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ASPECTS OF BUTTERFLY ZOOGEOGRAPHY OF SOME PANNONIAN ISLAND MOUNTAINS

ABSTRACT: Specific geology and tectonic history of the Pannonian plain is pointed out. Lower mountains and hills, specific to the area, are highlighted. They most likely were islands in the period of Paratethys and Pannonian Sea: Malé Karpaty, Vertes, Bakony, Mecsek, Medvednica, Papuk, Fruška Gora and Avala. A head-to-head comparison has been done as well as a comparison with the site Mohács, a typical plain habitat in the Pannonian plain. The existence of specific elements of butterfly fauna of island mountains is discussed. According to the existing data from literature, the list of 163 butterfly species in explored area has been made. The degree of similarity between the explored island mountains has been calculated. A conclusion has been made about their faunal and zoogeographical liaison.

KEYWORDS: Butterflies, Pannonian island mountains, zoogeography

INTRODUCTION

The area of the Pannonian plain was covered by sea in the past. Parts of the land which existed as islands today are known as *Pannonian island mountains*. During Miocene (~10 MY) the connection between Pannonian Sea and Mediterranean Sea was interrupted. Pannonian Sea became shallow and warm, gradually losing salinity during the Middle Miocene (Badenian) period. During Pontian period Pannonian Sea turned into individual freshwater lakes. The lakes existed throughout Pliocene (~ 5 MY), when they mostly exsiccated and made the Pannonian plain.

During Quaternary, in Pleistocene (~ 2 MY), the Ice Age commenced. Beside drastic climate changes, there was loess fall. Soon after the Ice Age ended, intensive human settlement commenced. Deciduous woods, as primary climax communities, were typical vegetation. During Boreal (~ 10 – 8.000 YBP) the Pannonian plain was covered by open forest with birch, elm, pine and oak trees

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as well as shrubs and herbaceous vegetation. Finally, during the Atlantic period farming communities prevailed. Two close types of pollen diagram confirmed these changes: Balaton in Hungary [Zólyomi 1953] and Keres river valley near Subotica [Gigov 1965].

Considering the turbulent history of the area, there is a question of the origin and genesis of butterfly fauna of *island mountains*, especially in terms of differences between that fauna and Pannonian plain fauna.

MATERIALS AND METHODS

In order to answer these questions, eight typical island mountains of the present Pannonian plain has been chosen. The data were taken from the existing literature, so the comparison of fauna composition in the chosen area was possible. Mohács (90 m) in Hungary, as a typical location in the central part of the Pannonian plain has been chosen. Therefore, the comparison was made between butterfly fauna of Pannonian island mountains and Pannonian plain.

For agglomerate clustering, UPGMA method and Jaccard's similarity coefficient were used [Sokal and Rohlf 1995]. Estimations were done using the program FLORA [Karadžić and Marinković 2009].

RESULTS

Geographical layout of the former Pannonian Sea and position of island mountains are shown in Fig. 1. In addition, the position of locality Mohács (90 m) in Hungary is shown as a typical one for the Pannonian plain. Literature data used for making faunal lists of nine selected sites are listed in Tab. 1. Butterfly faunal structure in nine explored sites is shown in Tab. 2. A total number of butterfly species in explored areas (N) is shown in Tab. 3 as well as the number of species common to compared fauna (C). The faunal composition for 61 species common to 9 locations is shown in Fig. 2, Tab. 4 and Fig. 3, where Jaccard's similarity coefficient (similarity measure) has been used to determine the degree of similarity of butterfly fauna in the analyzed area.

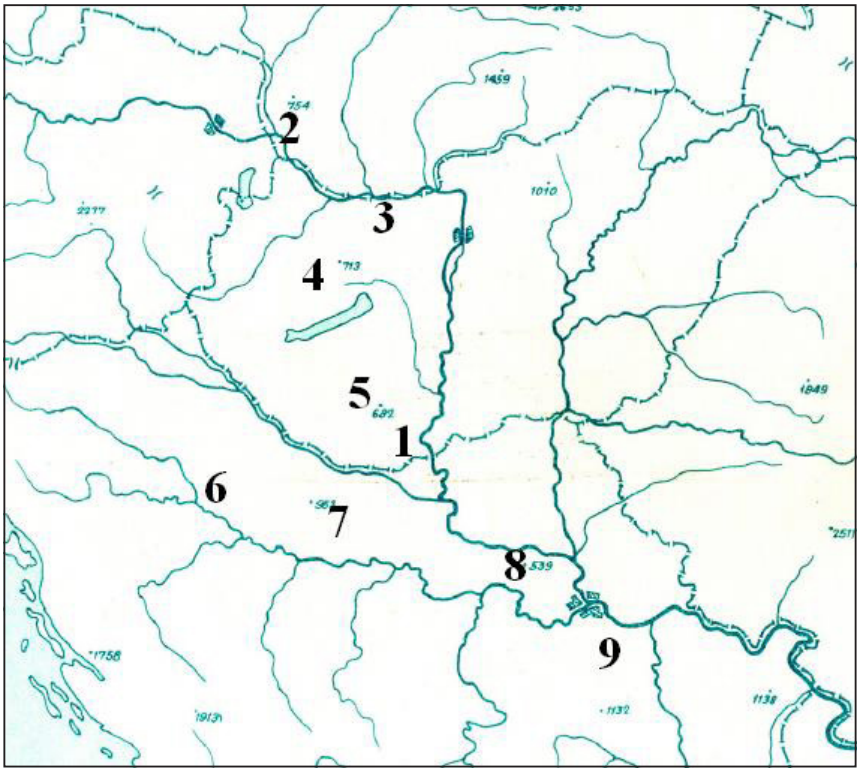

Fig. 1. *Investigated sites: 1) Mohács, 2) Malé Karpaty Mt., 3) Vertes Mt., 4) Bakony Mt., 5) Mecsek Mt., 6) Medvednica Mt., 7) Papuk Mt., 8) Fruška Gora Mt. and 9) Avala Mt.*

Table 1. List of localities and previous papers on distribution of butterflies.

No.	Localities	Data for Butterflies
1	Mohács, 90 m	Ábrahám [2008].
2	Malé Karpaty Mt., 754 m	Bálint *et al.* [2006]; Kudrna *et al.* [2011]; Kulfan [1982, 1983, 1992].
3	Vertes Mt., 480 m	Bálint *et al.* [2006]; Kudrna *et al.* [2011].
4	Bakony Mt., 704 m	Ábrahám [2001]; Abraham *et al.* [2007]; Bálint *et al.* [2006]; Fazekas [1983]; Dietzel [1997]; Kudrna *et al.* [2011], Levente [2001]; Papp [1968]; Szabóky [2009]; Takács [2009].
5	Mecsek Mt., 682 m	Bálint *et al.* [2006]; Kudrna *et al.* [2011].
6	Medvednica Mt., 1035 m	Jakšić [1988]; Koren and Zadravec [2010]; Kudrna *et al.* [2011]; Lorković [1989, 1996].
7	Papuk Mt., 953 m	Jakšić [1988]; Koča [1900]; Kudrna *et al.* [2011]; Mihoci *et al.* (2012].
8	Fruška Gora Mt., 539 m	Jakšić [1988, 2013]; Jakšić & Nahirnić [2014]; Kudrna *et al.* [2011].
9	Avala Mt., 506 m	Jakšić [1988]; Jakšić & Nahirnić [2014]; Kudrna *et al.* [2011]; Lorković [1989].

Table 2. List of butterfly species recorded in 8 island mountains in Pannonian area and Mohács as a control area.

