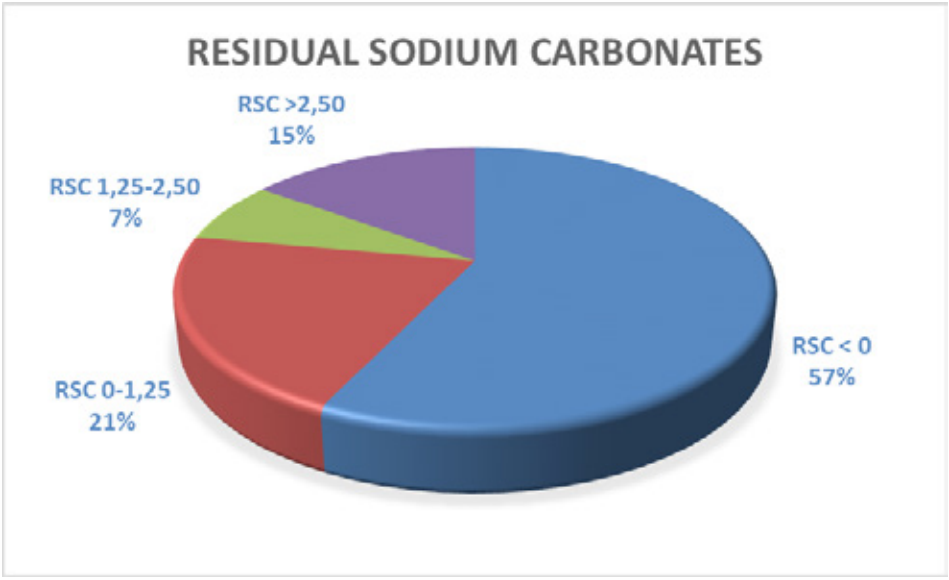
One of the important indicators of irrigation water quality is the content of carbonates (CO_3^{2-}) and bicarbonates (HCO_3^{2-}). The results of the study exhibited the average value of these ions at 0.24 (CO_3^{2-}) and 7.06 meq/l (HCO_3^{2-}), as shown in Tab 1. Significantly lowest bicarbonate content (HCO_3^{2-}) was found in water from the canal, while significantly lowest carbonate content (CO_3^{2-}) was observed in well water (Table 2). Over 83% of the observed samples have a bicarbonate content above 3 meq/l which can have a negative impact on crop production. At values above 3.3 meq/l, correction/treatment of such waters is necessary (Morgan and Graham, 2019) in order to reduce their negative effects. According to FAO (1994), when using overhead sprinklers, there is no restriction on use of waters having HCO_3^- less than 1.5 meq/l, but there is slight to moderate adverse impact on use of waters having HCO_3^- of 1.5-8.5 meq/l, and severe restriction for HCO_3^- greater than 8.5 meq/l (Capar et al., 2016) (Table 1). According to the results of Shahabi et al. (2005) bicarbonate in irrigation water is one of the factors that causes nutritional imbalances in plants disrupting the absorption and translocation of nutrients, particularly Fe and Mn, by the plant roots. When water containing dissolved HCO_3^- is applied to the soil surface in the presence of sufficient Ca^{2+} (and/or Mg^{2+}) ions, it can result in the formation of inorganic carbonates such as calcite ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$) (Suarez, 1999; Eshel et al., 2007; Sanderman, 2012).

The process decreases their reactive ability in competition with Na^+ ions towards the exchange complex of clay, leading to sodium permeability hazard. In such circumstances, the concentration of Na^+ ions in the soil solution increases and causes decomposition of structural aggregates, which significantly reduces water permeability of soil, nutrient uptake and root penetration, and intensifies soil degradation (Domenico and Schwartz, 1990; Todd and Mays, 2005). Water infiltration problems caused by excess sodium are easier to prevent than to remedy (Hopkins et al., 2007). Water sodicity can be mitigated through the judicious use of calcium-containing amendments such as gypsum ($CaSO_4.2H_2O$). Relative to other amendments, gypsum is cheap and easy to handle, and by far the most suitable amendment to bring down irrigation water sodicity (the ratio of sodium to calcium + magnesium), as reported by Hopkins et al. (2007) and Zaman et al. (2018).

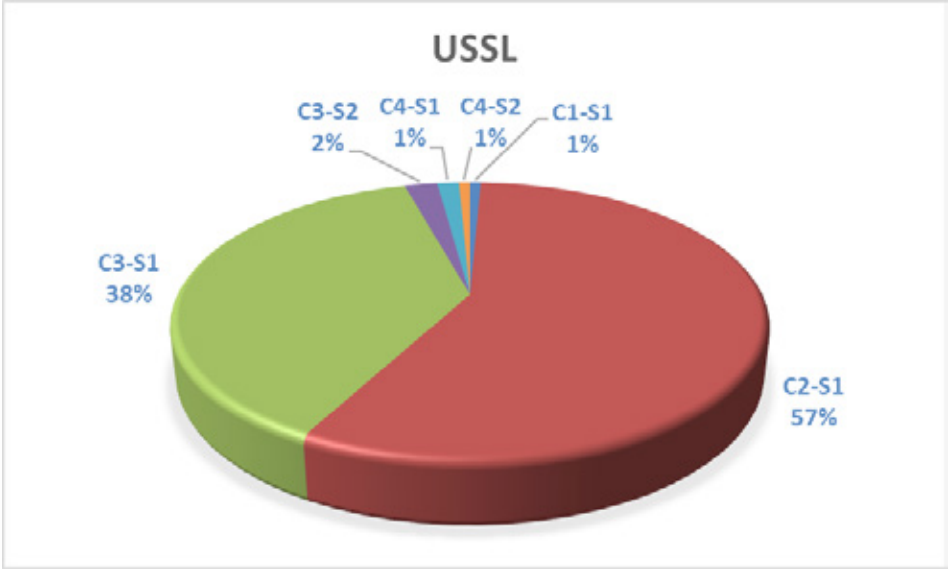
The characteristics of the tested water samples, i.e. the usability of water for irrigation were considered and compared according to the guidelines of the US Salinity Laboratory (Richards, 1954), Figure 2b. This classification considers the risk of salinization and alkalization based on two SAR and EC parameters. The USSL water classification diagram does not present an EC over 2.25 dS m^{-1} therefore, in order to accommodate higher water salinity levels, Shahid and Mahmoudi (2014) have modified the USSL Staff (1954) water classification diagram by extending water salinity up to 30 dS m^{-1}.

In terms of their usability for irrigation, most samples in the study (57%) are moderately saline, classified in class C2-S1, while 38% belong to class C3-S1. Saline (C3) to medium saline (C2) water, with a low content of sodium (S1), can cause salinization, but not alkalization, in poorly drained soils.

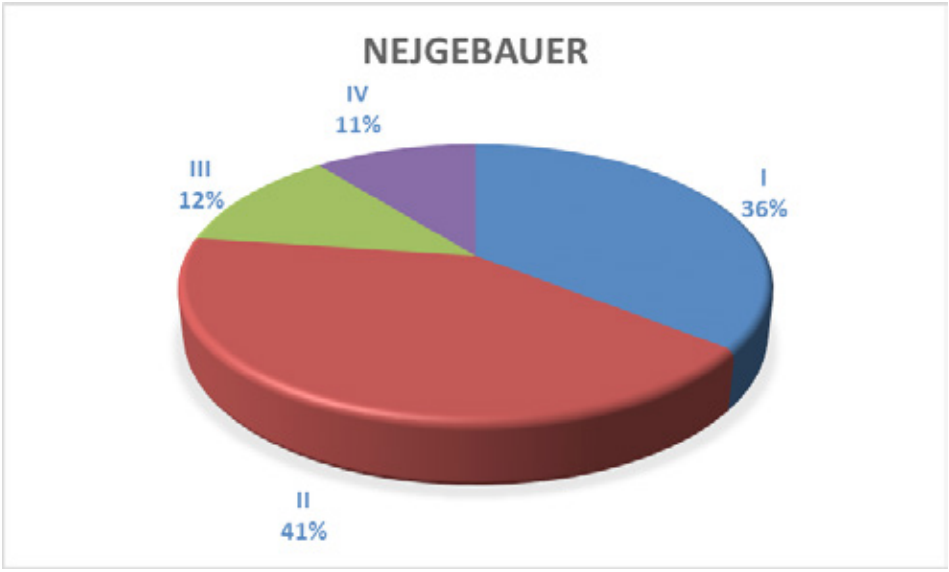
a)


RESIDUAL SODIUM CARBONATES
RSC >2,50
15%
RSC 1,25-2,50
7%
RSC 0-1,25
21%
RSC < 0
57%

b)


USSL
C3-S2
2%
C4-S1
1%
C4-S2
1%
C1-S1
1%
C3-S1
38%
C2-S1
57%

c)



d)

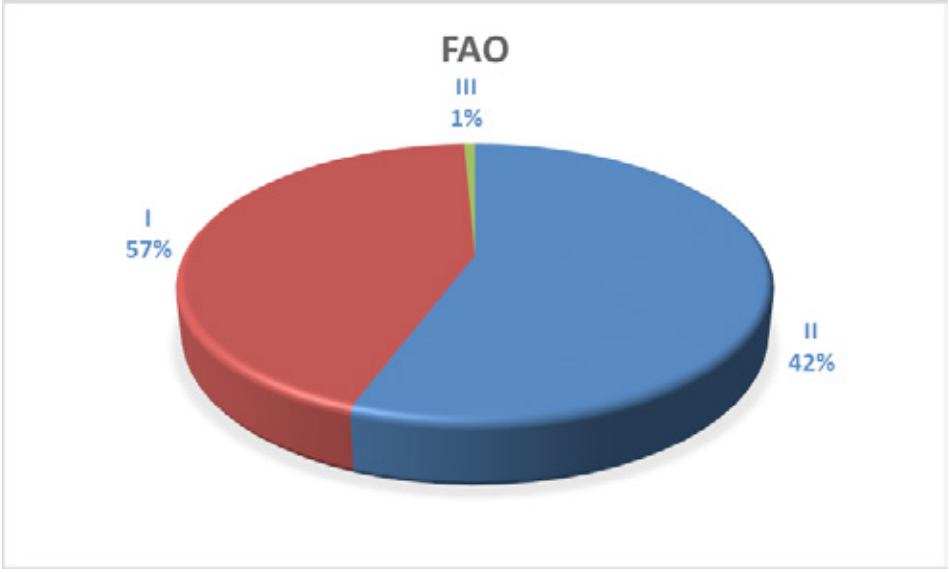


Figure 2. Proportion of water samples according to classifications: a) RSC; b) USSL; c) Nejgebauer; d) FAO

Table 2. Irrigation water quality parameters I and anionic content.

Water Source		pH value	EC (dS/m)	TDS (mg/l)	CO_3^{2-} (meq/l)	HCO_3^- (meq/l)	Cl^- (meq/l)	SO_4^{2-} (meq/l)	NO_3-N (mg/l)
Well	Average	7.71c	0.97ab	625.67ab	0.07c	8.93ab	1.13b	0.90a	5.92a
	Min	7.14	0.10	215.00	0.00	1.95	0.00	0.02	0.01
	Max	8.49	2.55	1699.00	0.90	21.80	6.11	7.82	96.76
	Stdv.	0.33	0.47	331.08	0.19	3.56	1.38	1.42	17.65
Canal	Average	7.95b	0.76c	459.68c	0.28b	5.85c	1.26c	1.11a	0.61b
	Min	7.21	0.26	112.00	0.00	1.63	0.07	0.07	0.01
	Max	8.61	3.50	2384.00	2.08	35.24	8.20	10.15	4.45
	Stdv.	0.38	0.53	340.30	0.44	4.54	1.40	1.42	0.68
Reservoir	Average	8.31a	0.92b	570.10b	0.83a	6.80ab	2.31ab	1.02a	0.54b
	Min	7.21	0.51	269.00	0.00	3.93	0.19	0.08	0.03
	Max	9.01	2.27	1319.00	1.70	11.62	9.69	3.68	1.51
	Stdv.	0.57	0.59	365.42	0.58	2.24	3.46	1.10	0.50

Table 3. Irrigation water quality parameters II and cationic content.

Water Source		Ca^{2+} (meq/l)	Mg^{2+} (meq/l)	K^+ (meq/l)	Na^+ (meq/l)	SAR	RSC
Well	Average	4.50a	4.40b	0.22a	3.60a	1.99a	0.10a
	Min	0.41	0.17	0.01	0.08	0.04	-12.82
	Max	13.80	13.58	4.60	15.98	16.52	14.76
	Stdv.	2.56	2.86	0.71	3.50	2.60	4.13
Canal	Average	2.64bc	2.66c	0.27a	3.25a	1.98a	0.83a
	Min	0.89	0.73	0.03	0.68	0.50	-3.50
	Max	5.00	10.02	1.94	16.62	11.71	21.58
	Stdv.	0.76	1.61	0.35	2.81	1.78	3.75
Reservoir	Average	2.45b	4.98ab	0.14a	3.34a	1.72a	0.20a
	Min	1.05	2.56	0.04	0.81	0.46	-2.80
	Max	5.99	6.65	0.35	9.77	4.82	4.41
	Stdv.	1.48	1.36	0.12	3.28	1.56	2.12

Figure 2. Proportion of water samples according to classifications: a) RSC; b) USSL; c) Nejgebauer d) FAO.

Similar results were reported by Nešić et al. (2003) who pointed out good quality of waters classified as C2-S1 according to the US Salinity Laboratory, while moderate restriction is proposed when using waters classified as C3-S1, due to higher EC and TDS. Only 3% of water samples tested in this study (C3-S2 and C4-S2) belong to high salinity and alkality classes (Figure 2b). Continuous use of such water quality over a long period of time can increase salinity and alkalinity in soils. Läuchli and Epstein (1990) pointed out the effect of salinity on growth and development of plants in different ways, such as osmotic effects, specificion toxicity and/or nutritional disorders.

Although the Nejgebauer classification was created 50 years ago, through the content of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} and the TDS, it simply and easily provides a reliable assessment of the usability of water for irrigation (Belić et al., 2003). According to this classification, the largest number of analyzed samples are placed in I and II class, excellent (36%) and good (41%), 12% are waters which need additional testing (III class), while 11% of tested samples are not suitable for irrigation (Figure 2c). Since Nejgebauer classification gives special emphasis on the ratio (Ca+Mg):Na, for the final evaluation of III class waters (waters that need additional testing) performing supplementary analyses and classification are justified (pH, chloride content, bicarbonates content, EC, sulfates content, FAO classification, RSC index, soil testing, etc.) in order to better understand water quality. In addition to the USSL classification, which is globally accepted, the analysis of water according to Nejgebauer significantly coincides with the classification of samples according to the RSC index. As the parameters of Vojvodina waters are analyzed according the two classifications, they can be, along with EC and SAR, a reliable and fast predictor of irrigation water quality.

