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NOVEL TRENDS IN FERMENTED DAIRY TECHNOLOGY

ABSTRACT: Novel trends in fermented dairy technology are presented in this review paper. The application of new starter cultures (probiotics, kombucha), as well as quality improving ingredients like transglutaminase (TGase), milk protein fractions, and functional components of plant origin have been investigated by the authors worldwide. New processing techniques such as: high-pressure processing (HPP), high pressure homogenization (HPH), and ultrasonic processing (USP) are interesting because of their potential to achieve a specific and/or novel functionality or to improve the efficiency. Novel trends in fermented dairy technology contribute to the creation of various products with high nutritive value, possessing also specific functional properties. Basic health benefits of functional fermented dairy products are: biologically active peptides – ACE inhibitors and antioxidative activity. Due to the mentioned functional characteristics, these dairy products are considered to be among the most precious functional foods.

KEYWORDS: ACE-inhibitors, antioxidative activity, fermented dairy technology, health benefits, kombucha, new techniques, transglutaminase.

INTRODUCTION

Consumption of fermented milk products, as a healthy and nutritious food has a long tradition. Milk fermentation was first used to extend the shelf life of the product, but many benefits were obtained along this process, like improving the digestibility and flavor, as well as the ability to produce a wide range of different products. Currently over 400 different commercial names exist for traditionally and industrially produced fermented milk products. The specificity of each type of product is defined by the applied starter cultures, milk quality, and process conditions (Iličić et al., 2015; Milanović et al., 2016; Milanović et al., 2017).

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The selection of the appropriate starter culture type, like traditional yoghurt culture, probiotic culture, or other combinations is of great importance in fermented milk products manufacturing. The addition of different ingredients (milk proteins, whey protein concentrate, whey protein isolates, etc.) is a good practice to improve starter culture growth during the fermentation period and enhance bacterial viability in the product. Inulin as a non-digestible ingredient beneficially affects humans, selectively stimulating growth and/or activity of bacteria in the colon. Recent studies have been dealing with possibilities of kombucha addition as non-conventional starter for milk fermentation.

The application of transglutaminase enzyme (TGase) and/or some sophisticated techniques (high-pressure processing, high-pressure homogenization, and ultrasonic processing) has enabled the improvement of the quality of fermented dairy products.

Novel trends in fermented dairy technology contribute to the creation of various products with high nutritive and less energy value, which possess functional properties. Main basic health benefits of functional fermented dairy products are: biologically active peptides – ACE inhibitors and antioxidative activity (Tamime et al., 2006; Korhonen, 2009; Surono and Hosono, 2011; Milanović, et al., 2016; Milanović et al., 2017).

INNOVATIONS IN FERMENTED DAIRY TECHNOLOGY

Kombucha

Kombucha is a symbiotic association of yeast (*Pichia, Zygosaccharomyces, Saccharomyces, Schizosaccharomyces, Saccharomycodes, Torulaspora, Candida*, and *Brettanomyces*), acetic acid bacteria (*Acetobacter* and *Gluconobacter*), and lactic acid bacteria (*Lactobacillus* population predominantly) (Marsh et al., 2014; Chakravorty et al., 2016). Traditionally, kombucha is cultivated on dark and green tea, but also it can be cultivated on a whey, lactose, wine, beer, etc. Kombucha tea beverage, as a consequence of fermentation on selected substrate, contains ethanol, carbon dioxide, a high concentration of acid (gluconic, acetic, and lactic), as well as a number of other metabolites and is thought to contain a number of health-promoting components (Malbaša et al., 2009; Jajabalan et al., 2014; Milanović et al., 2017).

Numerous studies have examined the possibilities of applying kombucha (native, concentrated by evaporation/microfiltration, or in a mixture with probiotic starter) in milk fermentation process. Malbaša et al. (2009) concluded that fermentation time was similar when evaporated kombucha inoculum at 10 and 15% was added to milk, while their fermentation time was two times longer compared to yoghurt starter.

The results of another study showed that kombucha cultivated at two different tea types: *Camellia sinensis* (black tea) and *Thymus serphyllum* (thyme tea) in combination with probiotics for milk fermentation at temperatures 37 °C and 42 °C, could also be used for production of new functional fermented

dairy products (Milanović et al., 2017). The combination of probiotic starter culture and kombucha cultivated at black tea and thyme tea lasted from 3.5 to 4.5 h respectively. Shapes of fermentation curves are sigmoidal and similar for both samples. Analysis of textural characteristics during ten days of storage revealed significantly better textural characteristics in samples produced with black tea kombucha inoculum than thyme tea.

The investigation of the effect of kombucha starter culture in comparison with yoghurt starter culture and probiotics, on rheological properties, texture and microstructure during milk fermentation at 42 °C at pH value of 5.4, 5.1, 4.8, and 4.6, as well as on protein profile was the objective of a comprehensive study (Hrnjez et al. 2014a, b; Vukić et al., 2014). Although the fermentation time of probiotic yogurt manufacture is two times shorter than in kombucha fermented milk production, application of kombucha in fermented dairy technology is justified by its nutritional advantages. Textural characteristics, viscosity, nutritive and sensory properties of kombucha fermented milk products classified it as a novel food of high nutritive value. Principal Components Analysis (PCA) methodology is applied to determine the differences among fermented dairy products obtained by yoghurt starter, probiotic starter, and a novel kombucha starter (Vukić et al., 2018). The Power-law model was used succesfully to describe the non-Newtonian fluid behavior of the examined fermented dairy products.

The effect of kombucha starter culture on the kinetics of the lactose transformation during milk fermentation from pH 5.8 to 4.6, at two different temperatures 37 ºC and 42 ºC, showed that the reaction rate passes through the maximum after 9 h and 30 min at 37 ºC and after 4 h at 42 ºC (Kanurić et al., 2018). The best model to present the curve of fermentation change of lactose is sigmoidal Boltzmann's function.The sigmoidal saturation curve indicates a complex kinetics of lactose fermentation by kombucha starter (Figure 1).

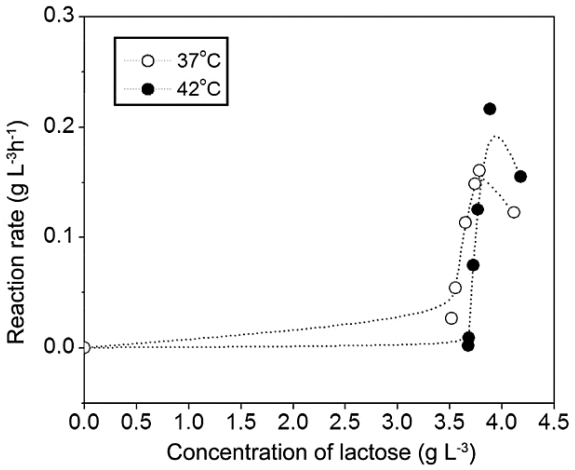

Figure 1. Saturation curves-reaction rates versus lactose concentration by kombucha fermentation (Kanurić et al., 2018).

Transglutaminase

Enzyme transglutaminase (TGase, EC 2.3.2.13) is isolated from the *Streptoverticillium* strains and commercially distributed by Ajinomoto Co. Inc. (Japan). TGase catalyzes the acyl transfer reaction between γ-carboxyamide groups of peptide-bound glutamine residues and the ε-amino groups of lysine residues. The result of this reaction is formation of intra- and intermolecular isopeptide bonds and formation of covalently cross-linked protein polymers.

The treatment of milk proteins with this enzyme induces their physical stabilization and structural modification as well as positive changes of physicochemical, textural, and rheological properties of fermented dairy products, particularly yoghurt with a reduced fat content.

Numerous authors have recently investigated the possible applications of TGase in food protein systems, especially in milk proteins. There are two ways of the application of TG in yoghurt manufacturing: (1) simultaneous addition with starter culture, or (2) prior to fermentation. In case (1) the enzyme is gradually inactivated, which is caused by acidification. Case (2) requires additional process time, as well as a thermal inactivation step. The advantage of case (2) is constant pH during the cross-linking reaction, which enables a wide range of incubation conditions. TGase pre-treatment is usually followed by a heating step to inactivate the enzyme at 80 °C for 1 min (Bönisch et al., 2007; Özer et al., 2007; Iličić et al., 2013, 2014; Milanović et al., 2009; 2017).

Protein ingredients such as milk powder, WPC, and sodium caseinates are often used to fortify the protein content of milk in order to obtain the desired yoghurt structure, which is markedly enhanced by TGase action (Iličić et al., 2016; Milanović et al., 2017).

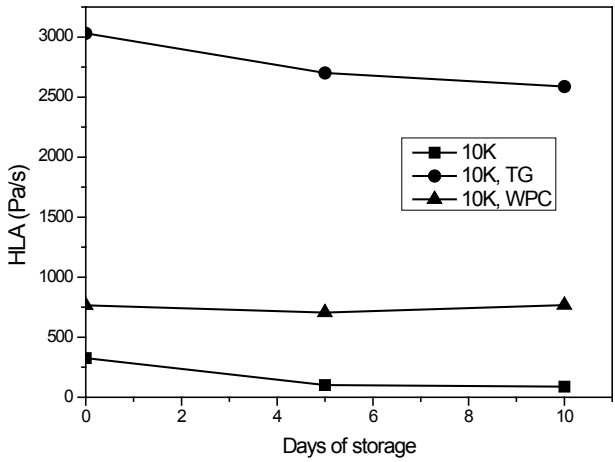

Figure 2. Rheological properties (hysteresis loop area) of kombucha fermented milk products during 10 days of storage (Iličić et al., 2016).

As consumer demand for additive-free and minimally processed food increases, innovative food technology is gaining interest as a processing technology and has been increasingly adopted within the dairy technology. New processing techniques, such as high-pressure processing-HPP, high pressure homogenization-HPH, and ultrasonic processing-USP are of interest beacuse of their potential to achieve a specific and/or novel functionality or improve efficiencies, including reduced chemical and water use (Udabage et al., 2010; Iličić et al., 2015; Milanović et al., 2017; Sfakianakis and Tzia, 2017; Gharibzahedi et al., 2018).

HPP is a physical method able to change milk components functionality. The operating pressure of 1,000 MPa and 400 MPa is applied in HPP and HPH processes, respectively. HP treatment in the dairy sector includes changing the properties of gels in yoghurt manufacture. Gelation at higher pH and improvements in the mechanical properties of acid gels (increase in gel rigidity and resistence to syneresis) prepared from HP-treated milk have been reported (Udabage et al., 2010).

Harte et al. (2002) characterised the yield stress, microstructure, water-holding capacity (WHC), and syneresis of plain full fat set yoghurt gels prepared from fortified milk subjected to the following processes: a) thermal process, 85 °C, 35 min, b) high hydrostatic pressure process (193 or 676 MPa for 5 or 30 min) (Figure 3; Table 1).

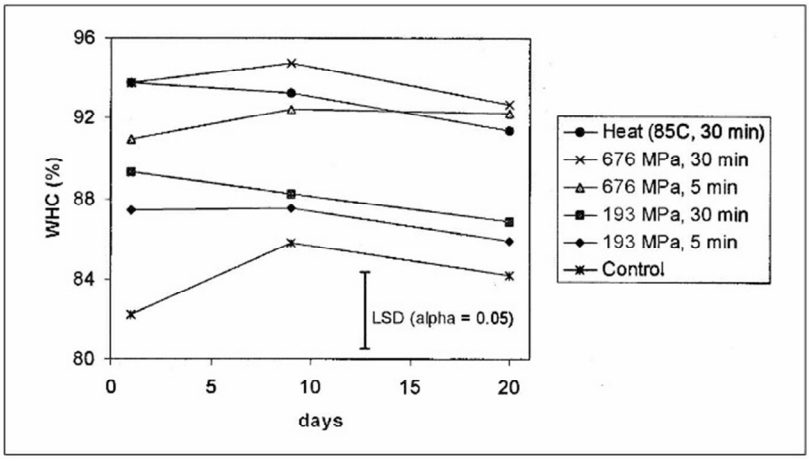

Figure 3. Water-holding capacity (WHC) for yoghurt prepared after milk treatments
(Harte et al., 2002)

Table 1. Mean pH and yield stress of yoghurts obtained from milk following selected treatments (Harte et al., 2002)

Milk treatment	pH	Yield stress (Pa)
Thermal, 85 °C, 30 min	4.54	63.5
676 MPa, 30 min	4.57	49.3
676 MPa, 5 min	4.54	22.9
193 MPa, 30 min	4.68	14.8
193 MPa, 5 min	4.69	13.7
Control	4.73	12.2

HPP treatmant of milk in combination with addition of TG can be useful for the dairy industry to achieve products of improved structure and desirable sensory characteristics (Tsevdou et al., 2013).

Utrasonic treatment is also an emerging, chemical free, novel method in food application, which involves the transmission of high intensity and high frequency (10–1,000 W/cm^2, 20–100 kHz) sound waves through the liquid food material. It could be successfully used in dairy systems, due to the ability to preserve food and modulate enzyme activity, to improve processing efficiency, the ability to manufacture products with tailored functionality, and the capability of improving microstructure through interactions between components.

The effect of ultrasonic treatmant with or without temperature control at 20, 40, 60, and 70 °C on the properties of heated (80 °C, 30 min) or non heated skim milk in the formation of acid gels has been investigated by Ngyen, Anema (2010). USP was performed at the frequency of 22.5 kHz and output power of 50 W during 0 to 30 min using a Microson XL200 Ultrasonic homogenizer/cell disruptor. Gels were formed by the acidification of the skim milk either by acidification with glucono-δ-lacton at 30 °C, or by addition of yoghurt culture at 42 °C until pH of 4.5 was obtained. The results showed that USP of skim milk for moderate times without temperature control could be used to produce acid gels with markedly increased final G' values and reduced gelation time.

Numerous studies revealed that the other properties of fermented dairy products prepared from ultrasound-treated whole milk were also improved. Textural characteristics and water holding capacity increased, while syneresis was weaker when compared with yoghurt prepared from conventionally heated whole milk. The USP of skim milk before fermentation by yoghurt cultures produced gels with higher firmness, shorter gelation time, higher gelation pH, and better sensory properties than those attained by conventional heating.

Sophisticated processing technologies could be an alternative to conventional heating by providing the improved functional properties and storage stability to fermented milk products. The application of new technology – high-pressure processing, high pressure homogenization, and ultrasonic processing – in fermented dairy processing could improve their nutritive characteristics, rheology, and sensory properties.

BIOLOGICAL POTENTIAL OF FUNCTIONAL FERMENTED DAIRY PRODUCTS

Basic health benefits

Fermented dairy products contain high levels of nutritionally important constituents like lactose, protein, fat, mineral matters, and vitamins. During the milk fermentation process, complex enzymatic biotransformations (glycolysis, proteolysis, and lipolysis) are underway, whereby organic acids, ethanol, EPS, GES, sphingolipids, and bacteriocins are formed. Fermented dairy products obtain different characteristics in relation to milk as the function of many processing factors (Milanović et al., 2017). The basic chemical composition of different types of yoghurt is shown in Table 2 (Tamime and Robinson, 2004).

Table 2. Chemical composition and energy value of milk and different types of yoghurt (Tamime and Robinson, 2004).

Constituent (units/100 g)	Milk		Yoghurt			
	Whole	Skim	Full fat	Low fat	Low fat/fruit*	*Greek-style*
Water (g)	87.8	91.1	81.9	84.9	77.0	77.0
Energy value (kcal)	66	33	79	56	90	115
Protein (g)	3.2	3.3	5.7	5.1	4.1	6.4
Fat (g)	3.9	0.1	3.0	0.8	0.7	9.1
Carbohydrate (g)	4.8	5.0	7.8	7.5	17.9	–
Calcium (mg)	115	120	200	190	150	150
Phosphorus (mg)	92	95	170	160	120	130
Sodium (mg)	55	55	80	83	64	–
Potassium (mg)	140	150	280	250	210	–
Zinc (mg)	0.4	0.4	0.7	0.6	0.5	0.5

* The nutrient levels in the fruit yoghurt vary with the type of fruit and added stabilizers.

The main change during milk fermentation is the transformation of lactose (20–30%) resulting from the activity of the appropriate starter culture that produces β-galactosidase enzyme, by transforming lactose to lactic acid. Lactic acid causes destabilization of casein micelles by conversion of colloidal calcium phosphate complex into soluble calcium phosphate, followed by casein coagulation at pH 4.6–4.7.

In adition to this essential effect of starter culture (lactose transformation) in fermented dairy products, many complicated biochemical transformations happen as well, not only connected with lactose fermentation, but also with proteolysis and milk fat degradation. As the result of these reactions, especially when probiotic starters are used in the process and functional ingredients added, biologically active peptides with ACE inhibitory activity (angiotensin-converting-enzyme-inhibitory peptides) and antioxidative activity have been observed. Due to the mentioned functional characteristics, these dairy products are considered to be among the most precious functional foods.

Biologically active peptides – ACE inhibitors

The therapeutic effect of fermented milk on cardiovascular diseases has been noticed long ago. The biologically active peptides are formed during the milk fermentation process due to the activity of the proteolytic enzymes of the starter culture and/or by using commercial enzymes like *in vivo* enzymatic hydrolysis in the gastrointestinal tract under the action of digestive enzymes. According to the function, these peptides can be divided into: ACE inhibitory peptides, antimicrobial peptides, antithrombin peptides, peptides that affect the immune system, casomorphins and other opioid peptides, calcium-binding peptides, and peptides with antioxidant properties (Beermann and Hartung, 2013; Hafeez et al., 2014; Milanović et al., 2017). ACE inhibitors reduce the activity of rennin angiotensin-aldostorene system (RAAS) which is the main cause of hypertension (Milanović at al., 2016).

The efficacy of the fermentation process during which certain ACE-inhibitory peptides are released depends on the proteolytic activity of the selected microorganisms (Table 3). It has been found that *L. helveticus* has the most effective proteolytic system among lactic acid bacteria.

Table 3. Examples of bioactive peptides released from milk proteins by various microorganisms and microbial enzymes (Korhonen and Pihlanto, 2006).

Micro-organisms used	Precursor protein[a]	Peptidne sequence	Bioactivity
L. helveticus, *Saccharomyces cerevisiae*	β-cn, κ-cn	Val-Pro-Pro, Ile-Pro-Pro	ACE* inhibitory, antihypertensive
Lactobacillus GG enzymes+pepsin & trypsin	β-cn, α_{s1}-cn	Tyr-Pro-Phe-Pro, Ala-Val-Pro-Tyr-Pro-Gln-Arg, Thr-Thr-Met-Pro-Leu-Trp	Opioid, ACE* inhibitory, immunostimulatory
L. helveticus CP90 proteinase	β-cn	Lys-Val-Leu-Pro-Val-Prp (Gln)	ACE* inhibitory
L. helveticus CPN 4	Whey proteins	Tyr-Pro	ACE* inhibitory
L. delbrueckii subsp. *bulgaricus* SS1 *Lactococcus lactis* subsp. *cremoris* FT4	β-cn, κ-cn	Many fragments	ACE* inhibitory
L. delbrueckii subsp. *bulgaricus* IFO13953	κ-cn	Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met	Antioxidative
L. rhamnosus + digestion with pepsin and Corolase PP	β-cn	Asp-Lys-Ile-His-Pro-Phe, Tyr-Gln-Glu-Pro-Val-Leu, Val-Lys-Glu-Ala-Met-Ala-Pro-Lys	ACE* inhibitory Antioxidative
L. delbrueckii subsp. *bulgaricus*	β-cn	Ser-Lys-Val-Tyr-Pro-Phe-Pro-Gly Pro-Ile	ACE* inhibitory
S. thermophilus+Lc. lactis subsp. *lactis* biovar. *diacetylactis*	β-cn	Ser-Lys-Val-Tyr-Pro	ACE* inhibitory
L. helveticus JCM 1004 cell free extract	Skim milk hydrolysate	Val-Pro-Pro, Ile-Pro-Pro	ACE* inhibitory

[a] cn-casein, * ACE-*angiotensin-I-converting-enzyme-inhibitory peptides.*

It has been shown that the ACE inhibitory activity increases the most during storage of the kombucha fermented milk-based beverage in comparison with the yogurt produced using the traditional or probiotic starter culture (Milanović et al., 2017; Hrnjez et al., 2014a). This confirms that proteinase and peptidase activity of starter cultures affects the milk protein breakdown to various levels resulting in a wide range of peptides with functional properties, such as the ACE inhibitory activity (Figure 4). The authors confirmed that kombucha starter culture is suitable for milk fermentation, contributes to ACE activity and should be further investigated as a potentially new starter in dairy industry.

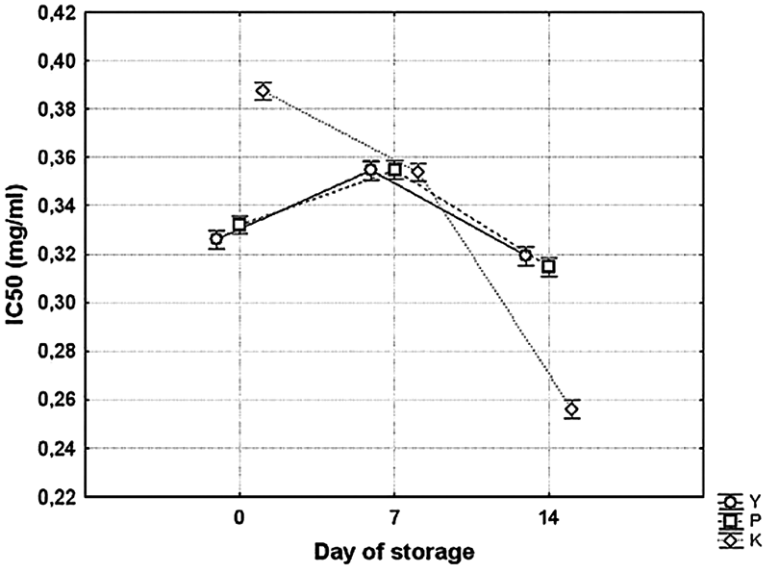

Figure 4. ACE inhibitory activity expressed as an IC_{50} value in yoghurt (J), probiotic yoghurt (P), and kombucha (K) fermented milk beverage during the storage period (Hrnjez et al., 2014a).

Antioxidative activity

The antioxidative activity of fermented dairy products depends on the content of antioxidant milk components: casein fractions and peptides derived from the hydrolysis of casein and additives used in the production process. The antioxidant activity of casein fractions ranges from the highest to the lowest in the following order: β-casein> α_{s1}-casein> α_{s2}-casein> α-lactalbumin> β-lactoglobulin (Rival et al., 2001a, b). It is known that the antioxidative capacity of milk increases with the content of milk fat, vitamins A and D, omega-3 polyunsaturated fatty acids, riboflavin, retinol, tocopherol, ascorbic acid, lactoferrin, and antioxidant enzymes (Milanović et al., 2017).

Numerous authors have investigated the addition of various components to fermented dairy products, giving them increased functional properties, especially antioxidative activity. Most of these functional components are of plant origin, such as: olives, grapes, soya, dill, mint, basil, green tea, and others.

However, it seems that green tea, among all mentioned components, deserves the greatest attention for its effect on functional properties of fermented dairy beverages. The main active polyphenolic compounds present in the tea are catechins and phenolic acids that can react with milk proteins and affect the functional properties, microbiological quality and oxidative stability of fermented dairy products (O'Connell and Fox, 2001). Najgebauer-Lejko et al. (2011) examined the possibility of producing yoghurt prepared from milk with the addition of 5, 10 or 15% of green tea. These authors identified a significant effect of the addition of tea on the antioxidant properties, microbiological characteristics, and stability of these products during storage. The average activity on free radicals (DPPH) is 31, which is 15 times higher than yoghurt without the addition of tea. In addition, green tea has antimicrobial effect against many pathogenic microorganisms (e.g. *S. aureus, E. coli, S. enteridis*) (Michalczik and Zavislak, 2008; Milanović et al., 2017).

The production of functional food with an antihypertensive effect has been steadily increasing worldwide. There are several fermented milk-based beverages which contain bioactive compounds on the market, such as *"Ameal &Calpis"* (produced by *Calpis Co, Japan*) and *"Evolus"* (produced by *Valio, Finland*). Besides the antihypertensive, other effects of the activity of biologically active peptides on human health are also intensively studied.

CONCLUSION

Fermented milk products, as a healthy and nutritious food, have a long tradition. There have recently appeared over 400 different industrial commercial products of fermented milks. The specificity of each type of product is defined by the applied starter cultures, milk quality, and process conditions.

The selection of the appropriate starter culture, traditional yoghurt culture or probiotic culture or other combinations like kombucha addition, is of great importance in fermented milk products manufacturing.

The application of transglutaminase enzyme (TGase) and/or some sophisticated new techniques (high-pressure processing, high-pressure homogenization, and ultrasonic processing) has enabled the improvement of the quality of fermented dairy products.

Novel trends in fermented dairy technology contribute to the creation of various products with high nutritive value, possessing also specific functional properties. Basic health benefits of functional fermented dairy products are: biologically active peptides – ACE inhibitors and antioxidative activity. Due to the mentioned functional characteristics, these dairy products are considered to be among the most precious functional foods.

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САВРЕМЕНА ИСТРАЖИВАЊА У ТЕХНОЛОГИЈИ ФЕРМЕНТИСАНИХ МЛЕЧНИХ ПРОИЗВОДА

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РЕЗИМЕ: Савремена истраживања у технологији ферментисаних млечних производа приказана су у овом прегледном раду. Примену нових стартер култура (пробиотици, комбуха), инградијената за побољшање квалитета производа као што су: трансглутаминаза, протеини и фракције протеина млека, функционалне компоненте биљног порекла, истраживали су бројни аутори у свету. Испитивања могућности примене нових софистицираних техника хомогенизације под високим притиском, високог хидростатичког притиска и ултразвука, значајна су за добијање производа специфичних и/или побољшаних функционалних карактеристика. Иновације у технологији ферментисаних млечних производа доприносе креирању различитих функционалних производа високе нутритивне вредности. Биолошки активни пептиди – АСЕ-инхибитори и антиоксидативна активност значајно доприносе здравственим бенефитима функционалних ферментисаних млечних производа. Имајући у виду наведене карактеристике, ферментисани млечни производи су најзначајнији представници функционалне хране.

КЉУЧНЕ РЕЧИ: антиоксидативна активност, АСЕ-инхибитори, здравствени бенефити, комбуха, нове технике, трансглутаминаза, ферментисани млечни производи

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Campylobacter IN FOOD PRODUCTION CHAIN IN VOJVODINA PROVINCE (SERBIA)

ABSTRACT: *Enteritis campylobacterialis* has an increasing trend in Serbia. Human illness usually appears as sporadic case, most commonly in children with obvious seasonality. In registered outbreaks, incriminated food was most frequently poultry meat. *Campylobacter* is one of the most important food borne pathogens, commonly underreported, mostly because isolation of this bacteria requires specific equipment. Since 1 January 2019, monitoring of *Campylobacter* in poultry carcasses is mandatory when it comes to poultry production facilities in Serbia. The aim of this paper was to analyze data from Autonomous Province of Vojvodina about the prevalence of *Campylobacter* spp. in poultry meat and risk for human illness. Our results indicate high prevalence of *Campylobacter* spp. in whole food chain: poultry farms, slaughterhouses, retail and, correspondingly, high risk for consumers in Vojvodina. Measures for risk reduction of disease incidence include better bio security measures on the farm level as a main source of pathogen but also introduction of *Campylobacter* diagnostic equipment in all human diagnostic and food control laboratories.

KEYWORDS: *Campylobacter*, poultry, food, outbreaks

INTRODUCTION

The genus *Campylobacter* now comprises twenty-eight member species and nine subspecies, most of which are microaerophilic, i.e. grow preferentially in low oxygen concentrations (Caceres et al., 2017). The majority of cases of acute campylobacteriosis are caused by two species: *C. jejuni* and the closely

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related *C. coli,* while *C. lari* and *C. concisus* may also play a role in enteritis (Kaakoush and Mitchell, 2012). *C. jejuni* and *C. coli* are often referred to as "thermophilic" or "thermo tolerant" *Campylobacter* as they grow preferentially at 42 °C (EFSA, 2010). Termophilic *Campylobacter* spp. is a leading cause of zoonotic enteric diseases in most developed countries. An upward trend of human campylobacteriosis was recorded in both developed and underdeveloped countries. Campylobacteriosis is considered endemic in Europe, North America, Australia, Asia and Africa, especially in children (Kaakoush et al., 2015). *Campylobacter* is most frequently reported cause of zoonotic diseases in European Union (EU). The overall incidence of 64.8 cases per 100,000 population was reported in EU countries in 2017. It was the second most common food borne illness in the USA (EFSA and ECDC, 2018; Ma et al., 2017).

Thermophilic *Campylobacter* is usually indirectly transmitted to humans through the consumption of food contaminated by faeces of infected animals. Contamination usually occurs during meat processing. Handling raw poultry and eating poultry products are the most important risk factors for sporadic campylobacteriosis, but also consumption of raw milk and contaminated water are important sources of infection (Kaakoush et al., 2015).

The surveillance of enteritis caused by *Campylobacter coli/jejuni* has been carried out since 1997 when disease/death registration was introduced in the Autonomous Province of Vojvodina, Serbia. Compulsory reporting of laboratory-determined *Campylobacter coli/jejuni* in biologic samples of human origin has been in place since 2005 (IPHV, 2017).

The aim of this paper was to determine the risk of *Campylobacter* contamination of food in a meat production chain and influence on human health in Vojvodina by linking our knowledge about spreading and dynamics of *Campylobacter* at farm level, slaughterhouses and retail with epidemiological data of human campylobacteriosis in Vojvodina.

Campylobacter AT FARM LEVEL

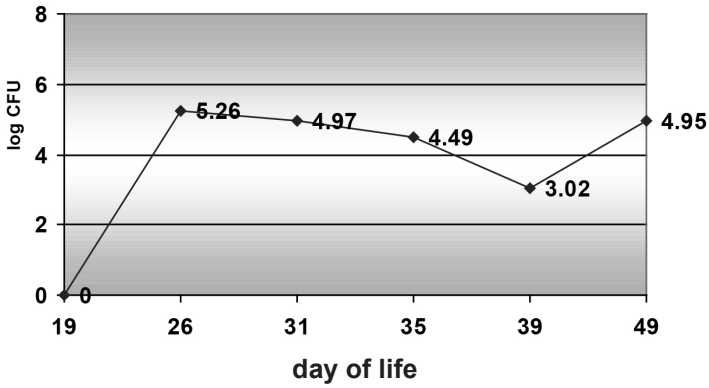
The occurrence of zoonotic pathogens in animals varies depending on the range of factors including the organism, geographical factors and farming practices. *Campylobacter* is present in farm surrounding environment, including the soil, water sources, dust, building surfaces and the air (Ellis-Iversen et al., 2012). *Campylobacter* usually colonises chickens in the third week of life. The level of colonisation and the spread of *Campylobacter* between animals depends on different factors: breeding conditions, flock size, hygiene measures, carry over from previous flock, flock thinning practices, contaminate air from adjacent poultry houses, contaminated water, immune response of animals, other infected livestock on the farm, mechanical transmission via insects and birds (Barrios et al., 2006; Stojanov et al., 2008).

Campylobacter colonisation in commercial poultry flocks is widespread in many countries. Studies in Europe indicate flock prevalence from 18% to 90%. Higher prevalence was obtained in southern countries (Barrios et al., 2006).

In Vojvodina *Campylobacter jejuni/coli* was identified at farm level in 73.3% of poultry samples, 66.6% calves samples and 58.3% pig samples of already ill or suspected cases (Stojanov et al., 2008).

Campylobacter may cause a disease, but also, in many cases, even when the bacteria were confirmed in laboratory, clinical symptoms were absent. Clinical manifestation of illness in birds can be expected if an additional factor is present, which affects the immune system and its ability to cope with the infection. In the symptomatic course of illness when diarrhoea occurs, it usually appears six hours after infection and lasts for 10 days. According to our experimental results (Stojanov et al., 2011) infection of one-day-old chickens induces diarrhoea, while infection of the three-days-old chickens with high doses (10^9 cfu per chicken) does not induce diarrhoea. The clinical symptoms usually depend of the strain, number of bacteria, stress and immunosuppression (Kazwala et al., 1992). Our experimental results (Stojanov et al., 2011) indicate that artificial infection of chickens with 4 log cfu *C. jejuni* and 4 log cfu *Salmonella* Enteritidis per chicken on day 14 of life leads to watery diarrhoea and traces of blood in first two weeks after the infection.

According to Petrović et al. (2012) artificial infection of healthy chickens with 6.77 log cfu *C. jejuni* per chicken on day 21 of life leads to 5.26 log cfu/g faeces after only five days, with a tendency to decrease during next 18 days. Tendency of decreasing was also found by Van Boven et al. (2003). In our experimental data, after initial decrease in count, the stabilisation of *Campylobacter* intestinal microflora and slight increase in count were noticed one month after the first infection (Graph 1). Reinfection or immunosuppression may be the resons.



Graphicon 1. Average number of *C. jejuni* in chicken by days of life (log cfu/g faeces)

Campylobacter AT SLAUGHTERHOUSE LEVEL

The presence of campylobacter on slaughtered chickens is affected by the contamination of live chickens and contamination of the feathers (Seliwiorstow et al., 2016). It was considered earlier that cross contamination is a significant

source of contamination of carcasses but the latest genotyping studies prove that carcass contamination originates from intestines of processed positive birds (Rasschaert et al., 2006; Sasaki et al., 2014). Although chilling, freezing, ultraviolet light, irradiation and chemical decontamination may significantly reduce *Campylobacter* contamination of carcasses, it cannot completely eliminate the initial contamination of live chickens (Seliwiorstow et al., 2016). According to our experimental results (Petrović et al., 2012) the prevalence of *Campylobacter* contaminated chickens from positive flock appears to drop from 100% in live birds (with 3.02 log cfu/g faeces) to 50% of chicken carcasses.

Increased contamination of the carcasses can occur due to poor hygiene practices: dump based unloading system, electrical stunning, lower scalding temperature, incorrect setting of plucking, vent cutter and evisceration machines (Seliwiorstow et al., 2016). Therefore, a high variability in contamination of carcasses can be considered; range from 11.43% to 90.00% carcasses prevalence was established in various slaughterhouses in Vojvodina (Petrović et al., 2008) as a result of slaughtering poultry batches contaminated with different loads of *Campylobacter* and the application of improper slaughter techniques (Table 1).

Table 1. Occurrence of *Campylobacter* in poultry samples (Petrović et al., 2008)

Occurrence of Campylobacter (%)	Abattoir mark						
	A	B	C	D	E	F	G
liver	40.00	5.00	8.56	6.00	34.28	2.86	5.71
carcasses	90.00	14.28	51.43	20.01	68.57	11.43	31.43

Campylobacter AT RETAIL

In Vojvodina, *Campylobacter* was detected in 18.8% and 10.0% samples of fresh poultry and other fresh meat respectively (Trajković-Pavlović et al., 2007). In Slovenia, a nation-wide genotyping study of *Campylobacter jejuni* isolates from humans, animals and food was performed by pulsed-field gel electrophoresis to determine the genetic profiles of more than 500 isolates obtained from humans (n=156), animals (n=133) and food (n=214). The isolates exhibited marked genetic diversity, however, identical PFGE profiles were identified for animal, human and food isolates, indicating the animals as a direct infection source for humans (Ocepek et al., 2011).

HUMAN CAMPYLOBACTERIOSIS

Human campylobacteriosis occurs sporadically or as a small-scale family outbreaks in the community. After incubation that usually takes 24 to 72 hours, *Enteritis campylobacterialis* commonly manifests with diarrhoea, fever,

abdominal pain, nausea and cramps. The illness can last 24 to 48 hours but it may have prolonged course (Blaser et al., 1997; Man, 2011). Dehydration, bacteraemia or sepsis and symptoms mimicking ulcerative colitis or acute appendicitis occur in a severe disease forms. Complications may include irritable bowel syndrome (9–13%), reactive arthritis (2–5% of patients), and Guillain-Barré syndrome (0.1% of patients) (CDC, 2018).

Between 2005 and 2017, an average annual incidence of *Enteritis campylobacterialis* in Serbia was 5.30 per 100,000 population (Table 2). The average incidence in Vojvodina in relation to the whole country and Central Serbia was not significantly different (p=0.87 and p=0.90, respectively). Mann-Kandell statisitcs prove that *Enteritis campylobacterialis* shows increasing trend in the whole country.

Between 2005 and 2017 overall 2,191 people suffered from *Enteritis campylobacterialis* in Vojvodina (IPHV 2018). The highest disease incidence was recorded in 2017 (n=294; Inc 15,2/100,000). The average annual number of cases was 179 with range from 91 to 294. Majority of the cases were laboratory confirmed, diagnosis was based on a constellation of clinical signs (e.g., fever, diarrhoea, abdominal pain) and isolation of *Campylobacter* from patients stool. Outbreak cases with clinical signs and epidemiological link (animal to man or interhuman transmission, exposure to a common source or exposure to contaminated foods/drinking water or environmental factors) were considered as probable cases (EC, 2018). The disease was confirmed in the laboratory using microbiological cultivation method, but in some outbreaks incriminated food was not found (food was not available for sampling).

The first outbreak of *Enteritis campylobacterialis* in Vojvodina was registered in 2004. Between 2005 and 2017, there have been 14 outbreaks recorded with a total of 70 cases, which makes up only 3.2% of the total of 2,191 patients notified in the observed period. Outbreaks were not registered every year. The annual number of outbreaks ranged one to three with 4–18 cases, most of them among family related patients (75%) with high attack rate (90.1–100.0%). Nine out of twelve outbreaks with known transmission pattern were caused by consumption of contaminated food, while direct contact was established in only one outbreak. Common incriminated food was chicken meat (eight outbreaks) and mixed grilled meat in one outbreak. Outbreak in school setting with 18 cases occurred in 2014 after consumption of row cow milk. Since patients in outbreaks belong to different age categories, it can be assumed that outbreaks were caused by high-virulent strains (WHO 2002).

One out of 5,212 patients that suffered from campylobacteriosis died in the period 2005–2017, in Serbia (mortality 0.01 per 100,000 population; lethality 0.2%) (IPHS, 2018).

The disease is registered all year round in Vojvodina, with a minimum number of cases recorded in January and February and the maximum in June and July (45% of patients). High temperatures in the summer months favor reproduction of thermophilic *Campylobacter*. In addition, in the summer months, grilled chicken meat is often consumed, which is the most common incriminated food that causes campylobacteriosis in Vojvodina.

Table 2. Incidence of campylobacteriosis in human population in Serbia

Year	Serbia	Central Serbia	Vojvodina
2005	1.9	4.5	6.2
2006	4.5	3.0	8.4
2007	4.1	3.7	5.2
2008	4.8	2.4	11.2
2009	5.4	4.2	8.5
2010	4.9	3.9	7.6
2011	4.1	3.4	5.9
2012	4.4	4.3	4.7
2013	5.3	4.8	6.6
2014	6.3	4.9	10.1
2015	6.6	5.8	9.4
2016	8.2	6.4	12.8
2017	8.4	8.3	15.2
avr	5.3	4.5	8.6
sd	1.8	1.5	2.5
min	1.9	2.4	4.7
max	8.4	8.3	13.2
CV	0.3	0.3	0.4
S	54	48	28
Confidence factor	> 99.9%	99.9%	95.0%
Trend	Increasing	Increasing	Probably increasing

CV – Coefficient of varriation; S – Mann-Kendall statistic

Enteritis Campylobacterialis in Vojvodina is mostly registered in children under four years of age. Compared to all other age categories, statistically significant difference in the number of ill children aged up to four years in relation to the number of cases in all other age categories, (p=0.00001) was evident (Table 3). The ratio of enteritis caused by *Salmonella* spp. and *Campylobacter jejuni/coli* at age below four years is about 2:1. For epidemiologically unknown reasons, male children were more likely to contract Campylobacter enteritis (2/3 patients) compared to females (IPHV, 2018).

Table 3. The age distribution of campylobacter enteritis cases presented by age specific incidence in the period 2010–2017 in Vojvodina

Age	2010	2011	2012	2013	2014	2015	2016	2017	Average	SD	Min	Max
0–4	85.3	61.6	55.2	76.6	109.3	104.8	135.2	146.5	96.8	33.1	55.2	146.5
5–9	22.2	15.8	9.5	16.8	31.6	24.3	43.2	32.7	24.5	10.9	9.5	43.2
10–14	10.7	12.3	9.6	7.5	9.6	10.6	14.9	36.2	13.9	9.3	7.5	36.2
15–19	2.2	2.9	4.6	5.5	12.7	5.5	17.3	24.6	9.4	8.0	2.2	24.6
20–59	1.9	1.6	1.3	2.3	3.1	2.7	3.8	4.4	2.6	1.1	1.3	4.4
60+	2.2	0.9	1.1	1.1	1.9	3.9	2.8	5.2	2.4	1.5	0.9	5.2

Underreporting influences assessment of the epidemiological patterns of the disease. The fact is that *Campylobacter* is a microaerophilic microorganism, very sensitive to environmental conditions. Thus, proper diagnosis requires specific equipment and education of laboratory staff influences availability of widespread laboratory diagnostics. Difficulties in diagnostics are not unique in our country; similar observations have come from authors from United Kingdom (Man, 2011). *Campylobacter jejuni/coli* carrier status in the human population is mandatory for reporting, but this is rarely done in practice, and campylobacter itself is not part of compulsory medical surveillance of coproculture in the food industry and catering staff.

CONCLUSION

Campylobacter is frequently found in poultry meat production chain in Vojvodina region. Despite high exposure of population to *Campylobacter*, the incidence of food borne human campylobacteriosis remains low, mainly because there is a lack of evidence i.e. laboratory confirmation of human campylobacteriosis. *Enteritis Campylobacterialis* is a sporadic disease; symptoms usually do not require hospitalisation and many patients do not seek medical advice. Even if they do, difficulties in laboratory diagnostics of this microorganism as well as underreporting are present. Another significant risk factor for contracting illness is cooking habits but in Vojvodina meat is usually well cooked.

Food borne campylobacteriosis in Serbia is important public health problem. In addition to measures needed to prevent the occurrence of pathogens in primary production, the reduction of contamination in slaughterhouses and measures for the control of campylobacteriosis should include better availability of laboratory diagnostics, education of all participants in food production chain and widespread health education of population.

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Campylobacter У ЛАНЦУ ПРОИЗВОДЊЕ ХРАНЕ У ВОЈВОДИНИ (СРБИЈА)

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РЕЗИМЕ: *Enteritis campylobacterialis* има растући тренд у Србији. Обољење људи обично се јавља у виду спорадичних случајева, најчешће код деце са очигледним сезоналитетом. У регистрованим епидемијама, најчешћи извор инфекције у исхрани било је месо живине. *Campylobacter* један је од најважнијих патогена који се преносе храном. Обољење се често не пријављује углавном зато што изолација ове врсте бактерија захтева специфичну опрему. Од 1. јануара 2019. мониторинг *Campylobacter*-а на труповима живине обавезан је у свим објектима за клање

живине у Србији. Циљ рада је да анализира податке из Аутономне Покрајине Војводине везане за преваленцу *Campylobacter* spp. у месу живине и ризик за настанак обољења код људи. Наши резултати указују на високу преваленцу *Campylobacter* spp-а у целом ланцу хране, од фарми живине, кланица, промета, као и висок ризик за здравље потрошача у Војводини. Мере за смањење ризика од настанка обољења укључују боље мере биосигурности на фармама, с обзиром да је главни извор патогена дигестивни тракт живине, али и увођење дијагностичке опреме за *Campylobacter* у све лабораторије које се баве дијагностиком хуманих обољења и контролом хране.

КЉУЧНЕ РЕЧИ: епидемије, живина, храна, *Campylobacter* spp.

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STUDY OF THE LOW INTENSITY SPECTRAL BANDS WITHIN THE INFRARED SPECTRA OF KERNELS OF HIGH-YIELDING MAIZE HYBRIDS

ABSTRACT: The nature, role, and significance of low intensity spectral bands contained within the infrared spectra of kernels of high-yielding maize hybrids: ZP 341, ZP 434, and ZP 505 were observed in this study. The observations were performed to identify organic molecules and their structural properties. The occurrence of unstable state of organic compounds and their functional groups are conditioned by such a process. The set hypothesis holds that there is a necessity to study the existence of many and low intensity spectral bands, not observed so far, occurring in different patterns (low intensity bands, single or grouped). They should be observed and the dynamics of their formation, caused by their different movements, including the possibility of their cancellation or amplification, should be explained. Such spectral bands most often appear in the wave number range of 400–950 cm^{-1}. They occur in several wave numbers up to 3,000 cm^{-1} and are caused by different types of vibration movements (valence and deformation vibrations) of organic compounds and their functional groups: primary, secondary, and tertiary amides, proteins, free amino acids, alkanes, alkenes, aldehydes, ketones, aromatic compounds, cellulose, carbohydrates, carboxylic acids, ethers, and alcohols. An unbiased analysis of low intensity spectral bands of maize hybrid kernels reveals that their occurrence is similar. Small differences, for some cases of the occurrence of low intensity spectral bands, can barely be ascertained. In this way, it is possible to establish not only the chemical composition of organic compounds of kernels of observed maize hybrids, but also it is possible to indicate their unstable, conformational, and functional properties.

KEYWORDS: *Zea mays* L, hybrid, kernel, infrared spectra, spectral bands

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INTRODUCTION

Nowadays, the fundamental improvement in the diagnosis of the state of organs and vital functions of the whole plant at the molecular level is achieved with modern methods of spectroscopy. The vibrational spectroscopy (infrared and Raman) is an unavoidable method in the analysis of infrared spectra of organic molecules resulting from molecular vibrations, thus it is possible to obtain many other results related to the structure of the studied systems. (Васильев и др., 2007; Свердлов и др., 1970; Тарасевич, 2012; Krim and Bandekar, 1986; Ribnikar, 1985).

In our previous papers (Radenović et al., 1994; Radenović et al., 1994a; Radenović et al., 1995; Radenović et al., 1998), studies are presented on the structural changes of carotenoid molecules in kernels of various maize inbred lines and hybrids. It was also shown that the structure of these molecules could be used as molecular markers in the evaluation of agronomic values of the maize inbred lines and hybrids. Our more recent papers (Radenović et al., 2015; Radenović et al., 2015a; Radenovich et al., 2016) discuss studies on the formation of infrared spectra, and in particular of 5–6 spectral bands that were most pronounced. No other, numerous and less developed spectral bands were analysed in these studies.

The method of infrared spectroscopy was applied in this study with the aim to analyse weak and insufficiently differentiated spectral bands, and thus, to identify the unstable state of the excited system and life functions of kernels of observed maize hybrids. It was shown that even by the use of infrared spectroscopy it was possible to determine the structure of organic compounds in kernels of studied maize hybrids (Macura and Radenović, 2016).