No.	SPECIES	Mohács (Hungary)	Malé Karpaty (Slovakia)	Vertes (Hungary)	Bakony (Hungary)	Mecsek (Hungary)	Medvednica (Croatia)	Papuk (Croatia)	Fruška Gora (Serbia)	Avala (Serbia)
	Fam. HESPERIIDAE									
1	*Erynnis tages* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
2	*Carcharodus alceae* (Esper, 1780)		1	1	1	1	1		1	1
3	*Carcharodus floccifera* (Zeller, 1847)	1	1	1		1	1	1	1	1
4	*Spialia sertorius* (Hoffmann., 1804)		1		1	1	1			
5	*Spialia orbifer* (Hübner, 1823)	1		1	1	1		1	1	1
6	*Pyrgus carthami* (Hübner, 1813)	1	1	1	1	1			1	1
7	*Pyrgus sidae* (Esper, 1784)								1	
8	*Pyrgus cacaliae* (Rambur, 1839)		1							
9	*Pyrgus malvae* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
10	*Pyrgus serratulae* (Rambur, 1839)		1	1	1					
11	*Pyrgus armoricanus* (Oberthur, 1910)	1	1	1		1	1		1	1
12	*Pyrgus alveus* (Hübner, 1803)		1	1		1	1	1	1	1
13	*Pyrgus trebevicensis* (Warren, 1926)								1	
14	*Heteropterus morpheus* (Pallas, 1771)	1	1	1	1	1	1	1	1	1
15	*Carterocephalus palaemon* (Pallas, 1771)	1	1	1	1	1	1	1		1
16	*Thymelicus lineola* (Ochsenheimer, 1808)	1	1	1	1	1	1	1	1	1
17	*Thymelicus sylvestris* (Poda, 1761)	1	1	1	1	1	1	1	1	1
18	*Thymelicus acteon* (Rottemburg, 1775)		1	1		1			1	1
19	*Hesperia comma* (Linnaeus, 1758)		1	1	1	1	1	1	1	1
20	*Ochlodes sylvanus* (Esper, 1777)	1	1	1	1	1	1	1	1	1
	Fam. PAPILIONIDAE									
21	*Zerynthia polyxena* (D. & S., 1775)	1	1	1		1	1	1	1	1
22	*Parnassius mnemosyne* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
23	*Parnassius apollo* (Linnaeus, 1758)			1						
24	*Iphiclides podalirius* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
25	*Papilio machaon* Linnaeus, 1758	1	1	1	1	1	1	1	1	1
	Fam. PIERIDAE									
26	*Leptidea sinapis* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
27	*Leptidea juvernica* Williams, 1946				1					
28	*Leptidea morsei* Fenton, 1881	1	1	1		1	1		1	
29	*Anthocharis cardamines* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
30	*Aporia crataegi* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
31	*Pieris brassicae* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1

32	*Pieris rapae* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
33	*Pieris ergane* (Geyer, 1828)			1	1		1			
34	*Pieris napi* (Linnaeus, 1758)	1	1	1	1	1		1	1	1
35	*Pieris balcana* Lorković, 1970						1			1
36	*Pontia edusa* Fabricius, 1777	1	1	1	1	1	1	1	1	1
37	*Colias erate* (Esper, 1805)	1	1	1	1	1	1		1	1
38	*Colias croceus* (Fourcroy, 1785)	1	1	1	1	1	1	1	1	1
39	*Colias myrmidone* (Esper, 1780)		1	1					1	1
40	*Colias chrysotheme* (Esper, 1781)		1	1						
41	*Colias hyale* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
42	*Colias alfacariensis* Ribbe, 1905	1	1	1	1	1	1		1	1
43	*Gonepteryx rhamni* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
	Fam. RIODINIDAE									
44	*Hamearis lucina* (Linnaeus, 1758)	1	1	1	1		1	1	1	1
	Fam. LYCAENIDAE									
45	*Lycaena phlaeas* (Linnaeus, 1761)	1	1	1	1	1	1	1	1	1
46	*Lycaena dispar* (Haworth, 1802)	1	1	1	1	1	1	1	1	1
47	*Lycaena virgaureae* (Linnaeus, 1758)	1	1	1		1	1			
48	*Lycaena tityrus* (Poda, 1761)	1	1	1	1	1	1	1	1	1
49	*Lycaena alciphron* (Rottemburg, 1775)	1	1	1	1	1	1	1	1	1
50	*Lycaena hippothoe* (Linnaeus, 1761)	1	1	1	1	1	1			
51	*Lycaena thersamon* (Esper, 1784)	1	1	1		1			1	1
52	*Thecla betulae* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
53	*Neozephyrus quercus* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
54	*Callophrys rubi* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
55	*Satyrium w-album* (Knoch, 1782)	1	1	1	1	1	1	1	1	1
56	*Satyrium pruni* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
57	*Satyrium spini* (D. und S., 1775)	1	1	1	1	1	1		1	1
58	*Satyrium ilicis* (Esper, 1779)	1	1	1	1	1	1	1	1	1
59	*Satyrium acaciae* (Fabricius, 1788)	1	1	1	1	1	1	1	1	1
60	*Lampides boeticus* (Linnaeus, 1767)	1				1	1		1	1
61	*Leptotes pirithous* (Linnaeus, 1767)		1				1		1	1
62	*Cupido minimus* (Fuessly, 1775)	1	1	1	1	1	1		1	
63	*Cupido osiris* (Meigen, 1829)							1	1	
64	*Cupido argiades* (Pallas, 1771)	1	1	1	1	1	1		1	1
65	*Cupido decolorata* (Staudinger, 1886)	1	1	1	1	1	1		1	1
66	*Cupido alcetas* (Hoffmannsegg, 1804)	1	1	1	1	1	1		1	1
67	*Celastrina argiolus* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
68	*Pseudophilotes vicrama* (Moore, 1865)	1	1	1		1	1	1	1	1
69	*Scolitantides orion* (Pallas, 1771)	1	1	1	1	1	1			
70	*Glaucopsyche alexis* (Poda, 1761)	1	1	1	1	1	1	1	1	1
71	*Iolana iolas* (Ochsenheimer, 1816)	1				1			1	1
72	*Phengaris arion* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
73	*Phengaris teleius* (Bergstrasser, 1779)	1	1	1	1	1	1	1		
74	*Phengaris nausithous* (Bergstr., 1779)		1	1	1	1	1			

75	*Phengaris alcon* (D. und S., 1775)	1	1	1	1	1	1			
76	*Plebeus pylaon* (Fischer, 1832)	1							1	
77	*Plebejus argus* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
78	*Plebejus idas* (Linnaeus, 1761)		1	1		1	1		1	1
79	*Plebejus argyrognomon* (Bergstr., 1779)	1	1	1	1	1	1	1	1	1
80	*Aricia eumedon* (Esper, 1780)		1	1						
81	*Aricia agestis* (D. und S., 1775)		1	1	1	1	1	1	1	1
82	*Cyaniris semiargus* (Rottemburg, 1775)	1	1	1	1	1	1	1	1	1
83	*Polyommatus dorylas* (D. und S., 1775)	1	1	1	1	1	1		1	
84	*Polyommatus amandus* (Schneider, 1792)	1	1	1		1	1	1	1	
85	*Polyommatus thersites* (Cantener, 1835)		1	1			1			
86	*Polyommatus icarus* (Rottemburg, 1775)	1	1	1	1	1	1	1	1	1
87	*Polyommatus eros* (O., 1808)		1							
88	*Polyommatus daphnis* (D. und S., 1775)	1	1	1	1	1	1	1	1	
89	*Polyommatus bellargus* (Rott., 1775)	1	1	1	1	1	1	1	1	1
90	*Polyommatus coridon* (Poda, 1761)		1	1	1	1	1	1		
91	*Polyommatus admetus* (Esper, 1783)					1			1	
92	*Polyommatus damon* (D. und S., 1775)		1							
	Fam. NYMPHALIDAE									
93	*Libythea celtis* (Laicharting, 1782)	1		1	1					
94	*Argynnis paphia* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
95	*Argynnis pandora* (D. und S., 1775)	1	1	1		1		1	1	1
96	*Argynnis aglaja* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
97	*Argynnis adippe* (D. und S., 1775)		1	1	1	1	1	1	1	1
98	*Argynnis niobe* (Linnaeus, 1758)		1	1			1		1	1
99	*Issoria lathonia* (Linnaeus, 1758)	1	1	1	1		1	1	1	1
100	*Brenthis ino* (Rottemburg, 1775)		1	1			1			
101	*Brenthis daphne* (D. und S., 1775)	1	1	1	1	1	1	1	1	1
102	*Brenthis hecate* (D. und S., 1775)		1	1	1	1	1	1	1	1
103	*Boloria euphrosyne* (Linnaeus, 1758)	1	1	1	1	1	1	1		1
104	*Boloria selene* (D. und S., 1775)		1	1		1	1		1	
105	*Boloria dia* (Linnaeus, 1767)	1	1	1	1	1	1	1	1	1
106	*Vanessa atalanta* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
107	*Vanessa cardui* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
108	*Aglais io* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
109	*Aglais urticae* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
110	*Polygonia c-album* (Linnaeus, 1758)	1	1	1	1	1	1		1	1
111	*Polygonia egea* (Cramer, 1775)									1
112	*Araschnia levana* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
113	*Nymphalis antiopa* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
114	*Nymphalis polychloros* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
115	*Nymphalis xanthomelas* (Esper, 1781)		1						1	1
116	*Nymphalis vaualbum* (D. und S., 1775)							1	1	1
117	*Euphydryas maturna* (Linnaeus, 1758)	1	1	1	1	1	1			
118	*Euphydryas aurinia* (Rott., 1775)		1	1	1		1	1		