The modified FAO guidelines for interpretation water quality for irrigation (Ayers and Westcot, 1985) include detailed analyses of the effect of salts dissolved in irrigation water on infiltration properties of soil and the toxic effects of certain ions, such as Na^{+} and Cl^{-}, on plants. According to the salinization and infiltration criteria, 57% of the tested samples have satisfactory quality without the need for restriction during use, while 42% of the observed samples are characterized with restrictions in the slight to moderate range. Only one test sample exceeds the values shown for severe restrictions, respectively user will experience soil and cropping problems or reduced yields using this water for irrigation. The FAO classification indicated that a majority of the samples do not have restriction regarding toxicity of Cl (91%) and Na (59%). The rest of samples belong to the group slightly to moderate restriction regarding both toxic elements. Similar findings were obtain by Vranešević et al. (2016) investigating irrigation water quality from artificial reservoirs on Fruška Gora. The FAO classification considered together produced similar results as the USSL water classification. Furthermore, Nejgebauer classification of water suitable for irrigation a bit overestimate FAO guidelines if bout classes are considered together (excellent and good). The results of the FAO classification of water suitability for irrigation obtained were not always in accord with the other estimates (Belić et al., 2003), especially when the hazards of salinization or disturbance of soil infiltration properties were analysed.

CONCLUSION

Based on the analysis of irrigation water quality in Vojvodina, the conclusion is that the vast majority of water samples are good quality and can be used for irrigation without concern. However, a small number of the tested irrigation water samples <10% can have an adverse impact in terms of soil

salinization and plant production. Sustainable use of these waters requires special soil management methods, good drainage, high leaching ability or water treatment. Significantly lower values of chloride content in water were found in waters originating from canals (1.26 meq/l) compared to well water and water from reservoirs. The water pH reaction in the study area is neutral to alkaline. HCO^{-3} dominates among anions, while the dominant cations are Na^{+} and Ca^{2+} and Mg^{2+}. Over 83% of the observed samples have a bicarbonate content above 3 meq/l which can have a negative impact on crop production. The USSL water classification produced similar results as FAO classification and RSC index <0, indicating that 57% of investigating samples are without concerns for irrigation use whereas Nejgebauers classification and RSC index 0–1.25 shows that over 75% of analyzed samples are suitable and safe for irrigation and soil properties.

Since the quality of irrigation water significantly affects plant productivity, as it determines the chemical and physical properties of agricultural land, monitoring of water quality for irrigation is of high importance. Further research should include examination of a larger number of parameters, including the content of hazardous and harmful substances.

REFERENCES

Ayers R, Westcot D (1985): Food, Water Quality for Agriculture, Irrigation and Drainage, Agriculture Organization of the United Nations (FAO), Rome: Paper No. 29. Rev. 1, M-56. ISBN 92-5- 102263-1.

Avakumović D (1994): Hidrotehničke melioracije. Navodnjavanje. Beograd: Građevinski fakultet [Hydrotechnical reclamation, Irrigation. Faculty of Civil Engineering, University of Belgrade, Belgrade].

Bauder TA, Waskom RM, Sutherland PL, Davis JG (2011): Irrigation water quality criteria. Colorado State University Extension Publication, Crop series/irrigation. Fact sheet no. 0.506,4 pp [URL: https://extension.colostate.edu/docs/pubs/crops/00506.pdf]

Belić S, Savić R, Belić A (2003): Upotrebljivost voda za navodnjavanje, *Vodoprivreda* 35: 37–49. [Usability of water for irrigation].

Belić S, Belić A, Vranešević M (2013): Water quality as a limiting factor for irrigated agriculture, Understanding Freshwater Quality Problems in a Changing World Proceedings of H04, IAHS-IAPSO-IASPEI Assembly, Gothenburg, Sweden, (IAHS Publ. 361, 2013) [URL: https://iahs.info/uploads/dms/15592.34-243-249-361-03-H04_Belic_SBABMV-Geothenburg-konacnoCORR.pdf]

Bošnjak Đ (1994): Osnove kriterijuma ocene kvaliteta vode za navodnjavanje – FAO 1985. *Zbornik radova*, Poljoprivredni fakultet, Institut za ratarstvo i povrtarstvo, 22: 109–117. [Basics of water quality assessment criteria for irrigation – FAO 1985].

Capar G, Dilcan CC, Aksit C, Arslan S, Celik M, Kodal S (2016): Evaluation of irrigation water quality in Gölbaşı District. *Tarim Bilimleri Dergisi – J. Agric. Sci.* 22: 408–421.

Čolić J, Petković A, Tončić J, Nenin T (2016): Mogućnost korišćenja vode akumulacija Međeš, Ljukovo i Borkovac za navodnjavanje sa aspekta kvaliteta vode. *Voda 2016*: zbornik radova

45. godišnje konferencije o aktuelnim problemima korišćenja i zaštite voda, Zlatibor, 15.–17. jun 2016. [Possibility of using water of reservoirs Međeš, Ljukovo and Borkovac for irrigation from the aspect of water quality, 45th conference on current topics of water use and protection "WATER 2016", 15–17. June 2016, Zlatibor, Publisher: Serbian Society for Water Protection, Belgrade], 339–348.

Domenico PA, Schwartz FW (1990): *Physical and chemical hydrogeology*. New York: John Wiley & Sons.

Dragović S, Ćirović M, Hadžić V (1993): Problem zaslanjivanja zemljišta u navodnjavanju i uticaj kvaliteta vode. *Zbornik radova* – Naucni institut za ratarstvo i povrtarstvo 21: 111–126 [The problem of soil salinization in irrigation and the impact of water quality. Proceedings of the Institute of Field and Vegetable Crops]

Dragović S, Božić M, Stević D, Rudić D, Vasić G (2007): Vodno-soni režim zemljišta u navodnjavanju sa aspekta problema zaslanjivanja u Surčinskom donjem polju. *Vodoprivreda* 39: 376–390. [Water-salt regime of soil in irrigation from the aspect of salinization problems in Surčin lower field]

Dragović S, Hadžić V, Maksimović L, Nešić Lj, Belić M (1994): Uticaj kvaliteta vode za navodnjavanje zemljišta u sistemima za navodnjavanje u Vojvodini. *Savremena poljoprivreda* 6: 132–135. [Influence of water quality for soil irrigation in irrigation systems in Vojvodina].

Eshel G, Fine P, Singer MJ (2007): Total soil carbon and water quality: an implication for carbon sequestration. *Soil Sci. Soc. Am. J.* 71: 397–405.

FAO (1994): Water quality for agriculture. FAO Irrigation and drainage paper. 29 Rev.1. Rome.

Follett RH, Soltanpour PN (2002): *Irrigation water quality criteria*. Colorado State University Publication No. 0.506 [URL: https://mountainscholar.org/bitstream/handle/10217/194958/AEXT_ucsu206220506.pdf?sequence=1&isAllowed=y]

Food and Agriculture Organization of the United Nations (1994): The state of food and agriculture 1994. FAO Avriculture Series, no 27, ISBN 93:5-103550-4, Rome.

Gajić B (1999): Influence of sprinkling irigation on the physico-mechanical properties of chernozem and humogley. *Zemljište i biljka* 48: 93–101.

Gao Y, Yu G, Luo C, Zhou P (2012): Groundwater nitrogen pollution and assessment of its health risks: A case study of a typical village in rural-urban continuum, China. *PloS One* 7: e33982. [https://doi.org/10.1371/journal.pone.0033982]

Ghassemi F, Jakeman AJ, Nix HA (1995): *Salinization of Land and Water Resources. Human causes, extent, management and case studies*. Center for Resource and Environmental Studies. Canberra: Australian National University.

Ghoraba SM, Khan AD (2013): Hydrochemistry and groundwater quality assessment in Balochistan Province, Pakistan. *Int. J. Res. Rev. Appl. Sci.* 17: 185.

Hadžić V, Nešić L, Sekulić P, Ubavić M, Bogdanović D, Dozet D, Belić M, Govedarica M, Dragović S, Verešbaranji I (2004): Kontrola plodnosti zemljišta i utvrđivanje sadržaja štetnih i opasnih materija u zemljištima Vojvodine. *Zb. rad. – Nauč. inst. ratar. povrt.* 40: 57–64. [Soil fertility control and determination of the content of harmful and dangerous substances in the lands of Vojvodina]

Hopkins BG, Horneck DA, Stevens RG, Ellsworth JW, Sullivan M (2007): *Managing Irrigation Water Quality for crops production in the Pacific Northwest*. Washington: Pacific Northwest Extension Publication. [URL: https://catalog.extension.oregonstate.edu/sites/catalog/files/project/pdf/pnw597.pdf]

Ilić M, Vranešević M, Bezdan A, Blagojević B (2019): Classification of Water Quality of Banat Watercourses in Serbia for the Needs of Irrigation. *J. Environ. Geogr.* 12: 51–57.

Läuchli A, Epstein E (1990): Plant responses to saline and sodic condi-tions. In: *Agricultural salinity assessment and management* (ed. KK Tanji), ASCE Manuals and Reports on Engineering Practice, ASCE, Reston 71: 113–137.

Maral N (2010): *Soil and water analysis techniques for agricultural production*. Master of Science Thesis, Middle East Technical University (Basılmamış), Turkey.

Mateo-Sagasta J, Burke J (2010): Agriculture and water quality interactions: a global overview. SOLAW Background Thematic Report—TR08. Food and Agriculture Organization of the United Nations, Rome, 46.

Miljković N (1986a): Klasifikacija površinskih i podzemnih voda u Vojvodini sa aspekta njihove primene za navodnjavanje. u: *Kongres o vodama Jugoslavije* (II), Ljubljana–Beograd: Jugoslovensko društvo za odvodnjavanje i navodnjavanje, 1360–1373. [Classification of surface and groundwater in Vojvodina from the aspect of their application for irrigation].

Miljković N (1986b): Savremeni pristup proceni kvaliteta vode za navodnjavanje. *Vodoprivreda* 21: 307–312.

Miljković N (1988): Appearance of the Secondary Salinization of Soils in Yugoslavia. *Procedings of the International Symposium on Solonetz Soils*, Osijek, 339–346.

Morgan KT, Graham JH (2019): Nutrient Status and Root Density of Huanglongbing-Affected Trees: Consequences of Irrigation Water Bicarbonate and Soil pH Mitigation with Acidification. *Agronomy* 9: 746. [DOI: 10.3390/agronomy9110746]

Nejgebauer V (1949): Upotrebljivost površinskih i podzemnih voda za navodnjavanje i njihova klasifikacija u prirodnim prilikama Vojvodine. *Radovi poljoprivrednih naučnoistraživačkih ustanova* [The Use of Surface and Groundwater for Irrigation and Their Classification in the Natural Conditions of Vojvodina].

Nešić Lj, Hadžić V, Sekulić P, Belić M (2003): Kvalitet vode za navodnjavanje i salinitet zemljišta u intenzivnoj povrtarskoj proizvodnji, *Letop. naučn. rad.*, Poljoprivredni fakultet u Novom Sadu, 27: 5–10. [Irrigation water quality and soil salinity in intensive vegetable production].

Ray C, Jain RK (1998): Nitrate pollution of wells: pollution prevention policy options. *Environ. Eng. Policy* 1: 165–173. [DOI:10.1007/s100220050018]

Richards LA (ed.) (1954): Diagnosis and improvement of saline and alkali soils. USDA Agriculture Hand Book No 60. [URL: https://www.ars.usda.gov/ARSUserFiles/20360500/hb60_pdf/hb60complete.pdf]

Sanderman J (2012): Canmanagement induced changes in the carbonate systemdrive soil carbon sequestration? A review with particular focus on Australia. *Agric. Ecosyst. Environ.* 155: 70–77.

Savić R, Belic A, Belic S (1997): Water quality of the Danube through the Vojvodina Province in view of the irrigation needs. *32 Konferenz der IAD*, Wien, 415–418.

Shahid SA, Mahmoudi H (2014): National strategy to improve plant and animal production in the United Arab Emirates. *Soil and water resources*. Annexes.

Shahabi A, Malakouti M, Fallahi E (2005): Effects of Bicarbonate Content of Irrigation Water on Nutritional Disorders of Some Apple Varieties*. *J. Plant Nutr.* 28: 1663–1678.

Shakoor A, Arshad M, Bakhsh A, Ahmed R (2015): GIS based assessment and delineation of groundwater quality zones and its impact on agricultural productivity. *Pak. J. Agri. Sci.* 52: 837–843.

Službeni list Republike Srbije [*Official gazette of RS*] (2017): Strategije upravljanja vodama na teritoriji Republike Srbije do 2034. godine, 3/2017. [Water Management Strategy on the territory of the Republic of Serbia until 2034].

Sreedevi PD, Sreekanth PD, Ahmed S, Reddy DV (2018): Evaluation of groundwater quality for irrigation in a semi-arid region of South India. *Sust. Water Res. Manag.* 5: 1043–1056.

Suarez DL (1999): Impact of agriculture on CO_2 as affected by changes in inorganic carbon. In: R. Lal, JM Kimble, H. Eswaran, BA Stewart (Eds.), *Global Climate Change and Pedogenic Carbonates*. CRC Press, Boca Raton, FL, 257–272.

Suthar S, Bishnoi P, Singh S, Mutiyar PK, Nema AK, Patil NS (2009): Nitrate contamination in groundwater of some rural areas of Rajasthan, India. *J. Hazard. Mater.* 171: 189–199.

Thompson M, Brandes D, Kney A (2012): Using electronic conductivity and hardness data for rapid assessment of stream water quality. *J. Environ. Manage.* 104: 152–157.

Todd DK, Mays LW (2005): *Groundwater hydrology*. New York: John Wiley & Sons, 3rd ed.

United Nations Development Program (UNDP) (2019): Crop heating – how to respond? Impacts of climate change on Serbian agriculture. United Nations Development Program in Serbia, Belgrade.

US Salinity Laboratory Staff (1954): *Diagnosis and improvement of saline and alkali soils*. Washington, DC: US Department of Agriculture Handbook 60.

Vranešević M, Blagojević B, Bezdan A, Savić R (2016): Kvalitet vode za navodnjavanje sa fruškogorskih akumulacija. *Letop. Naučn. Rad.* (Ann. Agr.), 40: 15–20. [Irrigation water quality from artificial reservoirs on Fruška Gora].

Vučić N, Vučić J (1965): Problems of evaluating irrigation water. *Arhiv za polj. n.*, 18: 67–76.