The objective of this study was to develop the methodology for analyses of numerous low intensity spectral bands of different shapes and kinetics, in order to identify organic compounds and their functional groups and to determine their stability and structure in kernels of analysed maize hybrids.

MATERIAL AND METHODS

Kernels of three highly productive maize hybrids, ZP 341, ZP 434, and ZP 505, developed at the Maize Research Institute, Zemun Polje, Belgrade, Serbia were used as a plant material in this study. Morphological and agronomic traits of observed maize hybrids, including those relevant to breeding and seed production, have been thoroughly described in our previously published papers (Radenović et al., 2015a; Radenovich et al., 2016).

The method of infrared spectroscopy applied to the grain of maize hybrids includes spectrophotometers used in the infrared spectrum region. They do not differ from spectrophotometers in the ultraviolet-visible spectrum regarding the sequence of components. Specificities, however, occur in the very principle of the work of the spectrophotometer. The fundamental differences

relate to the source of radiation, nature of samples, the principle of absorption of radiation, as well as to the use of various detectors (thermal and photo-detectors) (Васильев и др., 2007; Ribnikar, 1985).

Today, a special type of spectrophotometers is used, based on the principles of interferometry. Interferometers do not produce the spectrum itself, but an interferogram, which is then processed by a computer into a common spectral shape. This is co-called the Fourier transformation, and hence the name the Fourier Transform Spectroscopy (FTS). These devices are especially suitable for use in the far-infrared region and are characterised by good resolution (Radenović et al., 2015a; Radenovich et al., 2016).

In order to register the infrared spectrum of the observed maize hybrids, kernels were homogenised and compressed into tablets with the addition of potassium bromide (KBr). The spectrum was recorded with the Fourier Transform Infrared Spectrometer, Shimadzu IR-Prestige 21 (Instruction Manual User System Guide IRPrestige-21), within the spectral range of 400–4,000 cm^{-1}. This method has been described in details in our previous paper (Radenovich et al., 2016), including the modus operandi and the optical scheme of the device for registering infrared spectra.

Thirty kernels of each studied hybrid were randomly selected. Samples were prepared in the following way: kernels were ground and homogenised in a mortar and then mixed with potassium bromide (KBr) and rolled into samples – tablets with the component ratio of 1:100.

The primary processing of infrared spectra was done by the OriginPro software package, 2017 (OriginLab Corporation, USA).

The calculations were made by Microsoft Excel 2013 software package (Microsoft Corporation, USA).

The largest part of the statistical calculations was done using the program package Statistica, version 10 (StatSoft, Inc, USA).

RESULTS AND DISCUSSION

Kernels of the observed high yielding maize hybrids (ZP 341, ZP 434, and ZP 505) were homogenised and compressed into tablets and thus prepared for registering the infrared spectrum (Figure 1 a, b, c). Generally, the analysed infrared spectra for three maize hybrids were characterised by numerous spectral bands. The number of bands was up to 40 within the range of the wave number from 400 to 4,000 cm^{-1}. The registered spectral bands were of the unequal intensity, different shapes and kinetics.

Distinctively pronounced spectral bands (4–6) were analysed and presented in our recently published papers (Radenović et al., 2015a; Radenovich et al., 2016). However, in addition to these bands in the infrared spectrum of kernels of maize hybrids, a series of very different spectral bands appeared – high or low intensity bands, clearly separated or grouped, and spectral bands of a complex structure. Such spectral bands cannot be found in the literature and have not been analysed yet. Our study was actually focused on stated low

intensity bands with specific kinetic parameters. The careful consideration and analysis of kernel spectra of three studied maize hybrids (Figure 1 a, b, c) show a great number of spectral bands within the wave number range of 400–2,040 cm^{-1} for all three maize hybrids. According to their kinetic parameters, they differed considerably from one another. There were at least three classes. Similar spectral bands were formed within the range of the wave number of 400–950 cm^{-1}. Somewhat different spectral bands were formed within the range of the wave number of 1,200–1,600 cm^{-1}. Finally, there were spectral bands that were formed within the ranges of the wave numbers of 1,680–2,400 cm^{-1} and 1,680–2,800 cm^{-1} (Figure 1 a, b, c).

According to our hypothesis, the low intensity spectral bands with different kinetic parameters suggest an unstable (excited) state of the biological system (Macura and Radenović, 2016; Кольс и др., 1993; Radenović, 1998; Radenović et al., 2001). Furthermore, the excited state is expressed in certain functional groups that are presented in Table 1. It should not be forgotten that the unstable states of the biological system (tissue, cells, membranes) are a consequence of the excited state of molecules, radicals, atoms or ions and that they are inevitably caused by the occurrence of kinetic energy and their different movement modes (oscillation, vibration, rotations, and translations). This can cause the cancellation or the enhancement of the movement processes, which condition the different occurrence of low intensity spectral bands. The similar events happen in the process of the ion oscillatory transport through the excited membrane (Кольс и др., 1993; Radenović, 1998; Radenović et al., 2001). Moreover, today, beyond any doubt, great attention is paid to the contemporary study of the biological systems, seeking for information on genomes and proteomes and primarily their metabolomes, i.e. the concentration of all metabolites and their interactions. This is enabled by the application of infrared spectroscopy in studying of the structure and properties of organic compounds of kernels of maize hybrids (Radenović et al., 2015; Radenović et al., 2015a; Radenovich et al., 2016; Macura i Radenović, 2016).

Based on everything stated above, at least two questions may be posed. First, how to obtain reliable information on the existence of different biogenic organic molecules (substances), whose specificities regarding oscillations and deformations cause the occurrence of different spectral bands (Figure 1 a, b, c)? Second, are there any differences in kernels of the observed maize hybrids (ZP 341, ZP 434, and ZP 505) in relation to the integrity of the formation of spectral bands? If such differences exist, then it can be concluded that there are various structural properties of organic compounds in kernels of the studied maize hybrids.

The answer to the posed questions can be largely found out in our gained results presented in Table 1 and Figure 1 a, b, c. The careful analysis of literature data – the intensity, shape, kinetic values, as well as the range of the wave number of spectral bands (Volhardt and Schore, 1996; White and Johnson, 2003; Amir et al., 2013; Jackson and Mantsch, 2006; Skoog et al., 2007) – provides the identification of functional groups of organic compounds (Table 1). When the same parameters presented in Table 1 and Figure 1 a, b, c are compared,

it can be concluded that structural properties of organic compounds of all three maize hybrids are similar. Smaller differences occurred in the hybrid ZP 505.

Finally, according to the gained results the following can be added: the importance of intensity, shape, and kinetics of low intensity spectral bands that express unstable processes and states in biological systems and bioactive organic molecules contained in kernels of three studied maize hybrids, have been studied and emphasised for the first time.

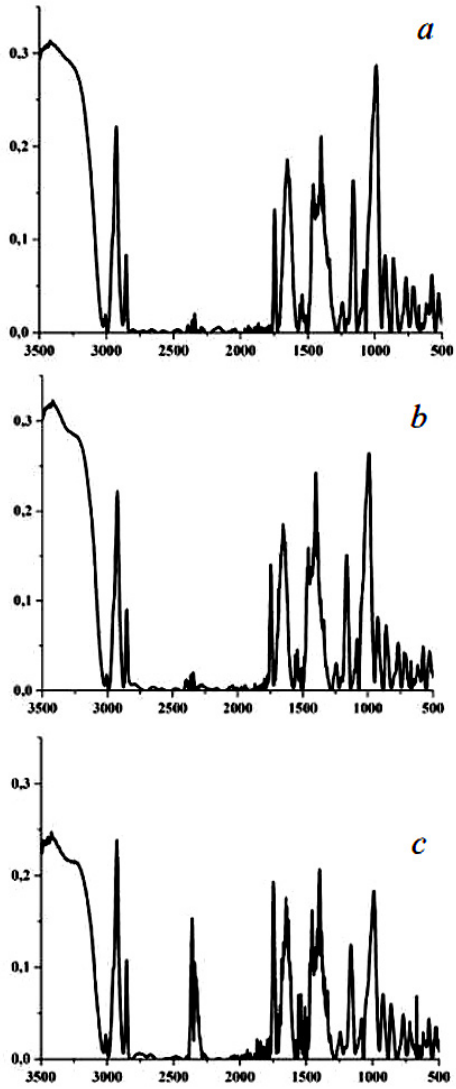

Figure 1 a, b, c. Infrared spectra of kernels of three maize hybrids – low intensity spectral bands were observed: a – ZP 341, b – ZP 434, c – ZP 505.
Abscissa: Wave number, cm^{-1}. *Ordinate*: Intensity, relative units

Table 1. Absorption bands of infrared spectra in kernels of maize hybrids: ZP 341, ZP 434, and ZP 505

Maize hybrids			Assignment of the IR absorption bands to functional groups in organic molecules
ZP 341	ZP 434	ZP 505	
Wave number of spectral band, cm^{-1}	Wave number of spectral band, cm^{-1}	Wave number of spectral band, cm^{-1}	
3,360 h	3,360 h	3,360 h	Alcohols, amides.[a, f, g] Valence vibrations of a free and bound OH group; valence vibrations of intramolecular and intermolecular H-bonds in dimers and polymers; valence vibrations of N-H bonds (primary and secondary amides); valence vibrations of free OH groups (water, carbohydrates, amino acids); valence vibrations of NH-groups (proteins, amino acids and their derivatives).[b, c, d, f, g]
3,010 l	3,010 l	3,010 l	
2,920 h	2,920 h	2,920 h	Valence vibrations of $-CH_2-$ bonds in alkanes (2,940–2,915 cm^{-1}).[c, f]
2,855 l	2,855 l	2,855 m	
2,350 m	2,350 m	2,350 m	Valence vibrations of O=C=O bonds in atmospheric carbon dioxide CO_2, used for graduation (2,349.3 cm^{-1}).[c] CO_2 is not contained in maize grains.
2,340 l	2,340 l	2,340 m	CO_2 bend.[h]
2,000–1,750 l	2,000–1,750 l	2,000–1,750 l	
1,720 l	1,720 l	1,720 l	
1,700 l	1,700 l	1,700 l	
1,680 m	1,680 m	1,680 m	Alkenes, aldehydes, ketones.[a, f]
1,750 m	1,750 m	1,750 m	Ethers, aldehydes, ketones, carboxylic acids (1,750–1,690 cm^{-1}).[a, f]
1,650 m	1,650 m	1,650 m	Valence vibrations of C=O bonds in primary, secondary and tertiary amides; stretching vibrations of N-H and C-N bonds in secondary amides, proteins, and free amino acids; vibrations of OH groups in crystal water of cellulose.[b, c, f]
1,645 m	1,645 m	1,645 m	
1,640 m	1,640 m	1,640 m	
1,610 l	1,610 l	1,610 l	
1,585 l	1,585 l	1,585 l	
1,565 l	1,565 l	1,565 l	
1,550 m	1,550 m	1,550 m	Amide II (protein N-H bending vibrations and C-N stretching vibrations).[e, f] 1,420 cm^{-1}, glucan[i] Vibration of C=C bonds in aromatic rings, vibration of NO_2 in nitro compounds.[e, f]
1,545 m	1,545 m	1,545 m	
1,520 m	1,520 m	1,520 m	

1,395 h	1,395 h	1,395 h	Valence vibrations of -C(CH_3)$_3$ bonds in alkanes, (1,395–1,385 cm^{-1}) and (1,365 cm^{-1}), Two spectral bands with absorption intensities of about 1: 2.[d, f]
1,380 m	1,380 m	1,380 m	
1,355 m	1,355 m	1,355 m	
1,310 l	1,310 l	1,310 l	
1,235 l	1,235 l	1,235 l	
1,150 m	1,150 m	1,150 m	Alcohols, ethers 1,150 cm^{-1} (1,000–1,260 cm^{-1}).[a] Valence vibrations of =C-O-C- bonds in simple ethers.[d, f]
1,100 l	1,100 l	1,100 l	
1,050 h	1,050 h	1,050 h	Stretching plane vibrations of C-H bonds in aromatic compounds.[b, c, d, f]
925 l	925 l	925 l	
850 l	850 l	850 l	
765 l	765 l	765 l	
710 l	710 l	710 l	
665 l	665 l	665 l	
615 l	615 l	615 l	
575 l	575 l	575 l	
525 l	525 l	525 l	

Intensity of spectral bands – Abbreviations:
l – low intensity (<0.1 rel. units), m – medium intensity (0.1–0.2 rel. units),
h – high intensity (>0.2 rel. units).
Source: [a] (Radenovich et al., 2016), [b] (Васильев и др., 2007), [c] (Свердлов и др., 1970), [d] (Тарасевич, 2012), [e] (Amir et al., 2013), [f] (Yu et al., 2004), [g] (Jackson and Mantsch, 2006), [h] (Chalmers, 2002), [i] (Yu et al., 2004).

CONCLUSION

The infrared spectroscopy was applied for the first time to register and study low intensity spectral bands contained in the infrared spectra in kernels of three high yielding maize hybrids: ZP 341, ZP 434, and ZP 505. According to the obtained results, the following can be concluded:

- Infrared spectra of kernels of the observed maize hybrids are characterised by up to 40 spectral bands within the range of wave number from 400 to 4,000 cm^{-1};
- Spectral bands can be differently pronounced, can be of unequal intensity, different shapes, and complex kinetics;
- Low intensity spectral bands (approximately 33 in the infrared spectrum of kernels of the observed maize hybrids) were studied. Besides being of low intensity, they can be single or grouped, can have different shapes and a complex kinetic structure.
- Although they are low intensity spectral bands they provide the identification of organic compounds and their functional groups: primary, secondary and tertiary amides, proteins, free amino acids, alkanes, alkenes,

aldehydes, ketones, aromatic compounds, cellulose, carbohydrates, carboxylic acids, ethers, and alcohols.

- An attempt has been made to clarify the nature, role, and significance of low intensity spectral bands that indicate an unstable (excited) state of the biological system (kernel) in which various movement modes occur, and thereby the possibility of the cancellation or the enhancement of the movement processes, which condition their different formation.

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ПРОУЧАВАЊЕ СЛАБО РАЗВИЈЕНИХ СПЕКТРАЛНИХ ТРАКА САДРЖАНИХ У ИНФРАЦРВЕНИМ СПЕКТРИМА ЗРНА ВИСОКОПРИНОСНИХ ХИБРИДА КУКУРУЗА

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РЕЗИМЕ: У овом раду чини се покушај изучавања природе, улоге и значаја слабо развијених спектралних трака, садржаних у инфрацрвеним спектрима зрна високоприносних хибрида кукуруза: ZP 341, ZP 434 и ZP 505. Ова проучавања вршена су ради идентификације органских молекула и утврђивања њихових структурних карактеристика. Оваквим процесом условљава се појава нестабилног стања органских једињења и њихових функционалних група. Износи се хипотеза да се постојање бројних и слабо развијених спектралних трака, које до сада нису изучаване, а које се појављују у различитој форми (слабог интензитета), појављују појединачно или груписано. Њих је неопходно посебно изучавати и објашњавати динамику њиховог настајања условљавану различитим карактером њиховог кретања, што укључује и могућност њиховог потирања или увећавања. Овакве спектралне траке најчешће се појављују у опсегу таласног броја од 400 до 950 cm^{-1}. Оне се успостављају, ту и тамо, на више места таласног броја све до 3.000 cm^{-1}, а настају различитим карактером вибрационог кретања (валенционо осциловање и деформационо вибрирање) органских једињења и функционалних група и то: примарни, секундарни и терцијарни амиди, протеин, слободне аминокиселине, алкани, алкени, алдехиди, кетони, ароматична једињења, целулоза, угљоводоници, карбоксилне киселине, етри и алкохоли. Непристрасном анализом слабо развијених спектралних трака зрна три проучавана хибрида кукуруза стиче се сазнање да је њихово појављивање слично. Мање разлике, за неке случајеве појављивања слабих спектралних трака, једва да се могу констатовати. На овај начин, могуће је утврдити не само структуру органских једињења у зрну проучаваних хибрида кукуруза, него и указати на њихова нестабилна, конформациона и функционална стања.

КЉУЧНЕ РЕЧИ: *Zea mays* L, хубрид, зрно, инфрацрвени спектри, спектралне траке

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EFFECTS OF OAK POWDERY MILDEW (*Erysiphe alphitoides* [GRIFFON AND MAUBL.] U. BRAUN AND S. TAKAM.) ON PHOTOSYNTHESIS OF PEDUNCULATE OAK (*Quercus robur* L.)

ABSTRACT: The aim of the present study was to evaluate the effect of one of the most important foliar diseases, powdery mildew, on the leaf physiological traits of *Quercus robur* L. using chlorophyll *a* fluorescence parameters in combination with parameters of leaf gas exchange. For this purpose, greenhouse semi-controlled experiment was conducted with 25 one-year-old seedlings kept in optimal conditions, and the same number of seedlings infected with the mentioned pathogen. Measurements were carried out when the coverage of epiphytic micelia visually reached more than 75% of the surface of leaves in the infected seedlings.

The results of gas exchange measurement showed that *Erysiphe alphitoides* (Griffon and Maubl.) U. Braun and S. Takam caused a significant reduction of net photosynthesis (A) and a significant increase in substomatal CO_2 concentration (Ci). Furthermore, considering the fast kinetics of chlorophyll *a* fluorescence, all of the observes parameters were significantly affected by oak powdery mildew. On the other hand, pulse amplitude modulated fluorescence parameters were mildly affected, with only minimal (Fo') and maximal (Fm') fluorescence of dark adopted leaves showing significant difference. This study presented the possibility of usage and the effectiveness of chlorophyll *a* fluorescence parameters in detection the severe stress conditions, on the example of leaves infected with oak powdery mildew over 75%. Some additional studies should be conducted in the future to determine the possibility of usage and the effectiveness of the observed fluorescence parameters of fast kinetics in detection of mild and early stress.

KEYWORDS: *Erysiphe alphitoides*, fast kinetics of chlorophyll *a* fluorescence, physiological traits, pulse amplitude modulated fluorescence, *Quercus robur*

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INTRODUCTION

Quercus robur L. is one of the most important deciduous tree species in European forests (Ducousso et al., 2004) due to its high-quality timber (Orlović et al., 2000; Stojnić et al., 2014). However, in the last few decades, this species has been recognised as vulnerable to different abiotic stresses, from which drought has been chosen as the most important threat (Urli et al., 2015; Bojović et al., 2017; Jevšenak and Lavanič, 2015). On the other hand, the most important biotic stress, spreading as a chronic desease throughout European oak forests, is oak powdery mildew caused by *Erysiphe alphitoides* U. Braun and S. Takam. (Pap et al., 2014; Dąbrowski et al., 2017). This obligate pathogen can cause severe damages, especially to young seedling in the phase of early ontogeny development (Hajji et al., 2009; Pap et al., 2012).

The negative effects of the above-mentioned pathogen are multiple and can be categorized as direct, since the fungi takes up nutrients from the host plant, and indirect as the epiphytic mycelium decreases assimilation by covering the leaf surface (Pap et al., 2013). Indeed, leaf shedding is one of the most extreme effect od powdery mildew, as it has been proven that 50% shedding leads to significant reduction of the infected leaves lifespan (Hajji et al., 2009). Further sympthoms, as necrosis and deformation of juvenile leaves, cause the reduction of functional leaf area (Marçais and Bréda, 2006). By damaging the leaves, oak powdery mildew negatively affects its physiological traits as well, causing the reduction of phostosynthesis and transpiration. In addition, the multiple negative effects of this fungi cause reduction of growth, and in the end mortality of young seedlings during late summer (Soutrenon, 1998).

Although it has been proven and well documented that oak powdery mildew has negative effects on the photosynthesis and transpiration of leaves (Hajji et al., 2009; Pap et al., 2014; Copolovici et al., 2014), there is a further need for better understanding the effects of this obligate leaf parasite on the photosynthetic apparatus, due to its complexity. For that purpose, chlorophyll *a* fluorescence measurement can provide useful additional information for understanding the primary events of photosynthesis and the effects of stress on photochemistry (Razavi et al., 2008; Ajigboye et al., 2016; Guo et al., 2016; Song et al., 2018). The main objective of this study was to access the impact of *E. alphitoides* on the leaf physiology, using non-destructive fluorescence parameters in combination with parameters of leaf gas exchange. Furthermore, the present study aims to identify chlorophyll fluorescence parameters that can quantify changes in PSII associated with plant responses to stress caused by the mentioned pathogen.

MATERIAL AND METHODS

Plant material and experimental conditions

For the purpose of the present experiment, in the second half of October 2017, one hundred acorns were randomly collected from eight pedunculate oak

(*Quercus robur* L.) genotypes, situated in an alley of Vojvoda Stepa Boulevard (N 45° 25' 61.75", E 19° 79' 73.47", elevation ca. 80 m above the sea level) in Novi Sad, Serbia. The above-mentioned genotypes were previously studied by Vaštag et al. (2017) and chosen accordingly to the desirable characteristics of the acorns (higher values of lenght, diameter and mass) from seventeen same aged genotypes situated at the described location.

The experiment was performed during the 2018 growing season at the greenhouse of University of Novi Sad, Faculty of Agriculture (45° 14' N, 19° 51' E) under semi-controlled conditions. The temperature in the greenhouse varied between 20 °C and 30 °C, while the lighting conditions depended on the outdoor conditions. After the acorns had been collected, they were air dried and stratified in peat, sand mixture (l:l, v/v, 5 L), throughout the winter. On the 30th March 2018, the acorns were soaked for 24h in water and sown in PVC pots (20 cm × 16 cm, height × diameter, 3 liters) afterwards, using Stender potting substrate S 200 (organic matter 20%, pH 5.5, electrical conductivity (EC): 670 µS/cm, dry matter 37.1%, fertilization: 1.0 kg NPK 14 + 16 + 28). Throughout the experiment the seedlings were watered close to field capacity every 2 days in order to ensure healthy seedling growth. After one month of growth under optimal conditions, 25 seedlings were infected with *E. alphitoides*, meanwhile the control seedlings were treated with fungicide to avoid infection during the experiment. Until the start of the measurement, the control seedlings were treated 3 times with fungicide Queen (active ingredient: azoxistrobin, in concentration of 0.075%) on the 4th May, 20th June, and 4th July 2018. The measurement started on the 6th July 2018, when the coverage of epiphytic micelia visually reached more than 75% of the infected seedlings leaves (FI>75%).

Leaf gas exchange measurements

Net photosynthetic rate (A [µmol/m^2/s]), rate of transpiration (E [mmol/m^2/s]), and substomatal concentration CO_2 (Ci [µmol/mol]) were recorded with the portable gas exchange system ADC Bioscientific Ltd. LCPro+. Twenty-five seedlings from each treatment with five replication per seedling (25 seedling x 1 leaf x 5 replicates) were chosen for the measurement of leaf gas exchange, as well as for the pulse amplitude modulated (PAM) fluorescence records and fast kinetics of chlorophyll *a* fluorescence. The measurements were carried out on the 3rd fully expanded leaf, aged 20 days or more, oriented towards the south-southwest. The leaves, which were not separated from the seedling, were measured in a sunny and clear weather from 09:00 AM to 11:00 AM, Central European Time. All measurements were made under constant photosynthetic active radiation (PAR) of 1,000 µmol m^{-2} s^{-1}, while the humidity, temperature, and the concentration of CO_2 were measured on the spot and varied as in ambient surroundings.

Fast kinetics of chlorophyll a fluorescence

Fast kinetics of chlorophyll *a* fluorescence measurements were recorded with PAM-2500 portable chlorophyll fluorometer (Walz, Germany) with high time resolution (10 μs). Before measurements were made, the leaves were put in dark adapted state for 30 minutes, using light exclusion clips. Afterwards, the OJIP transient was induced by red light of 3,000 photons μmol $m^{-1}s^{-1}$ produced by the PAM. Fluorescence intensity was measured under the fast kinetics function, between 10 μs and 320 ms (Kautsky curve). The OJIP transients were analyzed using JIP-test equation according to Strasser et al. (2000, 2010). The basic and derived fluorescence parameters recorded in dark acclimated samples are shown in Table 1.

Table 1. List of parameters of PSII photochemistry recorded and derived from the fast chlorophyll fluorescence kinetics.

Abbreviation and formula	Nomenclature and interpretation
Basic fluorescence parameters of OJIP-transient	
F_O (50 μs)	Minimum fluorescence level at 50 μs
F_K (300 μs)	Fluorescence level at K step (300 μs) of OJIP
F_M	Maximum fluorescence level at the peak of OJIP test
T_{FM}	Time needed to reach the maximum fluorescence
Derived fluorescence parameters of OJIP-transient	
F_O / F_M	Parameter related to changes in heat dissipation in the PSII anntena[1]
FV/F_O	Efficiency of the water-splitting complex on the donor side of PSII[1]
Φ_{Po} = FV/Fm = 1–(Fo/Fm)	Maximum quantum yield of primary PSII photochemistry[1]
$\Phi_{ET2o} = \Phi Po * \psi ETo$	Quantum yield of electron transport beyond reduced Qa[1]
$\Phi_{RE1o} = \Phi Po * \psi REo$	Quantum yield of electron transport from reduced Qa beyond PSI[1]
$\Psi_{ETo} = 1 - VJ$	Probability with which a PSII trapped electron is transferred from PSII beyond reduced Qa[1]
$\Psi_{REo} = 1 - VI$	Probability with which a PSII trapped electron is transferred from reduced Qa beyond PSI[2]
$\delta_{RE1o} = (1-VI)/(1-VJ)$	Probability with which a PSII trapped electron is transferred from PSII electron acceptor side to PSI electron acceptor side[2]

[1] (Strasser et al., 2000),[2] (Strasser et al., 2010)

Pulse amplitude modulated (PAM) fluorescence records

The photosynthetic activity of photosystem II (PSII) was measured using PAM-2500 portable fluorometer (Walz, Germany) connected to a computer with data acquisition software PamWin-3 from the same company. For the measurements, rapid light curve (RLC) function was used, with increasing intensities of actinic illumination from 144 to 2,443 μmol (photon) m^{-2} s^{-1} under uniform condition from 09:00 AM to 11:00 AM, on the 6th July 2018. Actinic illuminations lasted 10 s and each one was divided by saturating flash

of ~3,000 μmol m^{-2} s^{-1}, which lasted 0.8 s. The basic and derived fluorescence parameters recorded in light exposed samples are shown in Table 2.

Table 2. List of recorded or derived fluorescence parameters.

Abbreviation and formula	Nomenclature and interpretation
Basic fluorescence parameters	
Fo'	Minimum fluorescence level
Fm'	Maximum fluorescence level
Derived fluorescence parameters	
Y(II)=Fm'-F/Fm'	Effective photochemical quantum yield of PS II (Genty et al., 1989)
Y(NPQ)=F/Fm'- F/Fm	Quantum yield of non-regulated heat dissipation and fluorescence emission (Genty et al., 1996)
qN= 1- (Fm'-Fo'/ Fm-Fo)	Coefficient of non-photochemical fluorescence quenching (Schreiber et al., 1986 as formulated by van Kooten and Snel, 1990)
NPQ= Fm/Fm'-1	Coefficient of non-photochemical fluorescence quenching (Schreiber et al., 1986 as formulated by van Kooten and Snel, 1990)

Statistical analysis

A statistical evaluation of the differences between the measured parameters of control and fungal infected seedlings was made using t-test. After the values of t-test showed to be significant, comparison of means was performed (LSD test) to determine the level of significance. Furthermore, the differences between the control and treatment groups were shown in the form of boxplot diagrams. All statistical analyzes were performed using R 3.3.2. for Windows.

RESULTS AND DISCUSSION

Infection by oak powdery mildew reduces carbon acquisition (Percival and Fraser, 2002) and even a modest infection of leaves (10–20%) can cause a significant decrease (Copolovici et al., 2014). However, according to Pap et al. (2014) net photosynthesis is especially interrupted under the highest degree of leaf infection (>75%). Due to specific structures named haustoria (Divon and Fluhr, 2007), this obligate parasite absorbs nutrients from the tissues of its host plant, which benefits the fungi, but results in the deprivation of plant metabolism (Marçais and Desprez-Loustau, 2013; Bert et al., 2016). In this way, they alter photosynthetate for their own benefit and nutrition (Glawe, 2008).

In the present study, the results of t-test showed a highly significant decrease of net photosynthesis (A) and an increase of substomatal CO_2 concentration (Ci) in leaves infected with *E. alphitoides* above 75% (Figure 1). The decrease of net photosynthesis was found to be 43.9%, while the increase of substomatal CO_2 concentration was even greater, and amounted to 65.7%. On the other hand, the differences between the control group and severly infected

leaves, in terms of transpiration rate (E), were found to be not significant (Table 3). Our results are in accordance with the findings of Hajji et al. (2009) who demonstrated that the intensity of net photosinthesis declined progressively with the coverage of epiphytic micelia on the leaves, and that the highest infection level (85–100%) corresponded to a decrease of net photosynthesis by about 40–50%. According to the same author, severely infected seedlings of *Q. robur* did not experience any detectable change of transpiration. Furtermore, Pap et al. (2014) reported a greater decrease of net photosynthesis in leaves infected above 75%, which was about 70%.

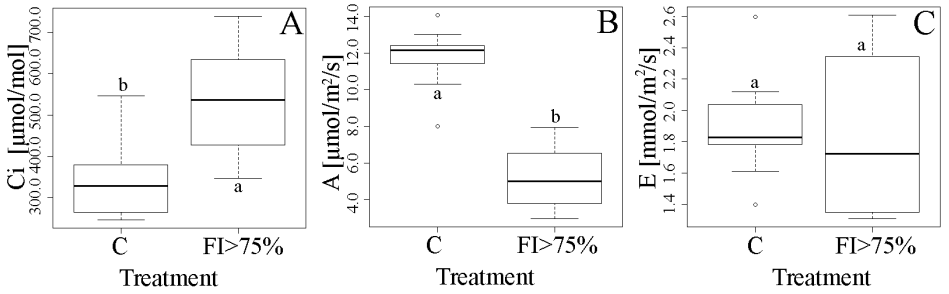

Figure 1. Observed physiological traits of control (C) and fungal infected (FI>75%) *Quercus robur* L seedlings. Different small letters above or below the error bars indicate significant differences between the values (LSD-test; $P \leq 0.05$).

The effect of oak powdery mildew on the physiologial traits were the subject of many studies (Percival and Fraser, 2002; Bassanezi et al., 2002; Desperez-Loustau et al., 2006; Hajji et al., 2009; Pap et al., 2014). However, to the best of our knowledge, this is the first research focusing on the detection of stress caused by the above-mentioned fungi with the combination of pulse amplitude-modulated method and the fast fluorescence kinetics measurments of chlorophyll *a* fluorescence. The chlorophyll *a* fluorescence methods were proven to be a reliable, non-invasive and non-destructive tools for understanding the responses of the photosynthetic apparatus to different physiological, genetic and environmental conditions (Razavi et al., 2008; Kalaji and Guo, 2008; Stirbet and Govindjee, 2011; Brestic and Zivcak, 2013; Kalaji et al., 2014). Until now, it has been successfully used for detection of drought stress (Falqueto et al., 2017; Wang et al., 2018), salt stress (Mehta et al., 2010; Lucena et al., 2012), light stress (Kalaji et al., 2012; Hazrati et al., 2016), nitrogen deficiency (Cetner et al., 2017), even the effect of atmospheric dust deposition (Ranjbar, 2017).

The results of fast kinetics of chlorophyll *a* fluorescence showed highly significant increase of Fo and similarly significant decrease of F_M parameters of leaves infected with powdery mildew above 75% (Figure 2). Furtermore, an additional peak was observed at Fk, which was in line with the findings of Oukarroum et al. (2012) and Martinazzo et al. (2012). Regarding the Fo and Fm parameters, the same trend was observed by Ranjbar (2017) in drought stressed leaves of *Pistacia vera* L., as well as by Martinazzo et al. (2012) in

temperature stressed leaves of *Prunus persica* L. The increase of the Fo value can be the consequence of impaired transfer of excitation energy which starts at reaction centers and ends at the antenna (Schreiber et al., 1998), and thus, indicate the damage of the photosystem apparatus; for example, the irreversible inactivation of reaction centers of PSII (Ranjbar, 2017). On the other hand, the decrease of F_M likely results from the rise of thermal or non-photochemical dissipation associated with the xanthophyll cycle, according to Müller et al. (2001).

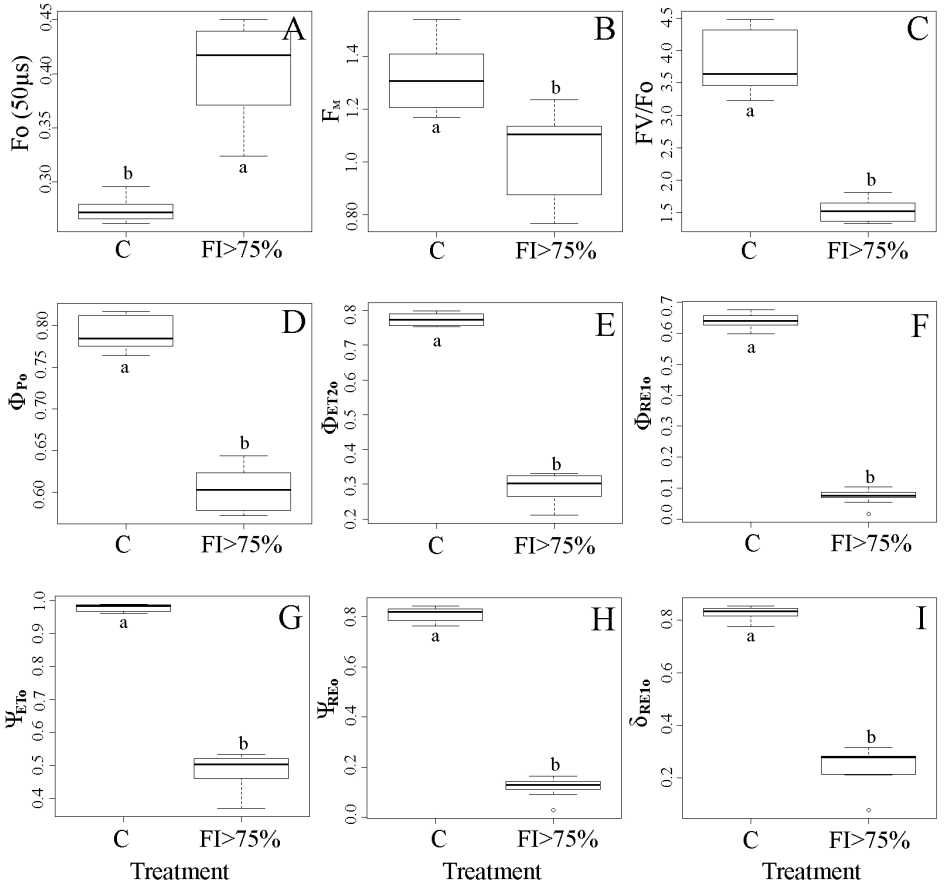

Figure 2. The parameters of PSII photochemistry derived from the fast chlorophyll fluorescence kinetics of control (C) and fungal infected (FI>75%) *Quercus robur* L seedlings. Different small letters above or below the error bars indicate the significant differences between the values (LSD-test; $P \leq 0.05$).

In comparation with the control plants, a highly significant reduction of T_{FM} was detected in leaves infected above 75% with oak powdery mildew. Kalaji and Guo (2008) reported that T_{FM} parameter is a good indicator of stress, which causes the F_M to be reached much earlier than in favorable conditions.

The results of the present study are in agreement with the findings of Kalaji et al. (2012), reporting a decrease of T_{FM} in leaves of barley under high light stress conditions, and Zhao et al. (2012) reporting a decrease of the same parameter in leaves of *Vitex negundo* var. *heterophylla* in conditions of drought stress. In terms of $Fo/F_{M,}$ significant increase was detected in severely infected leaves. Higher values of this parameters can occur due to the fact that the initial rate of the reduction of the primary quinone electron acceptor (Qa) is higher than the rate of secondary quinone electron acceptor (Qb) (Lucena et al., 2012) and due to the activity of photosystem I (PSI) when plants are exposed to stress conditions. According to Rohacek (2002) the rise of the above-mentioned parameter can be used as stress indicative, while the values between 0.14 and 0.20 corresponds to the plants under favorable conditions.

Under high stress conditions the capacity to repair the damages made on the PSII reaction center are minimal, and the result of the irreversible inhibition of PSII can be easily detected with reduction of Φ_{Po} fluorescence parameter (Misra et al., 2012). The results of this study showed a significant decrease of Φ_{Po} in leaves infected above 75% with oak powdery mildew. Moreover, this value ranged from 0.75 to 0.85, established by Bolhàr-Nordenkampf and Öquist (1993) for healty plants. According to Ranjbar (2017), lower values of Φ_{Po} in combination with higher values of Fo is a good destruction indicator of PSII, which decreases the efficiency of the transfer of absorbed light energy from the light-harvesting complex. Many studies about measurements on dark acclimated leaves are discussing only the Φ_{Po} values, although it provides only limited information about the function of the photosynthetic apparatus (Pšidová et al., 2018). An alternative parameter, similar to Φ_{Po}, is F_v/Fo (Krause and Weis, 1991). Although it does not give a direct measure of efficiency as Φ_{Po} does, it has got the advantage of being more susceptible to changes in efficiency at high values and because of that it can be a better way of expressing the data in some cases (Maxwell and Johnson, 2000).

In order to have a better insight and for better understanding the effect of powdery mildew on the light-harvesting apparatus, additional parameters were included in the present study. Ψ_{ETo} and ϕ_{ETo} parameters, which show the electron transport between PSII reaction centers and PSII quinone electron acceptors, were significantly limited in leaves infected above 75% (Table 4). The decrease of the two mentioned parameters was reported also by Pšidová et al. (2018) in leaves of *Fagus sylvatica* L. during the drought period, as well as by Cetner et al. (2017) in leaves of *Raphanus sativus* under the effect of nitrogen deficiency. Furthermore, according to the same author, the values of both parameters were lower in older leaves compared to young leaves. Ψ_{REo}, which reflects the efficiency of the electron transport from PSII to PSI, was similarly limited in leaves infected above 75%. The sensitivity of this parameter to environmental conditions was reported by many other authors (Busotti, 2004; Zivčak et al., 2014, Pšidová et al., 2018). The transfer of the electrons from plastoquinol to PSI, reflected in δreo parameter, was similarly affected by powdery mildew, although it is reported to be a less sensitive parameter to stress conditions (Yan

et al., 2013). Moreover, quantum yield of electron transport from reduced Qa beyond PSI (Φ_{REIo}) was also significantly decreased. According to Zhao et al., (2012), this could indicate that there was blocking of the electron transport from Qa.

The parameters of pulse amplitude modulated fluorescence were found to be mildly affected by the oak powdery mildew (Table 5). Statistically highly significant decrease was found only for Fo' and Fm' values. To our surprise, the other observed parameters showed no significant difference between leaves infected above 75% and control leaves. Many studies reported high sensitivity of NPQ to stress conditions (Szöllősi, 2008; Abdeshahian et al., 2010; Zhao et al., 2012). However, our results were in line with Pšidová et al. (2018) who found no significant differences between the leaves of *Fagus sylvatica* L., regarding the altitude of origin and the weather conditions. This finding is presumably the consequence of the long-term acclimatization of leaves on the effects of powdery mildew, due to the fact that NPQ serves as short-term protective response of PSII (Kramer and Evans, 2011).

Table 3. The effect of *Erysiphe alphitoides* on parameters of control (C) and fungal infected (FI>75%) leaves derived from pulse amplitude modulated (PAM) fluorescence records measured in the leaves of *Quercus robur* seedlings L.

Treatment	Intensity chlorophyll a fluorescence (relative unit)		Yield of non-photochemical quenching		Parameter of non-photochemical quenching	
	Fo'	Fm'	Y(II)	Y(NPQ)	qN	NPQ
C	0.112±0.008 a	0.294±0.046 a	0.090±0.013 a	0.390±0.084 a	0.521±0.108 a	0.783±0.252 a
FI>75%	0.088±0.009 b	0.216±0.026 b	0.088±0.023 a	0.440±0.059 a	0.584±0.061 a	0.954±0.232 a

Note: Differences between values followed by the same letter are not statistically significant ($p \leq 0.05$)

CONCLUSION

The results of the present study confirmed that most of the observed physiological traits were affected by *E. alphitoides*. Leaves infected above 75% showed significantly lower values of net photosynthesis rate, and higher internal CO_2 rate. Moreover, all observed parameters of fast kinetics of chlorophyll *a* fluorescence turned out to be significantly affected by the mentioned pathogen. This suggests that all observed parameters can be used as indicators of high stress conditions of plants. However, in terms of pulse amplitude modulated fluorescence, a significant difference was observed only for Fo' and F_M' parameters. Further studies should be conducted in order to investigate the effectiveness of the observed fluorescence parameters of fast kinetics in detection of mild and early stress.

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УТИЦАЈ ХРАСТОВЕ ПЕПЕЛНИЦЕ (*Erysiphe alphitoides* [GRIFFON AND MAUBL.] U. BRAUN AND S. TAKAM.) НА ФОТОСИНТЕЗУ ХРАСТА ЛУЖЊАКА (*Quercus robur* L.)

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РЕЗИМЕ: Циљ рада био је да се процени утицај храстове пепелнице, као једне од најзначајнијих фолијарних болести на физиолошке карактеристике листа храста лужњака (*Quercus robur* L.). Процена утицаја вршена је на основу параметара флуоресценције хлорофила и размене гасова. У складу с тим постављен је експеримент у полуконтролисаним условима, који се састојао од 25 једногодишњих садница гајених у оптималним условима и 25 садница заражених поменутим патогеном. Мерења су вршена када је прекривеност листа епифитном мицелијом визуелно достигла више од 75% површине покривености лишћа.

Резултати мерења размене гасова указали су на то да је храстова пепелница имала значајан утицај на смањење нето фотосинтезе (A), као и на повећање интерцелуларне концентрације CO_2 (Ci). Поред наведеног, статистички значајна разлика је утврђена за све посматране параметре брзе кинетике флуоресценције хлорофила *a* између здравих листова и листова заражених преко 75% храстовом пепелницом. Међутим, поменути патоген имао је слаб утицај на параметре импулсне амплитудне модулације флуоресценције. Једино су минимална (Fo') и максимална (Fm') флуоресценција замрачених листова показале значајне разлике између контролних и пепелницом заражених листова садница. У раду је приказана могућност и ефикасност коришћења параметра флуоресценције хлорофила *a* у детекцији јаког стреса на примеру листа зараженог пепелницом изнад 75%. Даља истраживања требало би усмерити ка испитивању могућности и ефикасности коришћења параметара брзе кинетике флуоресценције за детекцију почетног и благог стреса.

КЉУЧНЕ РЕЧИ: *Erysiphe alphitoides*, *Quercus robur*, брза кинетика флуоресценције хлорофила *a*, импулсна амплитудна модулација флуоресценције, физиолошки параметри

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EFFECT OF CADMIUM ON GERMINATION AND GROWTH OF WHEAT

ABSTRACT: In this study, the effect of Cd on the germination, growth of seedlings and composition of plants deriving from contaminated grains, grown in the field, was examined. Wheat grains were soaked in Cd-containing solutions: 0 (control, deionized water), 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M $CdCl_2$ during 24h. One portion of grains was used to test germination and seedling growth, while the other was sown in the field. The concentration of Cd in the grains almost linearly increased with the increase in the applied concentrations of Cd, which reduced the germination and energy of germination and increased the proportion of atypical seedlings. Larger concentrations of Cd significantly impaired the growth of seedlings (length of the shoots and roots, dry matter mass). In the grains of the field-grown plants the concentrations of N, P, and K were not affected by Cd, but their concentrations in the straw declined (especially of N). These results suggest that the emergence and development of plants on the soil polluted by Cd are likely to be significantly limited and yield reduced.

KEYWORDS: cadmium, concentration of N, P, and K, germination, grain, growth, straw, wheat

INTRODUCTION

Cadmium (Cd) belongs to a group of heavy metals without possessing a known biologically beneficial role. It is present in low concentrations in nature. Only in the era of industrial revolution, Cd became an important pollutant of the environment (soil, water, and air). Cadmium is equally toxic for living organisms – plants, animals, and humans (Gupta and Gupta, 1998). It jeopardizes key physiological processes in plants already at low concentrations (Kastori et al.,

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1997, Benavides et al., 2005). It affects the activity of enzymes (Kuriakose and Prasad, 2008), inhibits photosynthesis (Panković et al., 2000), induces oxidative stress (Ćosić et al., 2018), affects the uptake, accumulation and translocation of mineral elements (Jiang et al., 2004, Maksimović et al., 2007), affects water regime (Barceló et al., 1986), as well as plant anatomy (Maksimović et al., 2007; Luković et al., 2014).

Germination is the initial and crucial phase in the life cycle of higher plants. It is a very complex process (Mayer and Poljakoff-Mayber, 1989) during which significant physiological, biochemical and morphological transformations take place. Therefore, qualitative and quantitative changes induced by the action of external factors during the course of germination may have a negative effect on germination and sprouting. Having in mind that its phytotoxicity and the fact that Cd is a significant potential pollutant of agricultural soils, the aim of this study was to explore the effect of the increasing concentrations of Cd on the germination and growth of wheat seedlings under laboratory conditions, as well as on the concentration of nitrogen, phosphorus, and potassium in the grains and straw of next generation in the field.

MATERIAL AND METHODS

Winter wheat variety Pobeda was used in the experiments. It has been previously found that wheat grain soaked during 24h at 26 ^{o}C contains approximately 38% of water and after that period there is no statistically significant increase in water content in the seed. Wheat grains need about 30% of water to germinate. Therefore, during this experiment, Cd treatment was performed by soaking the wheat grains in water solutions of $CdCl_2$ at the following concentrations: 0 (control, deionized water), 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M Cd during 24h and then rinsed with deionized water. The effect of treatments was examined in the laboratory and in the field.

Examination of the effects of Cd on germination comprised the analyses of germination energy, germination percent, the portion of atypical seedlings and ungerminated grains, according to ISTA 2011 protocols. Germination took place on the filter paper, at 20 °C; germination energy was recorded on the fourth day after sowing and germination on the eighth day after sowing. Seedlings without root and with undeveloped coleoptiles were declared as atypical. The experiment was done in five replications.

The effect of Cd on the growth of seedlings was established on the tenth day after sowing, in the laboratory, at around 23 °C. The following parameters were recorded: length of shoots and roots, fresh weight and dry weight (after drying of plant material at 80 ^{o}C to constant mass). All measurements were taken in five replications, with 10 seedlings per replication.