119	*Melitaea cinxia* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
120	*Melitaea phoebe* (D. und S., 1775)	1	1	1	1	1	1	1	1	1
121	*Melitaea trivia* (D. und S., 1775)	1	1	1	1	1	1			1
122	*Melitaea didyma* (Esper, 1778)	1	1	1	1	1	1	1	1	1
123	*Melitaea diamina* (Lang, 1789)	1	1	1	1	1	1	1		1
124	*Melitaea aurelia* Nickerl, 1850	1	1	1	1	1	1	1		1
125	*Melitaea britomartis* Assmann, 1847	1	1	1	1	1	1			
126	*Melitaea athalia* (Rottemburg, 1775)	1	1	1	1	1	1	1	1	1
127	*Limenitis populi* (Linnaeus, 1758)		1				1	1	1	
128	*Limenitis camilla* (Linnaeus, 1764)		1	1	1		1	1	1	1
129	*Limenitis reducta* Staudinger, 1901	1	1				1	1	1	
130	*Neptis sappho* (Pallas, 1771)	1	1	1		1	1		1	1
131	*Neptis rivularis* (Scopoli, 1763)	1	1	1	1	1	1			1
132	*Apatura metis* (Freyer, 1829)	1			1	1			1	
133	*Apatura ilia* ([D. und S.], 1775)	1	1	1	1	1	1	1	1	1
134	*Apatura iris* (Linnaeus, 1758)		1	1	1	1	1	1	1	1
135	*Kirinia roxelana* (Cramer, 1777)									1
136	*Pararge aegeria* (Linnaeus, 1758)	1	1	1	1	1	1		1	1
137	*Lasiommata megera* (Linnaeus, 1767)	1	1	1	1	1	1	1	1	1
138	*Lasiommata petropolitana* (F., 1787)		1							
139	*Lasiommata maera* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
140	*Lopinga achine* (Scopoli, 1763)	1	1			1	1	1		
141	*Coenonympha tullia* (Müller, 1764)			1						
142	*Coenonympha arcania* (Linnaeus, 1761)	1	1	1	1	1	1		1	1
143	*Coenonympha glycerion* (Bkh., 1788)		1	1	1	1	1		1	1
144	*Coenonympha pamphilus* (L., 1758)	1	1	1	1	1	1		1	1
145	*Pyronia tithonus* (Linnaeus, 1767)	1		1			1	1		1
146	*Aphantopus hyperantus* (L., 1758)	1	1	1	1	1	1	1	1	1
147	*Maniola jurtina* (Linnaeus, 1758)	1	1	1	1	1	1	1	1	1
148	*Hyponephele lycaon* (Rott., 1775)		1	1						
149	*Hyponephele lupina* (O. Costa, 1836)		1	1						
150	*Erebia ligea* (Linnaeus, 1758)					1				
151	*Erebia aethiops* (Esper, 1777)					1	1	1		
152	*Erebia medusa* (D. und S., 1775)					1			1	
153	*Melanargia galathea* (L, 1758)	1	1	1	1	1	1	1	1	1
154	*Satyrus ferula* (Fabricius, 1793)								1	
155	*Minois dryas* (Scopoli, 1763)	1	1	1	1	1	1	1	1	1
156	*Hipparchia fagi* (Scopoli, 1763)	1	1	1	1	1	1	1	1	1
157	*Hipparchia hermione* (L., 1764)		1							
158	*Hipparchia semele* (Linnaeus, 1758)		1	1	1	1	1			
159	*Hipparchia statilinus* (Hufn., 1766)		1	1	1					
160	*Hipparchia volgensis* (M.-P., 1952)							1	1	
161	*Arethusana arethusa* (D. und S., 1775)	1	1	1	1	1			1	1
162	*Brintesia circe* (Fabricius, 1775)	1	1	1	1	1	1		1	1
163	*Chazara briseis* (Linnaeus, 1764)		1	1	1					

Table 3. An overview of a total number of butterfly species in the studied sites (N) and the number of species common to commpared fauna (C).

		C							
		Malé Karpaty	Vertes	Bakony	Mecsek	Medvednica	Papuk	Fruška Gora	Avala
Mohács	(N 110)	103	98	93	101	99	78	94	93
Malé Karpaty	(N 139)		127	107	112	119	86	105	103
Vertes	(N 134)			108	115	114	86	103	104
Bakony	(N 112)				100	99	78	89	91
Mecsek	(N 123)					111	82	103	100
Medvednica	(N 124)						86	92	99
Papuk	(N 92)							82	81
Fruška Gora	(N 119)								100
Avala	(N 112)								

DISCUSSION AND CONCLUSIONS

In Tab. 2 are presented 163 species of butterflies in the studied sites of the Pannonian plain. Comparing the number of species in individual sites (Tab. 3) we found absolute similarity. So, Malé Karpati with 139 established species has the highest similarity of 85%, while Papuk, with 92 species, shows only 56% of similarity. Relative similarity can also be analyzed. Analyzing results shown in Tab. 3, we can see that in site Mohács there are 110 butterfly species. Comparing the number of common species in other sites we can see that values range from 78 species (71%) in Papuk to 103 species (93%) in Malé Karpati site. According to these values we can conclude that there is no significant difference between butterflies of Pannonian island mountains fauna and Pannonian plain fauna of this group. In other words, both areas have identical origin and genesis of the fauna of this group. Analyzing common species in all 9 sites the nature of that fauna can become more familiar. Out of 163 identified species in all 9 locations, there are 61 species (37%) encountered at all locations. These are the following (the number of species relates to Tab. 2): 1, 9, 14, 16, 17, 20, 22, 24, 25, 26, 29, 30, 31, 32, 36, 38, 41, 42, 43, 45, 46, 48, 49, 52, 53, 54, 55, 56, 58, 59, 67, 70, 72, 77, 79, 82, 86, 89, 94, 96, 101, 105, 106, 107, 108, 109, 112, 113, 114, 119, 120, 122, 126, 133, 137, 139, 146, 147, 153, 155 and 156.

Analyzing their faunal origin (Fig. 2) we can see that among 6 faunal groups, Euro-Siberian species have a dominant position with 40 (66%), followed by Euro-Oriental species with 13 (23%). Thus, these two groups with 90% share make the basic faunal features of the Pannonian plain area.

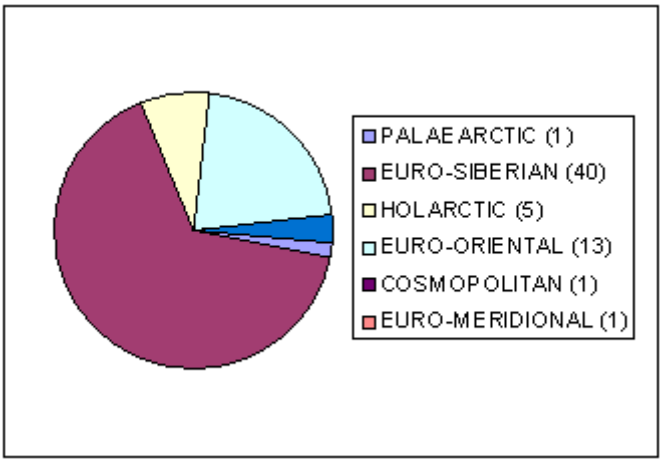

Fig. 2. *Faunistic composition of 61 species common to all nine sites*

Table 4. The degree of similarity coefficient (%) of the butterfly fauna in the analyzed area obtained by Jaccard's coefficient.