Vučić N (1976): *Navodnjavanje poljoprivrednih kultura: osnove dopunskog navodnjavanja*. Novi Sad: Poljoprivredni fakultet, Univerzitet u Novom Sadu. [Irrigation of agricultural crops].

Vučić N (1987): *Vodni, vazdušni i toplotni režim zemljišta*. Novi Sad: Vojvođanska akademija nauka i umetnosti [Water, air and heat regime of the soil].

Vuković A, Vujadinović M, Rendulić S, Đurđević V, Ruml M, Babić V, Popović D (2018): Global warming impact on climate change in Serbia for the period 1961–2100. *Therm. Sci.*, 22: 2267–2280.

World Water Assessment Programme (WWAP) (2013): The United Nations World Water Development Report 2013. United Nations World Water Assessment Programme (WWAP). Paris, United Nations Educational, Scientific and Cultural Organization.

Zaman M, Shahid SA, Heng L (2018): Irrigation Water Quality. *Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques*, 113–131. [DOI: 10.1007/978-3-319-96190-3_5]

ОЦЕНА КВАЛИТЕТА ВОДЕ ЗА НАВОДЊАВАЊЕ СА ТЕРИТОРИЈЕ АП ВОЈВОДИНЕ

Станко Б. МИЛИЋ[1], Душана Д. БАЊАЦ[1], Јовица Р. ВАСИН[1], Јордана М. НИНКОВ[1], Боривој С. ПЕЈИЋ[2], Ивана Б. БАЈИЋ[1], Бранка Љ. МИЈИЋ[1]

[1] Институт за ратарство и повртарство,
Институт од националног значаја за Републику Србију,
Максима Горког 30, Нови Сад 21000, Србија
[2] Универзитет у Новом Саду, Пољопривредни факултет,
Департман за ратарство и повртарство,
Трг Доситеја Обрадовића 8, Нови Сад 21000, Србија

РЕЗИМЕ: У интензивним системима гајења биљака, поред контроле физичких и хемијских особина земљишта, неопходно је вршити и континуирано праћење квалитета воде за наводњавање. У светлу надолазећих климатских промена, као и забрињавајућег опадања садржаја органске материје, не смеју се занемарити и неповољне последице примене воде неодговарајућег квалитета на наводњавано земљиште, гајене биљке и опрему за наводњавање. У раду су приказани резултати квалитета воде која се користи за наводњавање, прикупљени током 2018. и 2019. године. Истраживање је обухватило 140 узорака воде из различитих водозахвата пореклом из површинских бунара, каналске мреже и акумулација за наводњавање. За оцену квалитета воде анализирани су следећи параметри: pH вредност, електропроводљивост (EC), суви остатак, јонски биланс, као и коефицијент адсорпције натријума (SAR) и вредност резидуалног натријум-карбоната (RSC). Уобичајено је да се за ову намену користи и класификација према Америчкој лабораторији за слатине (USSL) као и FAO процена квалитета воде за наводњавање. За просторе АП Војводине развијена је и Нејгебауерова класификација коју смо такође искористили за потребе оцењивања. На основу резултата минерализације воде за наводњавање, утврђене су следеће вредности посматраних параметара: просечно израчуната pH вредност анализираних вода износила је 7,89 (min=7,14, max=9,01), вредности електропроводљивости кретале су се у опсегу од 0,10 до 3,50 dS/m, са просечном вредношћу 0,85 dS/m. У односу на вредности сувог остатака, испитиване вредности кретале су се у широком опсегу, од 112 mg/l до 2.384 mg/l, с просечном вредношћу 529,22 mg/l. SAR вредности варирале су у опсегу 0,04–16,52 и задовољавајућим просеком од 1,97. Класификација према Америчкој лабораторији за слатине (USSL) показује сличне резултате као ФАО класификација и RSC индекс <0, указујући на то да 57% истраживаних узорака није забрињавајуће за употребу у наводњавању. Нејгебауерова класификација и RSC индекс 0–1,25 показују да је преко 75% анализираних узорака погодно и за наводњавање и сигурно за очување физичко хемијских својстава земљишта. Будући да квалитет воде за наводњавање значајно утиче на продуктивност биљака, као и да значајно може утицати на хемијске и физичке особине пољопривредног земљишта, праћење квалитета воде за наводњавање од изузетне је важности.

КЉУЧНЕ РЕЧИ: EC, јонски биланс, квалитет воде за наводњавање, SAR

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 103—112, 2021

UDC 662.63
UDC 620.952
https://doi.org/10.2298/ZMSPN2140103R
ORIGINAL ARTICLE

Aleh I. RODZKIN[1], *Euhenia V. CHARNENOK*[1], *Borivoj Đ. KRSTIĆ*[2*]

[1] Belarusian National Technical University,
Nezavisimosty 65, Minsk 220123, Belarus
[2] University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology,
Trg Dositeja Obradovića 2, Novi Sad 21000, Serbia

THE USE OF DEGRADED PEATLANDS FOR BIOMASS PRODUCTION

ABSTRACT: The goal of this research is an assessment of the possible use of peatlands biomass and straw for the production of mixed bio-pellets. Peaty soils are a specific type of soils formed as the result of wetlands drainage. Peat mining is completed by the factory if the layer of the peat is less than 1–2 meters and not enough deep for effective mechanical excavation. After the peat excavation is finished, the biggest problem is the use of these lands for other purposes. One of the perspective directions is rewetting of post-mining peatlands to stimulate the growth of common reed, cattail, fescue, sedge, and other grasses which may grow in natural conditions, and also improve conditions for the cultivation of other plants, including trees. The yield of wetland grass in Belarus varies from 8.1 to 14 DM g ha^{-1} per year. The yield of willow wood can reach 8–10 of DM g ha^{-1} per year. The highest prime cost of biomass is obtained for willow wood, but it will be lower with the enlargement of the plantation area. Pellets with a high content of wood residues (sawdust) have the best technological parameters including calorific value when compared to pellets from only peat and peat plus straw. Biomass production on degraded peaty soils has also an ecological effect. The cost of carbon quotas on the market varies from 20 to 25 euro per ton, and biomass production can provide additional profit in the case of CO_2 emissions trading depending on the biomass content in the fuel.

KEYWORDS: peatlands, biomass production, short-rotation coppice, willow

INTRODUCTION

Soil is a fundamental natural resource and the basis for all terrestrial life. Soils are the foundation for agriculture and ecosystem functioning and they are responsible for 95% of the global food production (FAO, 2019). However, soils are a non-renewable resource as they are characterized by a high degradation

* Corresponding author. E-mail: borivoj.krstic@dbe.uns.ac.rs

potential and slow regeneration rates. As a result, 33% of soils are moderately to highly exhaust due to degradation and it has been estimated that every year approximately 12 million hectares of agricultural soils are lost.

Therefore, soil degradation is a serious environmental problem today because of its impact on the physical, chemical, and biological soil quality and deterioration of soil health.

According to FAO's "Save and Grow", the definition of soil health is "the capacity of soil to function as a living system. Healthy soils maintain a diverse community of soil organisms that help to control plant disease, insect and weed pests, form beneficial symbiotic associations with plant roots, recycle essential plant nutrients, improve soil structure with positive effects for soil water and nutrient holding capacity, and ultimately improve crop production. Healthy soil also contributes to mitigating climate change by maintaining or increasing its carbon content" (FAO, 2011).

The drivers of soil degradation may be the loss of organic matter, a decline in soil fertility and structural conditions (over compaction), erosion, adverse changes in salinity, acidity, or alkalinity, and the effects of toxic chemicals, pollutants, or excessive flooding (NSW, 2019). As a result, it is not possible to get high yields of demanding agricultural crops on degraded soils because of the low content of nutrients and wicked structure. In accordance, the fundamental task is recovering or recultivation of degraded soils. There are several basic directions of soil recultivation for further practical use: water reservoirs construction, horticulture, agriculture, and forestry. The effectivity of biological recultivation depends on two factors: requirements of crops regarding the soil fertility and the dynamic of biomass accumulation. Phytomass stimulates the activity of soil microorganisms and supplies sustainability of soil ecosystems.

Therefore, soil degradation is a reversible problem and the renewable biomass production is an effective solution for biological reclamation of soils. Sustainable management of degraded soils on the base of energy crops enables a gradual reclamation of soil fertility and gets additional biomass for energy necessary for present and future generations. There are several aspects of the preference of energy crops for degraded soils management. The first is that most of the energy crops are not so demanding to soil conditions as agricultural crops, and the second, biomass may be used as fuel despite the content of some soil pollutants, like heavy metals or radionuclides. For example, wood biomass for energy is largely produced in Europe from forest land as a result of silvicultural and management practices or from agricultural land in the form of fast-growing plantations. The total aggregated available potential in Europe is 76 Mm^3 of wood biomass from the forests, with an additional 90 Mm^3 from increasing the utilization of forest lands, and 98 Mm^3 from fast-growing plantations (Mola-Yudego et al., 2017). Of course, not every type of soil is suitable for energy crop production. This paper focuses on energy crop production on degraded peaty soils which are suitable for sustainable renewable biomass production, but not competitive for agricultural crops when compared to fertile soils. Peaty soils are a specific type of soils formed as the result of wetlands drainage. Drainage of wetlands is used mainly for two basic reasons: peat

mining or agricultural crops growing. The degraded peaty soils can be formed as a result of both types of activity. They are soils characterized by big loss of organic matter, but high content of sand, with bad structure and low fertility. Peat obtained after wetlands drainage and its mining can be used as fossil fuel, organic fertilizer, the substrate for greenhouse crops, and so on. Peat is not a renewable resource and peat mining is not an endless process. Peat mining is completed by the factory if the layer of the peat is less than 1–2 meters and not enough deep for effective mechanical excavation. After the peat excavation is finished, the biggest problem is the use of these lands for other purposes. Soils formed after peat mining (post-mining peaty soils) are very heterogenic but generally characterized by a high level of acidity, poor nutrient content, low fertility, and poor structure. As a result, it can be very problematic to grow traditional agricultural crops that have special requirement of soil fertility in those areas. Post-mining peaty soils are not favourable for growing any cultural plants for several years, with the most critical period being the time after planting crops (Mosiej et al., 2012). This type of soil covers hundreds of thousands of hectares and it is similar to deserts in terms of biodiversity for several years after peat mining (Figure 1).

Figure 1. Post-mining peatlands, Lida region, Republic of Belarus (Authors photo)

The drainage of wetlands for agricultural purposes is another reason for peaty soil degradation. Only in the Republic of Belarus, since the middle of

the 19th century, there has been drained about 2.9 million hectares of wetlands for crops growing (Rodzkin et al., 2012). Fertile peaty soils have been used mostly for growing of wide-row cultures like potato, maize, or beets. The practice of wide-row cultures growing implies a lot of inter-row machining. As the result of active oxygen admission, the organic compound of peaty soils was mineralized and fertile peaty soils were also gradually transformed into degraded sandy-peaty soils.

These areas can be used for several purposes, such as forestation, flooding and fishing, growing cranberries, and others. Of course, it is necessary to use additional measures for soil reclamation. For example, mineral fertilizers, pesticides, and plant growth regulators must be used for cranberries plantations. However, the resulting cranberries production might not be profitable, despite the high price of the product. One of the perspective directions is rewetting post-mining wetlands to stimulate the growth of common reed, cattail, fescue, sedge, and other grass which can grow in natural conditions, and also improve conditions for the cultivation of other plants, including trees. Biomass may be used for pellet production. The goal of this research is an assessment of the possible use of wetlands biomass for the production of mixed peaty bio-pellets. These kinds of pellets are of special interest because peat is a non-renewable recourse and the important task is to decrease its use for fuel by mixing with biomass.

MATERIAL AND METHODS

Our experiments were done on post-mining peaty soils in Grodno region, Lida district (Republic of Belarus), close to the Lida Peat Factory (LPF), the biggest peat briquette company production in the region. The degraded peaty soils are very heterogenic with different content of nutrients and different decomposition of the peat. It is necessary to apply cultural practice suitable for the concrete type of peaty soil. The experiments investigated the possibility of obtaining biomass from natural grass (common reed) and plantations of short rotation coppice (SRC) willow. Reed was harvested every year and willow every three years. The straw of cereals and rape from agricultural land close to the peatlands were also considered as a prospective source of biomass.

For the estimation of wetland biomass, five typical plots were chosen within the experimental field. The yield was calculated by weighting in the field on the base of 4 replications after manual mowing. Moisture and ash content and calorific value of biomass were estimated in the laboratory.

The content of metals in biomass (Pb, Cd, Zn, Cu, Ni, Cr) was determined by the X-ray fluorescence method (RFA).

Pellets from peat and biomass (peaty-bio) were produced using granulator Gemko Energy (China). Straw and wood chips were previously milled, peat and biomass were sieved into fractions of 1–3 mm and mixed later.

Kinds of pellets were given in Tabs.

As a control, variant peaty pellets were produced.

A completely randomized design was used for statistical analysis. The experimental data obtained as a result of the studies were processed using the methods of dispersion analysis based on the statistical programs Excel and Statistica 10.

RESULTS AND DISCUSSION

Common reed is the dominant (most spread) species on drained wetlands areas in Europe. It is a tall, thin, highly productive grass mostly distributed in Europe (Shurpali et al., 2010). Reed is interesting for the market in Europe and nowadays the efforts to rewet and restore drained wetlands increased the reed growing area (Wichmann and Köbbing, 2015). Common reed and other natural grasses are perspective sources of bioenergy from wetland areas that do not require new fertile arable lands. In agricultural practice, special machines and equipment must be used and adapted for wetlands areas. The significant fluctuation of wetland biomass productivity is directly connected with the natural conditions of the area. The yield of common reed biomass in Belarus varied from 8.1 to 14.0 DM g ha^{-1} per year and it is competitive with other results (Rodzkin et al., 2017). For example, in China dry biomass yield of common reed ranged between 3.8–36.0 DM g ha^{-1} per year depending on the location (Shuai et al., 2016). The yield achieved on non-agricultural land for reed canary grass in Scotland ranged from 4 to 7 DM g ha^{-1} per year (Lod, 2015). During this period two harvests were taken and there was no additional fertilization, apart from adding limited amounts of subsequent nutrients.

The perspective possibility for degraded peaty soil reclamation is the cultivation of energy crops for biomass. For instance, perspective culture for degraded peaty soils is short rotation coppice trees, including willow (Kuzovkina and Martin, 2005). Short rotation coppice is the term used for fast-growing trees that may reach up to 4–5 meters height in 3 years. The plantation of SRC trees can exist 20–25 years with the harvesting of wood every 3–5 years. The main use of SRC trees is biomass used for electricity and heating. Energy forests plantations have been already introduced in Sweden (Dimitriou and Aronsson, 2005), Poland (Mosiej et al., 2012), Germany (Schweier and Becker, 2012), and other European countries, and in North and South America (Abrahamson et al., 2002). The yield of willow wood on degraded peaty soils in our experiments can reach 8–10 of DM g ha^{-1} per year (Rodzkin et al., 2013). The ordinary yield of willow for mineral fertile soils varies from 10 to 15 DM g ha^{-1} per year (Rosenqvist and Dawson, 2005). It is higher than the yield of willow from wetlands, but post-mining peaty lands are not suitable for ordinary agricultural crops and the use of degraded soils for SCR crops also has a positive environmental effect.