To examine the effect of the imbibitions of wheat grains in the solutions containing Cd on the concentration of N, P, and K in the grains and straw of the progeny of imbibed grains, the other portion of imbibed grains were sown in the field, on the soil classified as a calcic, gleyic chernozem (Loamic,

Pachic-CH-cc.gl-Ip. ph [IUSS Working Group WRB, 2015]), of weak alkaline reaction, medium humus content, and optimal concentrations of N, P, and K. The concentration of Cd in the soil was significantly lower than the maximally allowed (2 mg/kg soil) and lower also than the usual concentration of Cd in the soil (0.2 to 1 mg/kg soil). In the topsoil layer (0 to 20 cm), where Cd accumulates the most, concentration in EDTA extracted fraction was 0.206, and a total content was 0.703 mg/kg of the soil. During the experiment, the usual management practices for wheat production were applied.

The concentration of N in the grains and straw of wheat was assessed by micro Kjeldahl method, of P spectrophotometrically using the vanadate-molybdate method, and of K by flame photometry. The concentration of Cd in grains was measured by inductively coupled plasma emission spectrometer, after digestion of grain wholemeal in a mixture of 10 ml HNO_3 (65%) and 2 ml H_2O_2 (30%) using microwave technique.

Statistical analyses were done by Statistica, version 13.3.

RESULTS AND DISCUSSION

Concentration of Cd in the grains imbibed in the solutions containing Cd increased almost linearly with an increase in the concentration of Cd in the solution, whereas concentration of Cd in the grains imbibed in the deionized water without Cd (control) was 0.032 mg/kg of dry mass (Figure 1), which

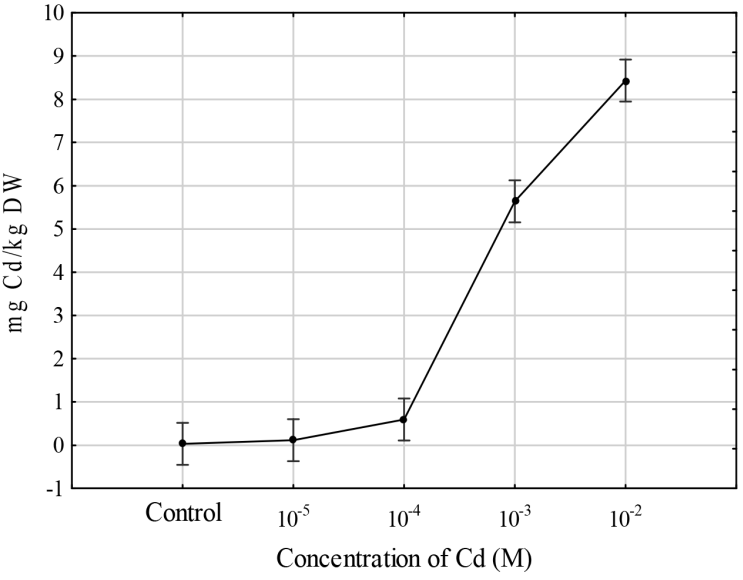

Figure 1. Accumulation of Cd in grains of wheat after imbibitions in solutions containing Cd

corresponds to the average concentration of Cd in uncontaminated grains of wheat (Kabata-Pendias, 2000). Rinsing of grains after exposure to the solutions containing Cd with deionized water served to eliminate Cd which was on the surface of the grain, attached to bran. According to Kuriakose and Prasad (2008), with an increase in the concentration of Cd the absorbed amount of Cd increases. Inhibitory effect of Cd on the germination of various plant species was recorded by a number of authors (Mrozek, 1980; Naquib et al., 1982; Rani et al., 1990; Chugh and Sawhney, 1996; Pandit and Prasannakumar, 1999; Kuriakose and Prasad, 2008) and it is in accordance with our results obtained in wheat. With an increase in the concentration of Cd in the imbibition solution, germination and energy of germination declined (Figure 2).

Germination in wheat commences with the absorption of water. The first phase in this absorption is dependent on the colloid system of the grains. Hydrophilic groups attract dipole molecules of water. By activation of hydrolytic enzymes, large organic molecules are hydrolyzed and the concentration of osmotically active molecules in the grain increase, which allows intensive absorption of water by the grains. Kuriakos and Prasad (2008) found that the content of water was significantly lower in the grains exposed to Cd. This leads to a conclusion that Cd reduces the uptake of water by the grains during germination, most probably in the phase in which absorption of water is directly dependent on the presence of osmotically active compounds. Their presence, however, depends on the activity of hydrolytic enzymes.

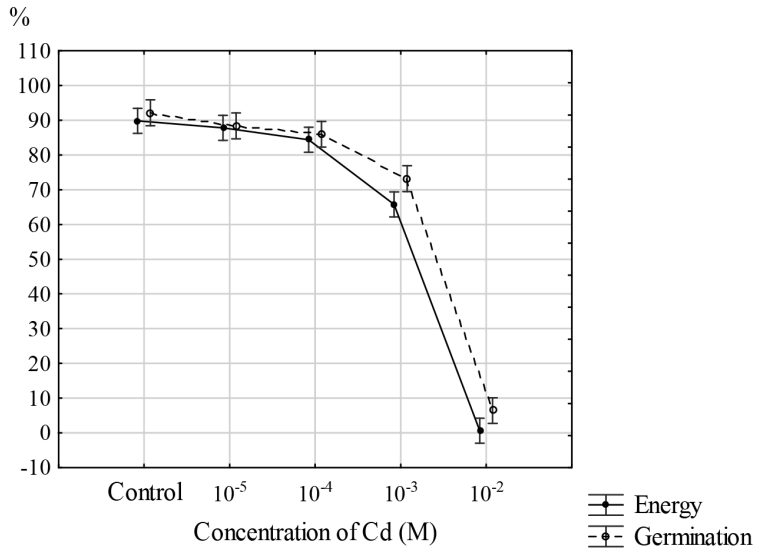

Figure 2. The energy of germination and germination after imbibition of wheat grains in solutions containing Cd

It was found that in seeds of pea exposed to the increasing concentrations of Cd during germination, total amylolytic activity and activities of α- and β- amylases, as well as respiration rate, declined (Chugh et al., 1992). Kuriakose and Prasad (2008) found that activities of acid phosphatase, protease, and α-amylase declined during germination of sorghum seeds exposed to the increasing concentrations of Cd. It is considered that Cd reduces not only the decomposition of insoluble sugars but also the transport of soluble sugars to the embryo, which is an important precondition for the development of the embryo and therefore for germination. Processes of decomposition of the organic compounds stored in the endosperm are controlled by phytohormones (Mayer and Poljakoff-Mayber, 1989). During germination, in the coleoptile and scutellum synthesis of gibberellins takes place. Gibberellins then diffuse to aleurone cell layer, where they induce synthesis and activation of hydrolytic enzymes. Hydrolytic enzymes are then released from aleuronic cell layer to endosperm. Besides α-amylase, gibberellic acid induces the formation of the other enzymes in aleuronic cell layer, such as proteases, carboxypeptidase, ribonuclease, arabinofuranozidaze, and acidic phosphatase (Jones and Jacobsen, 1991). Alpha-amylase is a key enzyme of the entire process of the mobilization of nutrients from the endosperm. In wheat grains, α-amylase, which commences the hydrolysis of starch, is synthesized *de novo* during germination, whereas β-amylase is present in the starchy endosperm in either free or bound state (Gallaeschi and Chapman, 1985). Gibberellic acid stimulates transcription and translation steps in the process of synthesis of α-amylase during germination (Ökkes et al., 2003). According to Maksimović et al. (2018) plant height, the number of spikes per m^2 and grain yield in wheat at harvest significantly declined with the increase in Cd concentration from 0 to 10^{-5} M in the solution in which the grains were imbibed prior to sowing. The height of plants deriving from grains treated with the highest concentration of Cd was reduced by 25%. This suggests that Cd may have reduced the activity of gibberellic acid and in this way the elongation of stems – in other words, Cd probably had a direct impact on gibberellic acid. This presumption is aided by the results of Ökkes et al. (2003) who found that Cd inhibited the activity of α-amylase and synthesis of gibberellic acid during pea seed germination. Details on the mechanism of the inhibition of synthesis of gibberellic acid remain to be further elucidated. The fact that the application of gibberellic acid reduces stress induced by Cd highlights the importance of the interaction between Cd and gibberellic acid (Ghorbanli et al., 1999; Hadi et al., 2014).

Besides the reduction in germination and energy of germination, with the increase in concentrations of Cd in the solution in which grains were imbibed the portion of atypical seedlings and ungerminated grains also increased (Figure 3), which confirms the complexity of the impact of Cd on plant development.

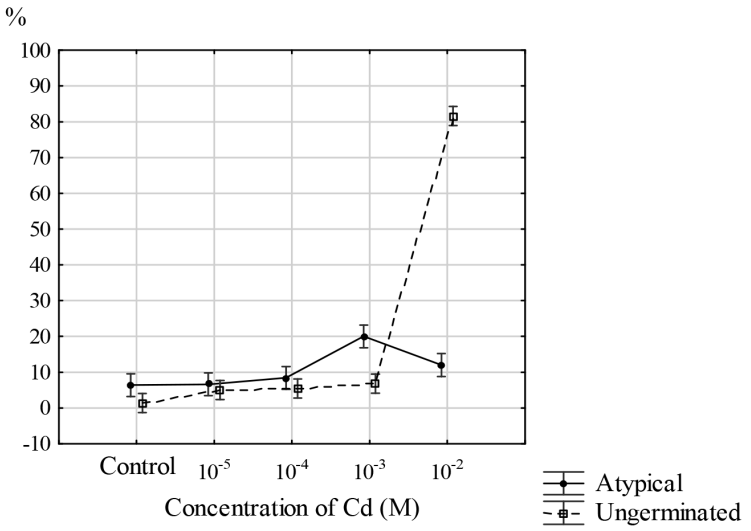

Figure 3. The portion of atypical seedlings and ungerminated grains after imbibition of grains in solutions containing Cd

Higher concentrations of Cd significantly reduced the length of shoots and roots (Figure 4), as well as their dry mass (Figure 5). The growth of shoots and roots was reduced more than dry mass, suggesting that Cd affected growth factors more severely than the accumulation of dry weight. Lux et al. (2011) showed that the presence of Cd in the rhizosphere inhibits elongation of roots and affects their anatomy.

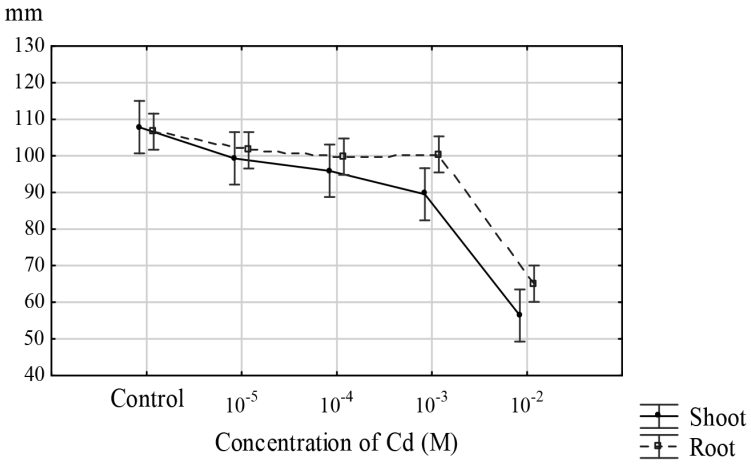

Figure 4. Length of shoots and roots of wheat seedlings exposed to Cd during germination

They also suggest that the concentration of Cd is most often higher in roots than in shoots because of restricted transport of Cd through xylem in the majority of analyzed plant species.

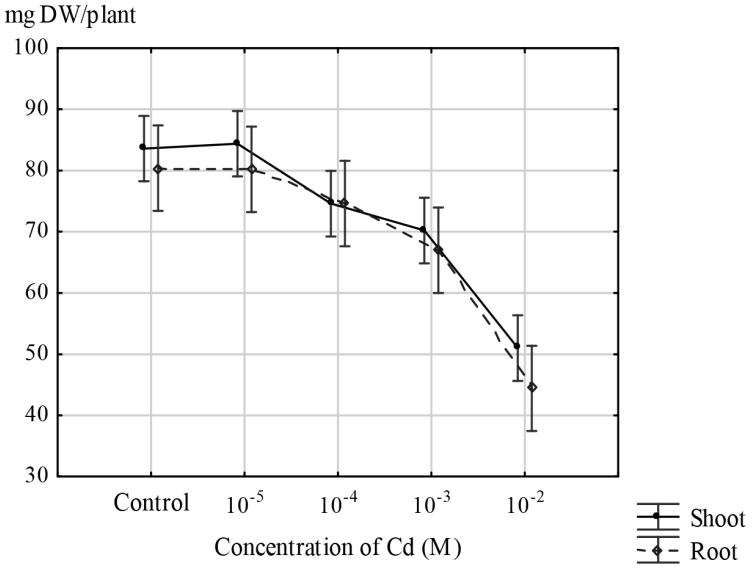

Figure 5. Dry mass of shoots and roots of seedlings exposed to Cd during germination and measured 10 days after sowing

Seedlings deriving from seeds exposed to the highest concentration of Cd were less hydrated that unexposed. The water content in the shoots was around 4% lower. Maksimović et al. (2007) observed significant changes in root anatomy of maize seedlings treated with Cd, which included also thickening of the cortex, and this may be the reason for the increased resistance to lateral transport of water from root surface to xylem vessels and therefore reduced water uptake. They also found that plants exposed to Cd were less hydrated. Kastori et al. (1992) found that sunflower plants exposed to Cd reduced transpiration intensity when compared to the control. All these findings lead to a conclusion that the toxic concentrations of Cd may affect the metabolism of young plants partly due to insufficient water provision, apart from the reduced transpiration. However, it is important to stress that the increase in the concentration of free proline, which is a reaction typical for water stress, occurs also in the presence of Cd in turgid plants (Kastori et al., 1992).

Higher concentrations of Cd may affect the accumulation and distribution of mineral elements in plant tissues (Maksimović el al., 2007; Putnik-Delić, 2013). Exposure of grains to the increasing concentrations of Cd, however, did not change the concentration of N, P, and K in the grains of the next generation (Figure 6), but it reduced the concentration of these elements, especially N, in the stems at physiological maturity (Figure 7). Damages of the root system of

young plants due to higher concentrations of Cd may have an unfavorable effect later, during vegetative growth (Chugh and Sawhney, 1996).

Cadmium is considered to influence nitrogen metabolism, but the effect may be direct and/or indirect (Kastori et al., 1997). Muhammad et al. (2008)

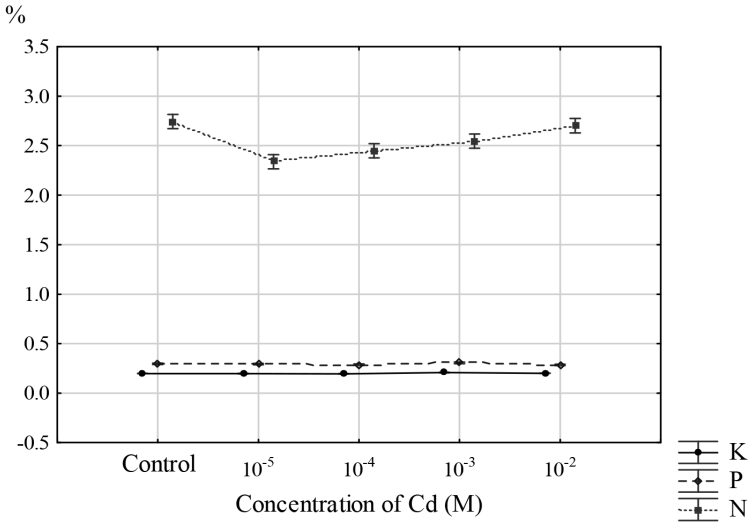

Figure 6. The concentration of N, P, and K in the grains of wheat which are the progeny of grains exposed to Cd during germination

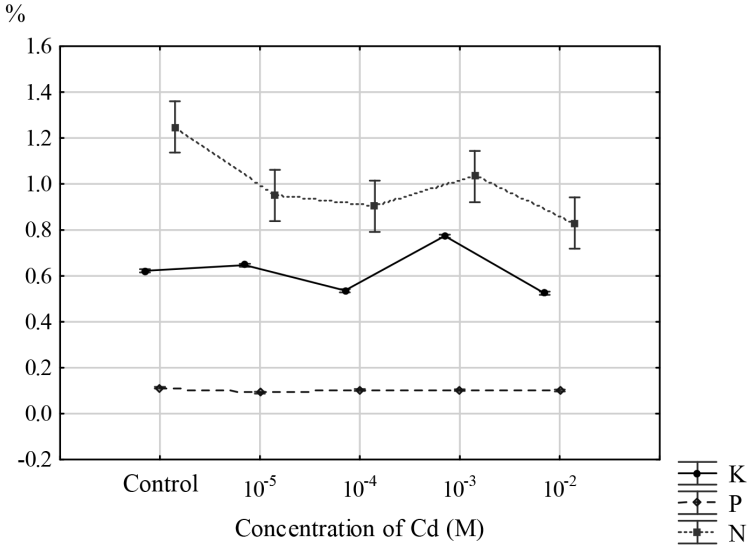

Figure 7. The concentration of N, P, and K in the straw of wheat which was derived from grains exposed to Cd during germination

found that Cd significantly reduced the concentration of N in roots and especially in stems of rice, as well as the activity of nitrate reductase. Similar changes in the activity of nitrate reductase, due to excessive concentrations of Cd, were also shown by Petrović et al. (1991). In addition, Panković et al. (2000) showed that optimal nitrogen nutrition reduces the unfavorable effects of Cd on the photosynthesis of sunflower.

All these results represent evidence of the complex interactions between Cd and plant nutrients.

CONCLUSION

Swelling of wheat grains, imbibed in solutions containing the increasing concentrations of Cd, nearly linearly increased the concentration of Cd in those grains and significantly reduced germination and energy of germination, and concomitantly increased the proportion of atypical seedlings. Higher concentrations of Cd significantly reduced the growth of seedling. The growth of shoots and roots was reduced more than the dry mass, suggesting that Cd affected the growth factors to a greater extent than dry biomass production. The highest applied concentration reduced the hydration of shoots. In the field, in the grains of plants which are the progeny of grains exposed to Cd, concentrations of N, P, and K were not changed but in the straw, at maturity, their concentration (especially concentration of N) was lower than in the control. These results suggest that germination is very sensitive to the presence of higher concentrations of Cd.

ACKNOWLEDGMENT

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УТИЦАЈ КАДМИЈУМА НА КЛИЈАЊЕ СЕМЕНА И РАСТ ПШЕНИЦЕ

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РЕЗИМЕ: Кадмијум (Cd) је потенцијални загађивач средине живих организама и при већим концентрацијама одликује се јаким фитотоксичним дејством. У раду је у лабораторијским условима проучено дејство различитих концентрација кадмијума на клијање и раст младих биљака пшенице, а у пољским условима његов утицај на концентрацију минералних материја у зрну и слами у фази пуне зрелости биљака, које су потекле од зрна третираног кадмијумом. С повећањем концентрације кадмијума у фази бубрења семена скоро се линерано повећала његова концентрација у семену. Утврђено је да веће концентрације кадмијума значајно смањује клијање и енергију клијања семена, а повећавају учешће атипичних клијанаца и непроклијалог семена. Веће концентрације кадмијума значајно су смањиле раст младих биљака као и масу њихове суве материје. Раст надземног дела и корена смањен је у већој мери од масе суве материје, на основу чега се може закључити да је кадмијум у већој мери утицао на факторе раста него на стварање суве масе. Највећа примењена концентрација кадмијума смањила је хидратисаност

надземног дела младих биљака. Бубрење семена у присуству различитих концентрације кадмијума није утицало на концентрацију азота, фосфора и калијума у зрну, а смањило је њихово присуство у слами, нарочито азота на крају вегетације. Добијени разултати потврђују налазе ранијих истраживања код других биљних врста на основу којих су физиолошки и биохемијски процеси клијања семена значајно инхибирани у присуству већих концентрација кадмијума. На основу наведеног може се предвидети да ће на земљишту загађеном кадмијумом ницање усева и раст младих биљака пшенице бити ограничено, што ће се неповољно одразити на принос усева.

КЉУЧНЕ РЕЧИ: кадмијум (Cd), клијање, концентрације азота (N), фосфора (P) и калијума (K), пшеница, раст, слама, зрно

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CHARACTERISTICS OF AGRICULTURAL ORGANIC MATTER DEGRADING BACTERIAL ISOLATES FROM DIFFERENT TYPES OF SOIL

ABSTRACT: A large amount of agricultural organic matter (AOM) comes into soil every day, through organic remains, and it is decomposed by bacteria, fungi and actinomycetes (Ugarković et al, 2011). The aim of this research was to select isolates of bacteria with the most organic matter degrading potential, by isolating the bacteria from five different types of soil. Isolation of bacteria was conducted from five types of soil – luvisol, cambisol, chernozem, forest land and meadow. Characterization of bacterial isolates was conducted based on morphological, physiological and biochemical features. Isolates with the most organic matter degrading potential could be used in the near future for conceptualizing microbiological preparation.

KEYWORDS: bacteria, characterization, degradation of AOM, isolation, soil

INTRODUCTION

Microorganisms in the soil have central role of synthesis and mineralization of organic matter (Vasilj et al., 2007). Mineralization (dehumification) and humification are two processes which take place parallelly, or one following the other. Both of them are of great significance for organic matter of the soil (Govedarica and Jarak, 1995). Microbiological decomposition of organic matter in the soil represents a process which results in releasing plant assimilatives and energy flow which determine the fertility of the soil and characteristics of an agro ecosystem. Isolation, determination and selection of effective isolates of bacteria, actinomycetes and fungi, which contribute to better and faster decomposition of AOM in soil, represent major steps in creating biofertilisers – microbiological fertilizers.

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Mineralization of organic matter is basically an oxidative process and represents the most important source of CO_2 in soil (about 800 kg/ha per year) for higher plants in the process of photosynthesis (Miljković, 1996). Out of the total amount of organic carbon in soil, there is 1–3% of the carbon in biomass of microorganisms (Sparling, 1992). Organic compounds of carbon come into soil mostly with plant remains. According to their chemical composition, they are: monosaccharides, polysaccharides (cellulose 15–60%, hemicelluloses 10–30%, starch 1.5% and pectin 1%), lignin 5–30%, lipids, waxes and resins up to 3%. Their transformation in soil takes place at different speed depending on the chemical composition, microbiological activity, type of soil and ecological conditions (Jarak and Čolo, 2007). A large amount of cellulose comes into soil every day, through organic remains, and it is decomposed by cellulolytic bacteria, fungi and actinomycetes (Ugarković et al., 2011).

The aim of this research was to isolate bacterial strains with the most AOM degrading potential, by isolating the bacteria from five different types of soil and their morphological, physiological and biochemical characterization. This will enable further research and formulation of microbiological preparation.

MATERIALS AND METHODS

Isolation of bacteria was conducted from five types of soil – luvisol, eutric cambisol, chernozem, forest land and meadow. The basic agrochemical characteristics of soil are given in Table 1. The morphological characteristics of a bacterial cell and bacterial colony were determined therein. Morphological characterization of cells included: shape, movability, Gramm staining and presence of endospores (Jarak and Djuric, 2004). Physiological and biochemical characteristics of bacteria were determined per Bergey's key for determination (Williams, 1994). Physiological characterization included the determination of carbon source utilization (Hugh and Leifson, 1953), temperature, pH, concentration of NaCl and heavy metals (Williams, 1994). Biochemical characterization included production of extracellular enzymes (hydrolysis of gelatin (Govedarica i Jarak, 2003), activity of lipase (Lanyi, 1987), amylase (Rodina, 1972), pectinase (Soares et al., 2001; Soriano et al., 2000), cellulase (Kasing, 1995), urease (Govedarica and Jarak, 1999) and capability of using the citrates as a source of energy (Simmons, 1926) and production of hydrogen-sulfide (Ćirić, 2014).

Table 1. Basic agrochemical characteristics of soil

Variant – depth	pH in KCl	pH in H_2O	$CaCO_3$ %	Humus %	Total N %	AL-P_2O_5 mg/100g	AL-K_2O mg/100g
Luvisol	6.03	4.87	0	3.65	0.18	10.58	23.07
Eutric cambisol	4.31	3.51	0	1.82	0.09	5.61	11.72
Chernozem	7.45	8.22	5.52	2.78	0.207	22.8	23.99
Forest land	4.19	4.81	1.25	3.66	0.18	0.36	12.98
Meadow	5.80	6.78	1.67	5.19	0.26	177.5	105.34

RESULTS AND DISCUSSION

Ten bacterial isolates, out of twenty investigated, have predominant irregular shape of the colony while eight have a round shape and they are almost all gigantic in size. They are divergent in the area, profile and border. Structure of most isolates is tiny granular or string-like. White colour dominates and all the colonies are from the surface, which points out that the isolates are aerobic. Changes in the medium are not present in any of the isolates. There is divergence in optical features and predominant slimy and dough-like consistency of the colony. Compared to morphological features of a cell, all isolates belong to rod shape, they are non-motile and Gramm positive, while 9 isolates of bacteria are sporogenous and 11 are asporogenous.

Table 2. Morphological characteristics of the bacterial colony

Isolates	Shape	Size	Area	Profile	Border	Structure	Optical property	Consistency
GB1	round	Gigantic	wrinkled/mat	raised	rhizoid	rhizoid	translucent	wax-like
GB2	irregular	Large	smooth/shiny	convex	curled	homogeneous	opaque	dough-like
RCB1	round	Gigantic	smooth/shiny	umbonate	large curled	filamentous	opaque	slimy
RCB3	round	Gigantic	wrinkled/mat	raised	rhizoid	large rhizoid	translucent	slimy
ŠB1	round	Gigantic	wrinkled/mat	raised	rhizoid	rhizoid	translucent	dough-like
ŠB2	irregular	Gigantic	wrinkled/great	umbonate	curled	grain	translucent	slimy
ŠB5	round	Gigantic	wrinkled/mat	convex	large curled	filamentous	opaque	dough-like
ŠB10	irregular	Gigantic	wrinkled/mat	raised	curled	tiny granular	translucent	slimy
ŠB11	irregular	Gigantic	wrinkled/mat	umbonate	filamentous	tiny granular	translucent	slimy
ČB1	irregular	Gigantic	wrinkled/mat	convex	curled	tiny granular	opaque	slimy
ČB4	round	Gigantic	smooth/mat	raised	curled	tiny granular	translucent	slimy
ČB5	irregular	Gigantic	wrinkled/mat	convex	curled	tiny granular	translucent	slimy
ČB7	ellipsoid	Gigantic	wrinkled/mat	raised	curled	curled granular	translucent	dough-like
ČB8	round	Gigantic	wrinkled/shiny	convex	curled	tiny granular	transparent	dough-like
ČB13	round	Gigantic	wrinkled/mat	raised	entire	homogeneous	transparent	slimy
LB1	filamentous	Gigantic	wrinkled/mat	raised	rhizoid	filamentous	translucent	dough-like
LB2	irregular	Large	wrinkled/mat	convex	large curled	homogeneous	opaque	dough-like
LB3	irregular	Gigantic	smooth/shiny	umbonate	large curled	string	translucent	slimy
LB4	irregular	Gigantic	smooth/shiny	umbonate	large curled	string	translucent	slimy
LB5	irregular	Large	wrinkled/mat	umbonate	curled	string	translucent	dough-like

Table 3. Basic physiological characteristics of bacterial isolates – in relation to different temperatures (T 5 ˚C T 15 ˚C, T 28 ˚C, T 37 ˚C, T 45 ˚C)

ISOLATES	T 5 ˚C	T 15 ˚C	T 28 ˚C	T 37 ˚C	T 45 ˚C
GB1	–	+	++	+++	–
GB2	–	+	+	+	+
RCB1	–	+	++	+	–
RCB3	–	+	++	+++	–
ŠB1	–	+	++	+++	–
ŠB2	–	+	++	+++	–
ŠB5	–	+	++	+++	–
ŠB10	–	+	++	+++	–
ŠB11	–	+	++	+++	–
ČB1	–	+	+	+++	++
ČB4	–	+	+	+++	++
ČB5	–	–	–	+++	–
ČB7	–	+	+	+++	+++
ČB8	–	+	+	+++	++
ČB13	–	+	+	+	–
LB1	–	+	+++	+++	+++
LB2	–	+	++	+++	+++
LB3	–	+	+++	+++	+++
LB4	–	+	+++	+++	+++
LB5	–	+	+++	+++	+++

– does not grow; + minimal growth; ++ optimal growth; +++ abundant

No isolates grew at low temperatures (5 ˚C); at 15 ˚C almost all examined isolates showed minimal growth while at 45˚C the growth of the isolates was minimal except for the isolates LB1, LB2, LB3, LB4, LB5 and ČB7 which showed huge growth at this temperature. Isolates from eutric cambisol, luvisol and forest land, showed an optimal growth at 28 ˚C, and meadow isolates showed abundant growth at the same temperature. Almost all the examined isolates show huge growth at 37 ˚C. It can be concluded that the examined isolates are mesophyll microorganisms.

Table 4. Relation to different pH values and different concentration of NaCl

ISOLATES	pH5	pH 7	pH 9	3% NaCl	5% NaCl	7% NaCl
GB1	+++	+++	++	++	–	–
GB2	++	+++	++	++	+	+
RCB1	+++	++	++	++	–	–
RCB3	++	++	+++	–	–	–
ŠB1	++	++	++	–	–	–
ŠB2	++	++	++	+	–	–
ŠB5	++	++	++	++	–	–
ŠB10	+++	++	++	–	–	–
ŠB11	+++	+++	+++	++	–	–

ČB1	++	++	+++	++	+	–
ČB4	++	++	++	++	++	+++
ČB5	++	++	+++	++	+	–
ČB7	++	++	+++	+++	+++	+++
ČB8	++	++	++	+	+	–
ČB13	+	++	+++	++	+	+
LB1	++	+++	++	+	–	–
LB2	++	+++	++	++	++	–
LB3	++	+++	++	+	–	–
LB4	++	+++	++	+	+	–
LB5	++	+++	++	+	+	–

– does not grow; + minimal growth; ++ optimal growth; +++ huge growth

Isolates from eutric cambisol, luvisol, chernozem and meadow soil, are predominantly neutrophils. As far as forest land is concerned, as is expected, the biggest growth is on the acidic pH. Isolates of eutric cambisol on the foundations which contained 3% NaCl showed optimal growth, while on other concentrations their growth was minimal. Most of the examined isolates can not stand high concentrations of salt, with exception of isolates ČB4 and ČB7. Almost all the isolates have optimum growth on the medium with 3% NaCl, while on the medium with 5% NaCl optimal growth is shown only by isolates ČB4 and LB2.

Table 5. Basic physiological characteristics – in relation to different sources of carbon

Isolates	Glucose	Galactose	Fructose	Lactose	Sucrose	Xylose	Citrates
GB1	–	–	–	–	–	–	–
GB2	+	–	–	–	+	–	–
RCB1	–	–	–	–	+	–	–
RCB3	+	–	–	+	–	–	–
ŠB1	–	–	–	–	+	–	–
ŠB2	–	–	–	–	–	–	–
ŠB5	–	–	–	–	+	–	–
ŠB10	–	–	–	–	–	–	–
ŠB11	–	–	–	–	–	–	–
ČB1	–	+	+	–	+	–	–
ČB4	–	–	–	–	+	–	–
ČB5	–	+	–	–	+	+	+
ČB7	–	+	–	+	+	–	–
ČB8	–	+	–	+	+	–	+
ČB13	–	–	–	–	+	–	–
LB1	+	+	–	+	–	–	–
LB2	+	–	–	+	+	–	–
LB3	+	–	–	–	+	–	–
LB4	+	–	–	–	+	–	–
LB5	–	–	–	+	+	–	–

+ positive reaction; – negative reaction

Only the isolate ČB5 as a source of carbon used xylose, while ČB1 used sugar fructose. Most isolates out of all 5 types of soil used sugar sucrose, while glucose is used by the isolates GB2, RCB3 and almost all isolates from meadow soil. Positive reaction on sugar galactose is shown by almost all isolates from chernozem and LB1 from meadow soil, while lactose is used by most isolates of chernozem and meadow soil. As a source of carbon, the examined isolates usually used sucrose, and only one isolate (ČB5) used xylose. Isolates ČB5 and ČB8 showed a capability to use citrates from the foundation/base.

Table 6. Basic physiological characteristics – in relation to different concentrations of heavy metals for selected sorts of bacteria

ISOLATES	Cd^{-2}	Cd^{-4}	Cd^{-6}	Pb^{-2}	Pb^{-4}	Pb^{-6}	Mn^{-2}	Mn^{-4}	Mn^{-6}
ŠB11	–	–	–	–	–	–	–	++	–
ČB4	+++	–	–	–	–	–	–	–	–
ČB5	–	–	–	–	+++	–	–	–	–
ČB7	+++	++	–	–	++	–	–	–	–
ŠB5	+++	*	+++	–	–	–	–	+	–
ČB8	+++	–	–	–	–	–	–	–	–
ŠB10	+	–	–	–	–	–	–	–	–
ČB13	+++	–	–	–	–	–	–	–	–
LB2	–	++	++	–	++	++	–	+	+
LB3	+++	+	+	–	–	–	–	+	+
LB4	+++	++	+++	+	–	–	–	+	+

– without zone (tolerant); + zone of inhibition 0–2 mm; ++ zone of inhibition 2–5 mm; +++ zone of inhibition larger than 5mm; *slowed down, reduced growth

Eleven isolates were chosen for examination of heavy metal tolerance. It was determined that cadmium, at the concentration of 10^{-2} mol/dm^3 affected as an inhibitor all isolates except ŠB11, ČB5 and LB2 which were tolerant to it in that concentration. Manganese at the same concentration did not have inhibitory influence on our examined isolates, while lead was noticed to have an inhibitory zone at the concentration of Pb^{-2} only with the isolate LB4. As far as cadmium, lead and manganese are concerned, at the concentration of 10^{-4} mol/dm^{3}they had inhibitory effect on less than a half of the examined sorts. The concentration of 10^{-6} mol/dm^3 had the most inhibitory effect on the examined sorts with cadmium, where isolates such as ŠB5 and LB4 had a zone of inhibition which was larger than 5mm.

Table 7. Basic biochemical characteristics – production of extracellular enzymes (pectinase, cellulase, amylase, urease, lipase and gelatinase)

ISOLATES	Pectinase	Cellulase	Amylase	Urease	Lipase	Gelatinase
GB1	–	+	–	–	+	+
GB2	–	–	–	+	+	+

RCB1	+	–	–	–	+	++
RCB3	–	+	–	–	+	+
ŠB1	–	+	++	–	–	+
ŠB2	–	+	–	–	+	+
ŠB5	++	–	++	–	+	–
ŠB10	+	–	+++	+	+	+
ŠB11	+	+++	++	–	+	++
ČB1	–	+	–	–	+	–
ČB4	–	+++	+++	–	+	+++
ČB5	++	+	++	+	+	+++
ČB7	+++	+++	–	–	+	+
ČB8	–	++	+	–	+	+
ČB13	+	–	+++	–	–	+++
LB1	–	–	–	–	+	+
LB2	+	+	+	–	+	+
LB3	–	+	+	–	+	+
LB4	–	+	+	+	+	+
LB5	–	–	+	+++	+	+

– negative reaction; + mildly positive reaction; ++ strongly positive reaction;
+++ the strongest positive reaction

Isolate ČB4 in a large quantity produces cellulase, amylase and gelatinase while isolate ŠB11 produces all three enzymes connected with the cycle of carbon. Pectinase and cellulose were produced by isolate ČB7, while ČB13 produces amylase for hydrolysis of starch and pectinase and gelatinase. This research determined that almost all isolates except ŠB5 and ČB1 have a capability to hydrolyze gelatin. All the examined isolates, except ŠB1 and ČB13 showed a capability of decomposing fats, that is production of extracellular lipases. Isolates such as ŠB11, ČB4 and ČB7 showed the biggest cellulase activity. All the isolates from forest land, chernozem and meadow soil had the capability to hydrolyze starch. Five bacteria isolates produce ureasa and decompose urea and carbon-dioxide. None of the isolates produces H_2S.

Regarding the fact that the increase of agricultural waste around the world is increasing every day, managing AOM has become a major problem which can result in pollution of the environment (Yi et al., 2017). AOM is an important part of organic solid waste which is treated through landfills, burning, pyrolysis, process of biogas and composting (Kulcu and Yaldiz, 2004). Composting is a complex process of biological decomposition, which can turn organic matter into humus and a stable product. Material can be used as a soil enhancer or organic fertilizer (Juardo et al., 2015, Kulcu and Yaldiz, 2004). Huang et al. (2010) followed changes in structure of microbiological population related to decomposition of lignin during composting of lignocellulose waste. Their results are going to improve understanding of microbiological dynamics and role in composting, which could be useful for development of technology of composting. Kushwaha et al. (2014) worked on a study with samples of soil from

India. Maximal production of cellulase was obtained after 48h of incubation at 45 ˚C in medium which contained 1.5% of carboxymethyl cellulose (CMC) as a substrate. Optimal pH for this enzyme is 6.5 to 7.5. Bacteriological studies showed that *Bacillus subtilis* is the most frequent cellulolytic bacteria found in agricultural fields. The purpose of the research was to list all species of Bacillus isolated from soil in order to examine its suitability in bioremediation. General findings of the research by Barman et al. (2011) show efficiency of *Moraxella sp. f*or bioconversion of solid organic waste. Their research has been done in order to learn about efficiency of cellulolytic bacteria for bio-decomposition of solid kitchen and agricultural waste as organic fertilizer or compost. The value of cellulose as a renewable source of energy has brought hydrolysis of cellulose into the focus of intense research and industrial interests (Bhat, 2000). A lot of research was dedicated to getting new microorganisms which produce enzymes with more efficiency (Subramaniyanet al., 2000). Isolation and characterization of bacteria which produce this enzyme are going to keep being an important aspect of research of biological fuels, biodegradation and bioremediation (Abedin, 2015). Pectinolytic enzymes have great importance in the existing biotechnology era with their comprehensive applications in the extraction of fruit juices, cotton cleaning, sewage treatment, extraction of vegetable oil, alcoholic beverages and the food industry (Ranveer et al., 2005).

CONCLUSION

Results of extensive scientific research have shown that fertilizers of chemical origin due to the content of toxic materials can be connected to unwanted effects which they have on people's health and have negative influence on the environment. It is the reason why the scientists directed their attention to natural alternatives, biofertilizers. Cellulases are one of the most widely used enzymes in various industries. The present study focused on isolation, characterization of AOM degrading bacteria and determination of their enzymatic potential. We expect that our isolates ŠB10, ŠB11, ČB4, ČB5, ČB7 and ČB8 (with most organic matter degrading potential) could be used in the near future for further examination and conceptualizing microbiological preparation. By applying microbiological fertilizers neither the soil nor groundwaters are polluted, and their use does not have a toxic effect on plants and environment. The fact that their use reduces the quantity of chemicals in agricultural production makes biofertilisation an eco-friendly process.

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КАРАКТЕРИСТИКЕ БАКТЕРИЈСКИХ ИЗОЛАТА, РАЗЛАГАЧА ПОЉОПРИВРЕДНЕ ОРГАНСКЕ МАТЕРИЈЕ, ИЗ РАЗЛИЧИТИХ ТИПОВА ЗЕМЉИШТА

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РЕЗИМЕ: У земљиште свакодневно кроз органске остатке доспевају велике количине органске материје из пољопривреде које разграђују бактерије, гљиве и актиномицете (Угарковић и сар., 2011). Циљ ових истраживања био је да се изолацијом бактерија из пет различитих типова земљишта и њиховим морфолошким, физиолошким и биохемијским карактеризацијама издвоје сојеви с највећим потенцијалом деградације органске материје. Изолација бактерија извршена је из пет различитих типова земљишта – ритска црница, гајњача, чернозем, шумско земљиште и ливада. Карактеризација изолованих сојева бактерија извршена је на основу морфолошких, физиолошких и биохемијских својстава. Изолати с највећим потенцијалом деградације органске материје могли би се у блиској будућности користити за конципирање микробиолошког препарата.

КЉУЧНЕ РЕЧИ: бактерије, деградација АОМ, земљиште, изолација, карактеризација

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ANTAGONISTIC POTENTIAL OF *Lactobacillus plantarum* AGAINST SOME POSTHARVEST PATHOGENIC FUNGI

ABSTRACT: *Lactobacillus plantarum*, one of the most widespread lactic acid bacteria, exert a strong antagonistic activity against many microorganisms. The present study was conducted to determine *in vitro* and *in situ* antagonistic potential of *L. plantarum* (DSM 20174) for control postharvest decay caused by phytopathogenic fungi: *Aspergillus flavus, Colletotrichum acutatum, Colletotrichum gloeosporioides*, and *Fusarium avenaceum.* The results obtained in *in vitro* assays showed that *L. plantarum* had a stronger inhibitory effect on spore germination than on mycelia growth of all tested fungi. After 3 days of incubation, the diameter of inhibition zones ranged from 11.67 mm for *C. gloeosporioides* to 14.67 mm for *C. acutatum*. The bacterial suspension of *L. plantarum* significantly inhibited conidial germination of all postharvest pathogens (89.62–97.61%). *In situ* assays showed that treatment with *L. plantarum* efficiently inhibited necrosis ranging from 42.54% for *C. acutatum* to 54.47% for *A. flavus*. The disease incidence in *L. plantarum* treated fruits was statistically significantly lower than in the positive control for all fungi tested ($P<0.05$). The presented data demonstrate the antagonistic potential of *L. plantarum* (DSM 20174) and indicate the possibility of using this bacterial strain as a biological agent to control postharvest fungal pathogens.

KEYWORDS: antagonistic activity, biocontrol**,** *Lactobacillus plantarum*, postharvest fungal pathogens

INTRODUCTION

The postharvest losses are mainly due to pathogenic fungi which usually infect fruits through wounds made during harvest, transportation, and processing (Vero et al., 2002). Some of the postharvest fungal pathogens cause serious problems in food by producing mycotoxins and potentially allergenic spores

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(Mushtaq et al., 2010). Several methods have been used to solve postharvest losses, such as fungicide treatment and modified controlled atmosphere (Montero et al., 2010; Romanazzi et al., 2012). The development of fungicide resistance by postharvest pathogens and increasing environmental concern over fungicide residues in food have stimulated the finding of alternative means for controlling postharvest decay (Holmes and Eckert, 1999). Biological control involves the use of naturally occurring nonpathogenic microorganisms, biocontrol agents (BCAs), that are able to reduce the activity of plant pathogens and thereby suppress diseases. Several strains of *Bacillus*, *Pseudomonas* and lactic acid bacteria (LAB), as well as yeasts, have been identified and commercialized for the control of postharvest decay caused by fungi in fruits (Janisiewicz and Korsten, 2002).

LAB form an ecologically heterogeneous group of Gram-positive bacteria, nonspore-forming, immobile and catalase negative that excrete lactic acid as the major product and are generally recognized as safe organisms (GRAS) (Konings et al., 2000). The antimicrobial properties of lactobacilli are of special interest in developing strongly competitive starter cultures for food fermentation (Harris et al., 1989). Today, LAB strains play crucial roles in the manufacturing of fermented milk products, vegetables, and meat, as well as in the processing of other products such as wine (Konings et al., 2000). These bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocin or bactericidal proteins during lactic fermentations (Lindgren and Dobrogosz, 1990). Lactobacilli are able to inhibit food-borne pathogens: *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli,* and *Listeria monocytogenes* (Jamuna and Jeevaratnam, 2004; Darsanaki et al., 2012). They are selected as probiotic, which are able to promote health and prevent infections against enteropathogenic bacteria (Fernandez et al, 2003). In addition, LAB strains are efficient in inhibition of mycotoxigenic fungi: *Penicillium expansum, Botrytis cinerea, Aspergillus niger, Aspergillus flavus*, and *Fusarium graminarum*, as well as phytopathogenic bacteria, such as *Xanthomonas campestris* and *Erwinia carotovora* (Lavermicocca et al., 2000; Trias et al., 2008).

One of the most widespread LAB strains used in food technology and biotechnology is *Lactobacillus plantarum*. This species synthesizes a number of substances, including benzoic acid, methylhydantoin, and mevalonolactone that have antifungal activity (Niku-Paavola et al., 1999). Lavermicocca et al. (2000) reported that the inhibitory activity of *L. plantarum* can be attributed to the organic acids phenyl-lactate and 4-hydroxy-phenyllactate.

The results of the previous investigation (Živković et al., 2014) indicated good antagonistic activity of *L. plantarum* (DSM 20174) against *P. expansum* and *Aspergillus ochraceus.* The present study was conducted to determine *in vitro* and *in situ* potential of this LAB against postharvest decay in apple fruits caused by *A. flavus, Colletotrichum acutatum, Colletotrichum gloeosporioides*, and *Fusarium avenaceum.*

MATERIAL AND METHODS

Pathogens and BCA

A. flavus, C. acutatum, C. gloeosporioides, and *F. avenaceum* were obtained from decayed apple fruits in storage and kept in the Culture Collection of Institute for Plant Protection and Environment. For conidial production, pathogens were grown on potato dextrose agar (PDA) at 25 °C. After a week, spores were harvested and suspended in 10 ml of sterile distilled water containing 0.05% (v/v) Tween 80. The concentration of spore suspension was determined with a Neubauer chamber and adjusted with sterile distilled water to 1×10^6 conidia/ml.

L. plantarum (DSM 20174) was obtained from the German Collection of Microorganisms and Cell Cultures. The bacterial strain was cultivated anaerobically in Man, Rogosa and Sharpe (MRS) broth for 72 hours at 30 °C.

In vitro assays of antagonistic activity

Antagonistic activity was determined in the dual culture overlay assays. Bacteria were inoculated in 2 cm lines on MRS agar plates and allowed to grow at 30 °C for 48 h in anaerobic jars. The plates were then overlaid with 5 ml of malt extract soft agar (2% malt extract; 0.7% agar) containing 1 x 10^6 spores of tested pathogens. After 72h of aerobic incubation at 30 °C, the zone of inhibition (ZI) was measured. The ZI was recorded as the distance between the fungal pathogen and the area of the antagonist.

For conidial germination test, 100 µl of the conidial suspension of each fungal pathogen (10^6 conidia/ml) and 100 µl of the bacterial suspension of *L. plantarum* (10^8 CFU/ml) were added into the glass tubes with 5 ml potato dextrose broth (PDB). The control consisted of suspensions of pathogens conidia in PDB. The tubes were then incubated in moist chambers for 24h at 25 °C. The percent of germination was determined by counting 100 conidia from each fungal pathogen under the microscope Olympus BX51 (Olympus Corporation Japan). Spores were considered germinated when germ tube length was equal to or greater than spore length.