	Malé Karpaty	Vertes	Bakony	Mecsek	Medvednica	Papuk	Fruška Gora	Avala
Mohács	70.54	64.90	72.09	76.51	73.33	62.90	69.62	72.09
Malé Karpaty		86.98	74.30	74.66	82.63	59.31	68.62	69.59
Vertes			78.26	80.98	79.16	61.42	68.66	73.23
Bakony				74.07	72.26	61.90	62.67	68.42
Mecsek					81.61	61.65	74.10	74.07
Medvednica						66.15	60.92	72.26
Papuk							63.56	65.85
Fruška Gora								76.33

The degree of similarity coefficient shows that location Papuk has the lowest similarity to butterfly fauna in other locations, this value is within 59.31–66.15. This results from the fact that Papuk has the lowest number of species common to other locations. This can be assumed as a result of lack of faunal exploration, since only 92 butterfly species have been determined in Papuk. On the other hand, location Mecsek shows the highest similarity to butterfly fauna in other locations, this value is within 61.65–81.61. This is due to a great number of common species, 100 or more common species in 7 different locations.

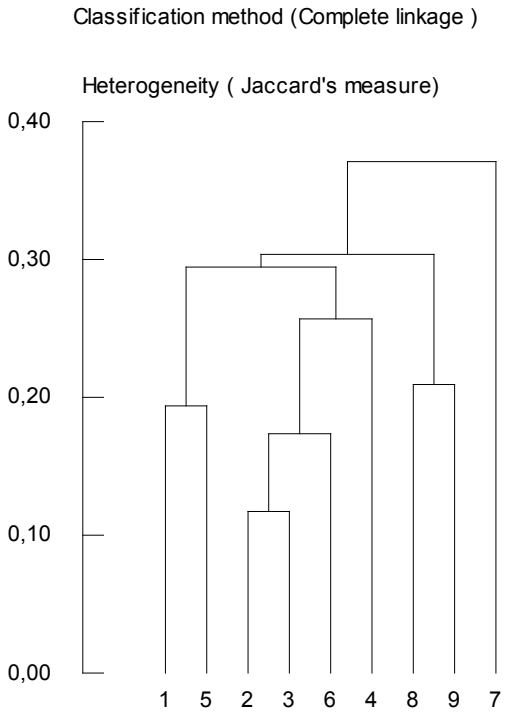

Fig. 3. *For agglomerate clustering, UPGMA method and Jaccard's similarity coefficient were used [Sokal and Rohlf 1995]. The estimation was done using the program FLORA [Karadžić and Marinković 2009].*

Jaccard's similarity coefficient analysis for agglomerate clustering shows interesting results. Location Mohács and Mecsek are clearly distinguished. It is understandable that they have similar faunal composition since Mohács lowland was the control site, and Mecsek is geographically closest to it (50 km).

The second cluster group consists of Malé Karpati (2), Vertes (3), Bakony (4), and Medvednica (6). All these sites represent the eastern branches of the unique mountain range the Carpathians-Alps. Specific and common fauna elements of these sites are the following species: 8, 10, 23, 40, 80, 148, 149, 159 and 163.

Finally, the third cluster consists of Fruška Gora (8) and Avala (9). What is common to them is that they are island mountains on the northern boundaries of the Balkan Peninsula. Therefore, the following group of species: 7, 13, 63, 111, 116, 154 and 160 are specific and common to them.

Papuk (7), in our opinion, is the most distinguished, primarily for not being faunaly explored enough. There have been only 92 species established, which is obviously not enough for making conclusions.

Based on these findings, we can come to conclusion that butterfly fauna in the Pannonian plain shows homogeneity in composition. This fauna originates from the areas of Siberia and Pontus. Specific elements of fauna are

newcomers from the Carpathians, Alps range and Balkan Peninsula. Considering fauna homogeneity, it is obvious that its genesis has lasted since the end of the Ice Age.

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АСПЕКТИ ЗООГЕОГРАФИЈЕ ДНЕВНИХ ЛЕПТИРА НЕКИХ „ОСТРВСКИХ ПЛАНИНА“ ПАНОНСКЕ НИЗИЈЕ

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РЕЗИМЕ: Простор Панонске низије претрпео је бурну геолошко-тектонску историју, праћену отицањем Панонског мора, наступањем глацијације са пратећим олеђивањем и навејавањем леса, као и постглацијалним формирањем вегетације и њеном антропогеном променом. Имајући у виду овако бурну историју поставља се питање порекла и генезе фауне дневних лептира овога простора, посебно у погледу постојања разлика у фауни између „острвских планина“ и Панонске низије.

У разрешавању овог питања компилирали смо постојеће литературне податке о фауни дневних лептира за осам „острвских планина“ и анализирали састав њихове фауне. Издвојили смо „језгро врста“, тј. 61 врсту које су присутне на свим истраженим локалитетима и утврдили да у фаунистичком смислу домини-

рају Евро-Сибирске врсте (њих 40, тј. 66%). У даљем поступку Жакаровом формулом и софтверским пакетом „Флора“ анализирали смо коефицијент степена сличности у саставу фауне одабраних подручја.

Добијени резултати показују да фауна дневних лептира Панонске низије, и „острвских планина“ у њој показује висок степен хомогености састава. Она води порекло из простора Сибира и Понта. Имајући у виду ову хомогеност извесно је да њена генеза тече од завршетка леденог доба. Специфични елементи фауне „острвских планина“ су придошлице са суседних планинских масива Карпата, Алпа и Балканског полуострва.

КЉУЧНЕ РЕЧИ: дневни лептири, Панонска низија, „острвске планине“, зоогеографија

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CONTRIBUTION TO THE BRYOPHYTE FLORA IN BEECH FORESTS OF VIDLIČ MOUNTAIN (SERBIA)

ABSTRACT: The research was conducted during 2011 and 2012 in Vidlič Mountain in southeastern Serbia. The aim of this research was to determine species composition of mosses and liverworts in beech forests. A total number of 48 taxa (6 liverworts, 42 mosses) was recorded. According to the Red Data Book of European Bryophytes there is one species in rare category. Also, there is one species with *low risk* threat status in Bryophyte Red List of Serbia and Montenegro. This research is a contribution to Serbian bryophyte flora study.

KEYWORDS: mosses, liverworts, Vidlič Mt., *Fagus*, forest, red listed species

INTRODUCTION

Mosses and liverworts are groups of plants with a special role in ecosystem functioning [Hallingbäck and Hodgetts 2000]. In most cases, mosses and liverworts are perennials, growing in almost all habitats, except extremely saline soils and marine ecosystems. There have been several bryophyte checklists for Serbia [Pavletić 1955; Gajić *et al.*, 1991; Sabovljević 2000; Sabovljević and Stevanović 1999; Sabovljević and Natcheva 2006; Sabovljević *et al.*, 2008]. According to the latest checklists published for the Mediterranean [Ros *et al.*, 2007; Ros *et al.*, 2013] there are 119 liverworts and 569 mosses taxa growing in Serbia. Some regions have been studied more intensively, therefore there are no records of bryophytes for certain places. Also, there are surprisingly few studies that deal with bryophyte flora in forest ecosystems in Serbia, despite their important role in forest ecosystems, like in nutrient cy-

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cling [Glime 2007], forest succesion, production, herbivory, etc. [Longton 1992]. The bryophyte flora of the Vidlič Mt. has never been surveyed. The nearest region with the data on bryophyte flora western Stara planina Mountain [Papp and Erzberger 2007].

The aim of this study was to determine the species composition and species richness of mosses and liverworts in beech forests in Vidlič Mt. This research is a contribution to the study of Serbian bryophyte flora and flora in general.

The investigated area

Vidlič is a trans-boundary mountain that stretches in Serbia and Bulgaria. In Serbia, Vidlič Mt. is located in southeastern part of the country (Figure 1), near the town of Pirot, between the rivers Nišava and Visočica. The highest peaks are Basarski Kamen, Velika Stena, Golemi Vrh and Smilovski Kamen, with altitudes of 1,377 m, 1,329 m, 1,371 m, and 1,348 m, respectively. Geologically, this is a very diverse area. The Early Paleozoic is represented by crystalline schist, gneiss, amphibolites, phyllite, quartzite, marbles, and slates. The higher mountain peaks are mostly made of Mesozoic limestone [Milovanović 2010].