The straw of barley and rape were obtained from agricultural soils around the peaty factory. The yield of barley straw varied from 3 to 3.5 DM g ha^{-1}, rape straw from 3.5 to 4 DM g ha^{-1}.

Biomass of wood or straw can be used for biogas production, for heat production employing direct firing, and for pellets production (Rodzkin et al., 2019).

Anyway, from the environmental point of view, an important characteristic is the content of heavy metals in biomass because metals will be emitted to the environment later.

The results of measuring the heavy metals content in biomass are presented in Table 1.

Table 1. Content of heavy metals in samples of biomass

Metal	Content in biomass, mg/kg DM				
	Willow wood	Reed hay	Barley straw	Rape straw	Wheat straw
Zn	1,598.7	46.3	147.7	83.2	61.5
Cu	154.7	61.5	34.2	18.2	20.2
Cr	4.6	10.8	41.7	9.2	8.7
Ni	3.7	2.8	10.9	1.3	7.3
Cd	0.5	1.2	2.8	0.1	1.1
Pb	2.4	2.7	16.5	3.8	4.6

Willow residues contain several times more Zn and Cu, but less Cd, Pb, and Cr if compared with reed hay and straw. The highest content of Pb, Cd, Cr, and Ni was in barley straw. Therefore, barley straw is more polluted biomass and willow residues cleaner. Nevertheless, the content of heavy metals in all samples of straw was similar and depended mostly on soil characteristics.

The calculation of the prime cost of energy from a different type of biomass have been done depending on the calorific value of feedstock, cost of harvesting, transportation, milling, and drying. All types of feedstock were transformed into identical biomass (contents of water and fraction of biomass) for real comparison. The results of the calculation are presented in Table 2.

Table 2. The prime cost of energy from a different type of feedstock

Feedstock	Content of water (%)	Fraction, mm	Calorific value (kJ/kg)	Cost of biomass (Euro/t)	Cost of energy (Euro/GJ)
Wood	10	5	18,500	30.5	1.64
Straw of cereals	10	5	16,000	14.4	0.90
Straw of rape	10	5	15,500	17.2	1.11
Hay	10	5	15,500	16.4	1.06

The highest prime cost of biomass was obtained for willow wood. Our calculations have been done for the area of willow plantation of 100 hectares. The prime cost will be lower with the enlargement of the plantation area. The market cost of wood chips is very different and not stable. In Belarus, for instance, it varied from 25–50 euro per ton. The highest prime cost of energy was also for wood.

The basic technological characteristics of pellets are the content of water and ash and calorific value. The results of our research for different types of pellets are presented in Table 3.

Table 3. Technological characteristics of pellets

Material of pellets	Characteristic			
	Content of water (%)	Ash (%)	Calorific value (kJ/kg)	Calorific value (kCal/kg)
Peat (100%)	14.2	2.81	17,565.7	4,193.17
Peat/straw (75/25)	11.0	2.95	17,083.6	4,078.09
Peat/straw (50/50)	10.5	3.00	17,190.0	4,103.48
Peat/straw (25/75)	8.4	4.15	17,434.8	4,161.93
Peat/sawdust (75/25)	10.9	2.04	17,459.5	4,167.82
Peat/sawdust (50/50)	9.8	2.04	17,720.0	4,230.00
Peat/sawdust (25/75)	7.7	1.30	18,374.8	4,386.31

The highest humidity had pellets from peat. It can be explained by the fact that the original humidity of peat was two times higher if compared with straw or sawdust. As a result, with the increase of the ratio of biomass in pellets the contents of water tends to decrease. The highest content of ash was in pellets with a high rate of straw (75%). Wood residues have the lowest content of ash and the content of ash in straw is higher than in peat. High humidity and ash content decrease the calorific value of the fuel. As our experiments have shown, the fuel with a high content of wood residues (sawdust) has the best technological parameters including calorific value.

One more advantage of peaty bio-pellets is that, in accordance with the Kyoto Protocol and Paris Agreements, the emissions of greenhouse gases in the process of renewable biomass utilization for energy are not taken into consideration for the definition of carbon quotas. Biomass, including grass residues and wood, is "neutral" fuel for climate change (Kundas et al., 2015). As a result of photosynthesis, the plants can accumulate carbon dioxide (CO_2) and an equivalent volume of carbon dioxide can be emitted into the air during biomass combustion for energy production. The cost of carbon quotas on the market varies from 20 to 25 euros per ton (Markets Insider, 2019). It means that biomass production can provide additional profit in the case of CO_2 emissions trading depending on the biomass content in the fuel.

CONCLUSION

Soil degradation is a serious environmental problem today because of its impact on the physical, chemical, and biological soil quality and deterioration of soil health. Nevertheless, it is a reversible process because sustainable management of degraded soils on the base of energy crops enables a gradual reclamation of soil fertility and gets additional biomass for energy. This paper focuses on energy crops production on degraded peaty soils and polluted soils which are suitable for sustainable renewable biomass production, but not for agricultural crops. Degraded peaty soils cover hundreds of thousands of hectares and they are not favorable for growing any cultural plants. One of the

perspective directions is the rewetting of post-mining peatlands to stimulate the growth of common reed, cattail, fescue, sedge, and other grasses that can grow in natural conditions, and also improve conditions for the cultivation of other plants, including trees. The yield of wetland grass in Belarus varies from 8.1 to 14,0 DM g ha^{-1} per year. Another possibility for degraded peaty land management is the cultivation of special energy crops for biomass including willow. The yield of willow wood on degraded peaty soils can reach 8–10 of DM g ha^{-1} per year. The highest prime cost of biomass was obtained for willow wood, and its production can also be profitable. Our calculations have been done for the area of willow plantation of 100 hectares, but the prime cost will be lower with the enlargement of the plantation area. The market cost of wood chips is very different and not stable. In Belarus, for instance, it varies from 25–50 euro per ton, and in Europe up to 100 euros per ton.

Biomass from wetlands and close agricultural lands (straw) can be used for the production of mixed peaty bio-pellets. These kinds of pellets are of special interest because peat is a non-renewable recourse and the important task is to decrease its use for fuel by mixing with biomass. As our experiments have shown, the pellets with a high content of wood residues (sawdust) have the best technological parameters including calorific value if compared with pellets from only peat and peat plus straw.

Biomass production on degraded peaty soils has not only economic but also ecological effects. The cost of carbon quotas on the market varies from 20 to 25 euro per ton and biomass production can provide additional profit in the case of emission trading depending on the biomass content in the fuel.

REFERENCES

Abrahamson L, Volk T, Smart L, Cameron K (2002): *Willow Biomass Producer's Handbook*. NY: State University of New York, Syracuse.

Dimitriou J, Aronsson P (2005): Willows for energy and phytoremediation in Sweden. *Unasylva* 221: 47–50.

FAO (2019): FAO register. Available: http://www.fao.org/fao-stories/article/en/c/1192794/. Accessed: 2020 May 1.

FAO (2011): *The state of the world's land and water resources for food and agriculture (SOLAW). Managing systems at risk*. Earthscan Rome FAO and London.

Kundas S, Wichtman W, Rodzkin A, Pashinsky V (2015): Use of biomass from wet peatland for energy purpose. International and renewable energy sources as alternative primary energy sources in the region. *8 Int. Scientific Conference, Lviv, 2–3 April 2015*, Proceedings.

Kuzovkina J, Martin F (2005): Willows beyond wetlands: uses of Salix l. species for environmental projects. *Water Air Soil Pollut*. 162: 183–204.

Lod R (2015): Reed canary grass (*Phalaris arundinacea*) outperforms Miscanthus or willow on marginal soils, brownfield and non-agricultural sites for local, sustainable energy crop production. *Biomass Bioenergy* 78: 110–125

Markets Insider (2019): CO_2 European Emission Allowances. Available: https://markets.businessinsider.com/commodities/co2-european-emission-allowances. Accessed: 2019 October 1.

Mola-Yudego B, Arevalo J, Díaz-Yáñez O, Dimitriou I, Frechwater E, Haapala A, Khanam T, Selkimäki M (2017): Reviewing wood biomass potentials for energy in Europe: the role of forests and fast growing plantations. *Biofuels* 8: 401–410.

Mosiej J, Karczmarczyk A, Wyporska K, Rodzkin A (2012): Biomass Production in Energy Forests. *Ecosystem Health and Sustainable Agriculture 3. The Baltic University Programme*, Uppsala University, 196–202.

NSW Government register (2019): Available: https://www.environment.nsw.gov.au/topics/land-and-soil/soil-degradation. Accessed: 2020 May 1.

Rodzkin A, Shkutnik O, Krstich B, Borisev M (2012): Environmental background of fast-growing willow production on different type of soil. *XVI International eco-conference, Novi Sad, 26–29 Sept. 2012*.

Rodzkin A, Orlovich S, Pilipovich A, Krstich B (2013): Ecological and economic importance of energy crops. Environmental protection of urban and suburban settlements. *XVII International eco-conference, Novi Sad, 25–28 September, 2013*.

Rodzkin A, Kundas S, Wichtmann W (2017): Life cycle assessment of biomass production from drained wetlands areas for composite briquettes fabrication. *Energy Proc.* 128: 261–267.

Rodzkin A, Khroustalev B, Kundas S, Chernenok E, Krstic B (2019): Potential of Energy Willow Plantations for Biological Reclamation of Soils Polluted by 137Cs and Heavy Metals, and for Control of Nutrients Leaking into Water Systems. *Environ. Clim. Technol.* 23: 43–56.

Rosenqvist H, Roos A, Ling E, Hektor B (2000): Willow growers in Sweden. *Biomass Bioenergy* 18: 137–145.

Rosenqvist H, Dawson M (2005): Economics of willow growing in Northern Ireland. *Biomass Bioenergy* 28: 7–14.

Schweier J, Becker G (2012): Harvesting of short rotation coppice – harvesting trials with a cut and storage system in Germany. *Silva Fenn*. 46: 287–299.

Shuai W, Chen N, Li B, Zhou D, Gao J (2016): Life cycle assessment of common reed (*Phragmites australis* (Cav) Trin. ex Steud) cellulosic bioethanol in Jiangsu Province, China. *Biomass Bioenergy* 92: 40–47.

Shurpali NJ, Strandman H, Kilpeainen A (2010): Atmospheric impact of bioenergy based on perennial crop (reed canary grass, *Phalaris arundinaceae* L.) cultivation on a drained boreal organic soil. *GCB Bioenergy* 2: 130–138.

Wichmann S, Köbbing J (2015): Common reed for thatching – A first review of the European market. *Ind. Crops Prod.* 77: 1063–1073.

УПОТРЕБА ДЕГРАДИРАНИХ ТРЕСЕТНИХ ЗЕМЉИШТА ЗА ПРОИЗВОДЊУ БИОМАСЕ

Олег И. РОДКИН[1], Евгенија В. ЧЕРНЕНОК[1], Боривој Ђ. КРСТИЋ[2]

[1] Белоруски национални технички универзитет,
Независности 65, Минск 220123, Белорусија
[2] Универзитет у Новом Саду, Природно-математички факултет,
Департман за биологију и екологију,
Трг Доситеја Обрадовића 2, Нови Сад 21000, Србија

САЖЕТАК: Производња биомасе на деградираним тресетним стаништима нема само економски ефекат него и еколошки значај јер доводи до рекултивације станишта после вађења тресета са тресетишта. Циљ овог истраживања била је процена могућности коришћења дрвне биомасе и сламе тресетњака за производњу пелета као обновљивих извора енергије. Након завршетка ископа тресета највећи проблем је освајање и коришћење ових земљишта за другу намену. Један од перспективних праваца је ревитализација тресетишта подстицајем раста трске и других врста трава које могу да расту у природним условима, а такође побољшавају услове за узгој других биљака, укључујући дрвеће, у првом реду засаде врбе. Пелет са високим садржајем дрвних остатака (пиљевина) има најбоље технолошке параметре, укључујући калоријску вредност у поређењу са пелетом само од тресета и тресета са сламом. Производња биомасе на деградираним тресетним земљиштима има и еколошки ефекат. Трошкови квота за угљеник на тржишту варирају од 20 до 25 еура по тони, а производња биомасе може пружити додатни профит у случају трговања емисијама CO_2 и зависи од садржаја биомасе у гориву.

КЉУЧНЕ РЕЧИ: тресетишта, производња биомасе, засади кратке ротације, врба

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 113—125, 2021

UDC 528.92(497.11)
https://doi.org/10.2298/ZMSPN2140113L
ORIGINAL ARTICLE

Polina LEMENKOVA[*]

Schmidt Institute of Physics of the Earth, Russian Academy of Sciences.
Department of Natural Disasters, Anthropogenic Hazards and Seismicity of the Earth.
Laboratory of Regional Geophysics and Natural Disasters (Nr. 303).
Bolshaya Gruzinskaya 10/1, Moscow, 123995, Russian Federation,
ORCID ID: https://orcid.org/0000-0002-5759-1089

SCRIPTING LANGUAGES FOR GEOMORPHOLOGICAL MODELLING AND TOPOGRAPHIC VISUALIZATION OF SERBIA

ABSTRACT: Scripting cartographic technique is a new method of geospatial data visualization – especially with thematic mapping such as geomorphological models. The purpose of this study was to explore the use of Generic Mapping Tools (GMT) and R for geomorphological and topographic mapping of Serbia using free open datasets (DEM, SRTM/GEBCO, OpenStreetMap). Current trend in education and research of distance-based and online-based education suggests that application of free high-resolution data for modelling and mapping by open source cartographic toolsets are more likely to result in deep geospatial analysis of the geomorphology of Balkans with associated geographic phenomena: hydrology, soils, vegetation, geology. Presented fragments of scripts aim to demonstrate the technical usage of R and GMT coding in cartographic workflow with a case study on Serbia. Using qualitative descriptive cartographic approach to visualize the slope, aspect and terrain elevations over the country and the 'raster' package provided by R, it was found that the geomorphology of Serbia spatially differs in southern and northern parts of the country resulting in regional geologic evolution and tectonic dynamics of the Balkan formation. The presented maps portrayed general distribution of the landforms in Serbian region of Balkans. The research contributes to the methodological development and testing of the cartographic techniques as well as geomorphological and environmental studies of Serbia.