In situ assays of antagonistic activity

Apple fruits (cv. Golden Delicious) were surface sterilized, wounded with a cork borer and then inoculated with 25 µl of the bacterial suspension of *L. plantarum* (10^8 CFU/ml). After 1 h, the wound was inoculated with 25 µl of the conidial suspension of *A. flavus, C. acutatum, C. gloeosporioides*, or *F. avenaceum* (1 x 10^6 conidia/ml). The positive control fruits were inoculated only with the fungal conidial suspensions, and the negative control with sterile distilled water. All apples were placed in a moist chamber and incubated at 25 °C. After 7 days the diameters of necrotic lesions were measured. The percentage

of necrosis inhibition (IN) was calculated using the formula: IN (%) = (KR-R/KR) x 100, where KR is the radius of necrosis in positive control fruit and R is the radius of necrosis in fruit treated with *L. plantarum*.

Statistical analysis

For all experiments, each treatment was done in triplicates and the entire experiment repeated twice. Data were analyzed by one-way analysis of variance (ANOVA). Mean values were compared using Tukey's multiple range test and significance was evaluated at $P<0.05$. Statistical analysis was performed using statistical software Minitab 18 (Minitab, Inc, USA).

RESULTS AND DISCUSSION

In the present study *L. plantarum* (DSM 20174) was evaluated *in vitro* and *in situ* for antagonistic activity against *A. flavus, C. acutatum, C. gloeosporioides*, and *F. avenaceum*. Results obtained in the dual culture overlay assays showed that *L. plantarum* had good antifungal activity against all tested fungi (Table 1). After 3 days of incubation, the diameter of inhibition zones ranged from 11.67 mm for *C. gloeosporioides* to 14.67 mm for *C. acutatum*.

The results of our study showed that *L. plantarum* had a stronger inhibitory effect on spore germination than *in vitro* mycelial growth of *A. flavus, C. acutatum, C. gloeosporioides*, and *F. avenaceum*. The conidia of all tested pathogens incubated in control treatment at 25 ºC were swelled and germinated, producing one germ tube. However, conidia of tested fungi were strongly limited in the co-cultivation assay with the bacterial suspension of *L. plantarum*. After 24h of co-cultivation, there was a significant inhibition of the conidial germination in all treatments with the antagonist (89.62–96.61%) (Table 1). Conidia that were ungerminated after 24h did not germinate afterward. Figure 1 (A-C) depicts the effect of *in vitro* bacterial suspension of *L. plantarum* against *A. flavus*.

Table 1. Antagonistic activity of *L. plantarum* against the postharvest fungal pathogens *in vitro.*

Pathogen	*L. plantarum*	
	Inhibition zone (mm)	Inhibition of spore germination (%)
A. flavus	12.67 ± 0.58* bc**	97.61 ± 0.60* a**
C. acutatum	14.67 ± 0.58 a	91.86 ± 0.59 b
C. gloeosporioides	11.67 ± 0.58 c	91.16 ± 1.02 bc
F. avenaceum	13.33 ± 0.58 ab	89.62 ± 1.04 c

* Data represented standard deviations of the means
** Means in columns followed by different letters are significantly different according to Tukey's multiple range test ($P<0.05$)

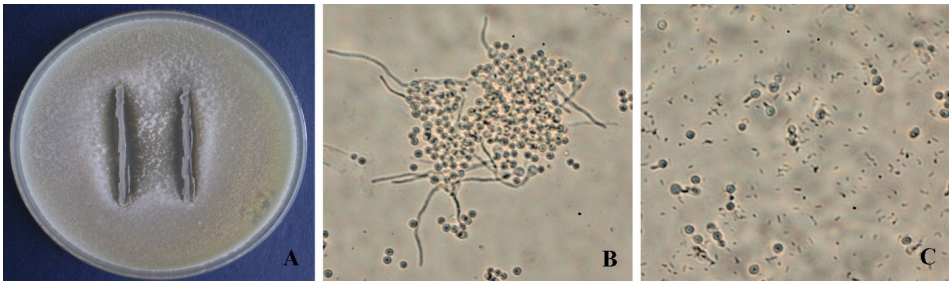

Figure 1. Effect of *L. plantarum* on *A. flavus in vitro*: A) inhibition zone; B) conidial germination of *A. flavus* in control: (magnification x400) C) inhibition of the conidial germination of *A. flavus* in treatment with *L. plantarum* (magnification x400)

The results of the previous investigation showed that *L. plantarum* (DSM 20174) had good antifungal activity against *P. expansum* (ZI =20 mm), and *A. ochraceus* (ZI =15 mm) *in vitro*. The bacterial suspension of this strain completely inhibited conidial germination of *P. expansum*, and significantly inhibited conidial germination of *A. ochraceus* (88%). In biocontrol assay, *L. plantarum* significantly reduced disease incidence caused by *P. expansum* (55%) in apple fruit. However, this LAB had moderate antifungal effect *in situ* on *A. ochraceus* (37%) (Živković et al., 2014).

The antifungal activity of *L. planatarum* has also been reported by other investigators. Trias et al. (2008) isolated *L. plantarum* from fresh fruits and vegetables and tested *in vitro* their potential as BCA against phytopathogenic fungi, *P. expansum*, *B. cinerea*, and *M. laxa*. All tested microorganisms except *P. expansum* were inhibited by one isolate of *L. plantarum*. Prema et al. (2010) investigated the antifungal activity of *L. plantarum* strain from grass silage. Agar plate assay showed that *Aspergillus fumigatus* and *Rhizopus stolonifer* were the most sensitive among molds. No inhibitory activity could be detected against *Penicillium roqueforti*. Sathe et al. (2007) tested the antifungal spectrum of LAB strains against *F. graminearum, R. stolonifer, S. oryzae, R. solani, B. cinerea*, and *S. minor* in the overlay method. The isolate identified as *L. plantarum* had a strong activity against all six spoilage fungi. Our results are in agreement with the results of Gerez et al. (2009) who reported that *L. plantarum* and other strains of lactobacilli were able to inhibit the conidial germination and mycelial growth of fungi from the genera *Aspergillus*, *Fusarium*, and *Penicillium*, the main contaminants in bread.

The antifungal activity of LAB strains are certainly a complex phenomenon and still partially unknown. There are few reports of low molecular weight of antifungal peptides synthesized by LAB, which inhibit spoilage and pathogenic fungi with insufficient information on their precise mechanism of action (Schnurer and Magnussion, 2005). Several studies have reported that the antifungal activity of LAB is not only related to the production of organic acids and hydrogen peroxide. Rather, it is a combined effect of several interrelated factors (Laitila et al., 2002). Cabo et al. (2002) have suggested that *in vitro* antifungal activity of LAB is due to a synergistic effect of lactic acid produced

by the bacteria and acetic acid from the MRS growth medium. This dual culture system is based on diffusion of the inhibitory substances into the agar, and consequently lactic acid will also contribute to the inhibition (Strom et al., 2002). The antimicrobial effects of different lactobacilli including *L. plantarum* against plant pathogenic fungi were greatly influenced by the substrate and pH of cultivation (Karunaratne et al. 1990; Gourama and Bullerman, 1995; Stiles and Holzapfel, 1997).

The results obtained in *in situ* assays showed that treatment with *L. plantarum* efficiently protected apple fruits from decay and inhibited necrosis ranging from 42.54% for *C. acutatum* to 54.47% for *A. flavus* (Figure 2). No lesion developed in negative control fruits inoculated with sterile distilled water. The disease incidence in *L. plantarum* treated fruits was statistically significantly lower than those in the positive control for all fungi tested ($P<0.05$). Figure 3 (A–C) presents the effect of the bacterial suspension of *L. plantarum* on apple fruits affected by *A. flavus* infection *in situ*.

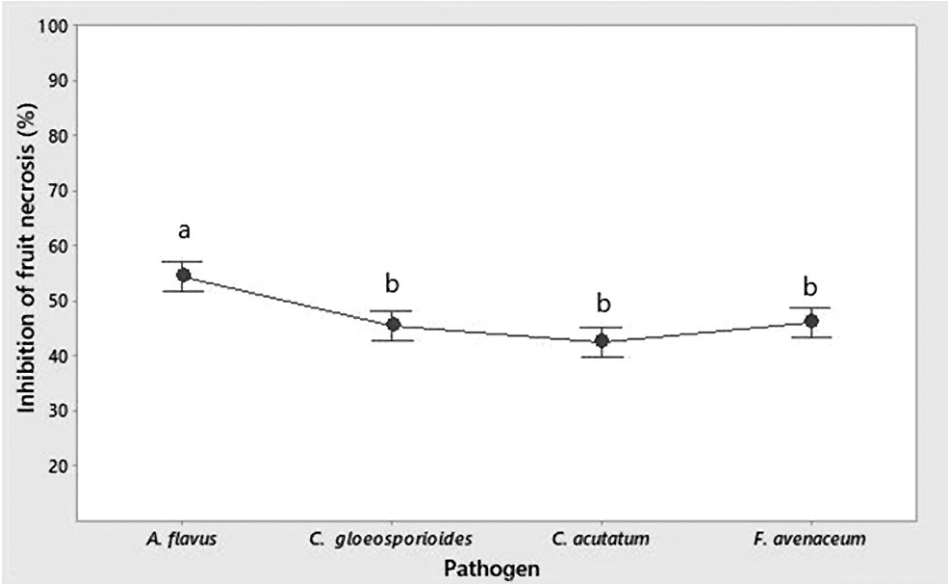

Figure 2. Inhibition of apple fruit necrosis induced by postharvest fungal pathogens using *L. plantarum.*

Sathe et al. (2007) reported that the suspension of *L. plantarum* delayed the growth of *A. flavus, F. graminearum, R. stolonifer*, and *B. cinerea* in cucumber. LAB isolated from yogurt and milk showed inhibitory activity against *F. oxysporum* and provided a protective effect to tomato plants (Hamed et al., 2011). Prusky et al. (2006) suggested that acidification of fruit tissue can reduce the postharvest decay caused by pathogens, such as *P. expansum* and *A. alternata*. The combination of different organic acids, such as lactic and propionic, has been reported to have a synergistic fungistatic effect (Adams and Hall, 1988).

Figure 3. Effect of *L. plantarum* on *A. flavus* decay on apple fruits *in situ*: A) positive control; B) treatment with *L. plantarum*; C) negative control.

In situ, the antimicrobial action is often the sum of many factors. In many cases, not only extracellularly produced compounds but also viable cells are needed for the maximum action.

CONCLUSION

Postharvest fungal pathogens are the main cause of substantial economic losses in stored fruits and might also be regarded as sources of mycotoxins, involving serious health problems. LAB strains are important organisms recognized for their fermentative ability as well as their health and nutritional benefits. One of the most widespread LAB, *L. plantarum,* produces several antimicrobial agents and exerts strong antagonistic activity against many microorganisms, including food spoilage organisms and pathogens. In this context, *L. plantarum* may be considered as an alternative for synthetic fungicides. The presented data exhibit *in vitro* and *in situ* antimicrobial activity of *L. plantarum* (DSM 20174) against *A. flavus, C. acutatum, C. gloeosporioides,* and *F. avenaceum*, and indicate the possibility of using this bacterial strain as a BCA to control these postharvest fungal pathogens in apple fruits.

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АНТАГОНИСТИЧКИ ПОТЕНЦИЈАЛ *Lactobacillus plantarum* ПРЕМА НЕКИМ СКЛАДИШНИМ ФИТОПАТОГЕНИМ ГЉИВАМА

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РЕЗИМЕ: *Lactobacillus plantarum* једна је од најраспрострањенијих млечнокиселинских бактерија која испољава антагонистичку активност према великом броју микроорганизама. Циљ студије био је да се у *in vitro* и *in situ* огледима утврди антагонистички потенцијал *L. plantarum* (DSM 20174) према складишним фитопатогеним гљивама: *Aspergillus flavus*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides* и *Fusarium avenaceum*. Резултати *in vitro* огледа показују да је

L. plantarum испољио јачи инхибиторни ефекат на клијање спора него на пораст мицелије тестираних гљива. Зоне инхибиције су варирале у распону од 11,67 mm за *C. gloeosporioides* до 14,67 mm за *C. acutatum*. Бактеријска суспензија *L. plantarum* је значајно инхибирала клијање конидија свих тестираних складишних патогена (89,62–97,61%). У *in situ* огледима *L. plantarum* је ефикасно инхибирао појаву некрозе у опсегу од 42,54% за врсту *C. acutatum* до 54,47% за врсту *A. flavus*. Инциденца појаве болести код плодова третираних овим биоконтролним агенсом била је статистички значајно нижа у односу на позитивне контроле свих испитаних патогена ($P<0,05$). Добијени резултати указују да *L. plantarum* (DSM 20174) има антагонистички потенцијал и да се може користити као биоконтролни агенс против складишних фитопатогених гљива.

КЉУЧНЕ РЕЧИ: антагонистичка активност, биоконтрола, *Lactobacillus plantarum*, складишне фитопатогене гљиве

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THE INFLUENCE OF NITROGEN AND GROWTH PHASE ON THE TOXICITY OF THE CYANOBACTERIAL STRAIN *Microcystis PCC 7806*

ABSTRACT: The worldwide occurrence of toxic cyanobacterial blooms and their numerous harmful effects have instigated extensive research into the environmental conditions promoting such events. Among the environmental factors which have been suggested to influence the increase in cyanobacterial proliferation, nutrient levels have been identified as one of the most prominent, affecting the growth and toxic metabolite production of cyanobacteria in freshwater ecosystems. In the present study, toxicity of the cyanobacterial strain *Microcystis PCC 7806* was evaluated after growth in media with three different nitrogen concentrations. The toxicity of intracellular extracts was analyzed during different growth phases (after 7, 21, and 35 days of cultivation) by observing mortality rates in the *Artemia salina* bioassay after 24h and 48h of exposure. The results have not shown significantly higher mortality levels between the test organisms exposed to extracts obtained from the cultures grown in the presence of higher nitrogen content (1.5 g/l and 0.8 g/l) and those grown in a nitrogen-free medium. A dose dependent effect, however, can be observed in most cases, with the most substantial changes observed in the high-dose groups. Also, the toxic effects and larval mortality increased during the exposure, suggesting the time-dependent toxicity. Extracts obtained after longer periods of cultivation (21 and 35 days) had stronger effects on the test organisms, which indicates that the toxicity of the tested cyanobacterial strain depends on the specific growth phase.

KEYWORDS: *Artemia salina*, Cyanobacteria, *Microcystis*, Nitrogen, Toxicity

INTRODUCTION

Cyanobacteria are a widely distributed group of gram-negative bacteria, which can be found not only in freshwater, marine and terrestrial ecosystems, but also in certain extreme environments such as hot springs and frozen glaciers (Vincent, 2009). In aquatic ecosystems, large masses of cyanobacteria can be

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formed due to extensive proliferation, which is made possible by nutrient pollution and the hydrological changes of the ecosystem (Štěpánková et al., 2011). These "blooms" can have various harmful effects on the living organisms and ecosystems in general, some caused by the release of toxic secondary metabolites (Wood, 2016). Cyanobacteria have the ability to produce a wide spectrum of secondary metabolites, but also several cytotoxic, neurotoxic, and hepatotoxic substances (Merell et al., 2013; Svirčev et al., 2008). These "cyanotoxins" are stored within bacterial cells and can be released into the water after cell rupture or death, after which they often come into contact with other living organisms, including humans (Wood, 2016). The effects of different environmental factors on the cyanotoxin production have been widely investigated (Neilan et al., 2013; Esteves-Ferreira et al., 2018). It is evident that the occurrence of such toxins in freshwater ecosystems is greatly influenced by the availability of specific essential nutrients, which dictate the rate of growth and metabolite synthesis of aquatic cyanobacteria (Kramer et al., 2018; Pimentel and Giani, 2014). One example of such nutrients is nitrogen, most commonly found in water in the form of nitrate or ammonium (Yin et al., 1997). Some of the most common toxigenic cyanobacterial genera are non-diazotrophic (e.g. *Microcystis*) and thus entirely dependent on readily accessible N_2 sources. When non-diazotrophic strains of the genus *Microcystis* are in question, an increase in nitrogen concentrations in the growth medium usually leads to a higher intracellular toxin content (Vaitomaa, 2006). Toxic cyanobacterial strains generally have higher demands in terms of nitrogen and phosphorus levels in their environment compared to the strains which are not toxic, possibly because of the higher energy and material requirements for the toxin synthesis (Vezie, 2002). As one of the known roles of nitrogen in cyanobacteria includes an increase in protein synthesis and intracellular protein concentrations, it is probable that the available nitrogen concentrations will influence the production of peptide based toxic metabolites (Ownby et al., 1979).

The aim of this study was to analyze the effects of different nitrogen concentrations, as well as growth phases, on the toxicity of aquatic cyanobacterial strain *Microcystis PCC 7806*, using the *Artemia salina* bioassay.

MATHERIALS AND METHODS

Cultivation and extract preparation of the tested strain *Microcystis* PCC 7806

The analyzed toxic strain of *Microcystis* genus, *PCC 7806* (purchased from the Pasteur Culture Collection) was maintained in a liquid mineral medium BG11 (Rippka et al., 1979). The culture was incubated under white fluorescent light (50 µmol m) with a light-dark cycle of 12:12h and cultivation temperature of 22–24 °C. Three variations of the BG11 medium were used for cultivation, one of which contained 1.5 g/l of nitrogen in the form of $NaNO_3$, the second

medium was prepared using $NaNO_3$ in the concentration of 0.8 g/l, and the third was a nitrogen-free medium.

Cyanobacterial biomass was separated from the growth medium after the 7th, 21st, and 35th day of cultivation, after which the wet biomass was freeze-dried (*Christ alpha 1-2/LD plus)* and weighed. Lyophilized biomass was used for the preparation of the intracellular methanolic extracts in three different concentrations (C1, C2, and C3). The release of active metabolites from the cells was induced by sonication (Branson 250 sonicator) for the duration of 2–5 minutes at room temperature and atmospheric pressure conditions. Afterwards, the samples were homogenized by gentle mixing for 30 minutes and centrifuged at 12,000 rpm for 5–10 minutes. The supernatants were analyzed for their acute toxicity in the *Artemia salina* bioassay.

Artemia salina bioassay

The bioassay consisted of three distinct phases: the initial phase which involved the incubation and hatching of the test organisms, then the preparation of the experimental solutions, and the measurement of mortality rates in the final phase. Artificial sea water (ASW) with total salinity of 35 g/l was used as a hatching medium, with the incubation period of 24–36h, constant light, and temperature of 30 °C. For the initial hatching phase, *A. salina* eggs (Carolina, USA) were introduced to a sterile ASW medium. After 32h, hatched nauplii were collected and used in the experiment. Toxicity of the cyanobacterial strain *Microcystis PCC 7806* was tested in different stages of growth – after 7, 21, and 35 days of cultivation. Three concentrations were prepared for each individual intracellular extract (on the 7^{th}, 21^{st}, and 35^{th} day), with the initial concentration (marked C1 – 100%) achieved by diluting the dried cyanobacterial biomass with 2 ml of 75% MeOH. The remaining two concentrations, C2 (50%) and C3 (10%), were prepared by diluting the initial C1 concentration with the appropriate volume of ASW medium. In every well which contained 100µl of the cyanobacterial extract, 10–20 live test organisms were added, after which the volume was filled up to 100 µl with the ASW medium. Another 100 µl of ASW was added to each well setting the total volume to 200 µl per well. ASW with brine shrimp without treatment were used as control. The mortality rates were calculated after 24h and 48h (Meyer et al., 1982).

Statistical analysis of data

A two-way analysis of variance (ANOVA) was used to test whether the effects of the nitrogen concentration in the medium and time of exposure to the cyanobacterial extracts on the survivability of the test organisms were significant in three different growth phases. The analysis was carried out in triplicates and the results were presented as the mean values ±SD. The data analysis was performed using GraphPad Prism software, version 6.

RESULTS

The results obtained in the *Artemia salina* bioassay were presented in three separate sets according to the nitrogen content in the growth media. Every set shows the results collected after analyzing the extracts of the strain cultivated for the period of 7, 21, and 35 days. The toxic effects of three different extract concentrations were analyzed after 24 and 48h and presented as mortality percentage values. Control replicates have shown mortality levels below 5% throughout the experiment.

The effects of intracellular extracts of *Microcystis PCC 7806* cultivated with 1.5 g/l $NaNO_3$

Figure 1 represents the mortality percentage of brine shrimp nauplii after the exposure to the intracellular extracts prepared from the strain cultivated in the medium with nitrogen ($NaNO_3$ concentration of 1.5 g/l).

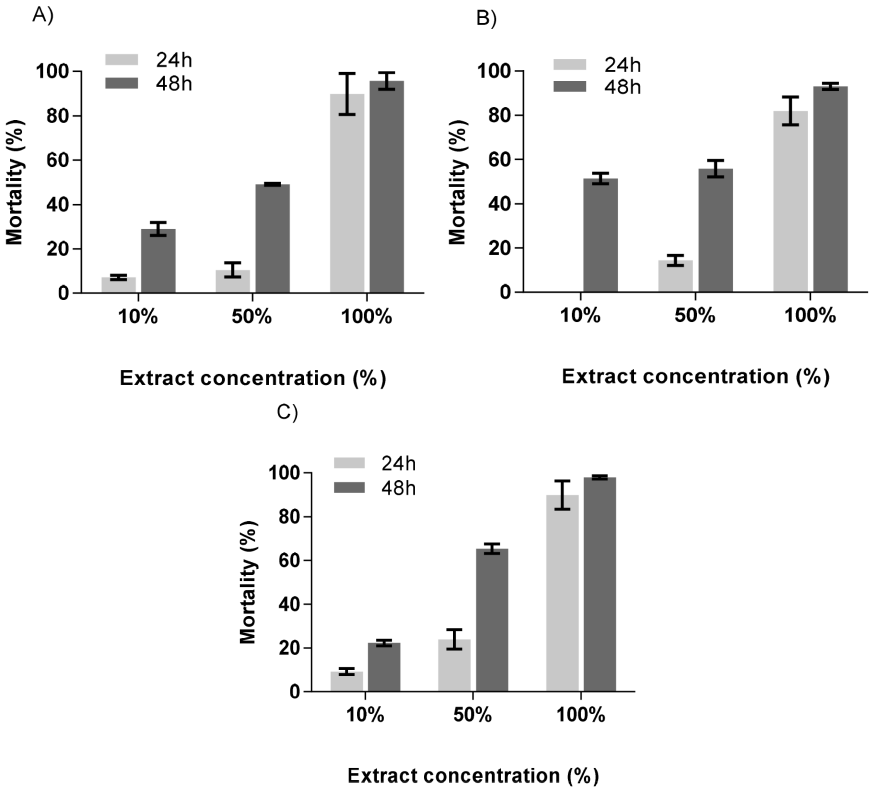

Figure 1. Mortality rates observed in *Artemia salina* test after 24 and 48h of exposure to the analyzed extracts obtained after 7 (A), 21 (B), and 35 (C) days of cultivation in medium with $NaNO_3$ content of 1.5 g/l

The observed mortality increased according to the applied extract concentration, suggesting a dose dependent toxicity. Also, a significantly higher response ($F_{1,15}$=42.67, R^2=0.699, p<0.0001) was observed in the cases where the test organisms had been exposed to the cyanobacterial extracts for a longer period of time, with the maximum larval mortality recorded after 48h. When examining the effects caused by the extracts obtained after 7 days of cultivation, it is visible that the intracellular extracts C3 (10%), C2 (50%), and C1 (100%) exerted their toxic effects after 24h, causing the 7.20%, 10.5%, and 90% mortality of the test organism, respectively (Figure 1A). Mortality rates were noticeably higher after 48h of exposure. The lowest concentration (C3) caused the mortality of 29%, the second concentration used (C2) caused the mortality of 49.1%, and the highest applied concentration (C1) caused the mortality of 95.8%.

After exposing the brine shrimp to the extracts prepared from the culture biomass developed after 21 days of cultivation (NaNO3 concentration was 1.5 g/l), the results have shown that the extracts manifested their toxic effects after 24h, as well as after 48h (Figure 1B). The highest mortality (82%) was recorded after the exposure of brine shrimp to C1 (100%) extract, while the lowest mortality was observed in the case of the lowest tested extract concentration C3 (10%), where there was no visible effect after 24h of exposure. As in the previous case, these intracellular extracts have also shown a substantial increase in larval mortality caused after prolonged exposure of the test organisms, with the highest toxicity (93%) recorded after 48h in the case of C1 (100%) extract concentration, while the lowest toxicity (51.4%) was detected in the case of C3 (10%) after 48h.

Figure 1C shows the mortality recoded after the exposure to extracts prepared from cultures which were cultivated for 35 days. All three used extract concentrations exerted their toxic effects in brine shrimp after 24h of exposure. The highest value of 89.9% was recorded for C1 (100%), and the lowest mortality of 9.25% was recorded after the test organisms were exposed to C3 (10%) extract concentration. The toxic effect increased after 48h of exposure, with 98% mortality caused by the C1 (100%) extract and 22.3% observed in the case of the C3 (10%) extract (Figure 1C).

The effects of intracellular *Microcystis PCC 7806* extracts cultivated with 0.8 g/l $NaNO_3$

When a lower concentration of $NaNO_3$ was used (0.8 g/l), in the case of extracts obtained after 7 days of cultivation (Figure 2A), the only intracellular extract concentrations that caused a toxic effect after 24h were C2 (50%) and C1 (100%), resulting in the mortality rates of 16.2% and 32,05%, respectively, while the remaining test concentration C3 (10%) had no effect. The results after 48h of exposure have shown a statistically significant increase ($F_{1,15}$=78.43, R^2=0.509, p< 0.0001) in the recorded mortality rates. The highest value of 73.7% was obtained from the sample exposed to the C1 (100%) extract concentration (Figure 2A).

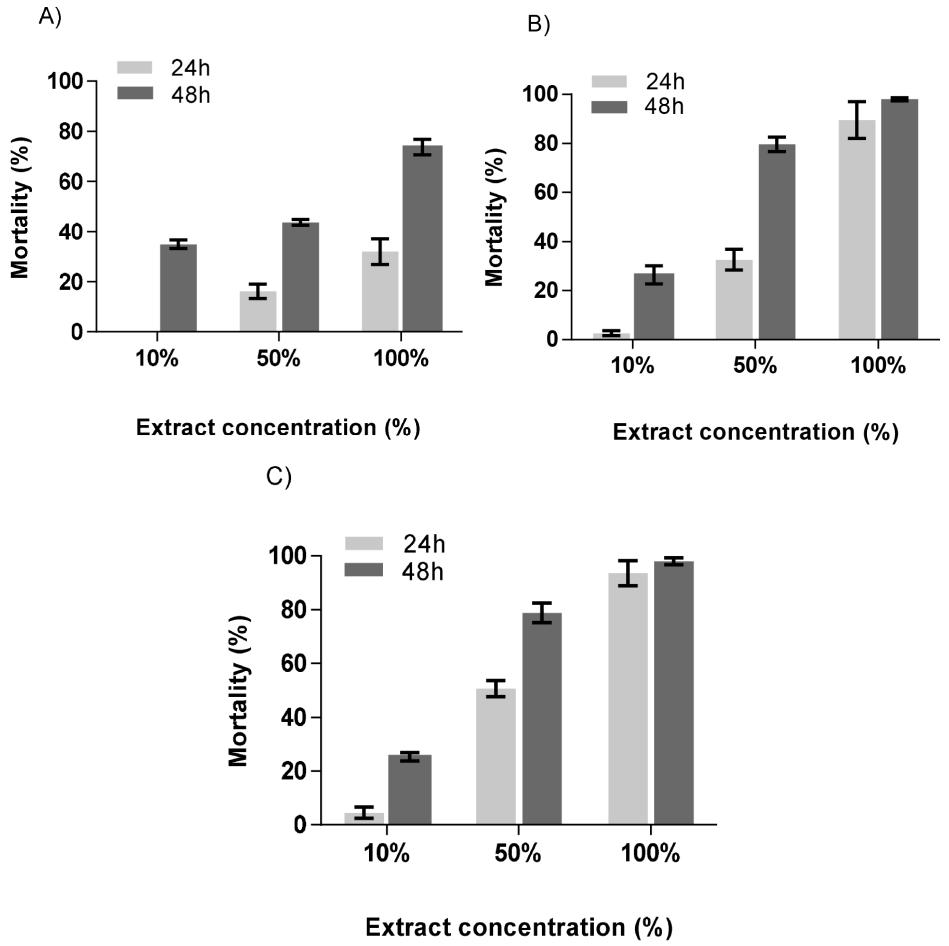

Figure 2. Mortality rates observed in *Artemia salina* test after 24 and 48h of exposure to the analyzed extracts obtained after 7 (A), 21 (B), and 35 (C) days of cultivation in medium with $NaNO_3$ content of 0.8 g/l

The values shown in Figure 2B represent the mortality rates caused by the cyanobacterial extracts obtained after 21 days of cultivation. The highest extract concentration C1 (100%) exerted its toxic effect causing the mortality of 89.5% even after 24h, and 98% after 48h. The remaining two concentrations, C2 (50%) and C3 (10%), caused larval mortality rates of 79.6% and 26.4%, respectively, after 48h of exposure. Extracts obtained after 35 days of cultivation caused high lethality of the brine shrimp (Figure 2C). The highest concentration C1 (100%) caused the mortality of 93.55% after 24h, reaching the value of 98% after 48h. The remaining concentrations C2 and C3 after 48h caused the mortality of 78.85% and 25.4%, respectively (Figure 2C).

The effects of intracellular *Microcystis PCC 7806* extracts cultivated in nitrogen-free media

The cyanobacterial extracts obtained after 7 days of cultivation in a nitrogen free medium had a slightly lesser effect on the mortality rates of the test organisms than the extracts from cultures grown in mediums with nitrate (Figure 3). Mortality rates after 48h were also significantly higher ($F_{1,15}$=130, R^2=0.667, p<0.0001) then those recorded after 24h of exposure. Apart from that, unlike the previous cases, no dose dependent response was observed when examining the results pertaining to the extracts obtained after cultivation of 7 days in the case of both time exposures. The highest mortality of 69.5% was observed after 48h in the case of C2 (50%) (Figure 3A). In the case of the extracts obtained after 21 days of cultivation, the strongest effect was found in the case of C1 (100%) and it reached the value of 89.5% after 48h, while the remaining two tested concentrations C2 (50%) and C3 (10%) caused the mortality of 46.4% and 25.2% of test organisms, respectively (Figure 3B).

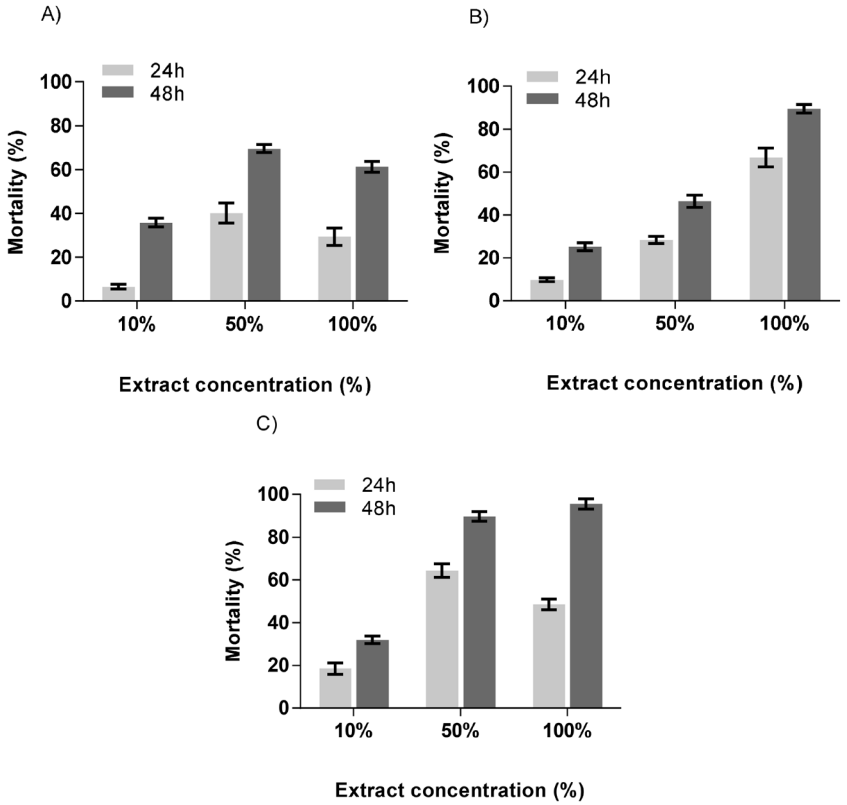

Figure 3. Mortality rates observed in *Artemia salina* test after 24 and 48h of exposure to the analyzed extracts obtained after 7 (A), 21 (B), and 35 (C) days of cultivation in the nitrogen-free medium

Figure 3C shows the results of *A. salina* bioassay where the test organisms were exposed to the extracts obtained after 35 days of culture growth. After 24h, the observed toxicity was no dose-dependent and the highest mortality (64.3%) was found in the case of C2 extract concentration. The results showed that after 48h, C1 (100%) extract had the strongest effect, causing the mortality of 95.5%, while the weakest effect was observed in the case of C3 (10%) extract with the value of 32%.

DISCUSSION

The importance of nitrogen as an environmental factor able to induce the production of certain primary and secondary metabolites in cyanobacteria has been proven and documented (Simeunović et al., 2013; Blagojević et al., 2018). However, the link between the available nitrogen and the cyanobacterial toxicity remains unclear. Several studies have shown that higher nitrate concentrations in the environment lead to an increase in growth rate and the microcystin content of toxic *M. aeruginosa* strains (Beversdorf et al., 2015; Downing et al., 2005). The results presented in this paper show that the intracellular extracts obtained from the cultures of *Microcystis PCC 7806*, grown in the presence of nitrogen, have exerted strong toxic effects, in most cases higher than those manifested when using the culture grown in a medium without nitrogen. Nevertheless, the observed difference in toxicity when using a nitrogen-free medium has not been statistically significant. Similarly, when observing the effects of nitrogen limitation on the toxin production in *Microcystis* sp. (Sevilla et al., 2010), no significant differences between the conditions of low and high nitrate concentration were detected. There are, however, reports which indicate that the cyanotoxin microcystin biosynthesis appears to be under direct control of nitrogen, increasing under nitrogen limitation (Neilan et al., 2013; Pimentel and Giani, 2014), thus warranting further investigation into the various environmental effects on the strain toxicity. Moreover, in the work of Kovač et al. (2014), cyanobacterial strains of *Anabaena* and *Nostoc* genera were highly toxic to *A. salina* larvae when cultured in the presence of nitrogen, causing up to 95% mortality, while the observed mortality rates were only as high as 10% after a nitrogen-free medium was applied.

It can also be concluded that the observed larval lethality increased gradually with the increase in applied dose, suggesting a dose dependent toxicity. By observing the toxicity of all three applied extract concentrations, obtained after 7, 21, and 35 days of cultivation, it can be deduces that the highest used concentration C1 (100%) was in fact the most potent, while the lowest concentration extract C3 (10%) generally had the weakest effect in terms of lethality of the brine shrimp. Such dose dependence was also reported in the paper published by Akin-Oriola and Lawton (2009), who used the *A. salina* bioassay to test the toxicity of *Microcystis* isolated from freshwater blooms.

By comparing the results of this analysis, a significant change in the mortality rates was observed after prolonged exposure to the analyzed extracts. Generally, the toxic effects increased during the period of exposition implying time-dependent toxicity of the tested strain. This trend can be an indication of a positive correlation between the rates of the mortality of test organisms and the length of exposure to the toxic cyanobacterial metabolites. The results also showed that the highest rate of toxic metabolite production can be attributed to the cultures grown for longer periods (21 and 35 days), which proved to have a stronger effect on the test organisms than their counterparts with shorter cultivation period (7 days). This observation is in accordance with the results of a previous study, where toxic strains of cyanobacteria have also shown to exhibit higher toxicity after longer cultivation periods (Tešanović et al., 2015). An explanation of such results may lie in the fact that the rate of secondary metabolite production, including cyanotoxins, depends on the growth phase of the culture. The maximum production rates are usually achieved when the culture enters the stationary growth phase, which happens in the case of the tested strain after 21 to 28 days of cultivation (Simeunović, 2009). Previous studies have also shown that the greatest intracellular toxin concentrations occur at maximum rates of cell division and in the late log or early stationary phase (Orr and Jones, 1998). Therefore, the potency of the extracts isolated after longer period of cultivation (21 and 35 days) can be explained by the stationary growth phase, which is characterized by the most intense secondary metabolite production.

CONCLUSION

The results of the toxicity analysis using *Artemia salina* bioassay have provided insight into the toxicity of the cyanobacterial strain *Microcystis PCC 7806* and have shown how the modulation of the growth conditions and cultivation parameters can influence the resulting toxicity of the strain. The effects of the different nitrogen concentrations have not been statistically significant for the toxicity of the tested strain. Higher nitrate concentrations in the growth medium caused only a slight increase in the observed toxicity. The toxicity differed according to the applied extract concentration and seamed to follow a dose dependent gradient with the highest lethality in most cases observed at the highest extract concentrations. Toxic effects of the cyanobacterial extracts have intensified with prolonged larval exposure, causing significantly higher mortality after 48h. These results suggest time-dependent toxicity of the tested strain. Also, the highest toxicity was recorded in the case of extracts obtained from older cultures (after 21 and 35 days of cultivation) suggesting that more intensive production of toxic compounds occurs during the stationary growth phase of the tested strain.

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УТИЦАЈ АЗОТА И ФАЗЕ РАСТА НА ТОКСИЧНОСТ ЦИЈАНОБАКТЕРИЈСКОГ СОЈА *Microcystis PCC 7806*

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РЕЗИМЕ: Учестала појава цијанобактеријског цветања широм света, као и бројни штетни ефекти који се јављају као последице овог феномена, подстакли су детаљна истраживања у циљу идентификације срединских услова који доприносе оваквим појавама. Међу основним факторима за које се сматра да подстичу убрзану пролиферацију цијанобактерија, могу се издвојити доступни нивои нутријената, чији се утицај директно може повезати с растом и продукцијом токсичних метаболита од стране цијанобактерија у слатководним екосистемима. У овом раду је анализирана токсичност соја *Microcystis PCC 7806* након узгајања у подлогама с три различите концентрације азота. Токсичност изолованих интрацелуларних екстраката је анализирана након 7, 21 и 35 дана култивације, на основу стопа морталитета у *Artemia salina* биоесеју након периода излагања од 24 и 48 сати. Резултати нису показали статистички значајно повећање морталитета тест организама након излагања дејству екстраката добијених из култура које су узгајане у присуству виших концентрација азота (1,5 g/l и 0,8 g/l), у поређењу са морталитетом који је узроковао екстракт соја гајеног у у безазотној подлози. Дозно зависан ефекат је међутим утврђен код већине експерименталних група, док су најзначајније промене у морталитету тест организама уочене у групама изложеним вишим концентрацијама екстраката. Поред тога, уочен је појачан токсичан ефекат и морталитет ларви *A. salina* током дужег периода излагања, указујући на испољену временски зависну токсичност соја. Такође, екстракти добијени након 21 и 35 дана култивације су имали већи утицај на тест организам, што указује на то да токсичност цијанобактеријског соја зависи од специфичне фазе раста.

КЉУЧНЕ РЕЧИ: *Artemia salina*, азот, цијанобактерије, *Microcystis*, токсичност

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OPTIMISATION AND APPLICATION OF POLYBROMINATED DIPHENYL ETHERS EXTRACTION METHOD

ABSTRACT: The optimal conditions for the extraction of eight polybrominated diphenyl ethers (PBDEs) were determined using 3^2 factorial design of experiments (DOE). The independent variables were coded at three levels and their actual values were selected on the basis of the preliminary experimental results. DOE consisted of nine runs with three replicates at the central point. A second-order polynomial model was used for predicting the response. After the optimization of the duration of extraction, the ratio of solvents and purification and instrumental parameters, the optimized conditions were applied on the samples taken from the surface layer of soil from 24 potentially contaminated locations (landfills, ex marshalling yard, dump of secondary raw materials and automotive waste). The gas chromatographic analysis with electron capture detection (GC-ECD) was used for the determination of PBDEs. The recovery values of BDE congeners at five concentration levels ranged between 80% and 115%. The total PBDEs concentrations ranged from 4.4 to 729 μg kg^{-1} of absolutely dry soil. The suitability of the experiment was proved by comparing the experimental and predicted values of the variable parameters.

KEYWORDS: Factorial design, GC-ECD, Landfills, PBDEs, Soil pollution

INTRODUCTION

The polybrominated diphenyl ethers (PBDEs) are new persistent organic pollutants (POPs). Three commercial technical mixtures of PBDEs, penta-BDE, octa-BDE and deca-BDE have been marketed under different trade names. They are composed of a mixture of congeners and used as a flame-retardants in the consumer goods such as electrical equipment, plastics, construction

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materials, coatings, curtains, textiles, automotive equipment and polyurethane foam (furniture padding). POPs which exist in the environment, bio accumulate through the food chain and pose a risk of causing adverse effects to human health and the environment. These chemicals are among the most toxic chemicals ever synthesized. There is evidence that they cause cancer, the damage to the central and peripheral nervous systems, diseases of the immune system, reproductive function disorders, functional disorders of the endocrine system and most importantly they negatively affect the development of the nervous system.

The improper management of these chemicals during the lifetime of the products which contain these substances, especially in the field of waste management, can lead to the emissions of POPs and their accumulation in the environment, humans and animals.

The poor, outdated and illegal waste management practice in urban environment and hazardous waste disposal still affect some local communities in the industrialized countries and represent an increasing problem in middle-low income countries. There are huge consequences of illegal cross-border trade of hazardous waste from the developed industrial countries with a special emphasis on electronic waste. The electrical and electronics waste, furniture, auto shredder residues etc. are potential sources of PBDEs in soil. The first steps in dealing with these POPs are the establishment of regular monitoring, determining sources and presenting their current state in the environment.

Because of all the aforementioned, the aim of this paper was to find the optimal conditions for the extraction of eight polybrominated diphenyl ethers (PBDEs) and to apply them on the soil samples collected near landfills, ex marshalling yards, dumps of secondary raw materials and automotive waste in order to determine the presence of PBDE in the potentially contaminated areas.

MATERIALS AND METHODS

Study area, sampling and preparation

Vojvodina is located in the northern part of Serbia. According to the census of the 2011 an area of 21.506 km^2 was populated by about 2 million inhabitants. A total number of 44 urban landfills and approximately 700 illegal dumps have been identified in the territory of Vojvodina. As it has been mentioned earlier, at almost all the landfills in Serbia, there is no systematically organized separate collection of waste, sorting and recycling. Thus, the presence of hazardous materials on the landfills is to be expected.

In order to investigate the presence of the PBDE congeners, twenty-four samples of surface soil were collected from five regions of Vojvodina (Figure 1). The samples were taken according to the template method. They were transported to the lab in portable refrigerator, where they were deposited in the freezer at the temperature of -20 °C until analysis.

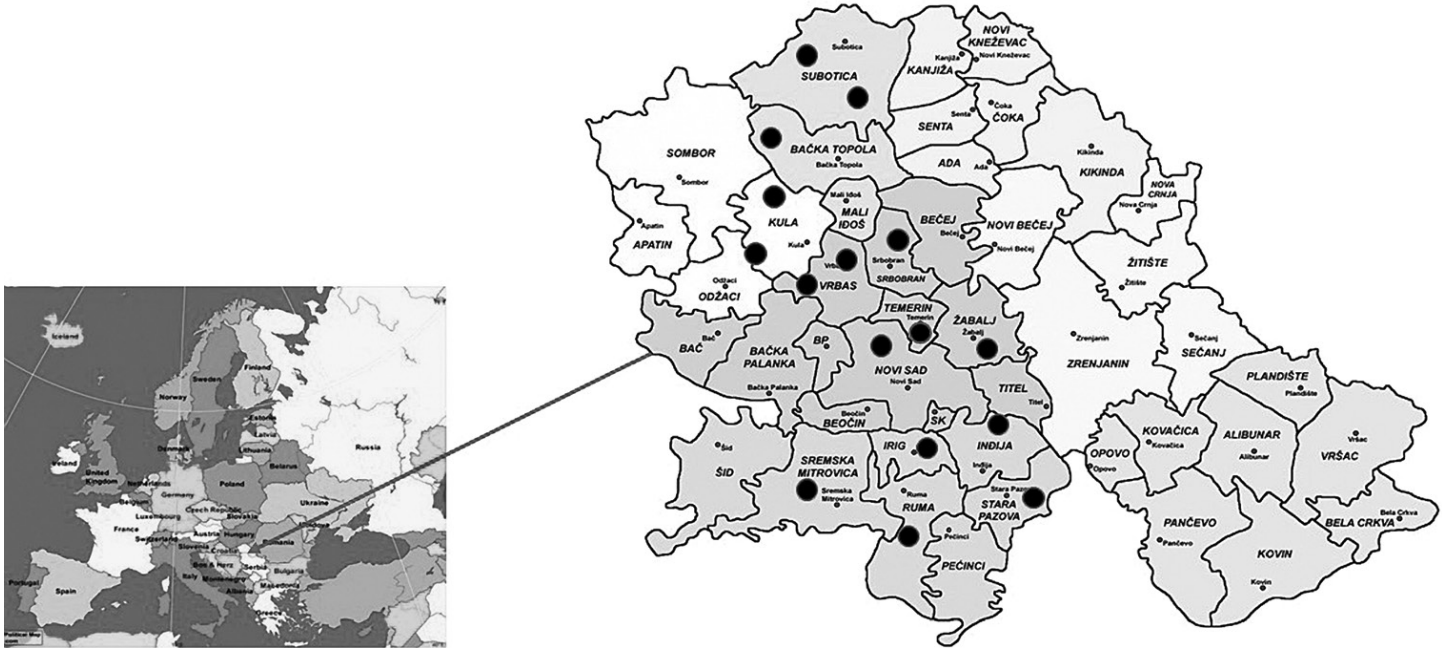

Figure 1. Map of the sampling locations

Chemical reagents and standard solutions

Hexane and dichloromethane for UV, IR, HPLC, ACS were purchased from Panreac (AppliChem, Germany). Silica gel 60 (Davisil grade 636, 35–60 mesh) and alumina (activated, neutral, Brockmann I, 58 Å) from Sigma Aldrich and SPE columns (54222-U) from Supelco were used for the extract purification. Silica gel was activated at 190 °C for 24 h. Acid silica gel was prepared by combining the concentrated sulphuric acid (30 ml) with activated neutral silica gel (50 g). PBDE standard solution (8 congeners of primary interest) was purchased from Accu Standard (USA).