The study site is located 1,200 m above the sea level in typical beech forest habitats. Beech forests in Vidlič extend to the elevation of 1,000 m. Soil testing in mono-dominant and poly-dominant mountain beech forests showed that in this area beech forests have been dominant for several thousand years [Marković 2013]. The northern part of Vidlič is characterized by the presence of Moesian beech forests, *Fagetum moesiacae montanum* Job. 1953 (non Rudski 1949) forming the old stands dominated by beech (*Fagus moesiaca*), which are in excellent condition.

Climate of this area is transitional humid-continental, between dry climate of Pirot region and humid climate of Stara Planina Mt. [Vidanović 1960]. In the higher parts there is a mountainous climate with short summers and long and cold winters, humid springs, and mild and long autumns [Marković 2013]. Considering that there is no weather station in Vidlič, climate observations can be obtained only from the nearest meteorological station in Pirot. The warmest month is July and the coldest month is January. The average annual temperature is 7.5 °C [Marković 2013]. Precipitation increases with altitude. An average precipitation in Vidlič at the altitude of 1,200 m is 946.5 mm [Marković 2013]. The highest precipitation is in May, June and December [Marković 2013].

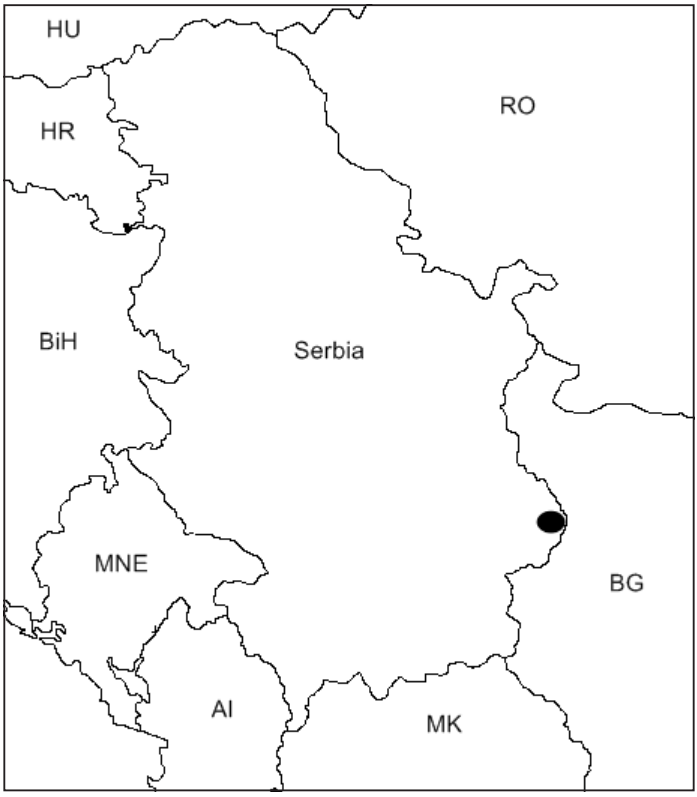

Figure 1. *Position of Vidlič Mt. on the map of Serbia (HU – Hungary; RO – Romania; BG – Bulgaria; MK – Macedonia; Al – Albania; MNE – Montenegro; BiH – Bosnia and Herzegovina; HR – Croatia)*

MATERIAL AND METHODS

This research was conducted during 2011 and 2012 in beech forest in Vidlič Mt. Specimens were collected along the transect line. The collection of plant material was performed in spring, summer and autumn in order to collect specimens from different parts of life cycle for easier and more accurate determination. Plant material was collected from tree bark, rotting trees, soil, rocks, etc. Specimens are deposited in BUNS (Herbarium of the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia). Species identification was performed with standard identification keys. Species nomenclature follows Hill *et al.* [2006] for mosses, Roskov *et al.* [2014] for liverworts, and for the genus *Metzgeria* nomenclature follows Grolle and Long [2000]. Classification is given according to Goffinet and Buck [2004].

RESULTS AND DISCUSSION

Total number of 48 taxa (6 liverworts, 42 mosses) was recorded.

Divisio Marchantiophyta

1. *Cephalozia* sp.
2. *Lophocolea heterophylla* (Schrad.) Dumort. 1835
3. *Jungermannia* sp.
4. *Marchantia polymorpha* L. 1753
5. *Metzgeria furcata* (L.) Dum. 1829
6. *Plagiochila porelloides* (Torr. ex Nees) Lindenb. 1840

Divisio Bryophyta

7. *Atrichum angustatum* (Brid.) Bruch & Schimp.1844
8. *Atrichum undulatum* (Hedw.) P.Beauv. 1805
9. *Brachytheciastrum velutinum* (Hedw.) Ignatov & Huttunen 2002
10. *Brachythecium geheebii* Milde 1869
11. *Brachythecium mildeanum* (Schimp.) Schimp.1862
12. *Brachythecium rutabulum* (Hedw.) Schimp. 1853
13. *Brachythecium salebrosum* (Hoffm. ex F. Weber & D. Mohr) Schimp. 1853
14. *Ctenidium molluscum* (Hedw.) Mitt. 1869
15. *Dicranum scoparium* Hedwig 1801
16. *Didymodon fallax* (Hedw.) R.H.Zander 1978
17. *Encalypta streptocarpa* Hedw 1801
18. *Encalypta vulgaris* Hedwig 1801
19. *Eurhynchium striatum* (Hedw.) Schimp. 1856
20. *Fissidens taxifolius* Hedwig 1801
21. *Grimmia pulvinata* (Hedw.) Sm. 1807
22. *Homalothecium philippeanum* (Spruce) Schimp. 1851
23. *Hygroamblystegium tenax* (Hedw) Jennings, 1913
24. *Hygrohypnum luridum* (Hedw.) Jenn. 1913
25. *Hypnum cupressiforme* Hedwig 1801
26. *Hypnum revolutum* (Mitt.) Lindb. 1867
27. *Isopterygiopsis pulchella* (Hedw.) Z.Iwats. 1987
28. *Isothecium alopecuroides* (Lam. ex Dubois) Isov. 1981
29. *Isothecium myosuroides* Brid. 1827
30. *Leptobryum pyriforme* (Hedw.) Wilson, 1855
31. *Leskea polycarpa* Hedwig 1801
32. *Leucodon sciuroides* (Hedw.) Schwägr. 1816
33. *Mnium spinosum* (Voit) Schwägr. 1816
34. *Mnium stellare* Reichard Ex Hedvwidg 1801
35. *Plagiomnium undulatum* (Hedw.) T.J.Kop. 1968
36. *Platygyrium repens* (Brid.) Schimp. 1851
37. *Polytrichastrum formosum* (Hedw.) G.L.Sm. 1971
38. *Pseudoleskeella catenulata* (Brid. ex Schrad.) Kindb. 1897
39. *Pseudoleskeella nervosa* (Brid.) Nyholm 1969
40. *Pterigynandrum filiforme* Hedwig 1801

41. *Pylaisia polyantha* (Hedw.) Schimp. 1851
42. *Sanionia uncinata* (Hedw.) Loeske 1907
43. *Schistidium* sp.
44. *Sciuro-hypnum starkei* (Brid.) Ignatov & Huttunen 2002 [2003]
45. *Syntrichia montana* Nees 1819
46. *Syntrichia ruralis* Weber & D. Mohr, 1803
47. *Tortella tortuosa* (Hedw.) Limpr. 1890
48. *Tortula subulata* Hedwig 1801

Among the recorded species, there is one species (*Brachythecium geheebii*) listed in Red Data Book of European Bryophytes [ECCB, 1995] in rare (R) category, and one species (*Leptobryum pyriforme*) listed in Bryophyte Red List of Serbia and Montenegro [Sabovljević *et al*., 2004) in low risk (LR) category. Species *B. geheebii* can be found only at two other localities in Serbia: Šaronje in Golija-Studenica Biosphere Reserve [Papp and Erzberger 2005] and Babin Zub in Stara Planina Mt. [Papp and Erzberger 2007]. It is a subcontinental mountain species [Natcheva 2011] growing on shaded rocks in forests. Species *L. pyriforme* was found on logs in forest. This is a cosmopolitan species [Rykovsky and Maslovsky 2004] and it was recorded in Serbia for the first time in Beočin [Stoizner 1870]. Also, it was found in Kopaonik [Jurišić 1900], Golija-Studenica Biosphere Reserve [Papp and Erzberger 2005], Temska [Papp and Erzberger 2007], and Metođe [Ilić 2012].