KEYWORDS: R, programming language, GMT, script, geomorphology, Serbia, mapping, cartography

INTRODUCTION

Qualitative cartographic data visualization and spatial analysis are the essential steps in geographic research. Plotting maps, graphs, and models representing

[*] Corresponding author. E-mail: pauline.lemenkova@gmail.com


Topographic map of Serbia
SRTM/GEBCO 15 arc sec resolution global terrain model grid
HUNGARY
Subotica
Tisza
Veliki Kanal
CROATIA
Novi Sad
Zrenjanin
ROMANIA
Belgrade
Sava
Danube
Smederevo
BOSNIA
AND
HERZEGOVINA
SERBIA
Velika Morava
Kragujevac
Karpatian Mts.
Zapadna Morava
DINARIDES
Kraljevo
Novi
Pazar
Juzna Morava
Leskovac
Balkan Mts.
(Stara Planina)
MONTENEGRO
Vranje
BULGARIA
ALBANIA
NORTH
MACEDONIA
Adriatic
Sea
Color scale 'geo': global bathymetry/topography relief [R=-6857/3206, H=0, C=RGB]
2021 May 27 15:05:11
Mercator projection. Scale: km

OpenStreetMap tile of Serbia. Plottiing: R package 'OpenStreetmap'

Figure 1. Map of Serbia. Left: GMT mapping. Right: OpenStreetMap, R. Source: author.

the phenomena of the Earth's objects present cartographic findings which in turn can significantly help in further research related to the Earth sciences (geological, environmental, geomorphological and landscape studies). However, it can be challenging, since there is not one accepted and universal cartographic method for data visualization and GIS mapping due to the wide variety of GIS. Analysis of territory is an essential part in environmental studies which involves such processes as data search and capture, data processing and visualization, data interpreting and modelling. At the same time, the process of cartographic data analysis is required to be selective and systematic while applying criteria for data quality with arising questions (Raster or vector? General or thematic? Topographic or environmental? Large-scaled or small-scaled? Fine-resolution or medium (or even coarse) resolution?) and GIS tools for data processing and mapping. Despite these challenges in the cartographic workflow, environmental mapping is always well worth the effort, since the results contribute to the increase of the general pool on research focused on the sustainable development and environmental modelling of the Earth.

There is no shortage of publications on the Balkan and Central European environment and ecology, land cover types and geology (see e.g. Marković et al., 2020; Cvetković et al., 2004; Gerzina & Djerić, 2017; Klaučo et al., 2013a, 2013b; Joó et al., 1981). There are also related papers on using GIS, spatial analysis and data modelling in environmental studies (Marović et al., 2002; Suetova, 2005; Lemenkova, 2020a, 2020b; Klaučo et al., 2014, 2017; Lemenkova et al., 2012; Lindh, 2004; Schenke & Lemenkova, 2008). However, there is not much of specific reference to using programming languages and scripting toolsets in cartographic data processing. There are various publications on using commercial GIS in geographic studies and environmental monitoring but none of them fulfill the requirements of the open source programming libraries and scripting tools which correspond to modern cartography.

In contrast with existing research on environmental studies of Serbia and Balkans (Bokhorst et al., 2009; Aufgebauer et al., 2012; Alex et al., 2019; Vidojević et al., 2020) this article first discusses cartographic issues of using the Generic Mapping Tools (GMT) relevant to high-quality data mapping. In addition, the code snippets used for visualization of the data are presented for repeatability with a detailed technical explanation on data processing. Furthermore, the reference is made to the GMT styles that were applied for topographic mapping of Serbia (specific codes of the most important GMT modules 'grdcut', 'psbasemap', 'grdcontour', 'pscoast' and 'pstext' are presented and explained in brief).

The R libraries for visualization of the free geodatasets and their geomorphological modelling are presented using R programming language (R Core Team 2020). These include such packages as 'tmap' (Tennekes, 2018), 'OpenStreetMap' and 'raster' as well as the dependent libraries ('ggplot2', 'maps', 'sp', 'sf'). Furthermore, some examples of functions presented in R flags were described and explained and cartographic results were compared. This is then followed by the discussion of results on geomorphological modelling of the slope, aspect and topographic elevation depicting the geomorphology of Serbia

by using scripting cartographic approach to qualitative spatial geographic research. Finally, there is a recommendation on further studies and consideration of the open source datasets applicability (such as OpenStreetMaps) in geomorphic studies and ways of cartographic visualizing and modelling of such data for spatial analysis.

DATA AND METHODS

A large number of approaches can be applied to cartographic data visualization and geomorphological modelling where the research focus and preferred choice of GIS will influence geospatial analysis. The geomorphological modelling depends on whether the data are used as a set of the raster grids that relate to a particular territory or vector files (e.g. ArcGIS ESRI .shp format). Another issue concerns a process of data collection, interpretation and geospatial analysis that may include additional set of data organized in layers for mapping layouts used for 3D and 2D terrain modelling. This paper presents a combination of quantitative and qualitative geomorphological research, where the input geodata are analyzed following data capture using scripting approaches of the GMT and R. There are following strategies using in this paper for geomorphological analysis.

The data capture has been performed from the two sources: 1) GEBCO/SRTM dataset (GEBCO Compilation Group, 2020) used for plotting Figure 1 (topographic map of Serbia); 2) Embedded datasets in R using packages 'OpenStreetMap' and 'raster'. Besides GEBCO grid, the ETOPO1 can also be used as a replacement (Lemenkova, 2020e) which has a lower file size comparing to GEBCO, although lower resolution as well. After data collection is complete, the set of data is focused upon, where data modelling and cartographic visualization is a separate step following data capture.

The topographic map (Figure 1) has been plotted by GMT cartographic scripting toolset (Wessel & Smith, 1995) using methodological approach discussed in details in existing relevant technical papers (Lemenkova, 2019a, 2019b). The mapping has been based on the sequence of codes by modules for plotting cartographic elements (Lemenkova, 2019c):

- Module 'grdcut' for selecting study area: gmt grdcut GEBCO_2019.nc -R18/24/41/47 -Gserbia_relief.nc
- Module 'grdimage' for visualizing the raster: gmt grdimage serbia_relief.nc -Cmyocean.cpt -R18/24/41/47 -JM6i -P -I+a15+ne0.75 -Xc -K > $ps
- Module 'psbasemap' for adding cartographic grid with ticks and title: gmt psbasemap -R -J -Bpx1f0.5a1 -Bpyg1f0.5a1 -Bsxg1 -Bsyg1 -B+t"Topographic map of Serbia" -O -K >> $ps
- Module 'grdcontour' for modelling isolines (topographic contours with a span every 1,000 m): gmt grdcontour serbia_relief.nc -R -J -C1000 -W0.1p -O -K >> $ps
- Module 'pscoast' for adding borders and rivers: gmt pscoast -R -J -P -Ia/thinner,blue -Na -N1/thickest,red -W0.1p -Df -O -K >> $ps

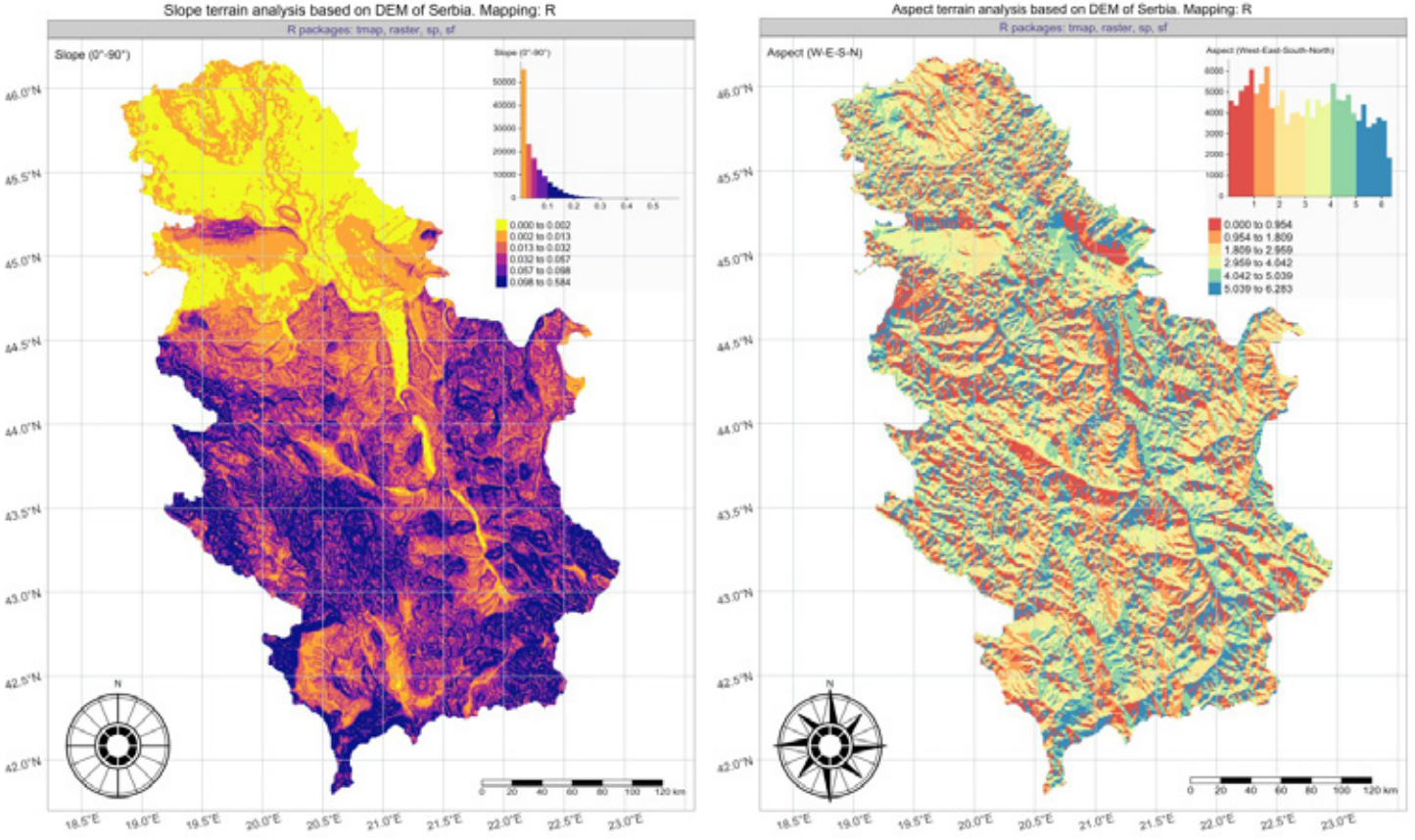


Figure 2. Left: slope modelling; Right: aspect modelling; Visualization: R; Source: author.

- Module ‘pstext’ for adding text annotations: gmt pstext -R -J -N -O -K -F+jTL+f14p,Helvetica−Narrow−Oblique,black+jLB -Gwhite@30 -Wthinnest >> $ps << EOF 20.0 44.1 Belgrade EOF
- Module ‘psxy’ for adding points (circle for capital city of Belgrade with coordinates in LON/LAT convention): gmt psxy -R -J -Sc -W0.5p -Gyellow -O -K << EOF >> $ps 20.0 44.0 0.3c EOF

The examples given above illustrate the principle of GMT functionality which performs mapping in a stepwise programmable manner similar to the programming language and scripting approaches used in geosciences for data analysis and processing (e.g. Lemenkova, 2019d, 2019d, 2019f). The final map made in GMT is presented in Figure 1 (left).

The next step in data analysis included the use of R library ‘OpenStreetMap’ for data visualization in Figure 1 (right). This has been plotted using libraries ‘maps’, ‘ggplot2’, ‘OpenStreetMap’ and dependent ‘sp’ of R. First, the data coordinates have been given using LAT/LON convention by the following two codes: ‘upper_left <- c(48, 18.0)’ and ‘lower_right <- c(40.0, 24)’ where the coordinates for the corners of the map were defined. Then the map in Figure 1 (right) was modeled on a screen using the following code: ‘map_osm <- openmap(upper_left, lower_right, type = c('esri-topo'))’. Afterwards the map has been visualized using command ‘plot(map_osm).

The third step in research methodology included geomorphological analysis using the two major libraries of R; ‘raster’ (used for modelling slope, aspect, hillshade and visualizing DEM based elevation), and ‘tmap’ used for cartographic mapping. The most important functions of the ‘tmap’ included the following ones. The general style of the layout was defined using the ‘tmap_style’ function. Then the raster was visualized using the ‘tm_shape’ function: ‘tm_shape(slope, name = "Slope", title = "Slope")’. The scale bar on all the maps has been added by the function ‘tm_scale_bar()’ with set up parameters (position, color, text).

The ‘tm_compass()’ function has been used for presenting compass oriented star in the lower left corner of the maps (Figure 2 and 3). The main cartographic visualization has been set up using the ‘tm_layout()’ function where a variety of parameters was adjusted (legend, position and text, title, histograms on data distribution, margins, among others). The ticks on the grid have been defined using the function ‘tm_graticules()’ with a set up text size, color, frequency of step. All the maps have been plotted in RStudio (RStudio Team 2017).