Extraction, cleanup and GC analysis

For the recovery testing, ten grams of blank soil matrix were spiked with 50 µl PBDE standard solution with the concentration of 2 µg ml^{-1}. USEPA Soxhlet extraction method was used for the extraction of PBDEs from the spiked soil. In order to determine the best ratio of solvents and optimum extraction time, nine extraction experiments were conducted using three different solvent ratios (hexane/dichloromethane 1:1, 2:1 and 3:1) and three different extraction periods (12, 18, 24 h). Each experiment was repeated twice.

After the completion of the extraction, the volume of the extract was reduced to 2 ml by rotary evaporation in the presence of the steam of nitrogen at the temperature of 45 °C. Purification was carried out by two columns using the procedure explained in the literature. The first column was loaded with acidic silica gel and the second one (multi-layered) contained activated aluminium oxide, neutral silica gel and acidic silica gel. Both columns were first washed with hexane. After the application of the extract, the elution was done in two steps, with hexane and hexane/dichloromethane (1:1) respectively. The total eluate was concentrated to 1 ml using a rotary evaporator at the temperature of 45 °C. The final eluate was transferred to the vial.

The determination of PBDEs was carried out with gas chromatography with the electron capture detector (Agilent 7890B) equipped with a capillary column DB5 (30 m x 0.32 mm i.d., 0.25 µm, Agilent J&W GC Columns). The working conditions of the GC system were set as follows: injector at 300 °C, detector at 310 °C, initial oven temperature at 90 °C, for 2.0 min, heating to 220 °C at the rate 50 °C min^{-1}, then to 300 °C at the rate 5 °C min^{-1}, with the final 20 min hold. Splitless injection with the flow of 60 ml min^{-1} was used. Chromatogram obtained under these conditions is shown in Figure 2.

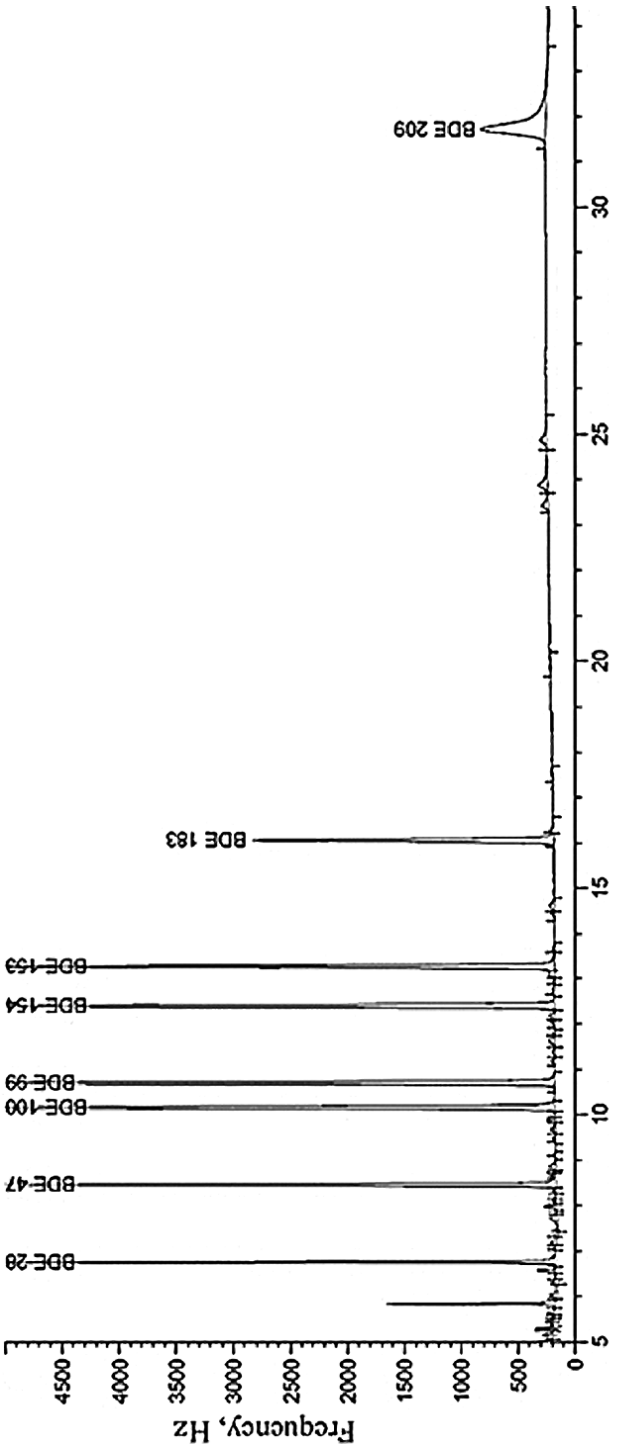

Figure 2. GC-ECD chromatogram of spiked blank soil matrix with mix of eight PBDE congeners

Experiment design

The obtained average recovery values for individual BDE congeners have been used for the examination of the influence of the extraction time and hexane/dichloromethane volume ratio by 3^2 factorial design of the experiments (DOE). The factorial DOE is a methodology used for the systematic evaluation of the influence of different factors (variables) on the studied system. At the same time, the remaining factors which might influence the system are kept as constant as possible. In this paper, a three-level factorial design with two factors was used in order to examine the influence of the extraction time and hexane/dichloromethane volume ratio on the recovery value. The number of the experiments was determined using the formula $n = 3^2$. Therefore, nine experiments for the factorial design with two factors, using 3^2 factor design are needed. During this study two replicates of the experiments were carried out. The schematic table of this factorial design is shown in Table 1.

Table 1. Schematic table of 3^2 factorial design

Experiment No.	Factors		Variables	
	X_1	X_2	Hexan:DCM, v/v	Time, h
1	-1	-1	1:1	12
2	0	-1	2:1	12
3	1	-1	3:1	12
4	-1	0	1:1	18
5	0	0	2:1	18
6	1	0	3:1	18
7	-1	1	1:1	24
8	0	1	2:1	24
9	1	1	3:1	24

RESULTS AND DISCUSSION

Analysis of a factorial design

As previously stated, the influence of the analysed factors on the PBDEs extraction efficiency (Table 2) was performed using 3^2 factorial design. Based on the literature data, it was decided that the factors of interest can be changed at the following intervals:

- Volume ratio of Hexane / DCM 1:1, 2:1 and 3:1
- Extraction time: 12 h, 18 h and 24 h

Table 2. The average of recovery values of eight BDE congeners

BDE congener	28	47	100	99	154	153	183	209
Recovery average, %	80.36	86.14	85.90	92.04	93.37	102.93	115.12	103.59

The modelling function used to examine the impact of these two variables on the recovery value was:

$$Y = b_0 + b_1 \bullet X_1 + b_2 \bullet X_2 + b_{12} \bullet X_1 \bullet X_2 \qquad \text{(Equation 1)}$$

In this equation X_1 and X_2 represent the coded variables (Table 1) for two factors (columns two and three in Table 1), b_0 represents the intercept from the axes of the dependent variable (in our case recovery, labelled as Y), b_1 represents the influence of the solvent ratio on the recovery, while the coefficient b_{12} represents the interaction between two factors.

The experiments were repeated twice. As previously stated, the volume ratio of the solvents (X_1) and time of extraction (X_2) were replaced with coded values from Table 1. Estimated coefficient values for eight congeners are shown in Table 3.

Table 3. Calculated effects for the 3^2 factorial design

	BDE 28	BDE 47	BDE 100	BDE 99	BDE 154	BDE 153	BDE 183	BDE 209
b_0 – main efects	78.47	88.15	86.62	91.36	92.13	95.54	106.2	82.96
b_1 – solvents ratio	-3.19	-8.39	-6.10	-5.75	-4.88	-4.72	-3.6	-10.12
b_2 – time	-1.58	-8.075	-4.95	-4.9	-2.9	-4.54	-7.28	-8.65
b_{12} – interaction	5.83	6.06	6.26	5.03	4.56	4.92	6.32	-12.51

Together with the estimated parameters of the model (b_0, b_1, b_2 and b_{12}) the confidence interval (at the level of significance of α=0.05) for each of these parameters is presented in Table 3. The confidence interval is important so as to recognize which of the parameters of the model have a significant influence on the recovery value. A detailed analysis of the estimated values for the main effects showed that the coefficients b_1 and b_2 have a significant influence on the results for most of the congeners. Both factors (X_1 and X_2) have a negative impact on the extraction efficiency. That means that a larger quantity of hexane in a mixture of solvents and a longer duration of the extraction reduce the recovery values of the reaction. Therefore, the ratio of the solvents of 1:1 and the extraction time of 12 hours were the best conditions for the extraction of PBDEs from soil.

PBDEs content

The optimized extraction conditions were applied to 24 soil samples. In all the samples the presence of PBDEs was confirmed and the total amount for

eight congeners varied between 4.4 and 723 μg kg^{-1} with a median value of 11.48 μg kg^{-1}. The concentrations in samples taken from the landfills ranged from 4.4 to 67.4 μg kg^{-1} with a median value of 7.97 μg kg^{-1}. The sample with the largest value of total PBDEs was the sample taken at the landfill receiving zone (500 m far from the residential area in Novi Sad). The highest values of PBDEs concentrations were detected in a sample taken at the outer edge of the land plot used for purchasing and storing of the recyclable waste materials. The company that owns the mentioned land parcel deals with purchasing of iron, car batteries, aluminium, stainless steel, brass, copper and electronic waste. The total content of PBDEs in sample 25, taken from mentioned location, was 723 μg kg^{-1}. The high concentration of the total PBDEs was also measured in a sample taken from ex marshalling yard located in a residential part of Novi Sad. Today this location is used as a repair yard for trains. Two samples were taken from this location. Sample 4 was taken close to the railway line where old unusable rolling stocks (manufactured in the 1970s) have been parked since 2006. The railway wagons, which were in vicinity of the second sample (sample 5), were placed there recently, in 2012.

Highest contributions to total sum of PBDEs in collected samples were those of BDE 209, BDE 47 and BDE 99. The concentrations of BDE 209 were in the range from 1.65 to 65.8 μg kg^{-1} of absolutely dry soil (a.d.s.) in soil samples from the landfills. It is less than the concentrations detected near decabromodiphenyl ether manufacturing factory in China, but in the range detected near a typical e-waste recycling site in South China. BDE 209 is always used in conjunction with antimony trioxide in polymers, mainly in high impact polystyrene (HIPS) which is used in the television industry for cabinet backs. BDE 209 is also used for polypropylene drapery and upholstery fabric by means of back coating and may also be used in some synthetic carpets. The high value of BDE 209 from 160.9 μg kg^{-1} was detected in a sample from secondary raw materials dump. According to Illinois Environmental Protection Agency, BDE 209 is bio accumulating in the environment, and the levels are increasing in some types of the samples (sediments, some top predators, and possibly human blood and breast milk). The most important health effects from the exposure to BDE 209 and/or lower-brominated congeners appear to be on the liver, kidney, thyroid gland, reproductive/developmental and neurological effects.

CONCLUSION

As a result of this study, the conditions for the extraction of PBDEs from the soil have been optimized. It was concluded that a larger quantity of hexane in the mixture with dichloromethane and the longer duration of extraction reduce the extraction efficiency. The information obtained during this part of the study was used as a guide to find the optimal conditions for performing the rest of the experimental procedure.

Optimized conditions have been applied to the 24 soil samples, collected from landfills, ex marshalling yard, dump of the secondary raw materials and automotive waste, in which the presence of eight BDE congeners was confirmed. Several potential sources of PBDE were detected. Mostly, they are the result of the bad practice of waste management. The measured PBDEs concentrations indicate the need to establish regular monitoring of those substances in the soil and map the potentially contaminated areas.

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ОПТИМИЗАЦИЈА И ПРИМЕНА ЕКСТРАКЦИОНЕ МЕТОДЕ ПОЛИБРОМОВАНИХ ДИФЕНИЛ ЕТАРА

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РЕЗИМЕ: Оптимални услови за екстракцију полибромованих дифенил етара (PBDE) одређени су коришћењем 3^2 факторског дизајна експеримента (DOE). Независно промењљиве величине биле су кодиране на три нивоа и њихове стварне вредности су одабране на основу прелиминарних експерименталних резултата. DOE се састојао од девет експеримената са по три понављања у централној тачки. Полиномни модел другог реда коришћен је за предвиђање одговора. Након оптимизације време трајања екстракције, односа растварача, начина пречишћавања и инструменталних параметара примењени су оптимизирани услови на узорцима узоркованих из површинског слоја земљишта са 24 потенцијално контаминираних локација (у близини депонија, бивше ранжирне станице, депонија секундарних сировина и ауто-отпада). За одређивање PBDE је коришћена гасна хроматографија с детектором са захватом електрона (GC-ECD). Вредности приноса BDE конгенера на пет нивоа концентрације кретале су се између 80% и 115%. Укупна концентрација PBDE била је у опсегу од 4,4 до 729 mg kg^{-1} апсолутно сувог земљишта. Валидност експеримента доказана је поређењем експерименталних и предвиђених вредности променљивих параметара.

КЉУЧНЕ РЕЧИ: GC-ECD, депоније, загађење земљишта, PBDE, факторски дизајн

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ORGANOCHLORINE PESTICIDES IN THE TISZA RIVER (SERBIA): DISTRIBUTION AND RISK ASSESSMENT

ABSTRACT: Paper provided the systematic data on the distribution and risk assessment status of organochlorine pesticides (OCPs) in sediment of the Tisza River (Serbia). The α-HCH, endrin ketone and methoxychlor are the most commonly found OCPs compounds. According to Serbian regulation concentrations of dieldrin, α-HCH, β-HCH and heptachlor were below limit values. In the Tisza River, sediment samples concentrations of aldrin, endrin, γ-HCH, endosulfans, heptachlor epoxide, p,p'-DDD, p,p'-DDE, p,p'-DDT were above limit values but below maximum concentration. Adverse effects are expected occasionally and slight potential health risks may exist to organisms based on the sediment quality guidelines. Upon exposure to organochlorine pesticides through non-dietary routes, results reported no potential cancer risk. The highest risk of cancer was through ingestion of contaminated sediments and minimal through inhalation routes.

KEYWORDS: chronic daily intake assessment model, distribution, incremental life time cancer risk, organochlorine pesticides, sediment

INTRODUCTION

Organochlorine pesticides (OCPs) are a large group of structurally different compounds. There is still an indication of excessive use of pesticides, regardless of the worldwide attempts to exclude or decrease releases of the pesticides (Kuranchie-Mensah et al., 2012).

The Tisza River (longest tributary of the Danube River) is facing a serious increase in the concentration of pesticides due to agricultural activities. The

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countries in the Tisza Basin (Ukraine, Romania, Slovakia, Hungary and Serbia) have taken great efforts to adopt, adjust and implement the EU Directives in support of implementing of measures to reduce the pressures from agricultural activities on water resources (ICPDR, 2007). The main initiatives are grouped around the Directive 2009/128/EC to establishing a framework for Community action to achieve the sustainable use of pesticides – by reducing the risks and impacts of pesticide on human health and the environment.

Valuing human health risk that may arise from exposure to pesticides is imperious. Some studies include estimates of the potential risk to human health from the consumption of pesticide contaminated food (dietary intake) (Jiang et al., 2005; Ezemonye et al., 2015). However, there is the need to estimate the risk to human health through non dietary exposures. These risks are predicted by chronic daily intake assessment model (CDI) (Huang et al., 2014; Ogbeide et al., 2016) and the incremental life time cancer risk (ILCR) (Qu et al., 2015; Ogbeide et al., 2016).

In this study, 20 compounds were analyzed in sediment samples collected from Serbian part of the Tisza River: α-HCH, β-HCH, γ-HCH, δ-HCH, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone, α-chlordane, γ-chlordane, endosulfan I, endosulfan II, endosulfan sulphate, DDT, DDD, DDE and methoxychlor.

The main objective of this study is to analyze distribution of the OCPs in sediments of the Tisza River, and to evaluate the risk assessment of OCPs in study area which has not been done so far.

MATERIALS AND METHODS

Study area

This study was carried out in the Tisza River (Serbia). The current investigations include four sediment profiles T, B, S and M. The sampling sites are presented in Figure 1.

Sediment samples were collected by Eijkelkamp core sampler from each sample point, according to standard method ISO 5667-12:1995. Core sediment samples, 80 cm long, were sectioned at 20 cm intervals (T1, B1, S1, M1 = 0–20 cm; T2, B2, S2, M2 = 20–40 cm; T3, B3, S3, M3 = 40–60 cm; T4, B4, S4, M4 = 60–80 cm). The standard USEPA Soxhlet extraction method (3540S) was used for OCP extraction from sediments. Analysis of sediment extracts was done with gas chromatography with electron capture detector (Agilent 7890B) equipped with capillary column HP-5 (Agilent J&W GC Columns, 30 m x 0.30 mm x 0.25 μm).

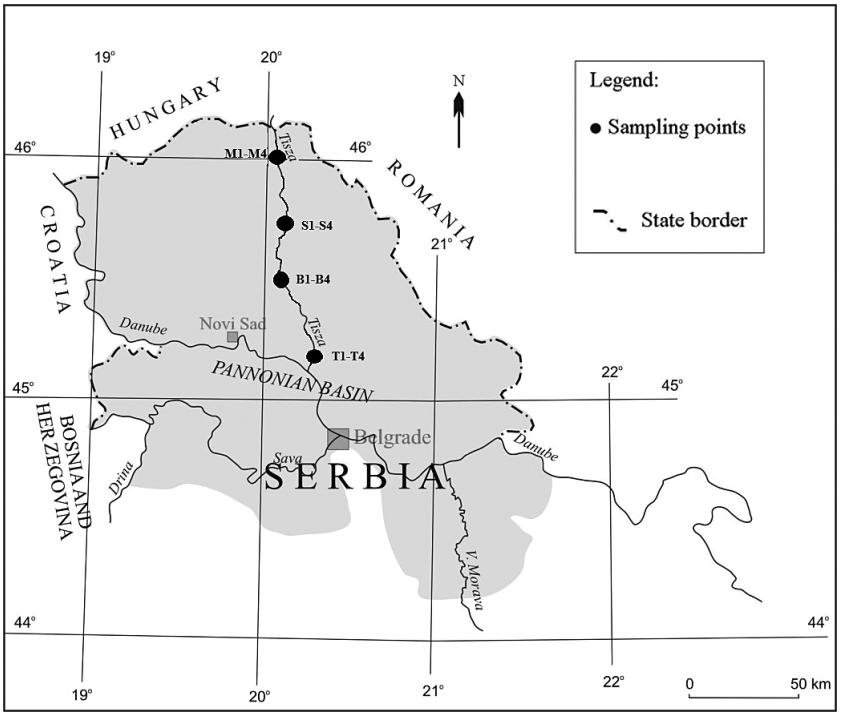

Figure 1. Study area

Risk assessment models

To determine whether the OCPs concentrations in the sediment samples from the Tisza River exceed the permitted values, the concentrations of OCPs were compared to Serbian regulation (*Off. Gazette of RS*, No. 50/12).

Sediment quality guidelines (Long and MacDonald 1998) were established to evaluate the ecotoxicology aspect of sediment contamination. Threshold effects level (TEL) and probable effects level (PEL) have been used to predict biological effects for sediments. Apart from that, effects range-low (ERL) and effects range-median (ERM) values have been used to predict potential impacts of contaminants in sediments (Yang et al., 2010). The non-carcinogenic and carcinogenic risk of detected pesticides to adults and children were calculated using a chronic daily intake (CDI) and the incremental life time cancer risk (ILCR) model. CDI are assessed for ingestion, inhalation and dermal contact (Ogbeide et al., 2016). The CDI was assessed using the following formulae (Ogbeide et al., 2016) (Equations 1–3).

$$CDI_{ingestion}=C_{(sediment)}\times IR_{(sediment)}\times CF\times EF\times ED/BW\times AT \quad (1)$$

$$CDI_{inhalation}=C_{(sediment)}\times (1/PEF)\times IAR\times EF\times ED/BW\times AT \quad (2)$$

$$CDI_{dermal}=C_{(sediment)}\times SA\times CF\times EF\times ED\times ABS\times AF/BW\times AT \quad (3)$$

ILCR for dermal, inhalation and ingestion pathways was calculated using the following equations (Ogbeide et al., 2016) (Equations 4–6).

$$ILCRs_{ingestion}=CS\times(CSF_{ingestion}\times\sqrt[3]{(BW/70)})\times IR_{sed}\times ED\times EF/BW\times AT\times CF \quad (4)$$

$$ILCRs_{dermal}=CS\times(CSF_{dermal}\times\sqrt[3]{(BW/70)})\times SA\times FE\times ABS\times AF\times EF\times ED/BW\times AT\times CF \quad (5)$$

$$ILCRs_{inhalation}=CS\times CSF_{inhalation}\times\sqrt[3]{(BW/70)})\times IRair\times ED\times EF/BW\times AT\times CF \quad (6)$$

The non-cancer and cancer risk was estimated for two age groups: childhood (0–10 y) and adulthood (19–70 y), since several exposure parameters, such as body weight, ingestion rate and inhalation rate, changed with age growth. The values used to derive a CDI and the ILCR and details of exposure parameters are presented in Table 1.

Table 1. Values of the parameters for the estimation of a chronic daily intake (CDI) and the incremental life time cancer risk (ILCR)

Exposure parameters	Unit	Childhood	Adulthood
Body weight (BW)	kg	13.95	58.75
Ingestion rate (IRsoil)	mg d^{-1}	200	100
Exposure frequency (EF)	d yr^{-1}	350	350
Exposure duration (ED)	yr	6	30
Average life span (AT)	d	LT × 365	LT × 365
Lifetime (LT)	yr	72	72
Surface area (SA)	cm^2 d^{-1}	2800	5700
Dermal exposure ratio (FE)	Unitless	0.61	0.61
Dermal surface factor (AF)	mg cm^{-1}	0.2	0.07
Dermal absorption factor (ABS)	Unitless	0.13	0.13
Inhalation rate (IRair)	m^3 d^{-1}	10.9	17.5
Particle emission factor (PET)	m^3 kg^{-1}	1.36 × 109	1.36 × 109

The carcinogenic slope factor of OCPs through ingestion, dermal contact and inhalation are listed in Table 2 (Qu et al., 2015).

Table 2. The carcinogenic slope factor (1/(mg/kg/d)) of OCPs through ingestion, dermal contact and inhalation

Compound	CSFingestion	CSFdermal	CSFinhalation
α-HCH	6.30E+00	4.49E+00	6.30E+00
β-HCH	1.80E+00	1.98E+00	1.86E+00
γ-HCH	1.30E+00	1.34E+00	1.80E+00
p,p′-DDE	3.40E–01	4.86E–01	NA*
p,p′-DDD	2.40E–01	3.43E–01	NA*
p,p′-DDT	3.40E–01	4.86E–01	3.40E–01

*NA indicated that a value was not available

The total risks were assessed as the totality of individual risk for the three exposure pathways in different age groups.

The contaminant level would exert an adverse human health effect or carcinogenic effect when CDI for a contaminant is more than the reference dose (RfD) (Huang et al., 2014; Ogbeide et al., 2016). The ILCR between 10^{-6} and 10^{-4} represents potential risk, while an ILCR larger than 10^{-4} indicates potentially high health risk (Chen and Liao, 2006; Ogbeide et al., 2016). An ILCR of 10^{-6} or less represents virtual safety (Chen and Liao, 2006; Huang et al., 2014; Ogbeide et al., 2016).

Statistical analysis

Principal component analysis (PCA) uses a correlation matrix to define a smaller set of computed values that reflect underlying shared variance in variables present in the original dataset (Wu et al., 2013). For PCA analysis Minitab 12 statistical software was performed.

RESULTS AND DISCUSSION

The α-HCH, endrin ketone and methoxychlor are the most commonly found OCPs compounds (maximal values contributed with 60%). Gamma-chlordane, DDT, and endrin aldehid contributed with 29 %. Concentrations of other OCPs such as β-HCH, γ-HCH, δ-HCH, DDD, DDE, heptachlor, drin, α-chlordane, and endosulfan contributed with 11 %, respectively. The highest level of ΣOCPs was found at site Martonoš (M1-4), while the lowest concentrations were found at site Senta (S1-4). The highest mean value of ΣOCPs concentrations was determined at a depth of 20–40 cm.

The ΣOCPs concentrations in the Tisza River sediments (11.6–21.34 μg kg^{-1}) were lower to those detected in Daya Bay, China (16.66–44.04 μg kg^{-1} Wang et al., 2008), similar to those detected in Honghu lake, China (17.88 μg kg^{-1} Yuan et al., 2013). Higher than those detected in Ariake bay, Japan (4.1 μg kg^{-1} Kim et al., 2007), Minjiang River Estuary, China (1.57–13.06 μg kg^{-1} Zhang et al., 2009), Da-han and Erh-jen River (0.57–14.1 μg kg^{-1} Doong et al., 2002).

Ration of α/γ HCH ranged from 1.50 to 5.49, indicating input of technical HCHs (Zhang et al., 2009; Yuan et al., 2013). HCH residues were resulting mainly from the historical inputs of technical HCHs (the ration of β-HCH/ΣHCHs ranged from 0.06 to 0.31 which was below 0.50) (Wu et al., 2013). Low levels of dieldrin show slow rate of aldrin degradation. Decrease levels of endrin in relation to its degradation products provide indication of the historical input of the OCPs (Kuranchie-Mensah et al., 2012). Average γ/α-chlordane was 5.15, indicating recent input of technical chlordane in the Tisza River sediment (Zhang et al., 2009; Yuan et al., 2013). Rations DDD/DDE was in the range 0.56–2.17, with 69% of the values greater than 1, indicating anaerobic biodegraded condition (Wu et al., 2013). Sediment contamination by DDTs in the

Tisza River came from the historical and recent input of DDT (rations (DDD+ DDE)/DDTs in 50 % of samples were greater than 0.5 and 50% of samples were smaller than 0.5). Ratio p,p'-DDT/p,p'-DDE was 0.17–28.14, respectively, also indicating fresh and old input of p,p'-DDT (Wu et al., 2013). Anaerobic biodegraded condition contributes to slower degradation of the parent compound. For this reason, the results indicate recent input of technical chlordane and fresh input of p,p'-DDT.

According to Serbian regulation concentrations of dieldrin, α-HCH, β-HCH and heptachlor in the Tisza River sediment samples were below limit values. On the other hand, concentrations of aldrin, endrin, γ-HCH, endosulfans, heptachlor epoxide, p,p'-DDD, p,p'-DDE and p,p'-DDT in the Tisza River sediment samples were above limit values but below maximum concentration. Therefore, the OCPs levels were categorized as moderate contaminations.

After performing PCA on the datasets, two principal components presented in Figure 2 account for 53.1% of the total variance.

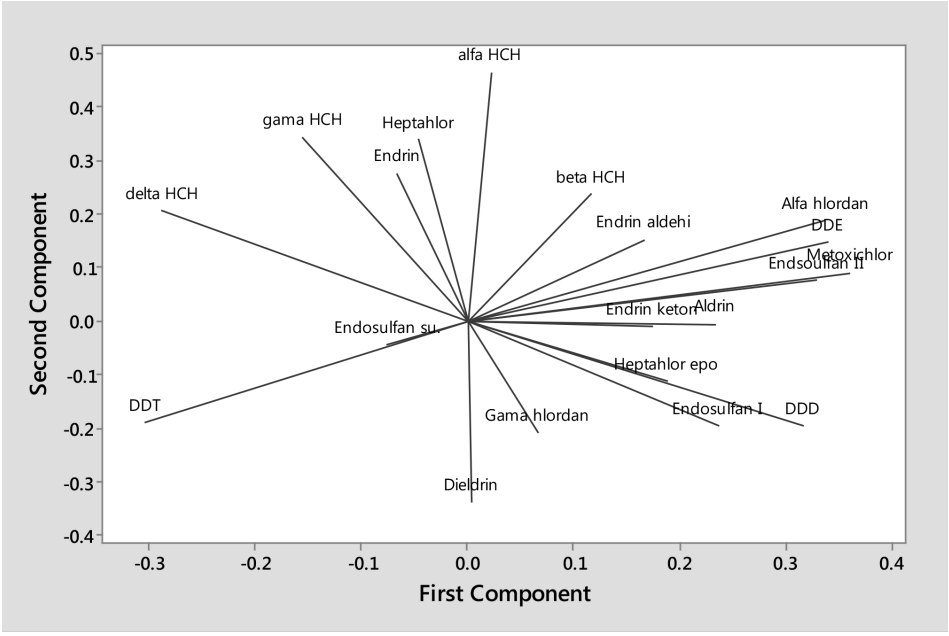

Figure 2. PCA plot of pesticide residues in sediment samples

Positive loadings of pesticides residues such as α-HCH, β-HCH, endrine aldehid, α-chlordane, endosulfan I, endosulfan II, aldrin, endrin ketone and γ-chlordane p,p'-DDD, p,p'-DDE, metoxichlor (Figure 2) imply that those pesticides undergo similar degradation and distribution patterns in sediment samples from the upper and lower reaches of the Tisza River.

Pesticides used many years ago such as DDT and γ-HCH are present in the sediment of the middle course of the river. The obtained results are expected

considering that agriculture in this region has been present for at least hundred years. Analyzing the results of PCA there can be determined the impact of agriculture on the presence of pesticides in the Tisza River sediments.

Adverse effects are expected occasionally and slight potential health risks may exist to organisms. Only concentrations of all HCH isomers were higher than PEL values (Table 3).

Table 3. Evaluations of potential ecotoxicological risks of selected OCPs in surface sediments

	Range	ERL	ERM	%< ERL	%ERL-ERM	%> ERM	TEL	PEL	%<TEL	%TEL-PEL	%>PEL
a-HCH	1.38–3.76	N.A.	N.A	–	–	–	0.94	1.38	–	–	100
β-HCH	0.26–1.51	N.A.	N.A	–	–	–	0.94	1.38	68.75	18.75	12.5
γ-HCH	0.34–2.22	N.A.	N.A	–	–	–	0.94	1.38	62.5	18.75	18.75
p,p' – DDT	0.17–1.99	1	7	50	50	–	1.19	4.77	50	50	–
p,p' – DDD	0.15–1.62	2	20	100	–	–	1.22	7.81	81.25	18.75	
p,p' – DDE	0.07–1.11	2.2	27	100	–	–	2.07	374.17	100	–	–
ΣDDTs	1.38–3.55	1.58	46.1	6.25	93.75	–	3.87	51.7	100	–	–
Aldrin	0.10–0.96	N.A	N.A	–	–	–	2	N.A.	100	–	–
Heptachlor	0.08–0.96	0.50	6	62.5	37.5	–	2.26	4.79	100	–	–
Heptachlor epoxide	0.04–0.99	N.A.	N.A.	–	–	–	0.6	2.74	93.75	6.25	–
Endrin	0.03–0.28	0.02	45	–	100	–	2.67	62.4	100	–	–

Results obtained from this study reported no potential cancer risk to humans upon exposure to organochlorine pesticides through non-dietary routes (Table 4).

Table 4. Estimated chronic daily intake (CDI) compared with reference dose for OCPs

	Ingestion		Dermal		Inhalation		Reference dose
	Children	Adults	Children	Adults	Children	Adults	
α-HCH	3.53E-05	5.24E-06	6.44E-06	3.14E-06	6.79E-09	5.18E-08	8.00E-03
β-HCH	1.13E-05	1.67E-06	2.06E-06	1.00E-06	2.17E-09	1.65E-08	8.00E-03
γ -HCH	1.23E-05	1.83E-06	2.25E-06	1.10E-06	2.37E-09	1.81E-08	3.00E-04
ΣDDT	3.25E-05	4.82E-06	5.93E-06	2.89E-06	6.24E-09	4.76E-08	5.00E-04

ILCRs estimates for α-HCH, γ-HCH, β-HCH DDE, DDD and DDT, where higher in children compared to adults when exposed via ingestion, dermally and through inhalation. Cancer risk was highest through ingestion of contaminated sediments and minimal when exposure routes were through inhalation (Table 5).

Table 5. Estimated incremental lifetime cancer risk (ILCRs) for OCPs

	Ingestion		Dermal		Inhalation	
	Children	Adults	Children	Adults	Children	Adults
α-HCH	1.32E-05	4.77E-06	2.04E-05	7.00E-06	5.17E-09	3.99E-09
β-HCH	1.20E-06	4.35E-07	2.88E-06	9.86E-07	4.88E-10	3.77E-10
γ -HCH	9.51E-07	3.44E-07	2.13E-06	7.30E-07	5.16E-10	3.99E-10
DDE	1.71E-07	6.19E-08	5.32E-07	1.82E-07	1.24E-09	9.61E-10
DDD	1.38E-07	5.01E-08	4.30E-07	1.47E-07	1.43E-09	1.10E-09
DDT	2.65E-06	9.60E-07	7.51E-06	2.83E-07	1.04E-10	8.04E-11

CONCLUSIONS

The α-HCH, endrin ketone and methoxychlor are the most commonly found OCPs compounds in the Tisza River sediments, Serbia. Ration of α/γ HCH indicates input of technical HCHs. The ration of β-HCH/ΣHCHs suggests that the HCHs were resulting mainly from the historical inputs of technical HCHs. Also, decrease levels of endrin indicate the historical input of the OCPs. Ration of γ/α-chlordane indicates recent input of technical chlordane in the Tisza River sediments. Sediments contamination by DDTs in the Tisza River came from historical and recent DDT input. Concentrations of dieldrin, α-HCH, β-HCH and heptachlor were below limit values. On the other hand, concentrations of aldrin, endrin, γ-HCH, endosulfans, heptachlor epoxide, p,p'-DDD, p,p'-DDE, p,p'-DDT in Tisza River sediment samples were above limit values but below maximum concentration. The OCPs levels are categorized as moderate contaminations. Adverse effects are expected occasionally and slight potential health risks may exist to organisms. Results obtained from this study reported no potential cancer risk to humans upon exposure to organochlorine pesticides through non-dietary routes. Cancer risk was highest through ingestion of contaminated sediments and minimal when exposure routes were through inhalation.

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ПЕСТИЦИДИ У РЕЦИ ТИСИ (СРБИЈА): ДИСТРИБУЦИЈА И ПРОЦЕНА РИЗИКА

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РЕЗИМЕ: У раду су дати системски подаци о статусу расподеле и процени ризика органохлорних пестицида у седименту реке Тисе (Србија). Најзаступљенији органохлорни пестициди у седименту су α-HCH, ендрин кетон и метоксихлор. Према националној регулативи концентрације диелдрина, α-HCH, β-HCH и хептахлора биле су испод граничних вредности. У узорцима седимента реке Тисе концентрације алдрина, ендрина, γ-HCH, ендосулфана, хептахлор епоксида, p,p'-DDD, p,p'-DDE, p,p'-DDT биле су изнад граничних вредности, али испод максимално дозвољених концентрација. Нежељени ефекти на организме очекују се повремено. Након излагања органохлорним пестицидима путем недијеталних путева потенцијални ризик од рака се не очекује. Највећи ризик од рака очекује се при гастроинтестиналном уносу седимената и минимално путем инхалације.

КЉУЧНЕ РЕЧИ: дистрибуција, модел процене хроничног дневног уноса, органохлорни пестициди, ризик од рака, седимент

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CHAROPHYTES OF GORNJE PODUNAVLJE PONDS: REVITALIZATION PROCESS ASPECT

ABSTRACT: Unique marshland area in the Danube floodplain – Special Nature Reserve Gornje Podunavlje is considered to be one of the last aquatic flora and fauna refuges in the region. The revitalization projects in SNP Gornje Podunavlje started in 2011. They were conducted in order to protect biodiversity and to provide the natural ecosystem. The aim of this study was to investigate and compare the present diversity and distribution of the charophytes in five ponds of SNR Gornje Podunavlje, depending on phases of the revitalization process within the ponds. The researched area includes the following ponds: Semenjača, Šarkanj, Široki rit, Sakajtaš and Ribolov. The Široki rit pond was under the process of revitalization during the field surveys, Semenjača and Šarkanj have already been revitalized, and Ribolov and Sakajtaš were not exposed to the revitalization processes at all. The field surveys were conducted monthly, from May to September 2016. Water quality parameters were measured in situ and in laboratory. Ponds differed considerably when it comes to water chemistry; Semenjača and Šarkanj were associated with highest nutrient concentrations, while Široki rit was characterized by highest conductivity and water hardness. The highest diversity was found in Široki rit pond, which was still in process of revitalization when the field survey was conducted. Charophytes were not detected within previously revitalized ponds (Semenjača and Šarkanj). Eight species of charophytes were detected: *Chara contraria*, *Chara globularis*, *Chara tenuispina*, *Chara vulgaris, Nitella confervacea*, *Nitella gracilis*, *Nitella syncarpa* and *Tolypella prolifera*.

KEYWORDS**:** charophytes, diversity, Gornje Podunavlje, revitalization, shallow ponds

INTRODUCTION

The Special nature reserve (SNR) Gornje Podunavlje is a protected natural resource of I category. It is situated in the north-west of Vojvodina, Serbia,

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along the left bank of the Danube river from 1.433rd km to the 1.367th km. SNR Gornje Podunavlje is one of the last large floodplains in Europe, a large marshland complex that makes a part of UNESCO Crossborder Biosphere Reserve "Mura-Drava-Danube", also known as Europe's Amazon. As of 2017, it has been designated as UNESCO Bačko Podunavlje Biosphere Reserve (www.unesco.org). SNR Gornje Podunavlje consists of Monoštorski and Apatinski marsh. Due to the anthropogenic pressure, near 80% of this fragile ecosystems have been lost in the last 150 years (www.amazon-of-europe.com).

Charophytes are macroscopic (mainly) freshwater algae, considered as basically thriving and dominate underwater freshwater vegetation (Vesić, 2016). Hence, charophytes play a crucial role in both ecosystem functions and providing ecosystem services (Lambert, 2011, Schneider et al., 2015). Apart from some sporadic records, diversity of charophyes in SNR Gornje Podunavlje was not systematically monitored.

The restoration projects in SNR Gornje Podunavlje started in 2011, as a part of the Living Danube Partnership among WWF, the Coca-Cola system and the International Commission for the Protection of the Danube River (ICPDR) (www.amazon-of-europe.com). This survey was also conducted under the patronage of the same project. The aim of this study was to investigate charophytes diversity in five selected ponds in SNP Gornje Podunavlje, including two revitalized ponds (Semenjača in 2011 and Šarkanj in 2015), one currently in the process of revitalization (Široki rit) and two ponds never exposed to this process (Ribolov and Sakajtaš).

MATERIALS AND METHODS

The studied ponds are situated in wetland area of Monoštorski marsh (Figure 1, Table 1). All the selected ponds (except Sakajtaš) are located at the flood protected zone (behind the flood bank), while Sakajtaš is located within the flooded area.

Basic water quality parameters (pH, temperature and conductivity) were measured *in situ* using digital field instruments Eutech Instuments Oakton®. Water samples for physical and chemical analyses were analyzed in the Institute of public health in Sombor.

Table 1. Comparative characteristics overview of five selected ponds in SNR Gornje Podunavlje.

Pond	Level of protection degree	Water supplying	Revitalization	Fishing
Semenjača	II	Tisa (groundwater)	2011	Permanent ban
Šarkanj	II	Tisa (groundwater)	2015	Permanent ban
Široki rit	II	Danube (through the channel)	2016	Permanent ban
Ribolov	II	Danube (through the channel)	/	Permanent ban
Sakajtaš	III	Danube (flooding)	/	Allowed

Charophytes sampling was performed monthly in the period May–September 2016, by wading shallow water. Three sampling sites were selected as representative for each pond, where belt transects method (Kolada et al., 2009) was used. Samples were collected manually or with the aid of rake and shafts. The charophyte samples were packed in plastic bags and transported to the Laboratory of the Department of Algology, Mycology and Lichenology, Faculty of Biology, University of Belgrade, where material was identified and deposited in the collection of wet specimens (BEOU).

Figure 1. Sampling sites – five selected ponds in SNR Gornje Podunavlje

Identification was conducted using STEMI DV4 stereomicroscope and Nikon YS100 microscope. Micrographs were taken with Reichert Diastar microscope, Canon PowerShot S40 camera and Remote Capture 2.1 software, while macro photos of charophytes *tali* were taken by Canon EOS 1200D, EF-S 18-55 III. Charophytes were identified using the keys proposed by Wood and Imahori (1964, 1965), Gollerbah and Krasavina (1983), Krause (1997), Mouronval et al. (2015), Urbaniak and Gabka (2014).

Statistical analyses were conducted by CANOCO for Windows, version 5.0 (ter Braak and Šmilauer, 2012). Linear unconstrained analysis (PCA) was performed for advanced visualization.

RESULTS AND DISCUSSION

PCA explained 60.07% of environmental data variability in the first two ordination axes (Figure 2).

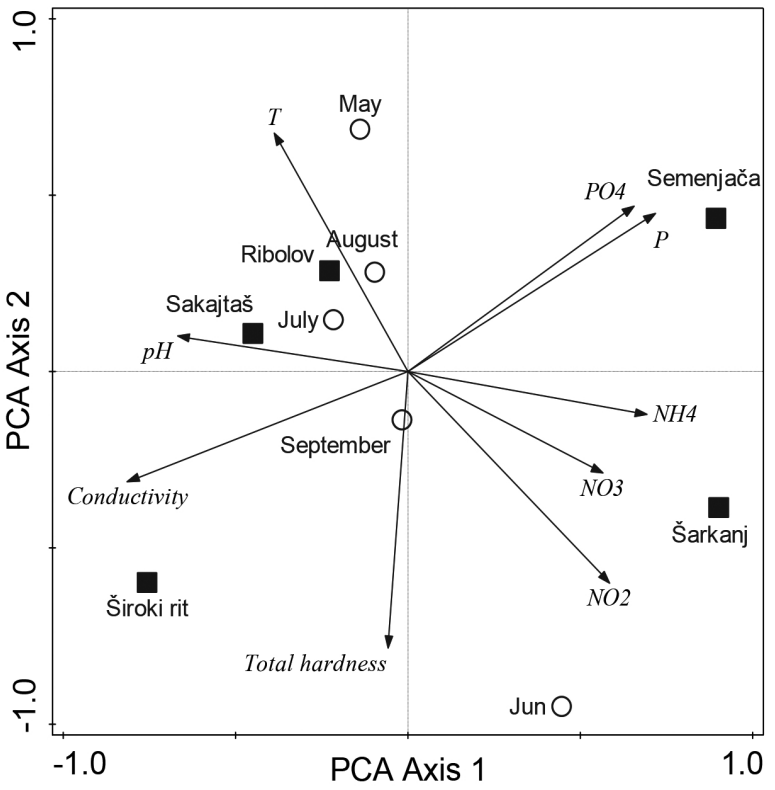

Figure 2. Principal component analysis (PCA) of the environmental variables in the explored ponds (Semenjača, Šarkanj, Ribolov, Sakajtaš and Široki Rit) during the study period.

The first PCA axis ordered the sampling ponds supplied by the underground water of the Tisza River on the positive side and sampling ponds supplied by the Danube River on the negative side. Semenjača and Šarkanj ponds, on the positive side, were associated with higher values of nutrients – total phosphorus (P), orthophosphates (PO_4), nitrates (NO_3), nitrites (NO_2), ammonium ion (PO_4) (Pearson correlation: r=0.72, 0.66, 0.57, 0.58 and 0.69, respectively), while the Široki rit, Ribolov and Sakajtaš ponds situated on the negative side of axis 1 were associated with higher conductivity (Pearson correlation: r=-0.81). The second axis of PCA distinguished sampling ponds according to total hardness (Pearson correlation: r=-0.78). These analyses implied that trophic status of Semenjača and Šarkanj, despite of being revitalized, is still higher compared to the pond under the current revitalization process Široki rit and two unrevitalized ponds Sakajtaš and Ribolov. Široki rit pond is characterized by the highest values for conductivity and total hardness.

Charophyta were recorded in three out of five selected ponds – Ribolov, Sakajtaš and Široki rit (Table 2), while in Šarkanj and Semenjača ponds charophytes were not recorded. The last two ponds have two main common characteristics – origin of water supplying from the Tisza River by the groundwater (Table 2) and relatively high nutrient levels (Figure 1). This is to be expected, as eutrophication is recognized as one of the principal drivers of charopytes population's declines (Rey-Boissezon and Auderset Joye, 2015). Blindow (1992) postulated that poor light conditions (as a consequence of eutrophication) is limiting factor for their growth.

Table 2. Diversity and dynamics of stonewort in three ponds where they were detected

Charophyta species	IUCN threat status in Serbia	Ribolov					Sakajtaš					Široki rit				
		may	jun	jul	aug	sep	may	jun	jul	aug	sep	may	jun	jul	aug	sep
Chara contraria A. Braun ex Kützing Kütz	LR	+			+		+						+			
Chara vulgaris Linne	LR				+							+	+			
Chara globularis Thuillier	VU				+							+	+			
Chara tenuispina A. Braun	CR												+			
Nitella confervacea (Brébisson) A. Braun ex Leonhardi	CR											+				
Nitella syncarpa (Thuillier) Chevallier	CR											+				
Nitella gracilis (Smith) Agardh	CR											+				
Tolypella prolifera (Ziz ex A. Braun) Leonhardi	CR											+				

The highest charophytes diversity value was recorded in Široki rit pond (Table 2). Rey-Boissezon and Auderset Joye (2015) suggested that the high conductivity is usually observed due to the high concentration of the calcium ions. The same authors proved that the conductivity represents one of the most important habitat charophytes selective. The results of this study confirm the Rey-Boissezon and Auderset Joye (2015) finding. During the survey period, Široki rit pond was under the revitalization process. It's interesting to point out the highest charophytes diversity in this particular pond in terms of verifying significance and success of restorations of shallow water bodies in SNR Gornje Podunavlje, as Rodrigo et al. (2015) already confirmed for Albufera de València Natural Park in Spain.

Charophytes are recognized as providers of a number of ecosystem services, such as maintaining clear water state in shallow water bodies, carbon sequestration and nutrient burial. Among others, providing shelter and food for various organisms could be underlined (Schneider et al., 2015). Auderset Joye and Rey-Boissezon (2015) estimated scenarios of charophytes success and distribution under the changing climate and detected groups of potential "losers" and "winners". These authors predicted that half of estimated species will become losers, prevalently the ones which colonize littoral zone of lakes, while colonizers of shallow and small water bodies were designated as winners.