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ПРИЛОГ ПОЗНАВАЊУ ФЛОРЕ МАХОВИНА У БУКОВИМ ШУМАМА ПЛАНИНЕ ВИДЛИЧ (СРБИЈА)

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РЕЗИМЕ: Флора маховина букових шума планине Видлич истраживана је током 2011. и 2012. године. Циљ истраживања био је сачинити списак врста маховина које расту у буковим шумама планине Видлич. На истраживаном подручју, које је обухватало букове шуме на око 1.200 m надморске висине, пронађено је 48 врста маховина (шест врста јетрењача и 42 врсте маховина). Увидом у Црвену листу флоре маховина Европе, утврђено је да се једна врста налази у категорији „ретке“. Такође, након увида у Црвену листу флоре маховина Србије и Црне Горе, утврђено је да се на истраживаном подручју налази једна врсте која припада „мање угрожене“ категорији.

КЉУЧНЕ РЕЧИ: маховине, јетрењаче, Видлич, *Fagus*, шуме, Црвена листа флоре маховина

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DIATOM SPECIES COMPOSITION IN THE RAŠKA RIVER (SOUTHWESTERN SERBIA)

ABSTRACT**:** The paper presents data on the composition of epilithic diatoms in the Raška River. Samples were collected by scraping stone surfaces with a brush from 5 localities along the Raška River in April, June, August and November 2011 and March and May 2012. Diatom frustules were cleaned using cold acid method, and mounted on permanent slides. An investigation of the Raška River resulted in description of 106 diatom taxa. The most species rich genera are *Navicula* (10), *Gomphonema* (10) and *Nitzschia* (9), while other genera are presented with one or more species. Detailed floristic analysis of the benthic diatom flora in this river has not been conducted before. Therefore, this paper provides a groundwork for future researches.

KEYWORDS: Diatoms, Raška River, species composition, taxonomy

INTRODUCTION

Diatoms are large and diverse group of single-celled algae [Round *et al*., 1990; Potapova and Charles 2002]. They are distributed throughout the world in nearly all types of aquatic systems and are one of the most important food resources in marine and freshwater ecosystems [Wichard *et al*., 2007; Medlin 2011]. A key issue in understanding diatom distribution is knowing the extent to which they are constrained by geographical factors that limit species dispersal vs. the extent to which they are limited only by the ability of the species

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to grow under a specific combination of environmental factors [Blanco 2014]. Ability of populations to compete and persist in a new local habitat depends on how well adapted they are to grow under its environmental conditions. Some of the factors most often found to be important for distribution of benthic river diatoms are water chemistry (particularly pH, ionic strength and nutrient concentrations), substrate, current velocity, light and grazing [Biggs 1996; O'Driscoll *et al*., 2012].

The Raška River is the left tributary of the Ibar River located in the southwestern part of Serbia. It belongs to the Black Sea drainage basin. It is 39 km long, 10–25 m wide with a catchment area of 1,040 km². The basin of the Raška River is located in Starovlaška – Raška highlands [Marković 1980]. There were 5 sampling sites along the river (RS1, RS2, RS3, RS4 and RS5) (Fig. 1).

There have not been any published data about diatom flora in the Raška River so far. An endangered red alga *Bangia atropurpurea* (Roth.) was found in the Raška River [Simić 2008]. Krizmanić *et al*. [2008] found *Hydrurus foetidus* (Vill.) in the same river. We sampled benthic diatoms to evaluate the floristic richness of the river. The main objective of the paper was to present 106 diatom taxa, which provides a groundwork for future researches.

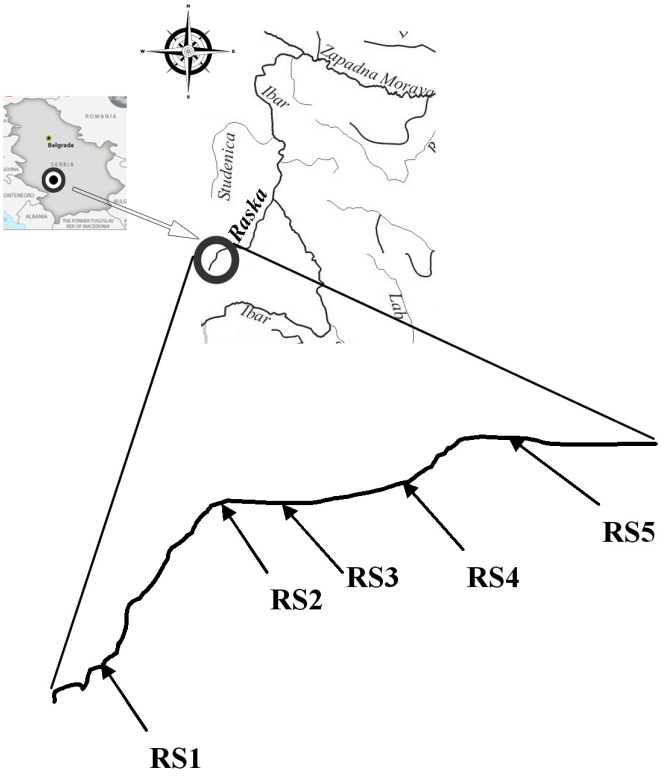

Figure 1. *Distribution of the sampling sites along the Raška River*

MATERIALS AND METHODS

The material was collected in April, June, August and November 2011 and March and May 2012 from 5 localities along the Raška River. Epilithic samples were scraped from the stone surfaces with a brush. Samples were fixed immediately with formaldechyde to a final concentration of 4%. Conductivity, oxygen, pH and water temperature were measured with a PCE-PHD at each sampling site. Chemical analysis of water was performed at the Institute of General and Physical Chemistry, University of Belgrade. The algological samples were treated in laboratory using standard methods to obtain permanent slides [Krammer and Lange-Bertalot 1986]. Permanent slides, prepared material and aliquots of the samples were deposited in the diatom collection of the Faculty of Biology, University of Belgrade. Light microscope observations and micrographs were made using a Zeiss AxioImagerM.1 microscope with DIC optics and AxioVision 4.8 software.

Terminology of valve morphology and identification were used according Hofman *et al*. [2013]. The abundance was estimated by counting 400 valves of each taxa present on a slide.

RESULTS AND DISCUSSION

Physico-chemical characteristics of water

According to Huet [1961], current velocity at the sampling sites along the Raška River ranged from moderate to very rapid (0.27–0.68 m/s). Low current velocity was caused by abundant vegetation. Water temperature of the Raška River ranged from 8 to 14 °C. The pH varied from neutral to slightly alkaline (7.02–8.23). The lowest pH value was recorded at RS2 sampling site in November and the highest was recorded at RS2 sampling site in March. Conductivity level had small variations (305–420 μS/cm). Total hardness was soft to slightly hard (6.6–121.0 mg CaCO3/l). The parameters providing information about a total content of organic matter are: biochemical oxygen demand (BOD_5), chemical oxygen demand (COD) and total organic carbon (TOC). BOD_5 was from 2.1 to 8.3 mg/l, COD from 4.2 to 18 mg/l and TOC was from 2.0 to 5.55 mg/l. Concentrations of other nutrients was low (ammonium ion, nitrates, nitrites, phosphates and orthophosphates) at all sampling sites along the Raška River.

Diatom species composition

A total of 106 diatom taxa have been recorded in the studied samples. We recorded two new taxa for diatom flora of Serbia: *Navicula jakovljevicii* and *N. catalanogermanica* [Vidaković *et al*., 2013]. Observed taxa were classified in 40 genera. The most abundant genera were *Navicula* (10), *Gomphonema* (10) and *Nitzschia* (9) (Tab. 1).

The presence of benthic diatoms in the Raška River for each of the six seasons is given in Tab. 2. Among the numerous species present in each season, *Achnanthidium minutissimum*, *Amphora pediculus* and *Navicula cryptotenella* were identified at all sampling sites along the Raška River. These taxa were abundantly developed and strongly predominated over other diatoms in the community.