The geospatial datasets are usually visualized into a digital form for closer study and analysis (Gohl et al., 2006; Josimović & Pucar, 2010; Keller et al., 2020; Kuhn et al., 2006). This concerns the cartographic workflow techniques as well. Datasets such as raster grids, OpenStreetMap tiles, vector layers, contour lines of rivers and country boundaries may not be geoprojected and therefore additional geodata processing may be directly interpreted and reprojected using GDAL (Geospatial Data Abstraction Library). An example would be a the projecting of the OpenStreetMap tile of Serbia by ‘openproj’ function which projects the open street map to an alternate coordinate system. For instance, selecting a Lambert Conic Conformal projection requires the following code:

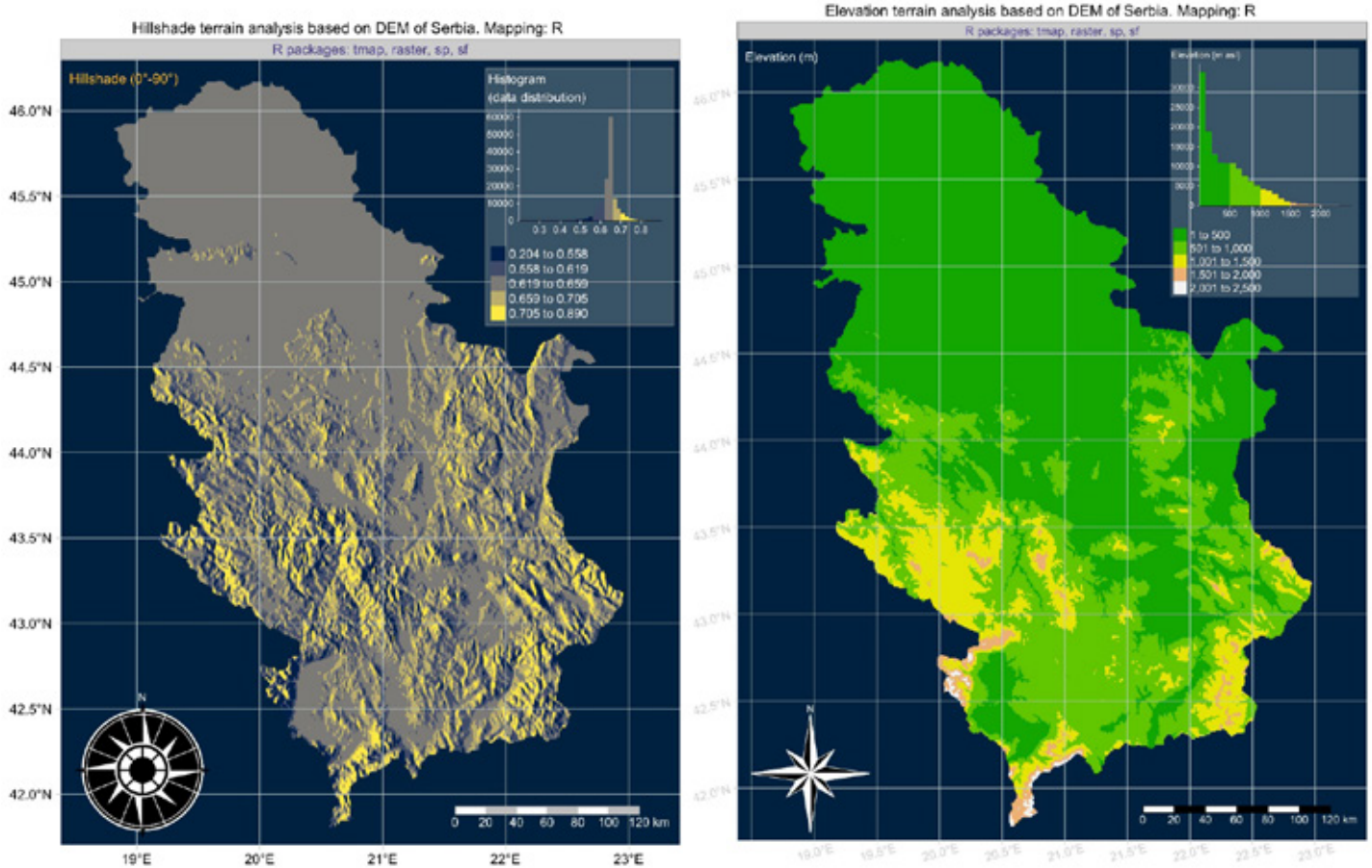


Figure 3. Left: Hillshade modelling; Right: Elevation DEM, R; Source: author.

map_serbia <- openproj(map_osm, projection="+proj=lcc +lat_1=41 +lat_2=47 +lat_0=44 +lon_0=21"). Then visualization of the map has been performed by the following code: plot(map_serbia, removeMargin=TRUE).

RESULTS AND DISCUSSION

The results shown in this manuscript demonstrated topographic data covering the territory of Serbia (SRTM, GEBCO, embedded datasets) processed in various ways by the scripting techniques of GMT toolset and R libraries. The results include visual geospatial data: cartographic layouts, plotted additional insert maps showing global location of the country, geomorphological models of slope, aspect and hillshade of the relief, visualized OpenStreetMap tiles. Six maps have been visualized using scripting cartographic techniques of R and GMT which shows a machine learning approach in contemporary cartography combining programming techniques of script writing with visualization of geospatial data.

Four of the maps were mapped for the geomorphological research (Figure 2 and 3), and two maps as topographic visualization (Figure 1). The map in Figure 1 (left) was plotted in GMT using presented techniques which can be repeated by the GMT learners and the datasets from GEBCO/SRTM grids using additionally available examples of GMT mapping (e.g. Lemenkova, 2020c, 2020d). The other maps (Figure 2 and 3) were plotted in RStudio and demonstrated the relief particularities in Serbia: slope steepness, aspect orientation by compass (West-North-East-South), hillshade relief which shows the relief with artificial illumination of the modeled light source which gives a visually effective way of the terrain representation and is widely used in the geographic studies or research. The maps are accompanied by the plotted histograms showing general statistical data distribution.

The paper is illustrated by the six cartographic images made by the author using free open source data and tools: the GMT scripting toolset and R (RStudio environment). The results are presented in form of topographic maps, geomorphological models histograms, which provide visualization of the topographic distribution of relief over the Republic of Serbia. The novelty of the research consists in the innovative cartographic methods that use GMT and R scripting for mapping the territory of Serbia which has not been published before. The study utilizes open source tools and data and presents automatization of the cartographic workflow through scripting which implies the repeatability of codes for similar geographic research in Serbia due to the free datasets and free instruments (R and GMT).

In this study, the data were captured freely from the open sources, which is a valuable point nowadays, since many researchers and scholars have a tendency for the online-based, home-based or distance-based studies and self learning. At the resulting research step, the data were visualized using a variety of modules and functions. These maps were digitally visualized on a screen and then saved. Plotting two maps side-by-side has been done: Twomaps <- tmap_arrange(map1,

map2). tmap_save (Twomaps, "Serbia_SlopeAspect.jpg", dpi = 300, height = 10, width = 15), where height and width determine map dimensions.

CONCLUSION

Existing studies on Serbian environment (Savic et al., 2021; Francke et al., 2016; Gachev et al., 2016; Kadović et al., 2012) identified that processing data reflected variability of the ecological and geographic processes that mirror its geomorphology which takes an important part of the land cover studies as a part of the complex ecosystems in Serbia. Hence, effective cartographic visualization of the terrain is a way of increasing our understanding of the landforms, visualizing possible correlations between the hydrological, botanical and soil factors in geomorphological context and increasing cartographic flexibility by using free data (SRTM, GEBCO, OpenStreetMap), supported by the open tools such as GMT and RStudio.

This research demonstrated that scripting methods are a means of more effective and flexible cartographic routine, since coding enables geographic data processing more quickly and transparent comparing to GIS. This research also pointed out factors associated with the use of R and GMT: the need of mastering scripting syntax as such and the access to the internet for data capture, installations and eventual look-up at technical tutorials. Geographic literature is also linked to the research on the geomorphology of the Balkans (Temovski et al., 2019; Toljić et al., 2013; Zagorchev, 2021) reflecting variability of Serbian geomorphology in regional context: mountains, valleys, highs, hills, and a variety of minor landforms.

ACKNOWLEDGEMENT

This research was supported by the project No. 0144-2019-0011, Schmidt Institute of Physics of the Earth, Russian Academy of Sciences. The author thanks the reviewers and the editor for the review, comments, corrections of the map of Serbia and editing of this manuscript.

REFERENCES

Alex B, Mihailović D, Milošević S, Boaretto E (2019): Radiocarbon chronology of Middle and Upper Paleolithic sites in Serbia, Central Balkans. *J. Archaeol. Sci.: Reports*, 25: 266–279.

Aufgebauer A, Panagiotopoulos K, Wagner B, Schaebitz F, Viehberg FA, Vogel H, Zanchetta G, Sulpicio R, Leng MJ, Damaschke M (2012): Climate and environmental change in the Balkans over the last 17 ka recorded in sediments from Lake Prespa (Albania/FYR of Macedonia/Greece). *Quat. Int.* 274: 122–135.

Bokhorst MP, Beets CJ, Marković SB, Gerasimenko N, Matvviishina Z, Frechen M (2009): Late Pleistocene geochemical variationsrecorded in Serbian and Ukrainian loess-paleosol sequences. *Quat. Int.* 198: 123–133.

Cvetković V, Prelević D, Downes H, Jovanović M, Vaselli O, Pécskay Z (2004): Origin and geodynamic significance of Tertiary postcollisional basaltic magmatism in Serbia (central Balkan Peninsula). *Lithos* 73: 161–186.

Francke A, Wagner B, Just J, Leicher N, Gromig R, Baumgarten H, Vogel H, Sadori L, Wonik T, Leng MJ, Zanchetta G, Sulpicio R, Giaccio B (2016): Sedimentological processes and environmental variability at Lake Ohrid (Macedonia, Albania) between 637 ka and the present. *Biogeosci.* 13: 1179–1196.

Gachev E, Stayanov K, Gikov A (2016): Small glaciers on the Balkan Peninsula: State and changes in the last several years. *Quat. Int.* 415: 33–54.

GEBCO Compilation Group (2020): GEBCO 2020 Grid. [DOI: 10.5285/a29c5465-b138-234d-e053-6c86abc040b9]

Gerzina N, Djerić N (2017): Chert blocks in the ophiolitic mélange of Zlatibor Mt. (SW Serbia): Age and geodynamic implications. *Geol. an. Balk. Pol.* 77: 13–21.

Gohl K, Eagles G, Udintsev G, Larter RD, Uenzelmann-Neben G, Schenke HW, Lemenkova P, Grobys J, Parsiegla N, Schlueter P, Deen T, Kuhn G, Hillenbrand CD (2006): Tectonic and sedimentary processes of the West Antarctic margin of the Amundsen Sea embayment and PineIsland Bay. *2nd SCAR Open Science Meeting*, 12–14 Jul, Hobart, Australia.

Joó I, Csáti E, Jovanović P, Popescu M, Somov VI, Thurm H, Thury J, Totomanov IN, Vanko J, Wyrzykowski T (1981): Recent vertical crustal movements of the Carpatho-Balkan region. *Tectonophysics* 71: 41–52. [DOI:10.1016/0040-1951(81)90045-7]

Josimović B, Pucar M (2010): The strategic environmental impact assessment of electric wind energy plants: case study 'Bavaniste' (Serbia). *Renew. Energy* 35: 1509–1519. [DOI: 10.1016/j.renene.2009.12.005]

Kadović R, Belanović S, Knežević M, Danilović M, Košanin O, Beloica J (2012): Organic carbon stock in some forest soils in Serbia. *Bull. Fac. Forest.* 105: 81–98.

Keller E, Adamaitis C, Alessio P, Anderson S, Goto E, Gray S, Gurrola L, Morell K (2020): Applications in geomorphology. *Geomorphology* 366: 106729.

Klaučo M, Gregorová B, Stankov U, Marković V, Lemenkova P (2013a): Determination of ecological significance based on geostatistical assessment: a case study from the Slovak Natura 2000 protected area. *Open Geosci.* 5: 28–42.

Klaučo M, Gregorová B, Stankov U, Marković V, Lemenkova P (2013b): Interpretation of Landscape Values, Typology and Quality Using Methods of Spatial Metrics for Ecological Planning. *Environmental and Climate Technologies*, October 14, 2013. Riga, Latvia. [DOI: 10.13140/RG.2.2.23026.96963]

Klaučo M, Gregorová B, Stankov U, Marković V, Lemenkova P (2014): Landscape metrics as indicator for ecological significance: assessment of Sitno Natura 2000 sites, Slovakia. *Ecology and Environmental Protection*, Minsk, March 19–20, 2014, 85–90.

Klaučo M, Gregorová B, Koleda P, Stankov U, Marković V, Lemenkova P (2017): Land planning as a support for sustainable development based on tourism: A case study of Slovak Rural Region. *Environ. Eng. Manag. J.* 2: 449–458.

Kuhn G, Hass C, Kober M, Petitat M, Feigl T, Hillenbrand CD, Kruger S, Forwick M, Gauger S, Lemenkova P (2006): The response of quaternary climatic cycles in the South-East

Pacific: development of the opal belt and dynamics behavior of the West Antarctic ice sheet. *Expeditionsprogramm Nr. 75* ANT XXIII/4. [DOI: 10.13140/RG.2.2.11468.87687]

Lemenkova P, Promper C, Glade T (2012): Economic Assessment of Landslide Risk for the Waidhofen a.d. Ybbs Region, Alpine Foreland, Lower Austria. *Protecting Society through Improved Understanding. 11th International Symposium on Landslides & the 2nd North American Symposium on Landslides & Engineered Slopes (NASL)*, June 2–8, 2012. Canada, Banff, 279–285. [DOI: 10.6084/m9.figshare.7434230]

Lemenkova P (2019a): GMT Based Comparative Analysis and Geomorphological Mapping of the Kermadec and Tonga Trenches, Southwest Pacific Ocean. *Geogr. Tech.* 14: 39–48.

Lemenkova P (2019b): Automatic Data Processing for Visualising Yap and Palau Trenches by Generic Mapping Tools. *Cartographic Lett.* 27: 72–89.

Lemenkova P (2019c): Geomorphological modelling and mapping of the Peru-Chile Trench by GMT. *Pol. Cartogr. Rev.* 51: 181–194.

Lemenkova P (2019d): Statistical Analysis of the Mariana Trench Geomorphology Using R Programming Language. *Geod. Cartogr.* 45: 57–84.

Lemenkova P (2019e): Testing Linear Regressions by StatsModel Library of Python for Oceanological Data Interpretation. *Aquat. Sci. Eng.* 34: 51–60.

Lemenkova P (2019f): AWK and GNU Octave Programming Languages Integrated with Generic Mapping Tools for Geomorphological Analysis. *GeoSci. Eng.* 65: 1–22.

Lemenkova P (2020a): Applying Automatic Mapping Processing by GMT to Bathymetric and Geophysical Data: Cascadia Subduction Zone, Pacific Ocean. *J. Environ. Geogr.* 13: 15–26.

Lemenkova P (2020b): SAGA GIS for information extraction on presence and conditions of vegetation of northern coast of Iceland based on the Landsat TM. *Acta Biol. Marisiensis* 3: 10–21.

Lemenkova P (2020c): GEBCO Gridded Bathymetric Datasets for Mapping Japan Trench Geomorphology by Means of GMT Scripting Toolset. Geodesy and Cartogr. 46: 98–112.

Lemenkova P (2020d): Using GMT for 2D and 3D Modeling of the Ryukyu Trench Topography, Pacific Ocean. *Misc. Geogr.* 25: 1–13.

Lemenkova P (2020e): GRASS GIS Modules for Topographic and Geophysical Analysis of the ETOPO1 DEM and Raster Data: North Fiji Basin, Pacific Ocean. *Geogr. Napocensis* 14: 27–38.