Figure 2. Habit (macroscopic *tali*) and gametangia of detected charophyta representatives, a) *Chara contraria*, b) *C. globularis*, c) *C. tenuispina* and d) *C. vulgaris*, e) *Nitella confervacea*, f) *N. gracilis*, g) *N. syncarpa* and h) *Tolypella prolifera*

It has to be mentioned that Auderset Joye and Rey-Boissezon (2015) based their survey on data collected only in Switzerland, while similar studies were never conducted in other areas. All the abovementioned highly accentuates importance of charophyta diversity monitoring, especially in the protected areas such as SNR Gornje Podunavlje, which are recognized as biodiversity treasures.

In total, eight species of charophytes were detected *Chara contraria, C. globularis, C. tenuispina, C. vulgaris, Nitella confervacea, N. gracilis, N. syncarpa* and *Tolypella prolifera* (Figure 2, a, b, c, d, e, f, g, and h, respectively). The IUCN status (Table 2) is given according to Blaženčić (2014).

All stonewort species detected were previously recorded in Vojvodina, Serbia (Vesić, 2016). Nevertheless, for the Monoštorski rit marsh, this is the first record of *Chara tenuispina, Nitella confervacea* and *Nitella gracilis*. Having been critically endangered (Table 2) it can be considered as highly important IUCN records.

CONCLUSION

In total, eight species of charophytes were detected *Chara contraria, C. globularis, C. tenuispina, C. vulgaris, Nitella confervacea, N. gracilis, N. syncarpa* and *Tolypella prolifera*. Charophyta were recorded in Ribolov, Šakajtaš and Široki rit ponds. The highest diversity was detected in the Široki rit pond, which was in the process of restoration during the survey. In Šarkanj and Semenjača ponds charophytes were not recorded, due to the advanced process of eutrophication, in spite of the prior restoration. Our results strongly recommend further research on charophyte diversity and distribution within the SRP Gornje Podunavlje, in terms of continuous monitoring and expanded research area.

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ПРШЉЕНЧИЦЕ У БАРАМА ГОРЊЕГ ПОДУНАВЉА: АСПЕКТ РЕВИТАЛИЗАЦИЈЕ ПЛИТКИХ ЕКОСИСТЕМА

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РЕЗИМЕ: Јединствено мочварно подручје у плавној зони Дунава – Специјални резерват природе „Горње Подунавље", сматра се једним од последњих уточишта бројних угрожених биљних и животињских врста у региону. Исушивање влажних станишта представља главни ризик од губитка биодиверзитета. Пројекти ревитализације плитких станишта у СРП „Горње Подунавље" започели су 2011. године с циљем заштите биодиверзитета и одрживости пружања екосистемских услуга. Циљ наше студије било је истраживање и упоредно представљање разноврсности и дистрибуције пршљенчица у пет бара СРП „Горње Подунавље", у зависности од фаза процеса ревитализације у овим барама. Изабране су баре Семењача, Шаркањ, Широки рит, Сакајташ и Риболов. Широки рит био је у процесу ревитализације током наших истраживања, Семењача и Шаркањ су ревитализоване раније, а Риболов и Сакајташ нису били изложени процесу ревитализације. Пршљенчице су узорковане месечно, од маја до септембра 2016. године. Параметри квалитета воде мерени су на лицу места и у лабораторији. Баре су се значајно разликовале у погледу еколошких параметара. Семењача и Шаркањ су се одликовале узнапредовалом еутрофикацијом, а Широки рит високим вредностима за тврдоћу и проводљивост воде. Највећи диверзитет пршљенчица забележен је у бари Широки рит, која је и била у процесу ревитализације током наше студије. У ранијим ревитализованим барама Семењача и Шаркањ пршљенчице нису детектоване. Укупно је нађено осам врста пршљенчица: *Chara contraria*, *C. globularis*, *C. tenuispina*, *C. vulgaris*, *Nitella confervacea*, *N. gracilis*, *N. syncarpa* и *Tolypella prolifera*.

КЉУЧНЕ РЕЧИ: пршљенчице, разврсност, Горње Подунавље, ревитализација, плитка станишта

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FLORISTIC ANALYSIS OF THE DANUBE'S SHORELINE FROM ČEREVIĆ TO ČORTANOVCI

ABSTRACT: Given the long and continuous history of human settlements on the territory of Novi Sad, the human impact on the Danube's coast configuration has been significant. Based on the field research and literature data regarding the Danube's shoreline, from Čerević to Čortanovci, a total number of 440 taxa classified into 224 genera and 68 families were registered. According to the number of species, the most abundant families were Asteraceae (51), Poaceae (49), Fabaceae (32) and Brassicaceae (28), while the most abundant genera were *Carex* (15), *Rumex* (10) and *Euphorbia* (8). Chorological analysis showed that 42% of the registered taxa belong to Eurasian floral element, 14% to Central European and 12% to Pontic-Southsiberian floral element, with special attention to alien plants with invasive character that included 24 taxa. The analysis of the life forms showed domination of hemicryptophytes (40%), followed by therophytes (27%) and phanerophytes (11%).

KEYWORDS: Invasive alien plant species, Pannonian plain, riparian vegetation

INTRODUCTION

Riparian zones are some of the most productive and heterogeneous ecosystems. They represent the interface between aquatic and terrestrial ecosystems and are characterized by high species richness. Flooding and deposition of alluvial soil highly affect these areas. Therefore, they are most suitable for pioneer species (Hood & Naiman, 2000; Richardson, 2007; Lair, 2009). Riparian vegetation controls water flow and streams water temperature. It provides a buffer zone that filters sediments and nutrients and stabilizes stream banks. What is most important, it serves as a corridor for spreading of various organisms (Pyšek and Prach, 1993; Hood & Naiman, 2000; Richardson, 2007; Schnitzler, 2007). Today, human impact on riparian zones is immense, and agriculture, damming,

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urbanization and different kinds of pollution are just a few of many disturbances, especially on large river systems such as the Danube River (Hood & Naiman, 2000; Richardson, 2007; Lair, 2009). Favorable conditions for high species richness can promote occurrence and establishment of non-native species, particularly in those parts with high anthropogenic influence. Once established, certain plants become invasive, spreading at the expense of indigenous species and altering the surrounding habitats (Pyšek and Prach, 1993; Hood & Naiman, 2000; Richardson, 2000; Tomanović 2004; Richardson, 2007; Schnitzler, 2007).

The Danube basin is distinguished by numerous important natural areas including primarily wetlands and flood zones. Apart from having great value due to its diversity of the environment, it also has a great economic and social importance (Vukov, 2008). According to Mölder & Schneider (2011), the number or percentage of Danubian hardwood floodplain forests on Upper, Middle and Lower Danube River increases downstream together with available nutrients, while there is a decrease in number or percentage of herb-layer. In Serbia, many important natural sites are associated with the Danube, but they represent only a small percentage of preserved habitats, compared to human-altered zones. Vojvodina (North Serbia) is characterized by great coverage of agricultural areas, which are in direct contact with riparian zones in most of the Danube's flow. Also, starting from the 20th century until the present day, there is a frequent occurrence of man-made poplar plantations, at the expense of natural flooded forests. The occurrence of invasive species accelerated the modification of natural habitats and today they pose a threat to protected areas. Given the long and continuous history of human settlements on the territory of Novi Sad, the human impact on the Danube's coast configuration in this area has been significant.

The aim of this study was to investigate the floristic structure of the Danube's shoreline from Čerević to Čortanovci, which is significantly marked by direct or indirect anthropogenic influence. Analyzing literature and fieldwork data, we were able to reveal the taxonomic structure and life form spectrum, as well as to answer which areal types are dominant in the investigated area and to see to how many adventitious plants there are in the total taxa count.

MATERIALS AND METHODS

Investigated area

The Danube is the second largest European river. Its flow through Serbia has a length of 588 km, as it enters on the 1.433rd km and exits on the 846th km (mouth of Timok). The Danube's flow is divided into three sections: Pannonian lowland Danube, Đerdap accumulation and West Pontian Danube (Bugarski, 1998; Vukov, 2008). High levels of precipitation during May 2014, in Balkan part of the Danube basin, resulted in a higher water level than usual for that time of the year (RHSS, 2014). The Danube's deposits are mainly represented

by young and undeveloped soil, formed by deposition of materials with different mechanical and mineralogical composition, such as sandy loam. Alluvial soils are characterized by the specific type of water regime, which is marked by extreme humidity and permeability (Živković et al., 1972; Miljković, 2001). Developed soil types appear only on old alluvial deposits and they have predominantly intrazonal character – humogley (Miljković, 2005). An important feature in urban areas of Danube's flow is man-made embankments, the first ones recorded in 1770, for the city of Novi Sad (Milošev, 2005).

The study of the Danube's left coastal zone includes the riparian and semi-natural parts of the Danube's coastline in municipality of Novi Sad from the Nature Park "Begečka jama" (N43°.13'; E19°.36') as the westernmost point, to the Special Nature Reserve "Koviljsko-petrovaradinski rit" (S45°.14'; E19°.55') to the east. Investigated territory of Danube's right coastal zone stretches between villages Čerević (N45°.13'; E19°.39') and Čortanovci (45°.10'; E20°.02'), including flooded forests and meadows of Beočin municipality and urban and semi urban coastal strip of Sremska Kamenica, Petrovaradin, Sremski Karlovci and Čortanovci settlements.

Analysis of floristic data

Plant material was collected in all growing seasons during 2014 and 2015. Plants were herbarized using standard method (Nikolić, 1996) and deposited in the main Herbarium collection of the University of Novi Sad (BUNS). Identification of plant material was done using referent national and regional floras and iconographies (Jávorka and Csapody, 1975; Josifović, 1972–1977; Király, 2009; Sarić, 1992; Stevanović, 2012; Tutin et al., 1964; Tutin et al., 1968-1980; Tutin et al., 1993). Taxonomic status was harmonized according to reference databases (Euro+Med, 2006; International Organization for Plant Information, 2012; The International Plant Names Index, 2012; The Plant List, 2013). Grouping of angiosperm, ferns, horsetails and gymnosperms taxa in higher systematic categories was done according to the selected authors (Christenhusz et al., 2011; Takhtajan, 2009). The life forms were defined according to Raunkier (1934), modified according to Mueller-Dumbois and Ellenberg (1974) and further adapted for Flora of Serbia by Stevanović (1992b). Floral elements were determined according to Gajić (1984) and then classified into defined areal types for the territory of Serbia according to Stevanović (1992a). Floral elements were analyzed by an areal type spectrum, while the percentual distribution of the corresponding life forms was presented by a biological spectrum (Janković, 1985). Invasive alien species where determined according to referent check-lists (Lazarević, 2012; Anačkov 2013). Besides collected plants at an investigated area, we included data from collection of BUNS Herbarium, gathered by L. Galamboš and V. Stanković, as their main topic was a floristic study of the Danube's shoreline on the territory of Novi Sad.

RESULTS AND DISCUSSION

Based on the field research and literature data regarding the Danube's shoreline, from Čerević to Čortanovci, 440 taxa on the level of species and subspecies were recorded (Table 1).

Table 1. Total number of registered taxa

Taxon	Life form	Areal type
Divisio: Equisetophyta		
Classis: Equisetopsida		
Family: Equisetaceae		
1. Equisetum arvense L. 1753	a Mes-Meg G rhiz	Hol
2. Equisetum palustre L. 1753	v-a Mes-Meg G rhiz	Hol
Divisio: Polypodiophyta		
Classis: Polypodiopsida		
Family: Marsileaceae		
3. Marsilea quadrifolia L. 1753	rhiz emer Hyd T	EAs
Divisio: Magnoliophyta		
Classis: Magnoliopsida		
Family: Aristolochiaceae		
4. Aristolochia clematitis L. 1753	v-a Mes-Meg G rad	MSm
Family: Ranunculaceae		
5. Caltha palustris L. 1753	v-a Mes-Meg G rad	Hol
6. Clematis integrifolia L. 1753	a Mes-Meg H Scap	EAs
7. Clematis vitalba L. 1753	Alt a S lig	SAt
8. Consolida orientalis (Gay) Schrod. 1909	v-a Mac T scap	PSs
9. Consolida regalis S. F. Gray. 1821 –	a Mes-Meg T scap	CEu
10. Helleborus odorus Waldst. & Kit. ex Willd. 1809	v Meg G rhiz	EAs
11. Ranunculus acris L. 1753	a meg H scap semiros	EAs
12. Ranunculus ficaria L. 1753	ver Mi-Mes G bulb/H scap	CEu
13. Ranunculus polyanthemos L. 1753	a Meg H scap semiros	PSs
14. Ranunculus repens L. 1753	v-a Mes-Mac H rept	EAs
15. Ranunculus sardous Crantz 1763	a Mes-Meg T scap	CEu
16. Ranunculus sceleratus L. 1753	v-a Mes-Mac T scap	Hol
17. Ranunculus trichophyllus Chaix 1786	rad sbm-nat Hyd T	CEu
18. Thalictrum aquilegifolim L. 1753	a Meg-Alt H scap	EAs
19. Thalictrum flavum L. 1753	a Meg-Alt H scap	EAs
20. Thalictrum minus L. 1753	Mes-Mac H scap	EAs
Family: Papaveraceae		
21. Chelidonium majus L. 1753	v-a Mes-Meg H semiros	EAs
22. Papaver rhoeas L. 1753	a Meg T scap	EAs
23. Papaver somniferum L. 1753	ver-a Mac-Alt T scap	Adv
Family: Fumariaceae		
24. Fumaria officinalis L. 1753	a Mi-Mes T scap	EAs
Family: Fagaceae		
25. Quercus pubescens Willd. 1805	fo dec Mes P scap	EAs
26. Quercus robur L. 1753	fo dec Mes P scap	MSm
Family: Juglandaceae		
27. Juglans regia L. 1753	v Mes P scap	MSm
Family: Phytolaccaceae		
28. Phytolacca americana Juss. 1789	a-aut Alt G rhiz	Adv
Family: Portulacaceae		
29. Portulaca oleracea L. 1753	a Mes-Mac T scap	Adv
Family: Caryophyllaceae		
30. Arenaria serpyllifolia L. 1753	v-a Mi-Mes T scap	EAs
31. Cerastium arvense L. 1753	a Mes-Mac H scap/Ch suffr	Hol

32. Petrorhagia prolifera (L.) P.W.Ball. et Heywood 1964	v-a Mes T scap	PSs
33. Petrorhagia saxifraga (L.) Link.	a Mes H caesp/fo dec Ch herb caesp	MSm
34. Sagina procumbens L. 1753	v-a Mi H caesp	Hol
35. Saponaria officinalis L. 1753	a Meg H scap	EAs
36. Silene latifolia subsp. *alba* (Mill.) Greuter & Burdet	a Meg H scap bienn	EAs
37. Silene vulgaris (Moench) Gracke 1869	a Mes H scap/G rad	EAs
38. Stellaria graminea L. 1753	a Mes-Meg H scap	EAs
39. Stellaria media (L.) Vill. 1789	v-aut Mi T rept	Cos
40. Stellaria palustris Ehrh. ex. Hoffm. 1791	v-a Mes-Mac H scap	EAs
Family: Amaranthaceae		
41. Amaranthus blitum L. 1753	a-aut Mes-Meg T scap	Adv
42. Amaranthus retroflexus L. 1753	a Mes-Alt T scap	Adv
Family: Chenopodiaceae		
43. Bassia scoparia (L.) A. J. Scott. 1978	a Mac-Alt T scap	Adv
44. Chenopodium album L. 1753	a Meg-Alt T scap	Cos
45. Chenopodium ambrosioides L. 1753	a-aut Mac-Meg T scap	Adv
46. Chenopodium botrys L. 1753	a Mes-MacT sca	EAs
47. Chenopodium polyspermum L. 1753	a Meg T scap	EAs
48. Chenopodium rubrum L. 1753 subps. *blitoides* (Lej.) Wartl	a-aut Mes-Meg T scap	Hol
Family: Polygonaceae		
49. Fallopia convolvulus (L.) Á. Löve 1970	v-a Mac-Alt Tscap	EAs
50. Polygonum aviculare L. 1753	a-aut Mi-Meg T rept	Cos
51. Polygonum hydropiper L. 1753	a Mes-Meg T scap	Hol
52. Polygonum lapathifolium L. 1753	a Mac-Alt T scap	Hol
53. Polygonum minus Huds. 1762	a Mac-Alt T scap	EAs
54. Polygonum mite Schank. 1789	a Mac-Alt T scap	CEu
55. Polygonum persicaria L. 1753	a-aut Mac-Alt T scap	EAs
56. Reynoutria japonica Houtt. 1777	a Alt H scap	Adv
57. Rumex confertus Willd. 1809	a Mac-Meg H scap	EAs
58. Rumex conglomeratus Murray 1770	a Meg H scap	EAs
59. Rumex crispus L. 1753	a Meg-Alt H scap	EAs
60. Rumex dentatus L. 1771	v-a Meg-Alt H scap	PSs
61. Rumex hydrolapathum Hudson 1778	v-a Mes H scap	CEu
62. Rumex maritimus L. 1753	a Mes-MacT scap	Cos
63. Rumex obtusifolius L. 1753	a Meg H scap	CEu
64. Rumex palustris Sm. 1800	a Mac T scap	CEu
65. Rumex patientia L. 1753	a Alt H scap	PSs
66. Rumex stenophyllus Ledeb. 1830	a Alt H scap	PSs
Family: Hypericaceae		
67. Hypericum perforatum L. 1753	a Mes-Meg H scap	EAs
Family: Primulaceae		
68. Lysimachia nummularia L. 1753	ver-a Mes-Mac H scap	CEu
69. Lysimachia vulgaris L. 1753	a Mac-Meg H scap	EAs
Family: Salicaceae		
70. Populus alba L. 1753	v Mi-Mes P scap	EAs
71. Populus deltoides Marshall 1785	v Mes P scap	Adv
72. Populus nigra L. 1753	fo dec Mes P scap	CEu
73. Populus tremula L. 1753	fo dec Mes P scap	EAs
74. Salix alba L. 1753	fo dec Mes P scap	EAs
75. Salix babylonica L. 1753	v Mes P scap	EAs
76. Salix fragilis L. 1753	v Mi P caesp/Mi-Mes P scap	CEu
77. Salix purpurea L. 1753	fo dec Mi P caesp	EAs
78. Salix triandra L. 1753	fo dec Mi P caesp	EAs
Family: Violaceae		
79. Viola arvensis Murray 1770	v-a Mes-Mac T scap	EAs
80. Viola odorata L. 1753	v Mi-Mes H ros	SAt

Family: Cucurbitaceae		
81. Echinocystis lobata (Michx.) Torr. & A. Gray 1840	a Alt T scap	Adv
Family: Brassicaceae		
82. Alliaria petiolata (M.Bieb.) Cavara & Grande 1913	v-a Meg H scap bienn	EAs
83. Armoracia rusticana P. Gaertn., B. Mey. & Schreb. 1800	v-a Mac-Meg G rhiz	Adv
84. Barbarea vulgaris R. Br. 1812	a Meg H scap	EAs
85. Berteroa incana (L.) DC 1821	a Mes H scap	PSs
86. Brassica napus L. 1753	v-aut Mac-Alt T scap/H scap	Adv
87. Calepina irregularis (Asso) Thell. 1905	a Mes-Meg T scap	MSm
88. Camelina microcarpa Andrz. Ex DC 1821	v-a Mac H scap bienn	EAs
89. Capsella bursa-pastoris (L.) Medik. 1792	v-aut Mi-Meg T ros/H ros bienn	Cos
90. Cardamine parviflora L. 1759	v-a-aut Mac T scap	EAs
91. Cardamine pratensis L. 1753	a Mes-Mac H scap	Hol
92. Descurainia sophia (L.) Webb. ex Prantl. 1892	a Mac-Meg T scap/H bienn	EAs
93. Erophila verna (L.) Chevall. 1828	v Mi T scap	Hol
94. Erysimum cheiranthoides L. 1753	v-a Mi-Mac T scap	Hol
95. Lepidium draba L. 1753	v v-a Mes-Mac G rhiz/H scap	PSs
96. Lepidium perfoliatum L. 1753	v-a Mes-Mac T scap/ H scap	PSs
97. Lepidium ruderale L. 1753	v-a Mes T scap	CEu
98. Lepidium virginicum L. 1753	v-a Mes-Mac T scap	Adv
99. Raphanus raphanistrum L. 1753	v Mes-Meg T scap	CEu
100. Rorippa amphibia (L.) Besser. 1822	rhiz emer Hyd G	EAs
101. Rorippa austriaca (Crantz) Besser 1822	v-a Mac-Meg H scap	PSs
102. Rorippa kerneri Menyh. 1877	v-a Mes H scap	PSs
103. Rorippa sylvestris (L.) Besser. 1821	a Mi-Mes H scap	EAs
104. Sinapis alba L. 1753	a Mac T scap	MSm
105. Sinapis arvensis L. 1753	v-a Mes-Meg T scap	EAs
106. Sisymbrium loselii L. 1755	a Meg T scap	PSs
107. Sisymbrium officinale (L.) Scop 1772	a Mac-Meg T scap	EAs
108. Sisymbrium orientale L. 1756	v-a Mi-Mes T scap semiros	PSs
109. Thlaspi perfoliatum L. 1753	v v-a Mes-Mac G rhiz/H scap	CEu
Family: Resedaceae		
110. Reseda lutea L. 1753	Mac-Meg H scap/T scap	CEu
111. Reseda luteola L. 1753	a-aut Mac-Alt H scap	EAs
Family: Tiliaceae		
112. Tilia tomentosa Moench. 1785	v-a Mes P scap	MSm
Family: Malvaceae		
113. Abutilon theophrasti Medik 1787	a Mac-Meg T scap	Adv
114. Alcea rosea L. 1753	v-a Alt H scap	Adv
115. Althaea officinalis L. 1753	v-a Mac-Alt H scap	PSs
116. Malva sylvestris L. 1753	a Meg-Alt H scap bienn	EAs
Family: Ulmaceae		
117. Celtis australis L. 1753	v Mes P scap	MSm
118. Ulmus laevis Oall. 1784	Mi-Mes P scap	CEu
119. UImus minor Miller 1768	v P caesp/Mi-Mes P scap	MSm
Family: Moraceae		
120. Morus alba L. 1753	fo dec Mi-Mes P scap	Adv
121. Morus nigra L.1753	v Mi-Mes P scap	Adv
Family: Cannabaceae		
122. Humulus lupulus L.1753	a Alt SH herb	EAs
Family: Urticaceae		
123. Urtica dioica L. 1753	a Meg-Alt H scap	EAs
Family: Euphorbiaceae		
124. Euphorbia amygdaloides L. 1753	v-a Mac Ch suffr	EAs
125. Euphorbia cyparissias L. 1753	a Mes-Meg H scap	EAs
126. Euphorbia esula L. 1753	v-a Mac-Meg H scap	EAs

127. Euphorbia esula L. 1753 subsp. *tomasiniana* (Bertol.) Kuzmanov 1979	a Mac-Alt H scap	EAs
128. Euphorbia helioscopia L. 1753	a Mi-Meg T scap	EAs
129. Euphorbia lucida Waldst. & Kit.1802	a Mac-Alt H scap	PSs
130. Euphorbia palustris L. 1753	v-a Mac-Alt G rhiz	EAs
131. Euphorbia platyphyllos L. 1753	a-aut Mes-Meg T scap	MSm
Family: Saxifragaceae		
132. Chrysosplenium alternifolium L. 1753	v-a Mes H scap	Bor
133. Saxifraga tridactylites L. 1753	v-a Mi T scap	CEu
Family: Vitaceae		
134. Parthenocissus quinquefolia (L.) Planchon 1887	ver P lian	Adv
135. Vitis longii W. R. Prince & Prince 1830	v-a Alt S lig	Adv
136. Vitis vinifera L. 1753	a Alt S lig	MSm
Family: Rosaceae		
137. Crataegus monogyna Jacq. 1775	fo dec Mi P caesp/Mi P scap	CEu
138. Crataegus nigra Waldst. & Kit. 1802	fo dec Mi-Mes P scap/caesp	PSs
139. Crataegus pentagyna Waldst. & Kit. 1802	fo dec Mi-Mes P scap/caesp	MSm
140. Filipendula ulmaria (L.) Maxim. 1879	a Meg H scap	EAs
141. Filipendula vulgaris Moench 1794	a Meg H scap	EAs
142. Geum urbanum L. 1753	v-aut Mac H perenn	EAs
143. Malus domestica Боркх. 1803	fo dec Mes P scap	Cul
144. Mespilus germanica L. 1753	fo dec Mi P scap/caesp	PSs
145. Potentilla anserina L. 1753	v-a Mes H rept	Hol
146. Potentilla argentea L. 1753	a Mes-Meg H scap	PSs
147. Potentilla reptans L. 1753	a Mi-Mes H rept	EAs
148. Potentilla supina L. 1753	a Mes-Mac T scap (H scap)	EAs
149. Prunus avium (L.) L. 1755	v Mes P scap	CEu
150. Prunus cerasifera Ehrh. 1758	fo dec Mi-Mes P scap/caesp	EAs
151. Prunus cerasus L. 1753	v Mes P scap	EAs
152. Prunus domestica L. 1753	v P scap (P caesp)	MSm
153. Prunus fruticosa Pallas 1784	fo dec Mi P caesp	EAs
154. Prunus mahaleb L. 1753	fo dec Mi-Mes P scap/caesp	EAs
155. Prunus spinosa L. 1753	fo dec NP caesp	PSs
156. Pyrus communis L. 1753	fo dec P scap	CEu
157. Rosa glauca Pourr. 1788	fo dec Mi P caesp	MSm
158. Rosa marginata Wallr. 1815	fo dec Mi P caesp	EAs
159. Rosa obtusifolia Desv. 1809	fo dec Mi P caesp	EAs
160. Rubus caesius L. 1753	v NP rept	EAs
161. Rubus koehlerii Weihe 1825	a Mac-Alt H scap	EAs
162. Rubus praecox Bertol. 1842	a Mac-Alt H scap	CEu
Family: Lytheraceae		
163. Lythrum salicaria L. 1753	a Meg-Alt H scap	PSs
Family: Onagraceae		
164. Epilobium hirsutum L. 1753	a Mes-Meg H scap	EAs
165. Oenothera biennis L. 1753	a Meg-Alt H scap bienn	Adv
Family: Fabaceae		
166. Amorpha fruticosa L. 1753	a fo dec NP caesp	Adv
167. Astragalus onobrychis L. 1753	a Mes fo des Ch suffr rept	PSs
168. Galega officinalis L. 1753	a Meg H scap	MSm
169. Gymnocladus dioica (L.) K. Koch 1869	v-a Mi-Mes T scap	Adv
170. Lathyrus aphaca L. 1753	a Mes ST herb/T scap	PSs
171. Lathyrus tuberosus L. 1753	v-a Mac-Meg H scap	EAs
172. Lotus corniculatus L. 1753	a Mes H scap	EAs
173. Lotus tenuis Waldst. & Kit. 1809	v-a Mes H scap	CEu
*174. Medicago arabica (*L.) Huds. 1762	a Mac T scap/rept	EAs
175. Medicago falcata L. 1753	a Mes-Meg H scap	EAs
176. Medicago lupulina L. 1753	a Mes T scap/H scap	EAs
177. Medicago sativa L. 1753	v-a Mac H scap	Adv
178. Melilotus albus Medic 1786	a-aut Meg T scap/H bienn	CEu

179. Melilotus altissimus Thuill. 1799	a Mac-Alt T scap	EAs
180. Melilotus officinalis (L.) Pall. 1776	a Meg-Alt H scap bienn	EAs
181. Ononis arvensis L. 1753	v-a Mac Ch frut caesp suffrut fo dec	CEu
182. Ononis spinosa L. 1753	a-aut Mes-Meg Ch frut caesp suffrut fo dec	CEu
183. Robina pseudoacacia L. 1753	fo dec Mes P scap	Adv
184. Securigera varia (L.) Lassen 1989	a Meg H scap	PSs
185. Trifolium campestre Schreb. 1800	v-a Mes T scap	CEu
186. Trifolium fragiferum L. 1753	v-a Mes-Mac H rept	CEu
187. Trifolium micranthum Viv. 1824	v Mi-Mac T scap	EAs
188. Trifolium pratense L. 1753	a Mes T scap	EAs
189. Trifolium repens L. 1753	v-aut Mi H rept perenn	EAs
190. Trifolium strictum L. 1755	v-a Mi-Mes T scap/rept	EAs
191. Vicia angustifolia L. 1753	v-a Mes-Meg T rept	EAs
192. Vicia cracca L. 1753	a Meg/Alt SH herb/H scap	EAs
193. Vicia grandiflora Scop. 1772	a Meg T scap rept	PSs
194. Vicia grandiflora Scop. 1772 var. *bierbersteiniana* (Besser) Grieseb	a Meg T scap rept	PSs
195. Vicia hirsuta (L.) Gray. 1821	a Mes-Meg T herb/scap rept	EAs
196. Vicia lutea L. 1753	v-a Mi-Meg T rept	MSm
197. Vicia tetrasperma (L.) Schreber 1771	v-a Mes T scap	EAs
Family: Oxalidaceae		
198. Oxalis acetosella L. 1753	v-a Mi G rhiz	Hol
199. Oxalis stricta L. 1753	a-aut Mi-Mes H scap	Adv
Family: Aceraceae		
200. Acer campestre L. 1753	fo dec Mes P scap	CEu
201. Acer negundo L. 1753	fo dec Mes P scap	Adv
202. Acer platanoides L. 1753	fo dec Mes P scap	CEu
203. Acer pseudoplatanus L. 1753	fo dec Mes P scap	CEu
204. Acer saccharinum L. 1753	fo dec Mes P scap	Adv
Family: Geraniaceae		
205. Erodium cicutarium (L.) L'Herit. 1792	v-a Mi-Mes T scap semiros	EAs
206. Geranium columbinum L. 1753	a Mi-Meg T scap	EAs
207. Gernaium dissectum L. 1755	ver-a Mes-Mac T scap	EAs
208. Geranium molle L. 1753	v-a Mi-Mes T scap/H bienn	EAs
209. Geranium palustre L. 1756	a Mes-Meg H scap	EAs
210. Geranium robertianum L. 1753	a Mi-meg T scap/H bienn	Hol
211. Geranium rotundifolium L. 1753	a-aut Mes T scap	EAs
212. Geranium sanguineum L. 1753	v-a Mes-Mac H rhiz	EAs
Family: Celastraceae		
213. Euonymus verrucosus Scop. 1771	fo dec Mi P caesp	PSs
Family: Cornaceae		
214. Cornus mas L.1753	fo dec Mi-Mes P caesp/scap	PSs
215. Cornus sanguinea L. 1753	fo dec Mi-Mes P caesp	CEu
Family: Sambucaceae		
216. Sambucus ebulus L. 1753	Alt a S lig	CEu
217. Sambucus nigra L. 1753	fo dec Mi-Mes P caesp/scap	PSs
Family: Valerianaceae		
218. Valeriana officinalis L. 1753	v-a Meg-Alt H scap	EAs
219. Valerianella carinata Loisel. 1810	v-a Mes T scap	PSs
220. Valerinaella locusta (L.) Laterrade 1821	a Mes T scap	MSm
221. Valerianella rimosa Bast. 1814	v-a Mes-Mac T scap	PSs
Family: Dipsacaceae		
222. Cephalaria transsylvanica (L.) Roemer & Schultes 1818	a Mi T scap	PSs
223. Dipsacus fullonum L. 1753	a Alt T scap/H bienn	CEu
224. Dipsacus laciniatus L. 1753	a Meg-Alt H scap bienn	PSs

225. Knautia arvensis (L.) Coult. 1823	a Mes-Meg H scap bienn	CEu
226. Scabiosa ochroleuca L. 1753	a Meg H scap	PSs
Family: Araliaceae		
227. Hedera helix L. 1753	aut semp Alt S lig	SAt
Family: Apiaceae		
228. Anthriscus cerefolium (L.) Hoffm. 1814	v-a Mac-Mes T scap	PSs
229. Aegopodium podagraria L. 1753	v-a Meg G rhiz	EAs
230. Chaerophyllum hirsutum L. 1753	v-a Mac-Meg H scap	CEu
231. Conium maculatum L. 1753	a Meg H scap bienn	EAs
232. Daucus carota L. 1753	a Meg H/T scap	EAs
233. Heracleum sphondylium L. 1753	a-aut Mac-Alt H scap	EAs
234. Pastinaca sativa L. 1753	a Mac-Meg H scap	EAs
235. Torilis arvensis (Huds.) Link 1821	v-a Mes-Mac T scap	EAs
Family: Campanulaceae		
236. Campanula rapunculoides L. 1753	a Meg H scap	EAs
Family: Asteraceae		
237. Achillea millefolium L. 1753	a Meg H scap	EAs
238. Ambrosia artemisiifolia L. 1753	a Mac-Meg T scap	Adv
239. Arctium lappa L. 1753	aut Meg-Alt H scap bienn	EAs
240. Arctium tomentosum Mill. 1768	a Mac-Alt H scap bienn	EAs
241. Artemisia vulgaris L. 1753	aut Meg-Alt H scap	Hol
242. Aster tradescanti Hoffm. 1803	a Mac-Alt H scap	Adv
243. Bellis perennis L. 1753	a Mes H ros	CEu
244. Bidens tripartita L. 1753	aut Mes-Alt T scap	CEu
245. Carduus acanthoides L. 1753	aut Mes-Alt T scap	CEu
246. Carduus nutans L. 1753	a Mac-Meg H scap bienn	EAs
247. Centaurea jacea L. 1753	a Mi-Alt H scap	EAs
248. Centaurea micrantha Hoffm.&Link	A Mac-Meg H bienn/perenn	PSs
249. Chondrilla juncea L. 1753	a Meg-Alt H scap	PSs
250. Cychorium intybus L. 1753	a-aut Meg-Alt H scap	EAs
251. Cirsium arvense (L.) Scop. 1772	a Meg-Alt G rad	EAs
252. Cirsium lanceolatum (L.) Hill 1769	a-aut Mac-Meg H bienn/H scap	EAs
253. Cota austriaca (Jacq.) Schultz-Bip. 1854	a Mes-MacT scap	PSs
254. Crepis foetida subsp. *rhoeadifolia* (M.Bieb.) Čelak 1808	a Mac T scap	PSs
255. Crepis setosa Haller 1797	a Mes-Meg T scap	MSm
256. Erigeron canadensis L. 1753	a-aut Mac-Alt T scap	Adv
257. Erigeron annuus (L.) Pers. 1807	a-aut Mes-Meg H scap	Adv
258. Gnaphalium uliginosum L. 1753	a-aut Mi-Mes T scap	EAs
259. Inula britannica L. 1753	a Mes-Meg H scap	CEu
260. Lactuca serriola L. 1756	a H bienn/T scap	EAs
261. Leontodon taraxacoides Hoppe & Hornsch. 1821	v-a Mes T/H scap	MSm
262. Matricaria chammomilla L. 1753	a Mes T scap	EAs
263. Matricaria suaveolens Koch 1843	a Mi-Mac T scap	Adv
264. Onopordum acanthium L. 1753	a Mi-Mes H scap bienn	PSs
265. Petasites albus (L.) Gaertn. 1791	v Mes-Meg H rhiz ros	EAs
266. Petasites hybridus (L.) Sch. 1801	v Mes-Alt H thiz ros	EAs
267. Pilosella officinarium Vaill. 1862	a Mi-Mac H ros	CEu
268. Podospermum canum C. A. Mey. 1831	a Meg-Alt G rad	EAs
269. Pulicaria dysenterica (L.) Bernh. 1800	a Mes-Meg H scap	SAt
270. Senecio leucanthemifolius Poir. 1789 subsp. *vernalis* (Waldst. & Kit.) Greuter 2003	v Mes-Meg T scap	PSs
271. Senecio vulgaris L. 1735	v-aut Mi-Mac T scap	EAs
272. Solidago canadensis L. 1753	a Mac-Alt H scap	Adv
273. Solidago gigantea Aiton 1789	a Mac-Alt H scap	Adv
274. Solidago serotina Retz. 1781	a-aut Mac-Alt T scap rhiz	Adv
275. Sonchus arvensis L. 1753	a Mac-Alt H scap	EAs
276. Sonchus asper (L.) Hill 1769	v-aut Mac-Alt T scap/H bienn	EAs

277. Sonchus oleraceus L. 1753	v-aut Mes-Meg T scap	EAs
278. Symphyotrichum salignum (Willd.) G. L. Nesom 1995	a Mac-Alt H scap	Adv
279. Taraxacum officinale Weber 1780	v-aut Mes H ros	Cos
280. Tragopogon dubius Scop. 1772	a Mes-Meg H scap bienn	PSs
281. Tragopogon pratensis L. 1753 subsp. *orientalis* (L.) Čelak 1871	v-a Mac-Meg H scap	EAs
282. Tripleurospermum inodorum (L.) Sch.Bip. 1844	a-aut Mac-Meg H scap	EAs
283. Tripleurospermum tenuifolium (Kit.) Freyn ex Freyn 1888	a Mac-Meg T scap	MSm
284. Tripolium pannonicum (L.) Greuter subsp. *tripolium* Greuter 2003	a Mac-Alt Hscap bienn	PSs
285. Tussilago farfara L.1753	v Mi-Mes G rhiz	EAs
286. Xanthium orientale L. 1763 subps. *italicum* (Moretti) Greuter 2003	a-aut Mac-Meg T scap	Adv
287. Xanthium strumarium L. 1753	a-aut Meg-Alt T scap	Adv
Family: Rubiaceae		
288. Cruciata glabra (L.) Opiz 1852	v-a Mes H scap	MSm
289. Cruciata laevipes Opiz 1852	v-a Mes-Mac H scap	CEu
290. Galium aparine L. 1753	v-a Mes-Meg T rept	EAs
291. Galium mollugo L. 1753	a Mac-Alt H scap	EAs
292. Galium palustre L. 1753	a Mes-Meg H scap	EAs
293. Sherardia arvensis L. 1753	v-a Mi-Mes T scap	Cos
Family: Asclepiadaceae		
294. Asclepias syriaca L. 1753	a Alt G rhiz	Adv
295. Vincetoxicum hirundinaria Medik subsp. *adriaticum* (Beck) Markgr. 1971	v-a Mac-Alt H scap	PSs
Family: Solanaceae		
296. Datura stramonium L. 1753	a-aut Meg-Alt T scap	Cos
297. Petunia hybrida Vilm. 1863	a MacT scap	Cul
298. Solanum dulcamara L. 1753	Meg-Alt a S lig	EAs
299. Solanum nigrum L. 1753	v-aut Mes-Meg T scap	Cos
Family: Convolvulaceae		
300. Calystegia sepium (L.) L.Br. 1810	v-a Mac-Alt H scand	EAs
301. Convolvulus arvensis L. 1753	a Alt G herb rhiz	Cos
Family: Boraginaceae		
302. Anchusa officinalis L. 1753	a Meg H scap bienn	CEu
303. Buglossoides arvensis (L.) I.M.Johnst. 1954	v-a Mes-Mac T scap	EAs
304. Lappula squarrosa (Retz.) Dumort. 1827	a Mi-Mac T scap/H bienn	EAs
305. Lithospermum officinale L. 1753	v-a Mac H scap	EAs
306. Myosotis arvensis (L.) Hill. 1764	a Mes H scap bienn	EAs
307. Myosotis laxa Lehm. 1818 subps. *caespitosa* (Schultz) Hyl. ex Nordh. 1940	a Mes-Mac T scap/H bienn	EAs
308. Myosotis palustris (L.) Nathh. 1756	a Mes-Mac H scap	EAs
309. Myosotis scorpioides L. 1753	a Mes-Mac H scap	EAs
310. Symphytum officinale L. 1753	a Mes-Meg H scap	CEu
311. Symphytum tuberosum L. 1753	ver G rhiz scap	EAs
Family: Oleaceae		
312. Fraxinus americana L. 1753	fo dec Mes P scap	Adv
313. Fraxinus ornus L. 1753	fo dec Mes P scap	CEu
314. Fraxinus pennsylvanica Marhsal 1785	fo dec Mes P scap	Adv
315. Ligustrum vulgare L. 1753	fo dec Mi P caesp	CEu
Family: Scrophulariaceae		
316. Gratiola officinalis L. 1753	a Mac H scap	Hol
317. Limosella aquatica L. 1753	rhiz emer Hyd T	Cos
318. Linaria angustissima (Loisel.) Borbás 1900	a Mac-Meg H scap	MSm
319. Linaria genistifolia (L.) Mill. 1768	a Mac-Meg H scap	PSs
320. Linaria vulgaris Mill. 1754	a-aut Mes-Meg H scap	CEu

321. Odontites vulgaris Moench 1794	a-aut Mi-Mac (Meg) T scap	CEu
322. Verbascum blattaria L. 1753	a Meg-Alt H scap bienn/T scap	EAs
323. Verbascum lychnitis L.1753	a Meg-Alt H semiros	EAs
324. Verbascum nigrum L. 1753	a Mac-Meg H ros bienn	PSs
325. Verbascum phlomoides L. 1753	a Meg H scap	PSs
326. Veronica anagalis-aquatica L. 1753	rhiz emer Hyd G	Hol
327. Veronica arvensis L. 1753	v Mi-Mes T scap	CEu
328. Veronica chamaedrys L. 1753	v-a Mi-Mes H scap	CEu
329. Veronica hederifolia L. 1753	v Mi-Mes T scap	CEu
330. Veronica persica Poir. 1808	v-aut Mi-Mac T scap	Adv
331. Veronica serpyllifolia L. 1753	v-aut Mi-Mes H rept	Hol
Family: Plantaginaceae		
332. Plantago altissima L. 1762	a-aut Mac-Meg H ros	MSm
333. Plantago lanceolata L. 1753	a Mi-Meg H ros	EAs
334. Plantago major L. 1753	v-a Mac-Alt H ros	EAs
335. Plantago media L. 1753	v-a H ros perenn	EAs
Family: Verbenaceae		
336. Verbena officinalis L. 1753	a Mes-Meg H scap	Cos
Family: Lamiaceae		
337. Ajuga reptans L. 1753	v-a Meg-Alt H rept	CEu
338. Ballota nigra L. 1753	a Meg H scap	PSs
339. Galeopsis speciosa Mill. 1768	a Mes-Mac T scap	CEu
340. Glechoma hederacea L. 1753	v-a Mes H rept	EAs
341. Glechoma hirsuta Waldst. & Kit. 1804	v-a Mes-Mac H rept	PSs
342. Lamium amplexiculae L. 1753	v Mi-Mes T scap	EAs
343. Lamium maculatum L. 1753	ver-a Mes-Mac H scap	CEu
344. Lamium purpureum L. 1753	v Mi-Mes T scap	CEu
345. Lycopus europaeus L. 1753	a Mes Meg H scap	EAs
346. Lycopus exaltatus L. 1782	a Mi-Mes H scap	EAs
347. Marrubium vulgare L. 1753	v-a Mes-Mac H scap	EAs
348. Mentha aquatica L. 1753	rhiz emer Hyd G	EAs
349. Mentha longifolia (L.) L. 1756	a Mes-Meg H scap	CEu
350. Mentha pulegium L. 1753	a Mi-Mes H scap	CEu
351. Prunella vulgaris L. 1753	a Mi-Mes H scap semiros	EAs
352. Salvia nemorosa L. 1753	a Mes-Meg H scap	PSs
353. Scutellaria hastifolia L. 1753	a Mes-Mac G rhiz	PSs
354. Stachys palustris L. 1753	a Mi-Mes H scap	Hol
Classis: Liliopsida		
Family: Butomaceae		
355. Butomus umbellatus L. 1753	a rhiz emer Hyd G	EAs
Family: Alismataceae		
356. Alisma plantago-aquatica L. 1753	a rhiz emer Hyd G	Cos
Family: Orchidaceae		
357. Spiranthes spiralis (L.) Chevall. 1827	aut Mi-Mac G tub	MSm
Family: Iridaceae		
358. Iris germanica L. 1753	v-a Mes-Mac G rhiz	MSm
359. Iris pseudoacorus L. 1753	rhiz emer Hyd G	CEu
Family: Hyacinthaceae		
360. Muscari racemosum (L.) Mill. 1768	v Mes-Mac G bulb	MSm
361. Ornithogalum umbellatum L. 1753	v Mes G bulb	CEu
Family: Amarayllidaceae		
362. Galanthus nivalis L. 1753	ver Mes G bulb	PSs
363. Leucojum aestivum L. 1759	v Mac G bulb	MSm
364. Leucojum vernum L. 1753	v Mac-Meg G bulb	CEu
365. Narcissus poeticus L. 1753	v Mes-Mac G bulb	Cul
Family: Convallariaceae		
366. Polygonatum odoratum (Mill.) Druce 1906	ver-a Mes-Mac G scap rhiz	EAs

Family: Ruscaceae		
367. Ruscus aculeatus L. 1753	v Mes-Meg fo semp Ch frut caesp/G rhiz	PSs
Family: Juncaceae		
368. Juncus bufonius L. 1753	a Mi-Mes G rhiz caesp	Cos
369. Juncus compressus Jacq. 1762	rhiz emer Hyd G	EAs
370. Juncus effusus L. 1753	a Mes G rhiz caesp	Cos
371. Juncus inflexus L. 1753	ver-a Mac-Alt G rhiz	Hol
Family: Cyperaceae		
372. Blysmus compressus (L.) Link 1827	a Mi-Mes G rhiz	EAs
373. Bolboschoenus maritimus (L.) Palla 1905	rhiz emer Hyd G	Cos
374. Carex acuta L. 1753	rhiz emer Hyd G	EAs
375. Carex distans L. 1759	a Mes-Meg G rhiz caesp	EAs
376. Carex divisa Hudson 1762	v-a Mes-Mac G rhiz	EAs
377. Carex elata All. 1785	v Mac-Meg H caesp	EAs
378. Carex flacca Schreb. 1771	v-a Mes-Mac G rhiz	EAs
379. Carex hirta L. 1753	a Mes-Meg G rhiz caesp	EAs
380. Carex hordeistichos Vill. 1787	v-a Mes-Mac He/G rhiz	CEu
381. Carex hostiana DC. 1813	a Mac H caesp	CEu
382. Carex leporina L. 1753	v-a Mac-Meg H caesp	Hol
383. Carex pendula Huds. 1762	v-a Mac-Meg G rhiz	MSm
384. Carex praecox Schreb. 1771	v Mi-Mes H scap	EAs
385. Carex pseudocyperus L. 1753	rhiz emer Hyd G	Hol
386. Carex riparia Curtis 1783	rhiz emer Hyd G	Hol
387. Carex spicata Hudson 1762	a Mes-Mac H caesp	CEu
388. Carex vulpina L. 1753	v-a Mac-Meg H caesp	EAs
389. Cyperus michelianus (L.) Delile 1813	rad emer Hyd T caesp	EAs
390. Eleocharis acicularis (L.) Roem. & Schult. 1817	rhiz emer Hyd G	Cos
391. Eleocharis palustris (L.) R. Br. 1810	rhiz emer Hyd G	Cos
392. Eleocharis uniglumis (Link) Schult. 1824	rhiz emer Hyd G	Cos
393. Schoenoplectus lacustris (L.) Palla 1888	rhiz emer Hyd G	EAs
394. Scirpoides holoschoenus (L.) Soják 1972	rhiz emer Hyd G	EAs
Family: Typhaceae		
395. Typha angustifolia L. 1753	rhiz emer Hyd G	Hol
Family: Poaceae		
396. Agrostis alba L. 1753	a Meg H caesp	EAs
397. Agrostis capillaris L. 1753	a Mes-Meg H caesp	Hol
398. Agrostis stolonifera L. 1753	v-a Mes-Mac H rept	EAs
399. Alopecurus myosuroides Huds. 1762	v-a Mes Mac T scap	EAs
400. Alopecurus pratensis L. 1753	v-a Meg-Alt H caesp	EAs
401. Anthoxanthum odoratum L. 1753	v-a Mac H caesp	EAs
402. Apera spica-venti (L.) P. Beauv. 1812	v-a Mac-Meg T scap	EAs
403. Arrhenatherum elatius (L.) J. Presl & C. Presl 1819	v-a Mac-Alt H caesp	CEu
404. Beckmania eruciformis (L.) Host. 1805	a Meg G rhiz	Hol
405. Bothriochloa ischaemum (L.) Keng. 1936	a Mac H – caesp	PSs
406. Brachypodium sylvaticum (Huds.) P. Beauv. 1812	a Mac H caesp	EAs
407. Bromus arvensis L. 1753	a Mes-Meg T scap	EAs
408. Bromus hordeaceus L. 1753	a MI-Meg T scap	MSm
409. Bromus inermis Leyss. 1761	a Meg-Alt H caesp	EAs
410. Bromus sterilis L. 1753	a Mes-Meg T caesp	EAs
411. Bromus tectorum L. 1753	v-a Mes T scap	EAs
412. Calamagrostis epigeios (L.) Roth 1788	a Meg-Alt H caesp	EAs
413. Catabrosa aquatica (L.) P. Beauv. 1812	rhiz emer Hyd G	Hol
414. Crypsis alopecuroides (Piller & Mitterp.) Schrad. 1806	a-aut Mi-Mac T scap	EAs
415. Cynodon dactylon (L.) Pers. 1805	a Mac G rhiz	Cos
416. Cynosurus cristatus L. 1753	a Mi-Mes T caesp	EAs
417. Dactylis glomerata L. 1753	a Meg H caesp	EAs
418. Echinochloa crus-gali (L.) P. Beauv. 1812	rhiz emer Hyd G	Cos

419. Eleusine indica (L.) Gaertn. 1788	a Mes T scap	MSm
420. Elymus repens (L.) Gould. 1974	v-a Meg G rhiz	EAs
421. Eragrostis cilianensis (All.) Janch. 1907	v-a Mes-Mac T scap	Cos
422. Festuca arundinacea Schreb. 1771	v-a Meg-Alt H caesp	EAs
423. Festuca pratensis Huds. 1762	v-a Meg-Alt H caesp	EAs
424. Holcus lanatus L. 1753	a Meg H caesp	EAs
425. Hordeum murinum L. 1753	v-a Mes T caesp	MSm
426. Lolium perenne L. 1753	a Mes H caesp	CEu
427. Pennisetum glaucum (L.) R. Br. 1810	a Mes-Meg T scap	Cos
428. Phalaris arundinacea L. 1753	rhiz emer Hyd G	Hol
429. Phleum pratense L. 1753	ver-a Mes-Meg H caesp	EAs
430. Phragmites australis (Cav) Steud. 1841	rhiz emer Hyd G	Cos
431. Poa annua L. 1753	v-aut Mi-Mes T caesp	EAs
432. Poa bulbosa L. 1753	a Mes-Meg H caesp	Hol
433. Poa nemoralis L. 1753	a Meg H caesp	Hol
434. Poa palustris L. 1753	v-a Mac-Alt H caesp	Hol
435. Poa pratensis L. 1753	a Mes-Meg H caesp	EAs
436. Poa trivialis L. 1753	a Mes-Meg H caesp	Cos
437. Setaria verticillata (L.) Beauv. 1812	a Mac-Meg T scap	Cos
438. Setaria viridis (L.) Beauv. 1812	a Mes-MacT scap	EAs
439. Sorghum halepense (L.) Pers. 1805	a Mac-Alt G rhiz	Cos
440. Vulpia ciliata Dumort. 1824	v Mi-Mac T caesp	MSm

Legend / **Life form**: a – summerly, ac – needle-leaved, alt – tall, aut – autumnal, bienn – biannual, bulb – bulbous, caesp – caespitose, Ch – Chamaephytes, dec – deciduous, fo – foliose, frut – fruticose, G – Geophytes, H – Hemicryptophytes, herb – herbaceous, lig – ligneous, Mac (Macro) – big/tall/long, Meg (Mega) – big, large, Mes (Meso) – intermediate, Mi (Micro) – small/low, N (nano) – dwarf, P – Phanerophytes, poik – poikilohydrous, pulv – pulvinate, rad – root-bud plant, rept – reptant, rhiz – rhizomatous, ros – rosulate, S – Scandentophytes, Sapr – Saprophytes, scap – scapose, Semipar – Semiparasitophytes, semiros – semirosulate, semp – evergreen, suffr – suffruticose, succ – succulent, T – therophytes, tub – tuberous, v – vernal; Areal type: AAl – Arcto-Alpian, Adv – Adventitious, Bor – Boreal, CEv – Central European, Cos – Cosmopolitan, EAs – Euroasian, Hol – Holarctic, MSm – Mediterranean-Submediterranean, PSs – Pontic-South Siberian, SEm – South European mountain

Taxonomic analysis

There was only one species – *Marsilea quadrifolia* that belongs to the phylum Polypodiophyta, and just two species from the phylum Equisetophyta. The rest of the taxa (437; 99.32%) from the total list were included in the most dominant phylum Magnoliophyta. Within this phylum, 350 taxa at species and subspecies level belong to class Magnoliopsida (79.59%), while class Liliopsida is represented with 87 taxa (19.76%).