Table 1. List of diatom genera with the number of taxa in the Raška River

Genus	No. of taxa	Genus	No. of taxa
Achnanthes	1	*Hantzschia*	1
Achnanthidium	5	*Hippodonta*	1
Amphora	3	*Luticola*	1
Caloneis	1	*Mayamaea*	1
Cocconeis	5	*Melosira*	1
Craticula	1	*Meridion*	1
Cyclotella	2	*Navicula*	10
Cymatopleura	3	*Neidiomorpha*	1
Cymbella	3	*Neidium*	1
Denticula	1	*Nitzschia*	9
Diatoma	4	*Placoneis*	3
Diploneis	2	*Planothidium*	3
Ellerbeckia	1	*Reimeria*	1
Encyonema	5	*Rhoicosphenia*	1
Fallacia	1	*Sellaphora*	4
Fragilaria	7	*Stauroneis*	1
Frustulia	1	*Staurosira*	1
Geissleria	1	*Staurosirella*	1
Gomphonema	10	*Surirella*	4
Gyrosigma	3	*Tryblionella*	1

The recorded composition of the benthic diatom communities is typical of running water and is similar to the diatom communities in other rivers in Serbia [Laušević 1993; Simić 1996; Nikitović and Laušević 1999; Krizmanić 2009; Andrejić *et al*., 2012]. *Achnanthidium minutissimum* is one of the most frequently occurring diatoms in freshwater benthic samples globally [Wojtal and Sobczyk 2006; Potapova and Hamilton 2007]. This species has been found in alkaline and acidic, oligotrophic and hypertrophic waters [Van Dam *et al*., 1994]. Various researches in Poland [Wojtal *et al*., 2011], South America [Potapova and Hamilton 2007] and Estonia [Vilbaste and Truu 2003] showed that *Achnanthidium minutissimum* was very abundant at the sampling sites. In the basin of the Zapadna Morava River, *Achnanthidium minutissimum* was also dominant taxon. In our study, this species was dominant at all sampling sites. Species such as *Amphora pediculus*, *Diatoma vulgaris*, *Navicula cryptotenella* and *Navicula tripunctata* are cosmopolitans and often dominant taxa in epilithic diatom community [Cremer *et al*., 2004; Wojtal 2009].

Table 2. List of the benthic diatoms in the Raška River (species names are listed in alphabetical order; '+' denotes the presence of a taxon).

Taxon	April 2011	Jun 2011	August 2011	November 2011	March 2012	May 2012
Achnanthes coarctata	+					
Achnanthidium affine	+	+	+	+	+	+
Achnanthidium biasolettianum		+	+	+	+	+
Achnanthidium exile		+	+		+	+
Achnanthidium minutissimum	+	+	+	+	+	+
Achnanthidium subatomus	+	+	+	+	+	+
Amphora inariensis	+	+	+	+	+	+
Amphora ovalis	+	+		+		+
Amphora pediculus	+	+	+	+	+	+
Caloneis bacillum	+	+	+	+	+	+
Cocconeis neodiminuta	+	+	+	+	+	+
Cocconeis pediculus	+	+	+	+	+	+
Cocconeis placentula var. lineata	+	+	+	+	+	+
Cocconeis placentula var. placentula	+	+	+	+	+	+
Cocconeis placentula var. pseudolineata	+	+	+	+	+	+
Craticula accomoda		+				
Cyclotella glabriuscula	+	+			+	+
Cyclotella meneghiniana	+		+		+	
Cymatopleura elliptica	+			+		
Cymatopleura solea		+				
Cymatopleura solea var. apiculata	+					
Cymbella compacta	+	+	+	+	+	+
Cymbella excisa	+	+	+			+
Cymbella parva	+	+	+	+		+
Denticula tenuis	+	+	+	+	+	+
Diatoma ehrenbergii	+	+		+	+	+
Diatoma mesodon		+			+	+
Diatoma moniliformis	+			+	+	
Diatoma vulgaris	+	+	+	+	+	+
Diploneis oblongela				+	+	
Diploneis parma	+					
Ellerbeckia arenaria		+	+	+	+	+
Encyonema lange-bertalotii	+	+	+	+	+	+
Encyonema minutum	+	+	+	+	+	+
Encyonema prostratum	+	+		+	+	+
Encyonema silesiacum	+	+	+	+	+	+
Encyonema ventricosum	+	+	+	+	+	+

Taxon	April 2011	Jun 2011	August 2011	November 2011	March 2012	May 2012
Fallacia subhamulata	+		+	+		+
Fragilaria acus		+				
Fragilaria biceps						+
Fragilaria capitellata				+	+	+
Fragilaria capucina	+			+	+	+
Fragilaria rumpens		+	+	+	+	+
Fragilaria ulna	+	+	+	+	+	+
Fragilaria vaucheriae	+	+	+	+	+	+
Frustulia vulgaris	+	+		+	+	+
Geissleria decussis	+	+	+	+	+	+
Gomphonema accuminatum	+	+		+	+	+
Gomphonema capitatum	+	+	+	+	+	+
Gomphonema micropus	+	+		+		+
Gomphonema minutum	+	+	+	+	+	+
Gomphonema olivacaeolacuum	+			+	+	+
Gomphonema olivaceum	+	+	+	+	+	+
Gomphonema parvulum	+	+	+	+	+	+
Gomphonema pumilum		+	+	+	+	+
Gomphonema sarcophagus	+			+	+	+
Gomphonema tergestinum	+	+	+	+	+	+
Gyrosigma attenuatum	+		+	+		
Gyrosigma obtusatum	+			+		
Gyrosigma sciotoense	+	+	+	+	+	+
Hantzschia amphioxys	+					
Hippodonta costulata						+
Luticola goeppertiana	+	+	+	+	+	+
Mayamaea atomus var. permitis			+	+		+
Melosira lineata	+	+	+	+	+	+
Meridion circulare	+	+	+	+	+	+
Navicula antonii	+	+		+	+	+
Navicula cari				+		
Navicula catalanogermanica			+	+	+	+
Navicula cryptocephala	+				+	+
Navicula cryptotenella	+	+	+	+	+	+
Navicula hintzii						+
Navicula jakovljevicii		+	+	+	+	+
Navicula lanceolata	+					
Navicula radiosa	+	+			+	+
Navicula tripunctata	+	+	+	+	+	+

Taxon	April 2011	Jun 2011	August 2011	November 2011	March 2012	May 2012
Neidiomorpha binodeformis	+			+		
Neidum dubium	+			+		+
Nitzschia dissipata	+	+	+	+	+	+
Nitzschia fonticola	+	+	+	+	+	+
Nitzschia heufleriana		+				
Nitzschia linearis	+	+		+	+	+
Nitzschia palea	+	+			+	+
Nitzschia pseudofonticola	+					
Nitzschia recta	+			+	+	
Nitzschia solita	+					
Nitzschia supralitorea	+			+	+	+
Placoneis paraelginensis	+					
Placoneis pseudanglica var. signata		+	+	+	+	+
Placoneis undulata		+	+	+	+	+
Planothidium dubium	+	+	+	+	+	+
Planothidium frequentissimum	+	+	+	+	+	+
Planothidium lanceolatum	+	+	+	+		+
Rhoicosphenia abbreviata				+	+	
Sellaphora bacillum	+	+				+
Sellaphora joubaudii			+	+	+	+
Sellaphora pupula	+	+			+	
Sellaphora seminulum						+
Stauroneis smithii	+					+
Staurosira binodis					+	
Staurosirella pinnata			+		+	
Surirella angusta	+	+	+		+	+
Surirella brebissonii var. kuetzingii	+	+		+	+	
Surirella linearis		+				
Surirella minuta	+	+				
Tryblionella angustata					+	

Seasonal dynamics of diatom species composition in the Raška River

At the first three sampling sites of diatom species composition in the Raška River, *Achnanthidium minutissimum* and *Amphora pediculus* appeared as dominant taxa. There was an exception in March 2013, when dominant taxon was *Diatoma vulgaris*. Besides these two taxa (*Achnanthidium minutissimum* and *Amphora pediculus*) at RS4 and RS5 sampling sites of diatom species composition there were present several other diatom taxa: *Achnanthidium affine*, *Gomphonema tergestinum* and *Achnanthidium subatomus*.