Lindh P (2004): *Compaction- and strength properties of stabilised and unstabilised fine-grained tills. PhD Thesis, Lund: Lund University.*

Marković S, Vandenberghe J, Stevens T, Mihailović D, Gavrilov M, Radaković M, Lehmkuhl F (2020): Geomorphological evolution of the Petrovaradin Fortress Palaeolithic site (Novi Sad, Serbia). *Quat. Res.* 1–14. [DOI: 10.1017/qua.2020.88]

Marović M, Djoković I, Pešić L, Radovanović S, Toljić M, Gerzina N (2002): Neotectonics and seismicity of the southern margin of the Pannonian basin in Serbia. *EGU Stephan Mueller Spec. Publ. Ser.* 3: 1–19.

R Core Team (2020): *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/

RStudio Team (2017): *RStudio: Integrated Development Environment for R. RStudio, Inc., Boston, MA*. URL: https://www.RStudio.com/

Savic R, Ondrasek G, Zemunac R, Kovacic MB, Kranjcec F, Jokanovic VN, Bezdan A (2021): Longitudinal distribution of macronutrients in the sediments of Jegricka watercourse in Vojvodina, Serbia. *Sci. Total Environ.* 754: 142138. [DOI: 10.1016/j.scitotenv.2020.142138]

Schenke HW, Lemenkova P (2008): Zur Frage der Meeresboden-Kartographie: Die Nutzung von AutoTrace Digitizer für die Vektorisierung der Bathymetrischen Daten in der Petschora-See. *Hydrogr. Nachr.* 25: 16–21.

Suetova I, Ushakova L, Lemenkova P (2005): Geoinformation mapping of the Barents and Pechora Seas. *Geogr. Nat. Resour.* 4: 138–142.

Temovski M, Milevski I, Madarász B, Kern Z, Ruszkiczay-Rüdiger Z (2019): Glacial geomorphology of the northeastern part of the Jakupica Mountain, Macedonia, Central Balkan Peninsula. EGU General Assembly 2019, Vienna, Austria. URL: https://meetingorganizer.copernicus.org/EGU2019/EGU2019-7822.pdf

Tennekes M (2018): tmap: Thematic Maps in R. *J. Stat. Softw.* 84: 1–39.

Toljić M, Matenco L, Ducea MN, Stojadinović U, Milivojević J, Đerić N (2013): The evolution of a key segment in the Europe-Adria collision: the Fruska Gora of northern Serbia. *Glob. Planet Change* 103: 39–82.

Vidojević DD, Manojlović MS, Đorđević AR, Nešić LM, Predić TM (2020): Correlations between soil organic carbon, land use and soil type in Serbia. *Matica Srpska J. Nat. Sci*. 138: 9–18.

Wessel P, Smith WHF (1995): New version of the Generic Mapping Tools released. *EOS* 76: 329–329.

Zagorchev IS (2021): Geology of the Balkan peninsula. In: Alderton D, Elias SA (eds.), *Encyclopedia of Geology* (2nd Ed), Academic Press, 382–407.

СКРИПТНИ ЈЕЗИЦИ ЗА ГЕОМОРФОЛОШКО МОДЕЛОВАЊЕ И ТОПОГРАФСКУ ВИЗУЕЛИЗАЦИЈУ СРБИЈЕ

Полина ЛЕМЕНКОВА

Институт Шмит за физику Земље, Руска академија наука,
Катедра за природне непогоде, опасности изазаване људским деловањем и сеизмолошку активност Земље,
Лабораторија за регионалну геофизику и природне непогоде (бр. 303)
Улица Бољшаја Грузинскаја 10/1, Москва 123995, Руска Федерација
ORCID ID: https://orcid.org/0000-0002-5759-1089

РЕЗИМЕ: Скриптна картографска техника је нов метод визуелизације геоспацијалних података – посебно са тематским мапирањем као што су геоморфолошки модели. Циљ овог проучавања је истраживање употребе Генеричког алата за мапирање (енгл. GMT) и R за геоморфолошко и топографско мапирање Србије користећи слободне отворене скупове података (DEM, SRTM/GEBCO, OpenStreetMap). Актуелан тренд у образовању и истраживање наставе на даљину и онлајн наставе указује на то да примена слободних података високе резолуције за моделовање и мапирање путем картографског алата из отворених извора, највероватније резултира дубоком геоспацијалном анализом геоморфологије Балкана са повезаним географским феноменима: хидрологија, земљишта, вегетација, геологија. Приказани фрагменти скрипта имају за циљ да демонстрирају техничку употребу R и GMT кодирања у картографском процесу рада где је Србија у фокусу истраживања случаја. Користећи квалитативни описни картографски приступ

да би се визуелизовали нагиб, положај и издизање земљишта широм земље и „растер” пакет обезбеђен путем R, откривено је да се геоморфологија Србије просторно разликује у јужним и северним деловима земље што резултира регионалном геолошком еволуцијом и тектонском динамиком балканске формације. Приказане мапе показале су генералну дистрибуцију рељефа у делу Балкана у којем је Србија. Истраживање доприноси методолошком развоју и тестирању картографских техника као и геоморфолошким изучавањима и изучавањима животне средине у Србији.

КЉУЧНЕ РЕЧИ: R, програмски језик, GMT, скрипт, геоморфологија, Србија, мапирање, картографија

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 127—135, 2021

UDC 574.1(497.113 Novi Sad)
https://doi.org/10.2298/ZMSPN2140127L
SHORT REPORT

Milena D. LAKIĆEVIĆ[1*], *Saša S. ORLOVIĆ*[1,2], *Ksenija J. HIEL*[1], *Srđan I. ŠEREMEŠIĆ*[1], *Radenka D. KOLAROV*[1], *Milena D. MAKSIMOVIĆ*[1]

[1] University of Novi Sad, Faculty of Agriculture,
Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia
[2] University of Novi Sad, Institute of Lowland Forestry and Environment,
Antona Čehova 13, Novi Sad 21000, Serbia

BIODIVERSITY INDICES FOR THE FUTOG PARK (NOVI SAD, SERBIA)

ABSTRACT: Biodiversity and biodiversity preservation are some of the most important topics in ecology. Protecting biodiversity in urban ecosystems is especially challenging. Urban parks are a valuable part of green infrastructure in cities, as they contribute to preserving natural habitats for many species. Biodiversity can be quantified by calculating different diversity indices, and in this paper, we have calculated alpha indices (Shannon, Simpson, Fisher, Berger-Parker and Margalef index) and beta indices (Sorensen index, Jaccard distance and Bray-Curtis index) for the Futog park in Novi Sad, Serbia, within two periods, 2005 and 2020. The goal of the paper is to analyze the current values of biodiversity indices in the park and compare them with the ones from 15 years ago. In addition to the analysis of biodiversity indices values, the paper provides the comparison of taxonomic structure of flora, and the abundance of native, endemic and invasive species in the park, in 2005 and 2020. The results show that the values of biodiversity indices have decreased in the indicated period of time, and one of the main causes can be related to the spread of invasive species within the park. For calculation purposes, we have used the R program and the R package "vegan".

KEYWORDS: biodiversity, species diversity, urban parks, Futog park

INTRODUCTION

Biodiversity encompasses variability and diversity of genes, species and ecosystems on Earth (Vujić, 2008). In order to quantify the species' diversity, there are many indices defined. Biodiversity indices can be split into alpha, beta and gamma indices; alpha indices measure biodiversity in a single area;

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

beta indices are used either to compare the similarity of flora composition in different areas or to compare the similarity of flora composition in the same area over different periods; while gamma indices measure biodiversity on a large spatial scale (Magurran, 2004). The most commonly used alpha diversity indices are Simpson and Shannon index (Lakićević and Srđević, 2018), but other indices such as Fisher, Berger-Parker, Margalef index are also analyzed and discussed in the prominent literature (Fedor and Zvaríková, 2019). Calculation of biodiversity indices can be performed by using different programs and computer tools, and nowadays programming language R, its interface RStudio and the R package "vegan" are commonly applied in ecological research (Lakicevic et al., 2020).

In the domain of urban ecology, it is highly important to calculate and keep track of species diversity in urban parks, as they present a habitat for many valuable and rare species (Srdjevic et al., 2019). In this paper, we analyze the biodiversity indices for the natural monument – the Futog park in Novi Sad, Serbia. There are two periods analyzed in the paper: 2005 and 2020, and the idea is to analyze the current values of biodiversity indices in the park and to compare them with the precedent ones. On one hand, calculating alpha diversity indices gives an insight into values of biodiversity, and its components, richness and evenness, in both periods separately. On the other hand, obtaining values of beta diversity indices quantifies (dis)similarities in plant species' composition between two analyzed periods, and directly reveals how much plant composition has changed over time. In this paper, the analysis of alpha and beta diversity indices are being supported with additional data such as analysis of the taxonomic structure of flora, the share of native, endemic and invasive species in both periods, etc. Gathering all of the listed data gives a detailed insight of the floristic elements in the Futog park for the period 2005–2020, and can serve as a starting point for future monitoring of changes in plant species diversity.

The main goals of the paper are: analysis of biodiversity indices in the Futog park in 2005 and 2020 and analysis of changes in the floristic composition that has occurred in the indicated time period. In addition to that, the paper aims to promote the application of the R program in urban ecology tasks, and particularly the application of the package "vegan" in calculating biodiversity indices. The paper also provides a detailed description of the most commonly used biodiversity indices that are important when keeping track of floristic elements in urban parks.

METHODS

Study area

The Futog park is situated in Novi Sad, Serbia (Figure 1). The park occupies approximately 12 ha, it was established in 1910 and therefore represents one of the oldest and the largest parks in Novi Sad (Lakićević and Srđević,

2017). The park was declared a natural monument in 2005 (*Službeni glasnik Republike Srbije / The Official Gazette of the Republic of Serbia*, 23/2005).

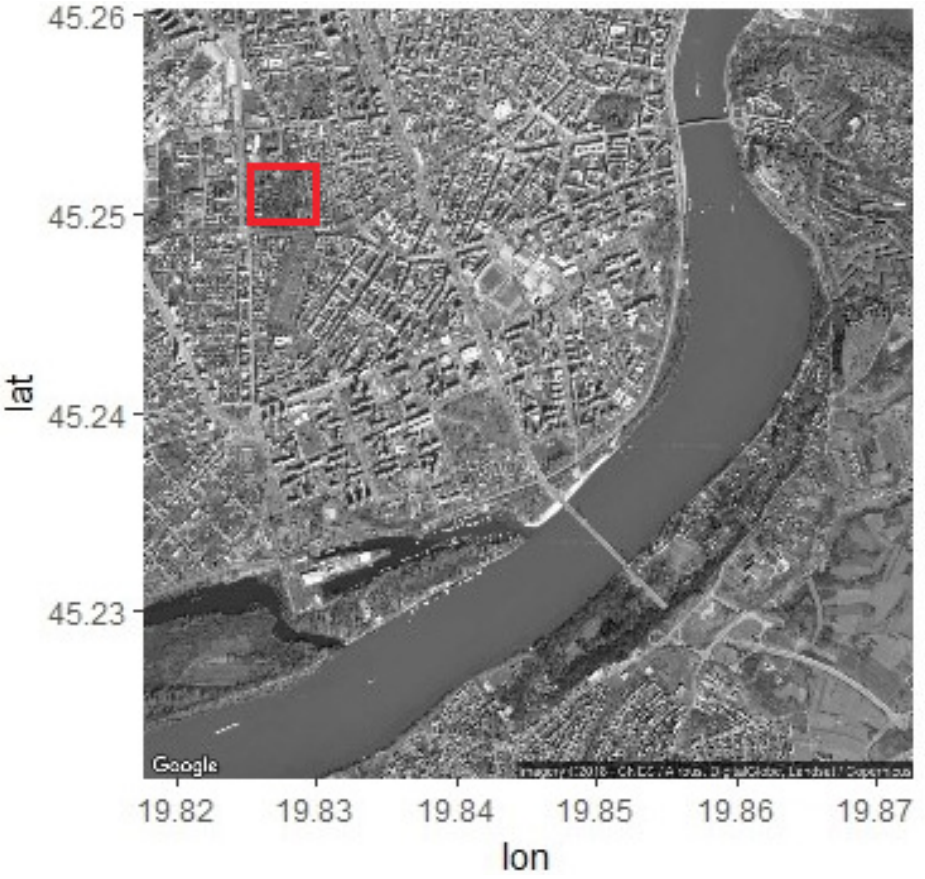


Figure 1. Location of the Futog park in Novi Sad

The Futog park was established in the first decade of the XX century, immediately after the construction of Jodna Banja. The original park design was the idea of the famous Hungarian landscape architect, Armin Pec Junior. The park was designed in a combined style that was dominant for landscape architecture projects of that period and implied mixing elements of French and English landscape style. The park was reconstructed in 1964 according to the designs of Ratibor Đorđević. Even though the outlook of the park has changed over time, the design concepts of both original and reconstruction projects are still present and recognizable.

One detailed survey of flora in the park was done in 2005 (Popović, 2005), followed by another one in 2008 (Ninić-Todorović et al., 2008). These data are compared with the current state, using a new survey from 2019 (Mlađenović, 2019), and the data collected at the site in 2020. Therefore, this research was

conducted in two phases: the first one implied fieldwork and determining all tree and shrub species that are present in the park, as well as their abundance. These data were input data for the second phase – further analysis and calculation of biodiversity indices for both time periods.

Biodiversity indices

In this section, we describe five alpha and three beta biodiversity indices used in this research. For each index, we provide a short description and a brief comment of its usual and threshold values.

Alpha biodiversity indices

We calculate one nonparametric index (Fisher index) and four parametric indices (Shannon, Simpson, Berger-Parker and Margalef index). More details about each index and corresponding formulas can be found in (Magurran, 2004).

Shannon index usually varies in the interval 1.5–3.5. Values of Shannon index over 3.5 and particularly over 4 suggest that an area is extremely valuable in terms of species' richness and evenness.

Simpson index measures evenness and varies in the interval [0,1], where the value of 0 reveals a monoculture, and higher values prove better evenness among the species.

Fisher index (α) is based on the assumption that the abundance of species follows the log series distribution. Fisher index is approximately equal to the number of individuals represented by a single species.

Berger-Parker index vary between 0 and 1, where smaller values correspond to higher diversity. This index quantifies the abundance of the most dominant species.

Margalef index is sensitive to sample size, but higher values of Margalef index prove higher biodiversity.

All of above listed alpha indices were calculated in program R, version 3.5.3. Fisher, Shannon and Simpson index were calculated by using the R package "vegan", while Berger-Parker and Margalef index were calculated by the R codes written by the first author of the paper.

Beta biodiversity indices

In this section, we present three beta biodiversity indices that have been used for a comparison of plant species composition in the Futog park in the periods 2005 and 2020. These indices are: Sorensen index, Jaccard distance and Brey-Curtis index, and a brief explanation for each of them is provided in the next paragraphs.