According to the number of taxa, the most abundant angiosperm families were Asteraceae (51), Poaceae (49), Fabaceae (32), Brassicaceae (28), Rosaceae (25), Cyperaceae (19), Lamiaceae and Polygonaceae (18). This taxonomic structure is contributed to the principle of the increase in the species number with a reduction in the degree of latitude in the northern hemisphere (Turril, 1929; Anačkov, 2013) and significantly corresponds to the floristic taxonomic structure of Serbia. However, it is important to emphasize that many non-native invasive species belong to family Asteraceae such as – *Ambrosia artemisiifolia*,

Erigeron canadensis, *E. annuus*, *Solidago gigantea* etc. The second most abundant family was Poaceae, which is according to Alekhine (1944) characteristic for steppes. In past, Danube's riparian zone was in contact with surrounding steppe areas (Budak, 1986), which are nowadays almost completely urbanized or used for agriculture. The embankments proved to be most suitable for the growth of grasses. Influence of the temperate part of the Holoarctic, Central Europe and Eurasia is reflected in a great number of species grouped in families such as: Brassicaceae (28), Rosaceae (25), Cyperaceae (19), Ranunculaceae and Scrophulariaceae (16).

Genera with the most species were *Carex* (15), *Rumex* (10) and *Euphorbia* (8), followed by *Geranium* and *Ranunculus* with seven, and a majority of these taxa are among the species-abundant genera in the taxonomic spectrum of the flora of Serbia (Stevanović et al., 1995).

Phytogeographical analysis

The phytogeographical analysis included a grouping of floral elements into nine areal types followed by an analysis of areal type spectrum (Figure 1). Results of this analysis showed domination of Eurasian areal type (42%), which includes species widespread in most of the Eurasian continent, indicating their wide ecological valence. Exceptional presence of Eurasian species favors the fact of vegetation succession in investigated area, caused by the human activities such as deforestation, flood protection measures, pollution and the pressure of non-native species. Due to the considerable terrain alternation, perfect conditions for colonization of wide-dispersed species were created. Central European areal type is the second most abundant in the context of the investigated area (14%). Since the taxa of this areal type are mostly related to deciduous forests of a temperate zone, relatively high number of Central European species can be explained by geographical location and similar ecological conditions prevailing in the natural willow-poplar and poplar clone flooded forests of the Danube's shoreline. A greater part of this areal type is represented by taxa belonging to the sub-Central European floral element. Pontic-Southsiberian areal type is represented with 53 taxa (12%), mostly belonging to Subpontic and Pontic-Pannonian floral element. Given the climate and biogeography nature of the investigated region, these results were expected, but also indicate the remaining of steppe forests and steppe areas, characteristic for this region in the past (Gajić, 1984).

More or less evenly represented areal types are Holarctic (7%), Cosmopolitan (6%) and Mediterranean (8%), the last being the only representative of Sub Mediterranean areal group. These results pointed out a similar floristic influence of the southern and northern parts of Europe on the investigated area. Small numbers of sub-Atlantic – 4 and Boreal – 1 species were expected due to the geographical location and limited presence of suitable habitats for these plants within the investigated area.

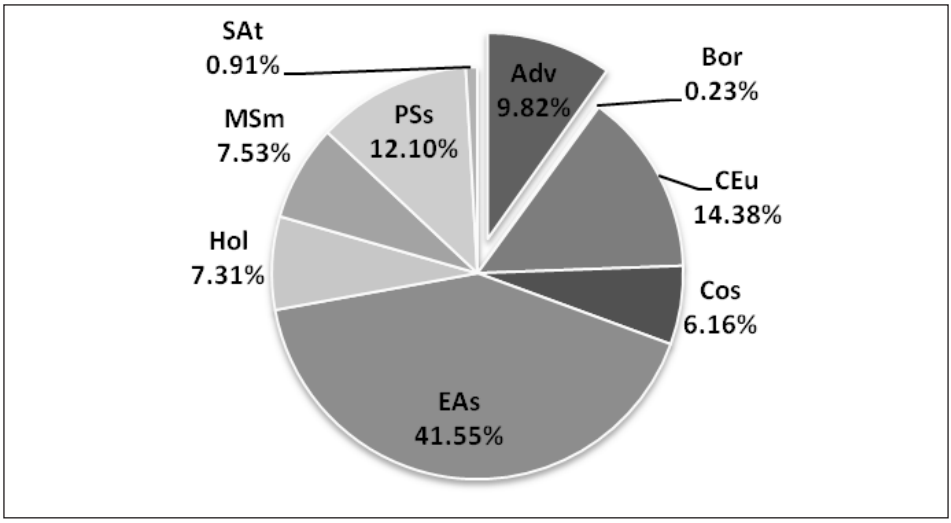

Figure 1. Areal types spectrum of the flora of Danube's shoreline from Čerević to Čortanovci

Legend: Adv – Adventive, Bor – Boreal, CEu – Central European, Cos – Cosmopolitan, EAs – Eurasian, Hol – Holarctic, MSm – Mediterranean-Sub Mediterranean, PSs – Pontic-South Siberian, SAt – Sub-Atlantic

Special attention was given to non-native (adventive) plants, which were represented by a significant percentage (10%), while 24 of these species showed invasive character (Table 2). Invasive species are generally characterized as naturalized in new habitats and produce abundant offspring with high dispersal ability (Pyšek and Prach, 1993; Richardson, 2000, 2007). A high prevalence of these plants can be explained by a combination of different factors, where the most important could be human impact and constant influence on natural habitats. For example, replacement of natural willow-poplar flooded forests with clone poplar plantations enabled the spreading of non-native plants, such as indigo bush (*Amorpha fruticosa*) in these man-made habitats (Pedashenko, 2012). Also, very important factor in dispersal and establishment of these plants is the Danube itself. Generally, large international river systems enable the introduction of propagules from a different region into a new one, where they can colonize new habitats, which are outside their natural range. (Pyšek and Prach, 1993; Hood & Naiman, 2000; Richradson, 2007: Schnitzler, 2007; Pedashenko, 2012). Additionally, a process of fluvial erosion-deposition provides new habitats for plant colonization, with a high nutrient content of freshly deposited sediments, while hydrology influences the vegetation through floods, droughts, and water table fluctuations (Schnitzler, 2007; Richardson, 2007). Plant invasions are amplified directly or indirectly by many ways of human-mediated disturbances to rivers and riparian zones (Richardson, 2007).

Table 2. Invasive alien plants of investigated area

Family	Species	Invasiveness
Aceraceae	*Acer negundo* L.	Highly invasive
Asteraceae	*Ambrosia artemisiifolia* L.	Highly invasive
Fabaceae	*Amorpha fruticosa* L.	Highly invasive
Asclepiadaceae	*Asclepias syriaca* L.	Highly invasive
Cucurbitaceae	*Echinocystis lobata* (Michx.) Torr. & A.Gray	Highly invasive
Asteraceae	*Erigeron canadensis* (L.) Cronq. 1943	Highly invasive
Asteraceae	*Erigeron annuus* (L.) Pers.	Highly invasive
Polygonaceae	*Reynoutria japonica* Houtt.	Highly invasive
Fabaceae	*Robinia pseudoacacia* L.	Highly invasive
Asteraceae	*Solidago gigantea* Aiton.	Highly invasive
Brassicaceae	*Armoracia rusticana* P. Gaertn., B. Mey. & Scherb.	Sporadically invasive
Oleaceae	*Fraxinus americana* L.	Sporadically invasive
Oleaceae	*Fraxinus pennsylvanica* Marh.	Sporadically invasive
Onagraceae	*Oenothera biennis* L.	Sporadically invasive
Phytolaccaceae	*Phytolacca americana* L.	Sporadically invasive
Asteraceae	*Solidago canadensis* L.	Sporadically invasive
Vitaceae	*Vitis vulpina* L.	Sporadically invasive
Asteraceae	*Xanthium italicum* Moretti.	Sporadically invasive
Amaranthaceae	*Amaranthus retroflexus* L.	Potentially invasive
Oxalidaceae	*Oxalis stricta* L.	Potentially invasive
Asteraceae	*Symphyotrichum* × *salignum* (Willd.) G.L.Nesom	Potentially invasive
Asteraceae	*Symphyotrichum tradescantii* (L.) G.L.Nesom.	Potentially invasive
Scrophulariaceae	*Veronica persica* Poir.	Potentially invasive
Asteraceae	*Xhantium strumarium* L.	Potentially invasive

Life-form spectrum

Recorded species of the investigated area were grouped into eight types of life forms, seven basic and one transitional. The analysis of biological spectrum showed (Figure 2) domination of hemicryptophytes – H (40%) which is in accordance with the biological spectrum of Serbia (Diklić, 1984). Therophytes – T (27%) are the second most abundant group followed by a transitional type of terophytes/hemicryptophytes – T/H (3%). Considering the fact that these species are predominantly xerophytic nature, general conditions of embankment itself are extremely favorable for their appearance. Sandy substrate makes the soil on the embankment significantly permeable to water, which makes it suitable for the development of therophytes; a fluctuation of the Danube's water level, as well as the ephemeral character of numerous investigated habitats, favor the plants with a short life cycle. A large difference between the biological spectrum of investigated area and Serbia was reflected in phanerophytes' percent. In Serbia, trees and large shrubs make 2.5% only, while in the studied area, they are quite present with 11%. This disproportion

can be explained with types of natural habitats, typical for the Danube's shoreline. The Danube as a lowland river is marked by natural gallery forests (*Salix alba*, *S. triandra*, *Populus alba*), mixed poplar forests (*P. alba* and *P. nigra*) and riparian mixed forests (*Quercus robur*, *Ulmus laevis*, *U. campestre* and *Fraxinus angustifolia*) (Parabućski, 1965; Gajić, 1984). These natural forests were eventually substituted by man-made poplar clone plantations and agricultural areas. Another important reason for this disproportion is a presence of numerous non-native phanerophytes (*Acer negundo*, *Aillanthus altissima*, *Fraxinus americana*, *Morus alba* etc). Generally, Geophytes – G (10%) are mainly associated with dry habitats in Serbia, and in the case of the Danube's coastal zone, embankment can be seen as corresponding/suitable one. Even though there is a suitable dry habitat for them, within floodplains and flooded forests there can only be found geophytes specialized for and marshy habitats (i.e. *Leucojum aestivum*). Hydrophytes (6%) were represented with helophytic species (i.e. *Ranunculus trichophyllus*, *Limosella aquatica*, *Butomus umbellatus*) which indicates the moisture of the substrate, frequency, and intensity of flooding within the investigated area. Although submerged and floating plants were not included in this research, we can assume that the general number is certainly higher. Scadentophytes (2%) and chamaephytes (1%) occurred in very small numbers. Despite the general richness of scadentophyta along the banks of major rivers, human alteration of natural habitats resulted in a decrease of phanerophytes, followed by a reduction in scadentophytes. However, it is important to emphasize the abundance of wild grape (*Vitis longii*) at most visited sites with developed shrub floor and presence of alien wines (*Parthenocissus quinquefolia*, *Echinocystis lobata*).

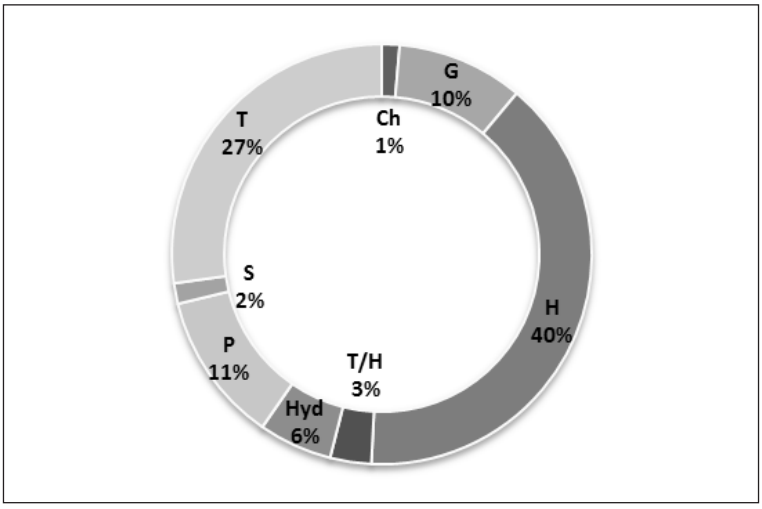

Figure 2. Life-form spectrum of the flora of Danube's shoreline from Čerević to Čortanovci

Legend: H – Hemicryptophytes, T/H – terophytes/hemicryptophytes, Hyd – Hydrophytes, P – Phanerophytes, S –Scadentophytes, T – Therophytes, Ch – Chamaephytes, G – Geophytes

Natural flooded forests, floodplains and meadows of the Danube's coastal zone from Čerević to Čortanovci are fragmented and under direct or indirect anthropogenic influence. Human activities represent a threatening factor due to the presence of heavy industry, waste water, illegal dumpsites and abandoned weekend resorts. Perhaps the most disturbing factor is poplar plantations, which are planted in large number along Danube's coastal belt, suppressing the natural floodplain forests. Additional threats to the indigenous flora are invasive alien species. The investigated region represents a very suitable area for a development and spreading of these plants, which are able to suppress native plant species from their natural habitats.

CONCLUSION

Based on the field work during 2014 and 2015 and literature data, 440 taxa of vascular plants at the level of species and subspecies were registered for the Danube's shoreline from Čerević to Čortanovci. The most species abundant families were Asteraceae, Poaceae and Fabaceae while the richest genera were *Carex, Rumex* and *Euphorbia*. Since a majority of investigated area is under direct or indirect human influence, it is important to emphasize the dominance of Eurasian and Central European areal type, especially in contrast to the cosmopolitan, Holarctic and adventitious species, which may indicate that native plants are retaining continuity of the established communities, thus resisting the pressure of the invasive alien species. The most prevalent life forms in this area are hemicryptophytes, therophytes and phanerophytes.

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ФЛОРИСТИЧКА АНАЛИЗА ОБАЛСКОГ ПОЈАСА ДУНАВА НА ПОТЕЗУ ЧЕРЕВИЋ–ЧОРТАНОВЦИ

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РЕЗИМЕ: С обзиром на дугу и непрекидну историју људских насеља на територији Новог Сада, утицај човека на обалску конфигурацију Дунава овог подручја је значајан. На основу литературних података и резултата двогодишњег теренског истраживања флоре обалског подручја Дунава на потезу Черевић–Чортановци, забележено је 440 биљних таксона, у оквиру 244 рода и 68 фамилија. Фамилије с највећим бројем таксона су: Asteraceae (51), Poaceae (49), Fabaceae (32), и Brassicaceae

(28), а родови најбројнији врстама су: *Carex* (15), *Rumex* (10), *Euphorbia* (8). У спектру ареал типова највише таксона припада евроазијском (42%), средњоевропском (14%) и понтско-јужносибирском ареалу (12%). Анализа биолошког спектра показује да су на испитиваном подручју најзаступљеније хемикриптофите (40%), терофите (27%) и фанерофите (11%).

КЉУЧНЕ РЕЧИ: инвазивне биљне врсте, Панонска низија, рипаријална вегетација

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ANTIOXIDANT POTENTIAL OF RAGWEEDS: *Ambrosia artemisiifolia, A. trifida* AND *Iva xanthifolia*

ABSTRACT: The purpose of this study was to analyze antioxidant systems among three invasive ragweed species, *Ambrosia artemisiifolia* L., *A. trifida* L. and *Iva xanthifolia* Nutt. Antioxidant capacity could be a possible marker of adaptation to variable environmental conditions, since change in amount of antioxidants represents one of the first responses to various environmental stimuli. Among investigated ragweeds, *I. xanthifolia* leaves had more pronounced guaiacol peroxidase activity (87.5 and 62.5%) and reduced glutathione content (2.3 and 28.8%) than *A. artemisiifolia and A. trifida*, respectively. However, superoxide dismutase activity was invariable in all investigated plants (234.1–247.5 U g^{-1} fresh weight). The highest content of total phenolics, tannins, flavonoids and proanthocyanidins were detected in *A. trifida* leaves (up to 3.7 – fold the amount of the others). According to antioxidant activity tests, investigated ragweed species could be presented in a scale: *A. trifida* > *I. xanthifolia* > *A. artemisiifolia*. Accumulation of non-enzymatic antioxidants and lower content of reduced glutathione point to different oxidative stress avoidance strategies of *A. trifida* when compared to *A. artemisiifolia* and *I. xanthifolia* within the same environmental conditions.

KEYWORDS: antioxidants, phenolics, ragweed, secondary metabolism

INTRODUCTION

Among *Ambrosia* species, only *A. maritima* L. is native to Europe, while others, such as: *A. artemisiifolia* L. (*Ambrosia elatior* L.), *A. trifida* L., *A. tenuifolia* Spreng. and *A. psilostachya* DC. (*A. coronopifolia* Torr. & Gray) are native to North America and were introduced to Europe (Smith et al., 2013). In the recent years, relatively reduced crop rotation, shallow tillage, inadequate

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pre-sowing cultivation and use of the same or identical herbicide groups enabled spread and domination of *A. artemissifolia* and *Iva xanthifolia* Nutt. in the Northern Serbia (Konstantinovic et al., 2006). *Ambrosia artemisiifolia* L. (Common ragweed), *A. trifida* L. (Giant ragweed) and *Iva xanthifolia* Nutt. (False ragweed) represent serious allergenic and agricultural weed in Vojvodina province. Higher prevalence of competitively advantageous traits (Matzek, 2012) and greater phenotypic plasticity that permits the species to survive the colonization period and to spread within a broad range of environments (Davidson et al., 2011) are main characteristics of functional traits which ensures the process of alien species becoming invasive. During invasive processes, native and alien species compete for the same resources within the ecosystem by means of the twists and turns in fortune that result from different environmental stresses (Pintó-Marijuan and Munné-Bosch, 2013). The same authors stated that the combination of reproductive success with high stress tolerance (through osmotic adjustment and antioxidants) is essential for invasion success, particularly in stressful environments in the frame of global change. Photosynthesis, photorespiration, and respiration processes are sources of reactive oxygen species (ROS) (Queval and Foyer, 2012). Although early research involving ROS metabolism focused on the potential toxicity of ROS and the different ROS-scavenging mechanisms, more recent studies have focused on the role ROS play as signaling molecules. To utilize ROS as signaling molecules, non-toxic levels must be maintained in a delicate balancing act between ROS production, involving ROS-producing enzymes and the unavoidable production of ROS during basic cellular processes, and the metabolic counter-process involving ROS-scavenging pathways (Mittler et al., 2004). The toxic effect of ROS is suppressed by a strong antioxidant system consisting of antioxidant enzymes (superoxide dismutase, catalase, peroxidases, glutathione reductase, etc.) and non-enzymatic components (proteins and peptides, phenolics, carotenoids, etc.) (Halliwell and Gutteridge, 2007). Some authors (Davis and Swanson, 2001; Lu et al., 2007) proposed that monitoring antioxidant system of weeds could provide an innovative approach to control their spread by overcoming its inherent resistence to environmental stresses. Identifying the genes that regulate some of the enzymes involved in regulating oxidative stress and finding ways to interfere with gene regulation may prove to be an economically viable way to control persistent perennial weeds in inaccessible locations or along waterways where the use of herbicides is undesirable (Davis and Swanson, 2001).

The purpose of this study was to determine possible differences in antioxidant contents and antioxidant ability among populations of ragweed species in Vojvodina province. Also, this would be the first report on comparison of antioxidant capacity of these important weeds and allergenic plant species. Information about these biochemical traits could provide useful information for models that predict establishment and spread of ragweed species in the same environmental conditions.

MATERIAL AND METHODS

Plant material

Plant material for this research represented plants from population of *Ambrosia artemisiifolia* L.*, Ambrosia trifida* L. and *Iva xanthifolia* Nutt. (number of plants per species, n=40). Plants were collected in June/July (2007 and 2017), in the area of Despotovo, South Backa District in the autonomous province of Vojvodina, Serbia (coordinates: N45°26.9', E19°31'). Semiarid conditions with dry, hot spring and summer, neutral autumn and moderately cold winter characterize this location (Figure 1). Plants were harvested by hand. A part of fresh collected leaves were immediately frozen in liquid nitrogen and the others were dried in a shaded and well-ventilated place.

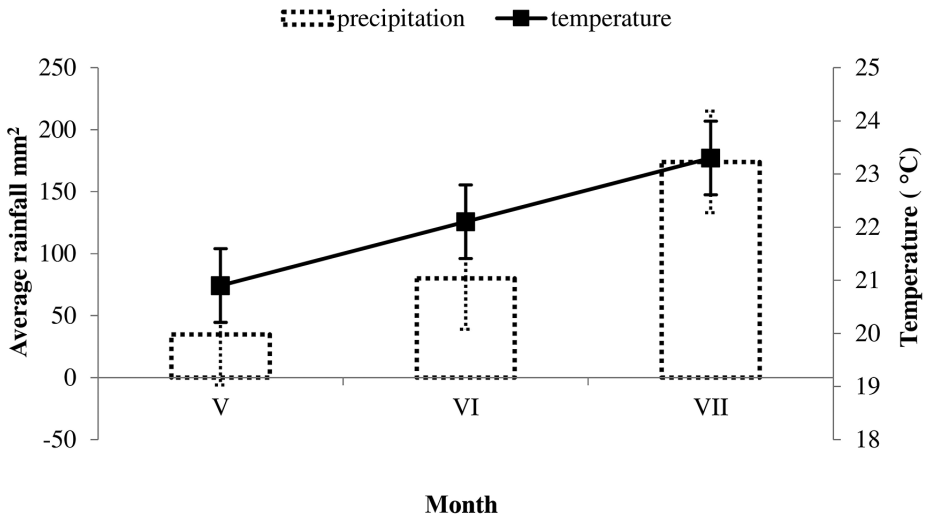

Figure 1. Average monthly air temperatures [AT (°C)] and precipitation [AP (mm)] sampling site in May, June, and July (2007–2017)

Preparation of extracts for biochemical analyses

One g of fresh leaves was ground in liquid nitrogen with cooled mortar and pestle and then homogenized with 10 ml of phosphate buffer solution (0.1M K_2HPO_4, pH 7.0). After centrifugation of 15,000 g for 10 min at 4 °C aliquots of the supernatant were used for measurements of antioxidant enzymes, reduced glutathione (GSH), superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical ($^{\cdot}OH$) scavenging tests. Dried leaves were ground to a fine powder in cooled mortar and pestle. Total phenolics, tannins, proanthocyanidins and DPPH-radical

scavenging activity were determined in 70% aqueous acetone extracts, and total flavonoids in MeOH : H_2O : CH_3COOH (140:50:10) extracts (1/50, w/V). The extracts were rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated until assayed.

Enzymatic antioxidant system and reduced glutathione (GSH) analyses

Biochemical analyses, quantification of investigated compounds and antioxidant ability of ragweed leaves extracts were performed using spectrophotometer UV/Visible Evolution 220 (Thermo Scientific, San Jose, USA). Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured by monitoring the inhibition of nitroblue tetrazolioum (NBT) reduction at 560 nm (Mandal et al., 2008). Peroxidase (EC 1.11.1.7) activity was measured using guaiacol (guaiacol peroxidase; GPX) as substrate according to Morkunas and Gmerek (2007). SOD and GPX activities were expressed as U g^{-1} fresh weight (U g^{-1} fw). Reduced glutathione (GSH) was determined according to Sedlak & Lindsay (1968) and expressed as μmol GSH g^{-1} fw.

Determination of total contents of phenolic compounds

Total phenolic content was determined by Folin-Ciocalteu method (Makkar, 2003). Total tannin content was determined by the Folin-Ciocalteu procedure, after removal of tannins by adsorption on an insoluble matrix (polyvinylpolypyrrolidone, PVPP). Calculated values were subtracted from total phenolic contents and both contents are expressed as gallic acid equivalents (GAE) in mg g^{-1} dry weight (dw) of leaves. Determination of total flavonoids content was performed according to Pękal and Pyrzynska (2014) with slight modifications. The amount of flavonoids was calculated as a rutin equivalent from the calibration curve of rutin standard solutions, and expressed as mg rutin g^{-1} dry weight (dw) of leaves. Proanthocyanidins were determined by a butanol-HCl assay (Makkar, 2003). Proanthocyanidins contents were expressed as mg leucoanthocyanidin g^{-1} dry weight (dw) of leaves.

Antioxidant activity tests

Superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical ($^{\cdot}OH$) scavenging tests represent assessment of total antioxidant activity (enzymatic and non-enzymatic) of fresh plant material extracts, i.e. ability of plant extracts to efficiently remove these ROS. The assay for superoxide anion ($O_2^{\cdot-}$) scavenging activity was based on a riboflavin-light-NBT system (Ahmed et al., 2013). Ascorbic acid was used as standard. Hydroxyl radical ($^{\cdot}OH$) scavenging activity of extracts was assayed

by the method of Sánchez-Moreno (2002). Total potential non-enzymatic antioxidant activity of investigated acetone extracts was assessed based on their scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals (Panda, 2012). All antioxidant activity tests were given as % of neutralized radicals.

Statistical analysis

All extraction procedures were performed in five replicas and measurements of the biochemical parameters were performed in triplicates and expressed as means ± standard error. Differences in analyzed biochemical parameters among investigated ragweed species were tested by ANOVA followed by comparisons of means by the Duncan test ($P<0.05$). To discover natural groupings of ragweed species, cluster analysis was done with Unweighted pair-group average analysis using Euclidean distance. All statistical analyses were performed using STATISTICA for Windows version 13.0.

RESULTS

Activity of SOD was invariable in all three ragweed species (76.1 U g^{-1} fw, on average). However, activity of GPx was markedly higher in *I. xanthifolia* leaves (2.5– to 9.7–fold) in comparison to *Ambrosia* species (Figure 2A). Also, *A. trifida* leaves had significantly higher GPx activity than *A. artemisiifolia* (26%). *A. artemisiifolia* and *I. xanthifolia* leaves had similar GSH content (13.05 μmol g^{-1} fw), while *A. trifida* had 1.3-fold lower GSH concentrations (Figure 2B).

Total phenolic compounds ranged from 30.0 to 111.1 mg GAE g^{-1} dw. *A. trifida* had pronounced contents of all measured phenolic compounds: total phenolics, tannins, flavonoids and proanthocyanidins in comparison to other two ragweed species, which did not differ only for total flavonoids and proanthocyanidins contents (Figure 2C). As for antioxidant activities, there is no difference in superoxide anion scavenging capacity between ragweed species, however, *A. trifida* leaves extracts had markedly higher hydroxyl and DPPH radical scavenging abilities than *A. artemisiifolia* and *I. xanthifolia* (on average, 1.0 – fold and 1.6 – fold, respectively) (Figure 2D). According to antioxidant activity tests, investigated ragweed species could be presented in a scale: *A. trifida* > *I. xanthifolia* > *A. artemisiifolia.* Despite the difference in genus, it can be clearly distinguished that *A. trifida* and *I. xanthifolia* can be separated from *A. aretmisiifolia* according to scavenging capacity.

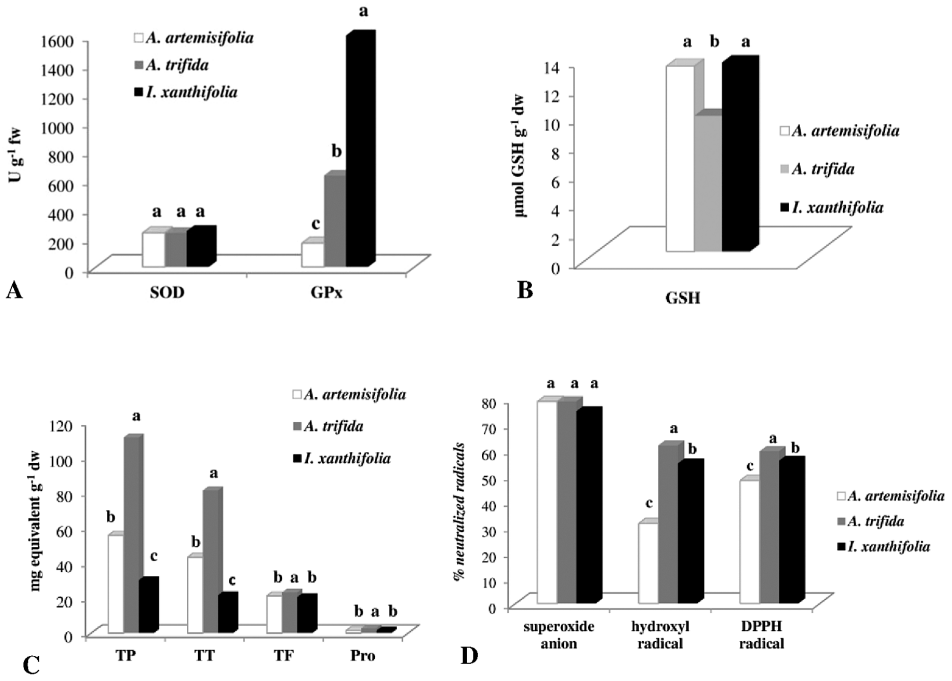

Figure 2. A – activity of antioxidant enzymes (A: SOD-superoxide dismutase, GPx – guaiacol peroxidase), B – reduced glutathione content (GSH), C – polyphenolics content (TP – total phenolics, TT – total tannin, TF – total flavonoids, Pro – proanthocyanidins) and D – antioxidant activities of ragweed leaves. Results marked with different letters differ significantly at $P<0.05$ (Duncan's test).

DISCUSSION

Antioxidant enzymes, total phenolics and total antioxidant capacity are some of the parameters that were employed in the research on the ecophysyological traits that are considered when it comes to invasive species (Pintó-Marijuan and Munné-Bosch, 2013). Deng et al. (2010) showed that during various environmental stresses including heat, cold, drought and flooding, activities of superoxide dismutase and guaiacol peroxidase increased in response to all stresses, while glutathione contents and anti-superoxide anion activities decreased in Common ragweed. However, Qin et al. (2013) showed that under high irradiance levels, *A. artemisiifolia* was able to scavenge oxygen radicals more efficiently by enhancing catalase and glutathione reductase activity and accumulating polyphenolics content, while superoxide dismutase activity was not greatly enhanced. Our results showed that *I. xanthifolia* had more enhanced enzymatic antioxidant system in comparison to *Ambrosia* spe-

cies, which points to a different response of their antioxidant systems when exposed to the same environmental conditions.

Environmental stresses selectively induce primary, as well as secondary, metabolic activities that are directly and indirectly involved in the accumulation of phenolic compounds (Cheynier et al., 2013). Phenylalanine ammonia lyase (PAL, EC 4.3.1.5) is the entry-point enzyme into the phenylpropanoid pathway responsible for synthesis of plant phenylpropanoids or phenolics, many of which play important roles in plant defense and present important non-enzymatic antioxidants (Gerasimova et al., 2005). Polyphenols and flavonoids suppress ROS formation by inhibiting enzymes and chelating trace elements involved in free-radical production, scavenging reactive species and up-regulating or protecting antioxidant defenses (Grassmann et al., 2002). Mueller et al. (2008) proved that *A. trifida* had significantly higher total phenolics content in comparison to *A. artemisiifolia* when treated with herbicide, however, our results showed that *A. trifida* had markedly high phenolics content constitutively.

Analyzed ragweed species occur primarily in ruderal habitats; whereas *I. xanthiifolia* and *A. artemisiifolia* had also increasingly invaded arable fields (Konstantinovic et al., 2006; Follak et al., 2013; Smith et al., 2013). When comparing these three species, Pajevic et al. (2010) concluded that *A. artemisiifolia* has the highest physiological potential (photosynthetic potential) which gives it significant advantage in colonization to new localities.

Results of performed antioxidant tests showed that *A. trifida* excelled in scavenging hydroxyl- and DPPH-radicals, which can be in correlation to high phenolics content. Accumulation of phenolic compounds is in significant correlation with high antioxidant capacity (Jacobo-Velázquez and Cisneros-Zevallos, 2009). The key for wide and rapid dispersal of ragweed species is due to the diversified resistant mechanism to various environmental stresses and ability to acclimatize itself to different habitats (Deng et al, 2010). However, *A. trifida* has a relatively low fecundity, a transient seed-bank and a high percentage of non-viable or low-survivorship seeds (Harrison et al., 2007), features which may have constrained its establishment and spread. Giant ragweed has remained largely restricted to ruderal habitats, which might be one of the reasons for its slow spread in Central and Eastern Europe (Follak et al., 2013). Outside of this region, *A. trifida* occurs in several (semi-) natural habitats (Lee et al., 2010). As for *A. artemisiifolia* and *I. xanthifolia*, they have both undergone a habitat shift and expansion during their invasion, which may have contributed to their more extensive colonization of Central and Eastern Europe (Follak et al., 2013; Essl et al., 2009).

We could conclude that *A. trifida* had the highest antioxidant ability of all investigated ragweed species. Accumulation of non-enzymatic antioxidants in leaves of these plants could be the mechanism of adaptation to ruderal habitat and compensation of slow spread with higher ability to endure in unfavorable conditions. Furthermore, activation of enzymatic antioxidant system and higher content of reduced glutathione in leaves of *A. artemisiifolia* and *I. xanthifoilia*, point to different oxidative stress avoidance strategies of these ragweed species when compared to *A. trifida*. Further research on correlation

among antioxidants contents and antioxidant ability of these weed species and their distribution could be of great importance to modeling experts to design accurate models for the prediction of future invasions of ragweed in similar environmental conditions.

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АНТИОКСИДАНТНИ ПОТЕНЦИЈАЛ КОРОВА: *Ambrosia artemisiifolia, A. trifida* И *Iva xanthifolia*

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РЕЗИМЕ: Сврха ове студије била је анализа антиоксидантих система три инвазивне врсте: *Ambrosia artemisiifolia* L., *A. trifida* L. и *Iva xanthifolia* Nutt. Антиоксидантни капацитет може бити потенцијални маркер адаптације на промењиве услове околине, јер промена у количини антиоксиданата представља један од првих одговора на различите еколошке факторе. Међу испитиваним коровима, листови *I. xanthifolia* имали су израженију активност гвајакол пероксидазе (87,5 и 62,5%) и садржај редукованог глутатиона (2,3 и 28,8%) у односу на *A. artemisiifolia* и *A. trifida*, респективно. Међутим, активност супероксид дисмутазе била је непроменљива у свим испитиваним биљкама (234,1–247,5 U g^{-1} свеже масе). Највећи садржај укупних фенола, танина, флавоноида и проантоцианидина детектован је у листовима *A. trifida* (до 3,7 пута више од осталих). Према испитиваним антиоксидантним активностима, дате врсте могу бити представљене скалом: *A. trifida* > *I. xanthifolia* > *A. artemisiifolia*. Акумулација неензимских антиоксиданата и мањи садржај редукованог глутатиона указују на различите стратегије избегавања оксидативног стреса код *A. trifida* у поређењу са осталим испитиваним коровима у истим условима животне средине.

КЉУЧНЕ РЕЧИ: антиоксиданти, феноли, амброзија, секундарни метаболизам

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SEROPREVALENCE OF PORCINE CIRCOVIRUS TYPE 2 INFECTION IN THE AREA OF VOJVODINA

ABSTRACT**:** The aim of this research was to determine seroprevalence of infection caused by PCV2 in different categories of pigs on the territory of Vojvodina. The research was conducted on 6 pig farms placed in different locations on the territory of Vojvodina (two farms from the territory of Bačka, Banat and Srem, respectively), with the capacity from 500 to 2,500 sows with intensive management, closed type. Blood samples from brachiocephalic plexus were collected from 540 pigs in three different categories, in suckling piglets between 3 and 4 weeks of age, in weaned piglets from 8 to 9 weeks of age, and in fattening pigs between 20 and 22 weeks of age. Specific anti-PCV2 antibodies are determined by using indirect ELISA. Based on our results, we conclude that PCV2 infections are widely spread on the territory of Vojvodina, with total seroprevalence of 74.8%. The highest value of seroprevalence is determined in category of fattening pigs 86%, in suckling piglets this was 74.5%, while in weaned piglets seroprevalence was 65.3%.

KEYWORDS**:** antibody, PCV2, pig, seroprevalence

INTRODUCTION

Circovirus infection of pigs is occupying a large number of world researchers today. Porcine circovirus type 2 (PCV2) is one of the most common pathogens in pig population worldwide, causing significant economic losses in commercial production. It belongs to the *Circoviridae* family and the *Circovirus* genus. Based on previous scientific information, for PCV2 virus-infected pigs there is no unique and final definition of the clinical picture and the disease because all of their most important virulence factors are not yet fully known and analyzed. PCV associated diseases have many different clinical manifestations.

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The most common are systemic diseases – post weaning multisystemic wasting syndrome (PMWS) and porcine dermatitis nephropathy syndrome (PDNS) (Segales, 2007). Beside these, PCV2 is associated with reproductive, respiratory and enteric disorders. After original discovery in 1974, PCV seroprevalence was determined in Germany (Tischer et al., 1986), Canada (Dulac and Afshar, 1989), England (Edwards and Sands, 1994) and the USA (Hines and Lukert, 1995). This initial study showed that PCV2 is widespread in pig population in these countries. Regarding PMWS presence today, European countries can be divided into 5 different categories (Segales, 2007). The Republic of Serbia belongs to countries that have reported presence of PMWS, but there is no sufficient data to establish the importance of the disease in their pig populations (Stevančević et al., 2014; Savić et al., 2012). The aim of this research was to determine seroprevalence of infection caused by PCV2 in different categories of pigs on the territory of the province of Vojvodina.

MATERIAL AND METHODS

Experimental animals

The research was conducted on 6 pig farms placed in different locations on the territory of Vojvodina (two farms from the territory of Bačka, Banat, and Srem, respectively) and ranging in the capacity from 500 to 2,500 sows, with intensive management and 'farrow to finish' type of production. Blood samples from brachiocephalic plexus were collected from 540 pigs in three different regions (Table 1).

Table 1. Number of samples by region

Region	Number of samples
Bačka	240
Banat	160
Srem	140
Total	540

Blood samples were collected from three different pig categories (Table 2). Tested animals were not vaccinated against PCV2.

Table 2. Number of samples by category

Pig category	Number of samples	Week of age
Suckling piglets	220	3–4
Weaned piglets	170	8–9
Fattening pigs	150	20–22
Total	540	

Accommodation and feed at all six tested farms were in line with the conditions of good animal practice and commercial pig production. Piglets are weaned at 28 days of life, from day 5 they start consuming concentrated food for pre-weaned and early weaned piglets. After 28 days of lactation, sows are transferred to service stables, and piglets are transferred to nurseries with cage fostering. Piglets stay in nurseries until 75 days of life, and then are transferred to growers/finishers stables where they remain until 6 months of age. Breeding pigs are transferred to reproduction center, while the rest of the animals stay in the fattening section until they reach slaughter weight.

Gilts and sows have no contact before transferring to farrowing sections. During farrowing and lactation, gilts and sows are held in farrowing crates, fed twice a day, and have free access to water. In each farrowing pen there is a heater, which provides adequate temperature for piglets (33–35 °C).

Blood sampling

Blood sampling was performed on 540 pigs in order to obtain blood serum and determine PCV2 antibodies. Blood sampling in suckling piglets was carried out between 3 and 4 weeks of age, in weaned piglets between 8 and 9 weeks of age, and in fattening pigs between 20 and 22 weeks of age. Sampling was made by brachiocephalic plexus punction. Blood samples were collected in the amount of 9 mL in vacutainers with coagulation activators and transported in hand cooler to the laboratory. Blood serum was collected after coagulation and centrifugation. These serum samples were stored at -20 ºC until being processed.

Determination of PCV2 antibodies using indirect ELISA method

Specific PCV2 antibodies were determined using indirect immunoenzyme test – INGEZIM CIRCO IgG (Ingenasa, Spain). Performing of the ELISA assay and interpretation of the results were made according to manufacturer's instructions.

RESULTS

From the total number of samples from all three categories of pigs from the territory of Bačka, a positive result was obtained in 77.5% of the tested samples. The highest percentage of 86.3% of positive animals was in the category of fattening pigs and the lowest 70.9% in weaned piglets (Table 3).

Table 3. Seroprevalence of PCV2 in different pig categories in the territory of Bačka

Pig category	Negative	Positive	Total
Suckling piglets	21 (23.8%)	67 (76.2%)	88 (100%)
Weaned piglets	23 (29.1%)	56 (70.9%)	79 (100%)
Fattening pigs	10 (13.7%)	63 (86.3%)	73 (100%)
Total	54 (22.5%)	186 (77.5%)	240 (100%)

Total percentage of positive animals in all three categories was 78.1% on the territory of Banat. Among these animals, the highest percentage (90.7%) of positive samples was in the category of fattening pigs and the lowest (64%) in the category of weaned piglets (Table 4).