Sorensen index takes into account the number of species that are common for both datasets (in this case both time periods), and the number of species that are present only in one dataset, i.e. one time period. The value of the Sorensen index falls into the interval between 0 and 1, where 0 means none, and 1 means a complete overlap in plant species composition in the analyzed time periods.

Jaccard distance uses the same input data as for the Sorensen index, but measures dissimilarities in plant species composition in two datasets. The value of the Jaccard index varies from 0 to 1, where values close to 0 mean that two datasets highly overlap in the plant species composition.

Bray-Curtis index takes into account different input data compared to the two previously described indices and the necessary data for calculation are: the sum of the lesser values of the number of specimens for the species that are common in both periods, and the total number of specimens present in each period. The value of this index can vary from 0 to 1, where 0 means that plant communities in two periods differ completely, and 1 means they are the same from the perspective of shared species and their abundance.

Calculation of all listed beta biodiversity indices has been done by using the R program, and its package "vegan". The paper presents additional data and analysis, such as the taxonomic structure of flora, the share of native, endemic and invasive species in the park in both time periods, etc.

RESULTS AND DISCUSSION

The first part of the results is related to the values of biodiversity indices in the Futog park in Novi Sad, in two time periods, 2005 and 2020. Table 1 shows the values for five main biodiversity indices.

Table 1. Alpha biodiversity indices in the Futog park (2005 and 2020)

Alpha diversity index	Value	
	Year 2005	Year 2020
Shannon index	3.592	3.578
Simpson index	0.957	0.947
Fisher index	16.116	20.599
Berger-Parker index	0.088	0.150
Margalef index	11.056	13.167

Shannon index is the principal alpha biodiversity index that quantifies both richness and evenness of species within an area. Based on the values in Table 1 it can be concluded that the value of the Shannon index for the Futog park has decreased in the analyzed time period. In order to get a better insight into the changes that have occurred, one should analyze the value of the Simpson index that quantifies the changes in the evenness of species in a plant

composition. Comparing the values of the Simpson index for 2005 and 2020 proves that there was a better distribution of species in overall species composition in the first analyzed period. However, it should be noted that the current value of the Simpson index (equal to 0.947) is still considered as high. Fisher index is interpreted as an approximate number of plant specimens that belong to one plant species, and the value of this index increased over the analyzed period of time, and this means that on average, each species has more specimens now than back in 2005. However, the value of this index should be associated with the value of the Berger-Parker index, which measures the share of the most dominant species in the plant composition. The value of this index severely increased in the analyzed time period, from 0.088 and 0.15, and this change also affects the higher value of the Fisher index in 2020. The most dominant species in the park now is *Mahonia aquifolium* (Pur) Nutt, and the value of the Berger-Parker index proves that it has a high share in overall plant species composition, equal to 15% (Table 1). The value of the Margalef index is more complex for a straightforward comparison, as being sensitive to the sample size, but its values for both 2005 and 2020 prove that the Futog park is valuable from the biodiversity perspective.

Apart from analyzing alpha biodiversity indices, the research included calculation of beta biodiversity indices, that compare the similarities in the plant species composition in the Futog park, in 2005 and 2020 (Table 2).

Table 2. Beta biodiversity indices in the Futog park (2005–2020)

Beta diversity index	Value
Sorensen index	0.893
Jaccard distance	0.193
Bray-Curtis index	0.614

Table 2 shows that the value of the Sorensen index is equal to 0.893 which proves that there were moderate changes in plant species composition in the Futog park in the period 2005–2020. The same conclusion can be drawn by analyzing the value of the Jaccard distance, equal to 0.193, which quantifies the dissimilarity in plant species composition in two time periods. Sorensen and Jaccard indices measure the overlap in plant species composition by taking into account the number of common species in both periods only, and not involving the number of their specimens. That is why it is useful to include additional indices, such as the Bray-Curtis index. The value of the Bray-Curtis index is equal to 0.614 and this means that number of specimens for common species has changed moderately.

The next analysis relates to the taxonomic structure of flora in the Futog park in 2005 and 2020 (Figure 2).

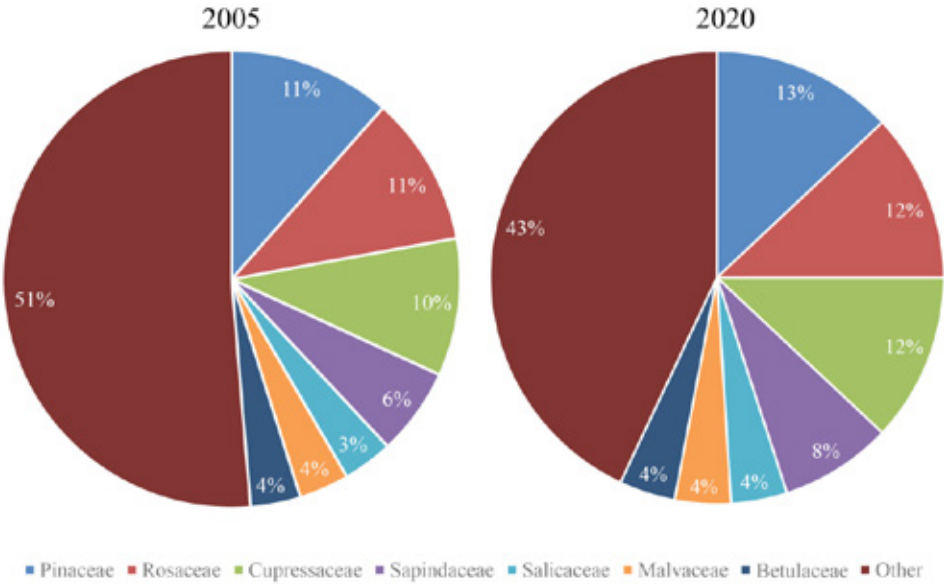


Figure 2. Taxonomic structure of flora in the Futog park

Figure 2 shows that the three most dominant plant families in 2005 were: Pinaceae and Rosaceae (each of them present with the share of 11%), and the family Cupressaceae (present with the share of 10%). In 2020, due to the changes in plant species composition, first of all, introducing the following species *Prunus avium* L., *Prunus cerasus* L., *Prunus cerasifera* Ehrh. and *Malus floribunda* Siebold., the family Rosaceae became the most dominant with a share of 13%. As in the previous period, the families Pinaceae and Cupressaceae have a high share, equal to 12%.

The next analysis considers native, endemic and invasive in the Futog park, in 2005 and 2020, by presenting the number of species and their specimens for each category (Table 3).

Table 3. Number of species and specimens for native, endemic and invasive species for the Futog park (2005 and 2020)

Description		Value	
		Year 2005	Year 2020
Native species	Number of species	86	97
	Number of specimens	7,893	4,273
Endemic species	Number of species	6	6
	Number of specimens	412	398
Invasive species	Number of species	10	10
	Number of specimens	173	277

Table 3 shows that in the past 15 years, the number of native species increased, but the number of their specimens significantly decreased – for 47%. Endemic species are present with the same number of species (5), but with a slightly smaller number of specimens. If one analyzes the invasive species, the conclusion is that the number of invasive species remained the same (10), but the number of their specimens significantly increased, for 60.1%.

The decrease in the number of native species' specimens and the increase in the number of invasive species specimens can seriously affect the structure of natural elements in the park. The spread of invasive species is one of the reasons for the slight decrease of biodiversity indices in the park in the period 2005–2020 and should be carefully monitored in the future.

CONCLUSIONS

This paper analyzes the biodiversity indices for the Futog park in Novi Sad, Serbia in the two time periods, 2005 and 2020. The park was declared a natural monument in 2005 and is one of the largest and the oldest parks in the city of Novi Sad. The flora of the park mainly consists of broadleaves (85%) and the most dominant plant families are Rosaceae, Pinaceae and Cupressaceae.

The values of five alpha biodiversity indices calculated in the paper (Fisher, Shannon, Simpson, Berger-Parker and Margalef index) prove that the flora diversity in the park is still high, including its both components: richness and evenness. However, the values of these same indices were more favorable in 2005. Since then, the plant species composition has moderately changed, encompassing both the number of common species and the number of their specimens. In the indicated period, the number of invasive species' specimens has increased by approximately 60%. In terms of expected climate change, it should be noted that invasive species have a high ability to adapt to the newly created environmental conditions, while at the same time, suppress the living niches of native and endemic species (Lakićević and Mladenović, 2018). Therefore, invasive species and their spread within the park should be carefully monitored in the future.

REFERENCES

Fedor P, Zvaríková M (2019): Biodiversity Indices. In: BD Fath (ed.), *Encyclopedia of Ecology*. Oxford: Elsevier Science Ltd.

Lakicevic M, Povak N, Reynolds KM (2020): *Introduction to R for Terrestrial Ecology*. Basel: Springer.

Lakićević M, Mladenović E (2018): Non-native and invasive tree species – their impact on biodiversity loss. *Matica Srpska J. Nat. Sci.* 134: 19–26.

Lakićević M, Srđević B (2017): Multiplicative version of PROMETHEE method in assessment of parks in Novi Sad. *Matica Srpska J. Nat. Sci.* 132: 79–86.

Lakićević M, Srđević B (2018): Measuring biodiversity in forest communities – role of biodiversity indices. *Contemp. Agric.* 67: 65–70.

Magurran AE (2004): *Measuring Biological Diversity*. London: Blackwell Publishing.

Mlađenović M (2019): *Spomenik prirode „Futoški park" u Novom Sadu, Revizija zaštite – Predlog za stavljanje pod zaštitu kao zaštićeno područje lokalnog značaja – III kategorije*. Novi Sad: Pokrajinski zavod za zaštitu prirode.

Ninić-Todorović J, Nešić L, Lazović R, Kurjakov A (2008): Futoški park as the preserved nature heritage. *Ann. Agron.* 32: 102–110.

Popović M (2005). *Spomenik prirode „Futoški park u Novom Sadu" – Predlog za stavljanje pod zaštitu kao značajno prirodno dobro*. Novi Sad: Pokrajinski zavod za zaštitu prirode.

Službeni glasnik Republike Srbije / The Official Gazette of the Republic of Serbia (23/2005).

Srdjevic B, Srdjevic Z, Lakicevic M (2019): Urban greening and provisioning of ecosystem services within hesitant decision making framework. *UFUG* 43: 126371. [https://doi.org/10.1016/j.ufug.2019.126371]

Vujić A (2008): *Zaštita prirode*. Novi Sad: Univerzitet u Novom Sadu, Prirodno-matematički fakultet.

ИНДЕКСИ БИОДИВЕРЗИТЕТА ЗА ФУТОШКИ ПАРК (НОВИ САД, СРБИЈА)

Милена Д. ЛАКИЋЕВИЋ[1], Саша С. ОРЛОВИЋ[1,2], Ксенија Ј. ХИЕЛ[1], Срђан И. ШЕРЕМЕШИЋ[1], Раденка Д. КОЛАРОВ[1], Милена Д. МАКСИМОВИЋ[1]

[1] Универзитет у Новом Саду, Пољопривредни факултет, Трг Доситеја Обрадовића 8, Нови Сад 21000, Србија
[2] Универзитет у Новом Саду, Институт за низијско шумарство и животну средину, Антона Чехова 8, Нови Сад 21000, Србија

РЕЗИМЕ: Биодиверзитет и очување биодиверзитета спадају у најзначајније теме у екологији. Очување биодиверзитета у урбаним екосистемима представља посебан изазов. Градски паркови су важан део зелене инфраструктуре у градовима, јер доприносе очувању природних станишта за бројне врсте. Биодиверзитет се може квантификовати рачунањем различитих индекса, и у овом раду израчунати су индекси алфа диверзитета (Шенонов, Симсонов, Фишеров, Бергер-Паркеров и Маргалефов индекс) и индекси бета диверзитета (Соренсенов индекс, Џакардово растојање и Брај-Куртисов индекс) за Футошки парк у Новом Саду, Србија, за два временска периода, 2005. и 2020. годину. Циљ рада је анализа садашњих вредности индекса биодиверзита у парку и поређење са стањем од пре 15 година. Осим анализе вредности индекса биодиверзита, рад приказује поређење таксономске структуре флоре и присуства аутохтоних, ендемичних и инвазивних врста у парку у 2005. и 2020. години. Резултати показују да су вредности индекса биодиверзитета смањене у анализираном временском периоду, и један од главних разлога може бити повезан са ширењем инвазивних врста у парку. За потребе прорачуна, коришћен је програм R и његов пакет „vegan".

КЉУЧНЕ РЕЧИ: биодиверзитет, диверзитет врста, градски паркови, Футошки парк

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 corre-

spondence 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

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. Review articles should not be longer than 12 pages, including references, tables, legends, and figures.

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

5. References

5.1. List the References alphabetically. Examples:

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

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

(c) Books: Last name VG, Last name CS (2009): Title of the cited book. Publisher, City

(d) Dissertations: Last name VA (2009): Title of the thesis. Doctoral dissertation, University, City

(e) Unpublished articles: designation "in press" should be used only for papers accepted for publishing. Unpublished articles should be cited in the same way as published articles except that instead of journal volume and page numbers should write "in press" information.

(f) Articles reported at scientific meetings and published in extenso or in a summary form: Last name FR (2011): Proceedings, Name of the meeting, Meeting organizers, Venue, Country, 24–29.

(g) World Wide Web Sites and other electronic sources: Author's last name, Author's initial. (Date of publication or revision). Title, In: source in Italics, Date of access, Available from: <Available URL>. Use n.d. (no date) where no publication date is available. Where no author is available, transfer the organization behind the website or the title to the author space.

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

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

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

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

6. Units, names, abbreviations, and formulas

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

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

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

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

7. Figures

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

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

8. Tables

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

8.2. Number the tables using Arabic numerals.

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

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

9. Electronic copy of the article

9.1. After the acceptance of the article, send a CD with final version of the manuscript and a printed copy to facilitate technical processing of the text. Articles should be written in Microsoft Word format and sent to the Editorial office of the Matica 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 by Matica srpska twice a year
Editorial and publishing office:
Matice Srpske 1, 21000 Novi Sad, Serbia
Phone: +381 21/6615798

E-mail: vnikolic@maticasrpska.org.rs
zmspn@maticasrpska.org.rs
www.maticasrpska.org.rs

The editors of the *Matica Srpska Journal for Natural Sciences*
completed the selection for Issue 140 (1/2021) on April 14, 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 May 2021
Computer set: Vladimir Vatić, GRAFIT, Petrovaradin
Printed by: SAJNOS, Novi Sad

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

CIP – Каталогизација у публикацији
Библиотека Матице српске, Нови Сад

5/6(082)

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

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

ISSN 0352-4906

COBISS.SR-ID 5845250