Table 4. Seroprevalence of PCV2 in different pig categories in the territory of Banat

Pig category	Negative	Positive	Total
Suckling piglets	13 (19.4%)	54 (80.6%)	67 (100%)
Weaned piglets	18 (36.0%)	32 (64.0%)	50 (100%)
Fattening pigs	4 (9.3%)	39 (90.7%)	43 (100%)
Total	35 (21.9%)	125 (78.1%)	160 (100%)

The percentage of positive animals in the tested samples from the territory of Srem was 66.4%, while the highest number of positive animals was in the category of fattening pigs with 79.4% (Table 5).

Table 5. Seroprevalence of PCV2 in different pig categories in the territory of Srem

Pig category	Negative	Positive	Total
Suckling piglets	22 (33.8%)	43 (66.2%)	65 (100%)
Weaned piglets	18 (43.9%)	23 (56.1%)	41 (100%)
Fattening pigs	7 (20.6%)	27 (79.4%)	34 (100%)
Total	47 (33.6%)	93 (66.4%)	140 (100%)

The percentage of positive animals in all 540 samples collected on the territory of the Vojvodina Province is 74.8%. The highest percentage of positive animals was in the category of fattener pigs (86.0%) and the lowest in the category of weaned piglets (65.3%), while there were 74.5% of positive blood samples in the category of suckling piglets (Table 6).

Table 6. Seroprevalence of PCV2 in different pig categories in the territory of Vojvodina province

Pig category	Negative	Positive	Total
Suckling piglets	56 (25.5%)	164 (74.5%)	220 (100%)
Weaned piglets	59 (34.7%)	111 (65.3%)	170 (100%)
Fattening pigs	21 (14.0%)	129 (86.0%)	150 (100%)
Total	136 (25.2%)	404 (74.8%)	540 (100%)

DISCUSSION

Porcine circovirus is one of the most important causative agents of the profit losses in farming pig population (Grau-Roma et al., 2009). A greater interest in this subject started after the first occurrence of PMWS. After the discovery of PCV2 and the detection of circoviral infections, seroprevalence was assessed in Germany (Tischer et al., 1986), Canada (Dulac and Afshar, 1989), England (Edwards and Sands, 1994) and the USA (Hines and Lukert, 1995). These studies had shown that circovirus was widespread in the pig population.

In our study, specific PCV2 antibodies were found in 74.8% of tested animals, and this result is very similar to the results from some other European countries, such as Spain, with seroprevalence of 72.7% (Rodriguez-Arrioja et al., 2003) and France with 80.2% (Blanchard et al., 2003). Seroprevalence determined in our study is lower compared to the results from Belgium with seroprevalence of 100% (Lefebvre et al., 2008), and higher than the results from Austria with seroprevalence of 60.0% (Schmoll et al., 2008) and Slovakia with 54.0% of animals tested positive (Csank et al., 2011). Seroprevalence determined in this study, however, is lower than in Canada with 82.4% (Liu et al., 2002) and the USA with 80.0% of specimens tested positive (Nawagitgul et al., 2002).

Results from our study indicate that the highest percentage of positive animals was detected in the category of fattening pigs with 86.0%, followed by suckling piglets with 74.5%, and the lowest percentage of positive animals (65.3%) was found in the category of weaned piglets. High level of maternal antibodies taken with colostrum can explain high percentage of piglets positive to specific PCV2 antibodies. What supports this is the fact that circoviral infections are rarely seen in piglets before 4 weeks of age (Segales, 2007), indicating possible protection from the infection by colostral antibodies. Estimated half-life of PCV2 antibodies is 19 days and level of passively received immunoglobulins goes under ELISA "cut off" level at the age of 5 weeks (Opriessnig et al., 2004), providing sufficient time for the significant loss of the passively transferred maternal antibodies via colostrum.

In research that was conducted at the PMWS positive and negative farms in France (Blanchard et al., 2003), seroprevalence of 100% was determined in the category of fattening pigs at 18–19 weeks of age. Similar research was conducted in Spain (Sibilia et al., 2004) and it was determined that the highest percentage of PCV2 antibodies was present in 1–5 weeks old piglets (suckling piglets/weaned piglets) and 16–20 weeks old pigs (fattening pigs).

In addition, our results are in accordance with the results from Spain and Denmark (Grau-Roma et al., 2009). High percentage of PCV2 positive pigs was determined in Spain at the age of 1–3 weeks. Thereafter, this percentage is rapidly decreasing to 7 weeks of age. From 7 to 11 weeks of age, the number of positive animals is in stagnation or slightly increasing, but from 11 weeks of age to the end of fattening it significantly increases. In the same research, similar results were obtained in Denmark. The number of positive pigs in Denmark is starting to rise from the 9th week of age to the end of fattening, but

percentage of positive animals is not higher than the one in the category of suckling piglets.

CONCLUSION

Based on our results, we can conclude that PCV2 infections are widespread among farmed pigs on the territory of Vojvodina Province, with the total seroprevalence of 74.8%. The highest value of seroprevalence was determined in the category of fattening pigs with 86.0%, followed by the category of suckling piglets with 74.5% and the lowest seroprevalence is in the category of weaned piglets with 65.3%.

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СЕРОПРЕВАЛЕНЦИЈА ИНФЕКЦИЈА СВИЊА ИЗАЗВАНИХ ЦИРКОВИРУСОМ ТИП 2 НА ПОДРУЧЈУ ВОЈВОДИНЕ

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РЕЗИМЕ: Циљ овог истраживања био је да се утврди серопреваленција инфекција изазваних са цирковирусом свиња типа 2 (PCV2) код различитих категорија свиња на подручју Војводине. Испитивање је рађено на шест фарми свиња с различитих локација на подручју Војводине (по две фарме са територије Бачке, Баната и Срема), капацитета од 500 до 2.500 крмача, с интензивним начином држања затвореног типа. Узорци крви од 540 испитиваних свиња узети су од три различите категорије свиња: прасади на вименима (између 3–4 недеље живота),

прасади у одгоју (између 8–9 недеље) и товљеника (између 20 и 22 недеље живота). Специфична анти PCV2 антитела одређена су применом индиректне ELISA методе. На основу наших резултата може се закључити да су PCV2 инфекције широко распрострањене на подручју Војводине, с укупном серопревалецијом од 74,8%. Највеће вредности серопреваленције утврђене су у категорији товљеника 86,0%, затим код прасади на вименима 745%, док је код прасади у одгоју серопреваленција износила 65,3%.

КЉУЧНЕ РЕЧИ: антитела, PCV2, свиња, серопреваленција

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URINARY TRACT INFECTIONS AND THEIR IMPORTANCE

ABSTRACT: Urinary tract infections (UTI) are one of the most common infectious diseases, primarily caused by bacteria present in the intestinum, affecting the entire urinary tract or only a part of it. If the urinary bladder and urethra are affected then they are considered "lower" urinary tract infections, and the affected kidneys and ureter are considered "upper" urinary tract infections. There is a division into uncomplicated and complicated UTIs. Approximately 15% of all prescribed antibiotics in the United States are prescribed as therapy of UTIs. The UTI data from other countries are similar. The costs incurred in the treatment of these infections are significant – in the United States, the direct costs of UTI treatments are estimated at $ 1.6 billion per year.

KEYWORDS: infections, urinary tract, bacteria

PATHOGENESIS OF URINARY TRACT INFECTIONS

The urinary tract has a normal defense mechanism that protects it from infections. Urine is an adverse environment for bacterial growth due to low pH, high urea concentration, presence of organic acids, and high osmolarity. The bladder has bactericidal properties and creates an immunoglobulin A that reduces bacterial adherence to the uroepithelium. There are differences in the general and local resistance of the host to the urinary tract infections. This is the reason why some children get sick once or never, while in others the infections are often repeated, in a lighter or more severe form. The most important mechanism of defense against urinary tract infections is normal urodynamics. The disorder leads to urinary retention and the production of residual urine in which the bacteria multiply smoothly. These disorders lead to obstruction of the urinary

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tract and functional disorders of the lower part of the urinary tract. Characteristics of microorganisms, their number, ability to reproduce, and virulence are also significant.

Using the concept of bacterial virulence, we come to the conclusion that not all bacteria are equally capable of stimulating and developing infections. If in any way the human immune defense mechanisms are weak, then the bacteria that are less virulent can cause an infection. This fact is supported by well-documented in-vitro studies that bacteria isolated in patients with complicated urinary tract infection often fail to express virulence factors. The concept of virulence also suggests that certain strains of bacteria are uniquely equipped with specialized virulence factors, e.g. different types of fimbriae (saw), which facilitate the rise of bacteria from the fecal flora, vagina or perianal area in the bladder, or rarely can reach the kidneys and cause systemic inflammation.

The most common causes of urinary tract infections are bacteria from the intestinal flora that colonize the outer part of the urethra and accidently enter the urethra, the urinary bladder, and sometimes the upper parts of the urinary tract. In boys, pathogens can come from the bacterial flora beneath the prepuce. In neonates, urinary tract infections are most commonly due to hematogenesis in relation to gram negative septicemia. Hematogenic infections of the urinary tract are also possible in older children in systemic bacterial infections.

In addition to hematogenous, microorganisms can enter the urinary tract by lymphatic spread, but there are not many clinical and experimental evidences that the introduction of urinary tract microorganisms leads to the formation of urinary tract infections, especially organisms of enteric origin, e.g. *Escherichia coli* and other intestinal bacteria. This is a logical explanation for the higher incidence of urinary tract infections in women than in men and for the increased risk of a bladder infection after catheterization. One catheter insertion into the urinary bladder will lead to urinary infection in 1–2% of ambulatory patients. When using the catheters with an open drainage system, bacteriuria occurs in almost 100% of cases.

By using drainage systems, which include the use of a valve that prevents retrograde flow, the occurrence of the infection is only delayed, but cannot be prevented. Bacteria are thought to migrate into the mucopurulent space between the urinary tube and catheter and this leads to the development of bacteriuria in almost all patients within four weeks (Abbo and Hooton, 2014).

CLASSIFICATION OF URINARY TRACT INFECTIONS

Clinical signs of a urinary tract infection depend on asymptomatic bacteriuria, or a process in the form of acute pyelonephritis or urosepsis. It all depends on the localization and the severity of the infection. Further, it is necessary to differentiate between a complicated and uncomplicated urinary tract infection, in order to properly choose the treatment.

There is a general consensus that the species and number of wet organisms, the presence of bacteriuria, and complicated host factors must be considered in determining whether the presence and number of bacteria are pathological.

Urinary tract infections are classified according to the following criteria: by localization: urethritis, cystitis, pyelonephritis, glomerulonephritis, and urosepsis; by repeat tendency: recurring infections or occasional infections; by symptomatology: symptomatic infections or asymptomatic infections; by the complication factor: uncomplicated infections or complicated infections.

CAUSES OF URINARY TRACT INFECTIONS

Causes of urinary tract infections are predominantly gram negative bacteria. Escherichia coli is isolated in more than 80% of cases. However, not all strains of this species are virulent for the urinary tract. The process of bacterial cell adhesion is the key factor for the infection. Virulent strains possess a whole series of specific fimbria that are classified according to the epithelial cell receptor, and by their structure they are glycosphinolipids. Specific bacterial adhesins or fimbrial peak proteins determine the strength and binding site. The adhesion of the bacterium begins with the production of cytokines, which are released into the urine or systemic circulation, and is followed by proliferation, invasion, and initiation of the inflammatory reaction. The result is the release of the protective layer of the glycoprotein, which covers the uroepithelium, and finally the colonization of the bacteria. *Klebsiella* species and *Proteus mirabilis* are next by frequency of occurrence. *Enterobacter* species and *Pseudomonas aeruginosa* are isolated in less than 2% of children with urinary tract infections. According to some data, *Proteus* is as common as *Escherichia coli* in boys over one year old. Gram-positive *Enterococcus* species and *Streptococcus* B groups are rarely isolated, except for newborns. *Staphylococcus aureus* is the most commonly isolated bacteria in urinary tract infections in adolescent girls. In urinocytosis of patients with malformations or dysfunction of the urinary tract, *Pseudomonas aeruginosa*, *Klebsiella species*, and *Enterobacter species* are the most common.

ABNORMALITIES THAT CAN CAUSE INFECTION

Many children with urinary infections have normal kidney and bladder. However, if a child has a deformity of the urinary tract, it needs to be detected as soon as possible to protect the kidneys from damage. The most frequent abnormalities are:

- Vesicoureteral reflux (VUR) – it is normal that urine flows from the kidney down the ureter to the urinary bladder in one direction. However, with VUR, when the bladder is full, the urine starts to return from the bladder through the ureter to the kidney. This deformity is common in children with urinary infections (Alatoom et al., 2017);

- Urinary obstruction – blockage of urine flow may occur at different sites in the urinary tract. Ureter or urethra can be too narrow or kidney stones can block the urination from the body. Sometimes, the ureter is coupled with the kidney or urinary bladder in the wrong place and thus prevents normal renal urine output;
- Dysfunctional discharge – some children regularly postpone going to the toilet because they do not want to leave the game. They can make such a strong effort to squeeze the sphincter's muscles to forget how to relax when needed. As a consequence, it is impossible to empty the bladder completely when needed. Some children cramp during urination, causing pressure in the urinary bladder and thus pushing urine back into the ureter. Dysfunctional discharge can lead to vesicoureteral reflux, unconscious urinary incontinence, and urinary infections.

Risk factors for the development of urinary tract infections are numerous, many of which are host dependent. Some of them are: gender – girls are more susceptible to infection; the age of the child – younger children are more susceptible to infection; anatomical structure – urethra in girls is shorter than in male children, and bacteria easily reach the urinary bladder and infect it. Every condition that obstructs the urine flow sets the prerequisites for infection. The infections are frequently caused by catheters or tubes that are placed in the urinary bladder, urinary or fecal incontinence, anomalies that should be detected in children as early as possible in order to prevent kidney damage, immunity disorders, bacterial virulence, hereditary predisposition to bladder infection, injuries, and so on (Angelescu et al., 2016).

SYMPTOMS OF URINARY TRACT INFECTION

Clinical characteristics of urinary tract infection are variable and partly dependent on the age, gender, and frequency of repetition. Clinical picture in newborns can be very unspecific in the form of drowsiness or anxiety, rejection of food with the usual presence of elevated body temperature. The urine has an unpleasant smell, and it is possible to have flatulence, vomiting and, diarrhea. Pediatricians know that if during the examination of a child aged up to 2 years with an elevated body temperature the cause of the infection cannot be detected, the urinary tract infection should be suspected. Infant and small child (1–2 years): occasional episodes of febrile, urinary incontinence, unpleasant smell of urine, anorexia, abdominal pain, vomiting, and febrile convulsions in 4% of children. In pre-school and school children with urinary tract infections dysuria is the most common symptom, and the other signs are: frequent urination, immediate urination, recurrence of night urination, intense smell urine, abdominal pain after urinating, back pain, fever, and so on. High body temperature, fever, lateral back pain, and leukocytosis suggest possible acute pyelonephritis. In older children, signs and symptoms of urinary tract infection are usually limited to the lower urinary tract with minimal systemic symptoms. These are: frequent urination, drip, poor ureteric jet, abdominal pain during

urination, intense smell, blurred urine, and so on. Unusual somnolence can arise as a result of hyperammonemia caused by the infection of urea hydrating organisms, such as *Proteus*. Although fever may occur, it is less common than its prevalence in younger children.

If present, it may indicate renal parenchymal infection or infection in congestive urinary tract (Weinick et al., 2010).

Generally speaking, the symptoms of urinary tract infections in elderly children and adolescents are related to the lower urinary tract and are the consequence of instability of the detrusor and mucosal irritation of the urethra. Vesicoureteral reflux (VUR) is likely to have a minimal role in generating such symptoms, although overall reflux with significant ureteric dilatation can cause a high volume residual urine after urinary tract and stimulate urinary infection and instability of the detrusor.

METHODS OF URINE COLLECTION FOR ANALYSIS

For the diagnosis of urinary infection, the most important is the analysis of urine. Most parents are aware that the collection of urine in children is very difficult, especially in young children. The older children who control the sphincter should bathe in the evening, and the sample could be taken in the morning. It is a mid-stream urine specimen collected in a sterile container. For urine culture, small amounts of urine are sufficient (about a milliliter of urine), while the analysis of urinary sediment requires about half a sterile container (David et al., 2005).

Urine can be collected in the urine bag, which has to be fixed to the skin around the outer opening of the urethra and can stand until the child spontaneously urinate. The result of the urinary culture for which the urine is collected in a sterile bag can be considered valid only if it is negative. However, it is positive in 70% of cases because of contamination, so this method of collecting urine is not recommended. The bag should not stand for more than half an hour, and if the child does not urinate during that time, the whole procedure of washing, wiping, and positioning of the new sterile bag is repeated.

The problem with the bags is that they move with the child's movements, so there are frequent contaminations of urine with feces, when false positive results are obtained. For this reason, a group of Spanish pediatricians have recently proposed a technique of stimulating urinary miction. It is a fast, simple and safe way to urinate with newborns. It takes two people to participate and the first step is to feed the newborn with breast milk or milk formula. After that, the genitals are washed with warm water and soap, wipe with a sterile gauze, and prepare a sterile container (Gupta et al., 2011).

One person takes the newborn under the armpits, so that the legs hang. The other person starts gently and quickly tapping the pubic part (above the genitals) for about half a minute. The third step is a massage of the lower back of the spine along the spinal column (paravertebral lumbar region) for about half a minute with mild circular movements. The procedure can be repeated

several times and the urine should appear within 5 minutes. Unfortunately, the technique cannot be applied to older children, because the function of the detrusor muscle that helps the urine passage is no longer a reflex type but is controlled by the cerebral cortex. If this technique does not produce any results, catheterization or urinary bladder access for urine collection can be performed in hospital conditions (Ferry et al., 2004).

Urinalysis directly on the sterile surface (URICULT) is done only exceptionally, if it is not possible to submit a sample to laboratory for examination within 30–60 minutes. Of course, it is far easier for boys to collect a midstream specimen of urine than girls, so the patience is needed. Namely, it is of paramount importance to carry out urinary culture before initiating therapy, the implementation of which should not be delayed (Christiaens et al., 2002).

LABORATORY ANALYSIS OF URINE

The urine sample obtained should be taken quickly to the laboratory, because the analysis should be done in about 1 hour if the urine sample is kept at room temperature or after a maximum of 4 hours if it is kept in the refrigerator. A single urine sample can serve for several analyses. The fastest and simplest dipstick method, which is done by placing a tape in a glass of urine, reads straight away by comparing the color with the standard pattern. In this way, different biochemical parameters are determined, first of all the leukocyte esterase indicating purulent inflammation (pyuria), as well as nitrites, resulting from the conversion of nitrate from the urine due to the action of bacteria. The combination of these two parameters is highly sensitive and specific, and supports urinary infections, while the presence of glucose, protein, and blood in the urine is less specific (Christiaens et al., 2002).

Another analysis that can be done from the sample is the sediment of urine – the urine is centrifuged, Gram-stained, observed under the microscope, and the results are completed within a few hours. If it is necessary to determine what type of bacteria is in urine the urinoculture is done, and the reading of the results is after two days. *Escherichia coli* is most frequently isolated from the urinary tract, and then other bacteria such as *Enterobacter* or *Proteus mirabilis*, which enter the urogenital tract from the bowel. The presence of some bacteria strains, such as *Pseudomonas aeruginosa*, indicates the possibility of having a disrupted function or anatomic abnormality. If they are isolated from urine, due to the type of bacteria the therapy will include a list of antibiotics that the bacteria are sensitive to (antibiogram). The concentration of bacteria in the urinary culture is expressed in colony-forming units of bacteria per ml (CFU/ml). It should be emphasized here that there is no sterile urine and that the presence of bacteria up to 50,000 CFU/ml indicates contamination of urine with feces, and the finding of over 100,000 CFU/ml implicates the urinary infection (Warren et al., 1999).

OTHER ANALYSES

In addition to urine analysis, it is useful to make blood counts and biochemical analysis, as well as ultrasound examination of the urinary tract, to establish the diagnosis of urinary infection. The latest recommendations suggest the use of inflammatory indexes, such as C reactive protein (CRP) and procalcitonin (PCT) to confirm kidney inflammation (pyelonephritis). The level of inflammatory cytokines IL-6 and IL-8 in the urine can be used in diagnosis of urinary infection, but it is in the testing phase. Anatomical disorders are detected by invasive methods of microcirculation or DMSA scintigraphy.

CONCLUSION

Urinary tract infection is a common bacterial infection of childhood, which, if not diagnosed and treated in a timely manner, can lead to permanent damage of the kidneys and the occurrence of subsequent complications. In the clinical approach, the accuracy of UTI diagnosis based on urine culture is important, with the determination of the localization of the infection using certain laboratory tests and similar searches. In this way, the selection of risky patients with recurrent infections, in the first place of pyelonephritis, is ensured. Considering that the prognosis for most children with UTI is good, and bearing in mind the invasiveness, it is crucial to select children with structural anomalies and dysfunctional urination.

Antimicrobial therapy should only be prescribed if necessary to obtain effective results. Reproductive tract infections should be prevented by self-injection or prophylaxis, and not immediately treated as an emergency. Health care workers need to be constantly aware of the risk of cross-infection among catheterized patients. Also, the hand hygiene protocol should be obeyed and the usage of single-use gloves should be considered. These guidelines provide easy access to uncomplicated urinary tract infections, resulting in a good clinical outcome.

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ИНФЕКЦИЈЕ УРИНАРНОГ ТРАКТА И ЊИХОВА УЛОГА

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РЕЗИМЕ: Инфекције мокраћног тракта, односно инфекције уринарног тракта (ИУТ) спадају у једне од најучесталијих инфективних болести, првенствено изазване бактеријама присутним у интестинуму, које погађају цео уринарни

тракт или само један његов део. Уколико су захваћене мокраћна бешика и уретра, тада се оне сматрају „доњим“ инфекцијама уринарног тракта, а када су захваћени бубрези и уретери сматрају се „горњим“ инфекцијама уринарног тракта. Такође се користи подела на некомпликоване и компликоване ИУТ. Апроксимативно 15% од свих преписаних антибиотика у САД се преписује у терапији ИУТ. Подаци других земаља приказују сличну ситуацију. Трошкови настали у терапији ових инфекција су значајни – у САД директни трошкови лечења ИУТ процењују се годишње на 1,6 милијарди америчких долара.

КЉУЧНЕ РЕЧИ: инфекције, уринарни тракт, бактерије

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ASSESSING LANDSCAPE PLANS WITH ABBREVIATED PAIR-WISE COMPARISONS IN THE AHP (ANALITIC HIERARCHY PROCESS)

ABSTRACT: This paper demonstrates the application of the Analytic Hierarchy Process (AHP) in assessing landscape plans using the option of abbreviated pair-wise comparisons to simplify the weight elicitation process for decision makers. Whereas the standard AHP elicitation procedure requires a full set of pairwise comparisons among all criteria at each node of the decision hierarchy in order to derive criterion weights for the decision model, the abbreviated pairwise method uses a minimal spanning set of pairwise comparisons, and remaining comparisons are then derived by transitivity rules. In this paper is presented the abbreviated pairwise method with a case study in which alternative management plans are evaluated for the Zvezdarska forest of Belgrade, Serbia. The analysis was performed with the Criterium DecisionPlus software, which fully implements the AHP methodology, and provides useful diagnostics on AHP decision models. As a conclusion, some of the key advantages and disadvantages of the abbreviated pairwise variant of the AHP method are demonstrated. One of the key qualities of the Criterium DecisionPlus software is a clear and easy graphical representation of the results.

KEYWORDS: AHP (Analytic Hierarchy Process) pairwise comparison, abbreviated pairwise, landscape plan

INTRODUCTION

Decision making in landscape management and planning can be supported by numerous decision support methods (Kangas et al., 2015; Lakićević and Srđević, 2017). Among them, one of the most commonly applied is the

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Analytic Hierarchy Process (AHP), which has been demonstrated in numerous publications in the natural resource literature (Mendoza and Martins, 2006; Lakićević et al., 2016). This paper deals with the problem of assessing alternative landscape plans for the urban Zvezdara Forest located in Belgrade, Serbia.

The standard AHP method begins with a specification of 1) an overall goal (e.g., select the best alternative plan), 2) a set of criteria that define the requirements for satisfying the goal, and 3) a set of alternatives (plans in this case) to be considered in the analysis. The simplest possible specification for an AHP decision model has a single set of criteria beneath the goal level, but, in the more general case, the model structure may be further elaborated with additional levels of subcriteria supporting each criterion. In what we shall refer to as the classic AHP method, weights on criteria subtending any higher level node in the model hierarchy are derived by first comparing the importance of all possible pairs of criteria using an importance scale of 1 to 9. In the case of five criteria, for example, the comparisons are entered into a 5 by 5 matrix, in which the diagonal of self-comparisons always take a value of 1, and values in the lower left portion are the inverses of comparisons in the upper right. Practically, this means that a decision maker needs to provide 10 comparisons [(5*5 – 5)/2] for a 5 by 5 matrix. Given the completed comparison matrix, criterion weights are derived as an eigenvector solution, which is described in more detail in the Methods.

In order to reduce the burden on decision makers with respect to the weight elicitation step of model design, an alternative to the classic AHP full pairwise comparison process employs a variant referred to as the abbreviated pairwise method, which is also described in more detail in the Methods. The application of the abbreviated pairwise approach is illustrated with the example of selecting the best management plan alternative for Zvezdara Forest, and the software Criterium DecisionPlus – CDP (InfoHarvest, Inc., 1996–2018) is used, which implements the abbreviated pairwise variant in addition to the classic full pairwise method.

The main goals of this research are to (1) demonstrate the use of the abbreviated pairwise AHP comparison method for evaluating landscape planning alternatives, and (2) introduce the CDP software as a support for AHP computations and graphical presentation of the results.

METHODS

Study area

The selected area is Zvezdara Forest in Belgrade, Serbia (Figure 1). The forest occupies approximately 137 ha and was originally established by planting in the 1950s, but the potential vegetation, based on physiographic characteristics of the site, is *Quercetum-farnetto cerris* forest. This urban forest area is well-equipped with sport and recreation facilities and is one of the most visited urban forests in Belgrade.

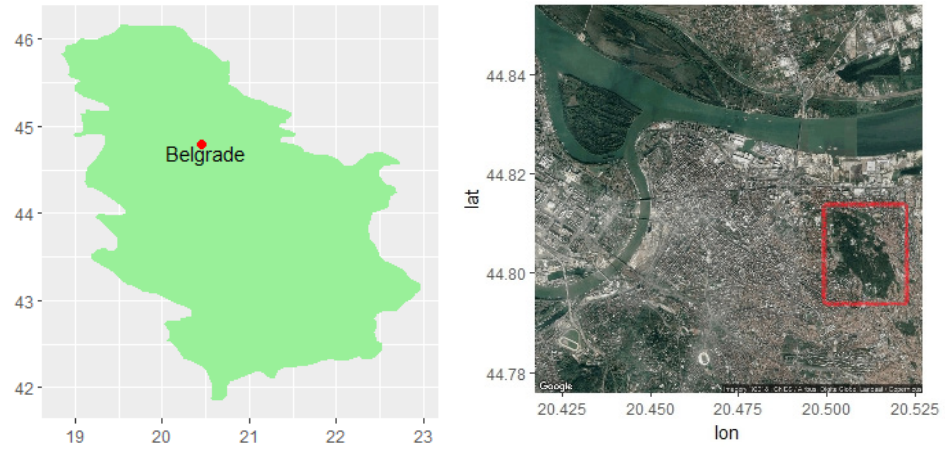

Figure 1. Location of Zvezdara Forest

For management of the Zvezdara Forest area, five alternative management plans have been proposed (Table 1). Lakićević et al. (2014) provide detailed descriptions of the five plans.

Table 1. A brief description of management plans for Zvezdara Forest

Management plan	Description
MP 1	Maintain the current management plan
MP 2	Enhance tourist appeal and maintain biodiversity
MP 3	Biodiversity conservation
MP 4	Promote scenic values
MP 5	Promote tourist appeal

The AHP process models a decision-making problem in the form of a hierarchy as we have briefly described in the Introduction. The AHP model structural specification for the Zvezdara Forest plan selection problem, in particular, shows the overall goal in the leftmost column, the five criteria in the center column, and the five plan alternatives (descriptions in Table 1) in the rightmost column (Figure 2).

In order to complete the model specification, weights need to first be derived for the criteria. The method for deriving the weights, originally developed by Saaty (1980), begins by eliciting judgments from a decision maker (or group of decision makers) regarding the relative importance of criterion *i* compared to criterion *j* (Table 2), for all pairs *i, j* with respect to their parent node. In our case, the five criteria have the goal node as their parent, so, in our example from the Introduction, 10 judgments need to be elicited from the decision maker in the case of the full pairwise method involving five criteria.

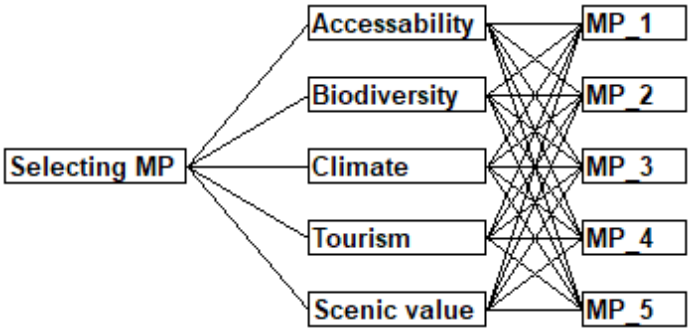

Figure 2. AHP analysis with the abbreviated pairwise method in CDP

Table 2. Saaty's (1980) scale of relative importance for pairwise comparisons

Definition	**Assigned value**[a]
Equally important	1
Weak importance	3
Strong importance	5
Demonstrated importance	7
Absolute importance	9
Intermediate values	2, 4, 6, 8

[a] Importance values are defined on a geometric scale in which the integer value indicates the number of times criterion *i* is more important the criterion *j*. Self-comparisons on the matrix diagonal are set to 1, and element *j,i* is the inverse of *i,j*.

Pairwise comparisons are placed into a pairwise comparison matrix. Given the filled comparison matrix, weights on criteria are calculated as the eigenvector solution to the following formula:

$$Aw = \lambda w, e^T w = 1 \quad (1)$$

in which: A is the matrix of pairwise comparisons, w is the first principal eigenvector of weights, λ is the first principal eigenvalue of matrix A, and e is a unit vector.

Obtaining the vector of weights, w, from eq 1 requires that the comparison matrix, A, is completely filled. However, the abbreviated pairwise method is a special case of an incomplete A matrix, in which we have comparisons for all elements of the diagonal above the main diagonal of A (Figure 3). Given the elements x, the remaining elements of the upper right triangle of A can be calculated by applying the transitivity rule presented by Ishizaka and Lusti (2004), and the lower triangle element j,i is computed as the inverse of i,j. The CDP software, which implements the abbreviated AHP pairwise method, was used in our application.

	E_1	E_2	E_3	…	E_n
E_1	**1**	x			
E_2		**1**	x		
E_3			**1**	x	
…				**1**	x
E_n					1

Figure 3. Initial matrix required for the abbreviated pairwise comparison method

The final step needed to complete the specification of the AHP model (Figure 2) involved repeating the pairwise comparison process at the level of the five plan alternatives, MP1 to MP 5, comparing the plans to each other with respect to each of the model criteria. Whereas criteria were compared to each other with respect to their relative importance for satisfying the overall model goal, comparisons among alternatives with respect to a criterion are expressed in terms of preference. For example, with respect to the criterion for accessibility, how much more preferable is MP 1 to MP 2, using a numeric scale analogous to that for importance (Table 2). Comparisons among plan alternatives were again elicited using the abbreviated pairwise process, which was repeated for each of the five criteria, and weights on each plan alternative were again derived by the eigenvector method (eq 1).

The five alternative management plans (Table 2) were evaluated with CDP, with the overall goal to select the most appropriate management plan with respect to the five criteria (Fig. 2). Pairwise comparisons needed to complete the abbreviated pairwise process were elicited from a decision maker, a senior author of the paper, and all the authors processed the evaluations in the CDP software. Analyses conducted in CDP included the overall performance of each management plan with respect to the goal (Figure 2) and the contributions of the criteria to the overall performance of each management plan. In addition, a sensitivity analysis was performed to assess the robustness of the model with respect to changing weights on the criteria. Specifically, sensitivity was assessed as the absolute change in a criterion required to replace the top-ranked plan alternative with another alternative. An AHP model is considered sufficiently robust if the most sensitive criterion requires an absolute weight change of at least 10% in order to alter the ordering of alternatives (Saaty, 1980, 2008).

RESULTS

The decision model for choosing the best management plan (Figure 2) requires 10 full pairwise comparisons for the criterion level (criteria versus goal), and 50 full pairwise comparisons for the alternative level of the hierarchy (alternatives vs. criteria). However, by employing the abbreviated pairwise

comparison method, the number of comparisons that need to be elicited from the decision maker for our decision model is significantly reduced, requiring only four pairwise comparisons at the criterion level and 20 pairwise comparisons at the alternative level.

Figure 4a presents the matrix with abbreviated pairwise comparisons for the criterion level of the hierarchy, initially provided by the decision maker. These data were entered in the CDP software, and, by using the transitivity rule (Ishazaka and Lusti, 2004), missing elements of the matrix were computed by CDP (Figure 4b).

Crit.	**C_1**	**C_2**	**C_3**	**C_4**	**C_5**
C_1	**1**	1/4			
C_2		**1**	1		
C_3			**1**	4	
C_4				**1**	1/2
C_5					1

Figure 4a. Matrix with abbreviated pairwise comparisons provided by the decision maker

Crit.	**C_1**	**C_2**	**C_3**	**C_4**	**C_5**
C_1	**1**	1/4	1/4	1	½
C_2		**1**	1	4	2
C_3			**1**	4	2
C_4				1	½
C_5					**1**

Figure 4b. Matrix with full pairwise comparisons computed by CDP using the transitivity rule

Evaluation of pairwise comparisons at the alternative level was similarly performed by using abbreviated pairwise comparisons (Figures 5–9).

C_1	**A_1**	**A_2**	**A_3**	**A_4**	**A_5**
A_1	**1**	2			
A_2		**1**	1		
A_3			**1**	1/3	
A_4				1	1/2
A_5					1

Figure 5a. Matrix with abbreviated pairwise comparisons provided by the decision maker

C_1	**A_1**	A_2	A_3	A_4	A_5
A_1	**1**	2	2	1	1/3
A_2		**1**	1	1/3	1/6
A_3			**1**	1/3	1/6
A_4				**1**	1/2
A_5					1

Figure 5b. Matrix with full pairwise comparisons computed by CDP using the transitivity rule

C_2	A_1	A_2	A_3	A_4	A_5
A_1	**1**	1/5			
A_2		**1**	1/2		
A_3			**1**	5	
A_4				**1**	2
A_5					1

Figure 6a. Matrix with abbreviated pairwise comparisons provided by the decision maker

C_2	**A_1**	**A_2**	**A_3**	**A_4**	**A_5**
A_1	**1**	1/5	1/9	1/2	1
A_2		**1**	1/2	2	5
A_3			**1**	5	9
A_4				**1**	2
A_5					1

Figure 6b. Matrix with full pairwise comparisons computed by CDP using the transitivity rule

C_3	A_1	A_2	A_3	A_4	A_5
A_1	**1**	1/4			
A_2		**1**	1		
A_3			**1**	5	
A_4				1	2
A_5					1

Figure 7a. Matrix with abbreviated pairwise comparisons provided by the decision maker

C_3	A_1	A_2	A_3	A_4	A_5
A_1	**1**	1/4	1/4	1	2
A_2		**1**	1	5	9
A_3			**1**	5	9
A_4				**1**	2
A_5					1

Figure 7b. Matrix with full pairwise comparisons computed by CDP using the transitivity rule

C_4	A_1	A_2	A_3	A_4	A_5
A_1	**1**	3			
A_2		**1**	2		
A_3			**1**	1/5	
A_4				**1**	1/2
A_5					1

Figure 8a. Matrix with abbreviated pairwise comparisons provided by the decision maker

C_4	A_1	A_2	A_3	A_4	A_5
A_1	**1**	3	6	1	1/2
A_2		**1**	2	1/2	1/5
A_3			**1**	1/5	1/9
A_4				**1**	1/2
A_5					1

Figure 8b. Matrix with full pairwise comparisons computed by CDP using the transitivity rule

C_5	A_1	A_2	A_3	A_4	A_5
A_1	**1**	2			
A_2		**1**	2		
A_3			**1**	1/5	
A_4				1	2
A_5					1

Figure 9a. Matrix with abbreviated pairwise comparisons provided by the decision maker

C_5	A_1	A_2	A_3	A_4	A_5
A_1	**1**	2	4	1	2
A_2		**1**	2	1/2	1
A_3			**1**	1/5	1/2
A_4				**1**	2
A_5					1

Figure 9b. Matrix with full pairwise comparisons computed by CDP using the transitivity rule

Given the complete set of full pairwise comparisons (Figures 4b to 9b), the eigenvector solution (eq 1) was then calculated in CDP for the criterion and alternative levels of the decision model (Figure 10). Notice that the sum of the weights (w_i) at each level of the hierarchy sum to 1 per the specification of the eigenvector solution (eq 1).

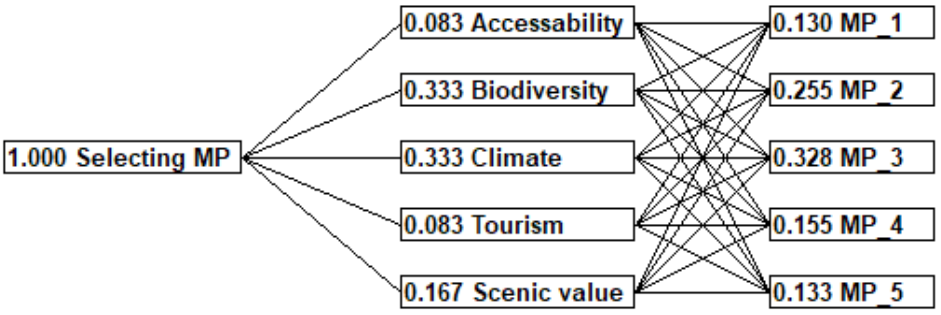

Figure 10. Weights on criteria and overall weights (scores) of alternatives

The key conclusion from the evaluated CDP model (Figure 10) is that the management plan MP 3 is clearly the preferred alternative with an overall score of 0.328. Recall that this management plan was designed to promote the conservation of biodiversity as a primary concern in the analyzed study area (Table 1). In addition to providing this basic result, the CDP analysis also provides additional supporting documentation that is useful to the analysis.

First, the overall score received by each alternative can be more fully explained in terms of how the criteria contribute to the overall score of an alternative (Figure 11). MP 3 strongly outperforms all other alternatives on the biodiversity criterion, which might be expected, and it strongly outperforms all other alternatives except the closely related MP 2 (the second-ranked alternative) with respect to performance related to climate. Furthermore, it can be seen that management plans MP 1, MP 4, and MP 5 outperform plans MP 2 and MP 3 on the criteria of scenic value, accessibility, and tourism, but the weights on these criteria are relatively low compared to those for biodiversity and climate (Figure 10), hence the dominating influence of biodiversity and climate in the overall results (Figure 11).

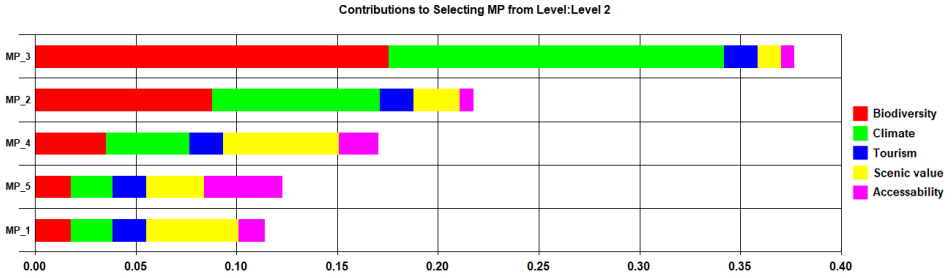

Figure 11. Contributions of criteria to the final score of each alternative

An additional informative analysis that CDP provides is an evaluation of model robustness, commonly referred to as sensitivity analysis in the AHP context (Murphy, 2014). CDP displays a graph for each criterion that illustrates how, in this example, the score on each alternative changes as the weight on the criterion is changed (Figure 12). Our example shows the case for the biodiversity criterion. The red vertical line in the figure shows the weight currently assigned to biodiversity, which is 0.333 (Figure 10). The point at which the red line intersects the lines for the five alternative management plans is the score (read from the vertical axis) for the alternative. In CDP, this graphic is interactive and the user can slide the vertical bar left or right to get a rough idea of how the scores on the alternatives change as the weight on the criterion is changed. In our example (Figure 12), if the bar is slid to the left, eventually it crosses the intersection of the score lines for MP 2 and MP 3. Notice that if the bar is slid past the latter intersection point, the decision score for MP 2 is now greater than that for MP 3. The CDP documentation refers to this as the cross-over point; that is, the weight on biodiversity at which the top-ranked MP 3 alternative is replaced by MP 2 as the top-ranked alternative.

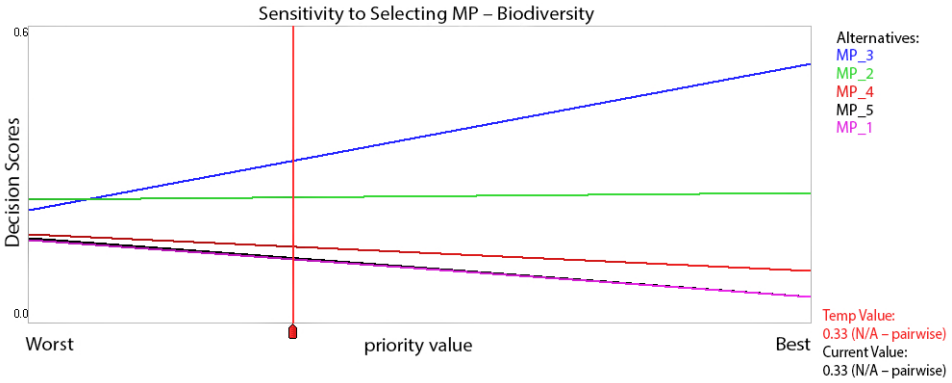

Figure 12. Sensitivity analysis for the criterion of biodiversity conservation

As mentioned above, the graphics analysis of weight sensitivity (Figure 12) can be viewed for all decision criteria. In addition, CDP provides a summary table that shows the absolute change in weight on a criterion (its criticality) that is required to reach the cross-over point (Table 3). In our analysis, the most sensitive criterion is biodiversity, which has a criticality of 25.5%. In our comments in the Methods section, this result is indicative of a very robust decision model. In other words, very substantial changes in the criterion weights are required before another alternative would become selected as the preferred alternative.

Table 3. Sensitivity analysis for the set of criteria

Goal – Criteria	Criticality [%]
Selecting MP – Biodiversity	25.5
Selecting MP – Accessibility	30.8
Selecting MP – Tourism	31.4
Selecting MP – Scenic value	32.0
Selecting MP – Climate	66.7

DISCUSSION

The AHP method is widely used in landscape planning tasks (Srdjevic et al., 2013; Lakicevic et al., 2016). Usually, it is applied by considering complete pairwise comparisons, but it can also be applied when there are missing elements in the comparison matrix. If only pairwise comparisons that are placed parallel to, and above, the main diagonal are available, this represents a special case that is suitable for applying the abbreviated pairwise comparison method demonstrated in our example. The abbreviated form cannot be considered as preferred over the full pairwise method but can be applied occasionally, for

example when the evaluation requires a large number of comparisons or including decision makers who are not familiar with AHP methodology. The complete pairwise comparison method offers the possibility of checking the internal consistency of judgments provided by decision makers and to obtain more detailed input data from the decision maker (Saaty, 1980, 1992). On the other hand, applying the abbreviated form offers an expedited elicitation process for completing the model specification, but without the possibility for the internal consistency check, because the abbreviated method fills the missing elements to create a perfectly consistent matrix (*CR*=0), or at most only slightly inconsistent matrices due to rounding numbers to Saaty's scale and having value of 9 as the maximum threshold.

In this paper, the primary objective was to demonstrate the evaluation of landscape plans for Zvezdara Forest, based on input from one decision maker (the senior author of the paper), using abbreviated pairwise comparisons. Calculations were performed with the CDP software, which proved to be an efficient and effective decision support tool for applying the AHP methodology to our example. The main strengths of the software are a user-friendly interface, a clear graphical representation of results, an easy performance of sensitivity analysis, and other useful model diagnostics.

CONCLUSIONS

This research provides a simple demonstration of abbreviated AHP comparisons in a real case study example from Serbia. Using abbreviated AHP pairwise comparisons expedites the decision-making process (Harker, 1987), which can make this method more appealing to people participating in the elicitation process in some situations. It should be noted that most AHP research still supports the full AHP pairwise comparison method, but the abbreviated form is suggested as a good alternative in some cases, usually related to having participants not experienced with the AHP method, or when the AHP hierarchy is more complex and consists of many criteria and subcriteria. The paper also analyzes the suitability of using the CDP software as a support for the AHP calculations, and clear graphical representation of the results is recognized as one of the main strengths of the software.

DISCLAIMER

The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

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ПРИМЕНА СКРАЋЕНИХ АХП (АНАЛИТИЧКИ ХИЈЕРАРХИЈСКИ ПРОЦЕС) ПОРЕЂЕЊА У ВРЕДНОВАЊУ ПЛАНОВА ПРЕДЕЛА

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РЕЗИМЕ: Рад приказује поступак примене Аналитичког хијерархијског процеса (АХП) у вредновању планова предела коришћењем скраћених поређења у паровима, у циљу поједностављења поступка за доносиоце одлука. Стандардни АХП поступак захтева да за сваки ниво хијерархије одлучивања постоје потпуна поређења у паровима, док поступак скраћених вредновања подразумева спровођење најмањег могућег броја поређења у паровима и попуњавање преосталих поља применом правила транзитивности. У раду је поступак примене скраћених АХП вредновања приказан на студији случаја која се односи на вредновање више различитих планова предела за Звездарску шуму у Београду (Србија). Анализа је извршена коришћењем програма Criterium DecisionPlus који у потпуности подржава АХП методологију. Као закључак приказане су кључне предности и недостаци поступка скраћених АХП вредновања. Један од основних квалитета програма Criterium DecisionPlus је лако читљиви графички приказ резултата.

КЉУЧНЕ РЕЧИ: АХП (аналитички хијерархијски процес), поређења у паровима, скраћена поређења у паровима, план предела

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