Percentage of valves for each taxon at the sampling sites from the six seasons is available on request from the authors.

The values of standard structure metrics (diversity indices and evenness) during all seasons are generally high (Fig. 2). In May 2012, the diatom community had high diversity indices (3.78) which influenced high evenness (0.78).

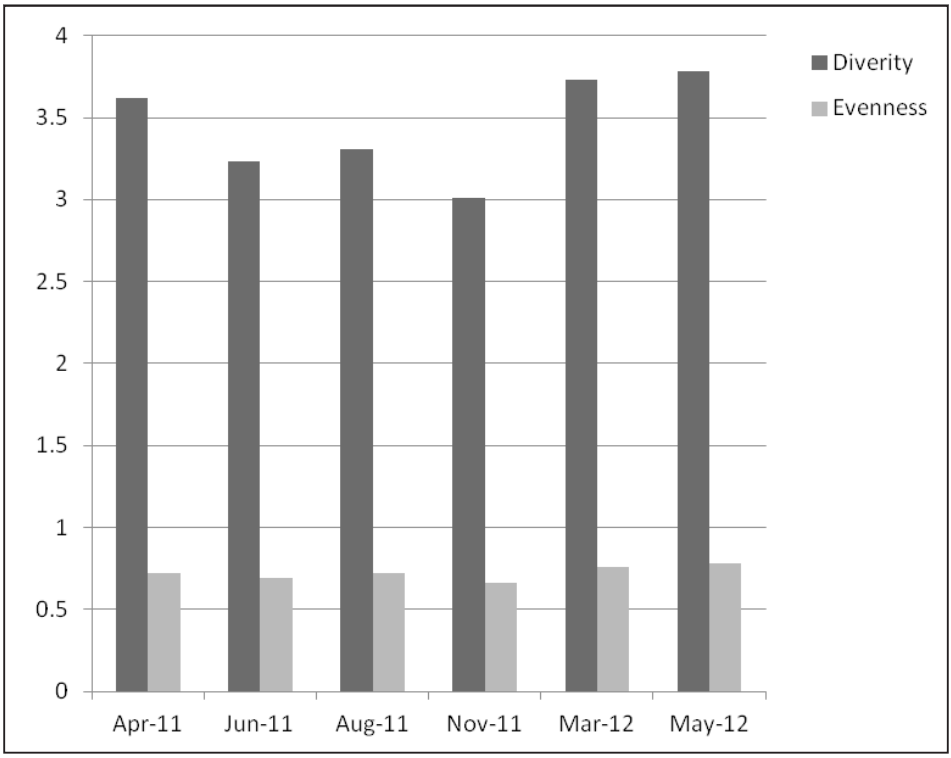

Figure 2. *The values of diversity indices and evenness during six seasons in the Raška River.*

The structure of the diatom assemblages in the Raška River agrees with the findings in the literature, which indicate that diatom communities are dominated by a few species that occur frequently and a large number of rare species that occur occasionally or sometimes only once [Kelly and Whitton 1995; Chatháin and Harrington 2008].

The diatom community was very similar at all sampling sites along the Raška River. In general, the most abundant diatom species were common at all investigated sites.

Some of the reported taxa were not frequent in the studied material (*Achnanthes coarctata*, *Craticula accomoda*, *Hippodonta costulata, Navicula hintzii*, *Nitzschia heufleriana*, *N. pseudofonticola*, and *Placoneis paraelginensis*) (Tab. 2). They are probably not ecologically significant for this river, but they are noticeable for floristic studies.

The presence of many diatom taxa could give evidence of a wide range of environmental possibilities for their development within the studied area [Wojtal *et al.*, 2005]. The study of diatom communities in rivers dates back many decades, therefore much information exist about species sensitivity to changes in the environment due to different pressures, particularly anthropogenic ones [Kelly and Whitton 1998; Kwandrans *et al.*, 1998; Gómez and Licursi 2001; Ivanov *et al.*, 2003; Vilbaste and Truu 2003; Ács *et al.*, 2004; Newall and Walsh 2005]. Evaluation of the floristic richness of diatoms in the river is a necessary, further step.

CONCLUSION

The main purpose of this study was to collect records on the floristics and the characteristics of the diatom assemblages inhabiting the Raška River.

There have not been any published data about diatom flora in the Raška River so far. These findings indicate the necessity for further fundamental investigations, since the diatom microflora in this part of the Balkan Peninsula remains poorly investigated regardless of a relatively long period of research. These new information increase our knowledge of the river system, which is important for further prediction of diatoms as bioindicators, as well as for monitoring programs.

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САСТАВ СИЛИКАТНИХ АЛГИ РЕКЕ РАШКЕ (ЈУГОЗАПАДНА СРБИЈА)

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РЕЗИМЕ: Рад садржи податке о саставу епилитских силикатних алги реке Рашке. Узорци су сакупљани у априлу, јуну, августу и новембру 2011. и у марту и мају 2012. године са пет локалитета дуж реке Рашке, стругањем четком са површине камена. Узорци су третирани стандардном лабораторијском методом и направљени су трајни препарати силикатних алги. Прегледом препарата идентификовано је 106 таксона силикатних алги. Родови најбројнији врстама су: *Navicula* (10), *Gomphonema* (10) и *Nitzschia* (9). Детаљна флористичка анализа бентоских силикатних алги реке Рашке није рађена раније па тиме овај рад даје основу за будућа истраживања.

КЉУЧНЕ РЕЧИ: река Рашка, силикатне алге, таксономија

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BIOREMEDIATION POTENTIAL OF FIVE STRAINS OF *PSEUDOMONAS* SP.

ABSTRACT: Because of their huge biodiversity and metabolic capabilities, the application of microorganisms as bioremediation agents is a way to enhance pollutant degradation. The aim of this research was to investigate the potential of five strains of *Pseudomonas* sp. as possible bioremediation agents. Strains are from the Collection of the Microbiology Department, Faculty of Agriculture, Novi Sad. Bacterial strains were cultivated in King's B liquid medium and incubated in shaker at 28 ^{0}C. Starter culture was obtained after 24h, CFU 10^8. This 24h old bacterial culture was used for the analysis of influence of five different natural naphthenic acids. Bacterial growth was determined spectrophotometrically through optical density, after 24h and 48h of growth. Our results showed that two bacterial strains (PS V1 and PS2) had better growth after 48h as they used C from the petroleum derivates. The growth of these strains was increased by 72% and 25% with derivates concentration of 10^{-5} mol/cm^3 and 10^{-6} mol/cm^3, respectively. The results of this research showed the potential of certain bacterial strains as bioremediators.

KEYWORDS: bioremediation, degradation, petroleum acids, *Pseudomonas* sp.

INTRODUCTION

With the increase of industrialization, environmental problems such as soil and groundwater contamination have become global issues [Ward *et al.*, 2003; Albers 2007]. Most components of crude oil are toxic to humans and wildlife in general, as they easily incorporate into the food chain. This fact has increased scientific interest in examining the distribution, fate and behavior of crude oil and its derivates in the environment [Stroud *et al.*, 2009]. Oil spills in the environment cause long-term damage to aquatic and soil ecosystems, human health and natural resources. Contamination of soil with crude

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oil and its derivates are causing numerous problems and hazards. These soils need to be remediated before any further use.

Bioremediation can be briefly defined as the use of biological agents, such as bacteria, fungi, or green plants (phytoremediation), to remove or neutralize hazardous substances in polluted soil or water. According to Diaz [2008], a way to enhance pollutant degradation is the application of microorganisms as bioremediation agents, because of their huge biodiversity and metabolic capabilities. Many different enzymes and metabolic pathways are required to degrade components of crude oil [Nilanjana *et al*., 2011]. Different species of microorganisms including bacteria, yeasts and fungi obtain both energy and tissue-building material from petroleum. The dominant genera of microorganisms that utilize petroleum hydrocarbons are: *Nocardia, Pseudomonas, Acinetobacter, Flavobacterium, Micrococcus, Arthrobacter, Corynebacterium, Achromobacter, Rhodococcus, Alcaligenes, Mycobacterium*, *Bacillus, Rhodotorulla, Candida, Sporobolomyces, Aureobasidium, Fusarium, Aspergillus, Mucor, Penicillium, Trichoderma* and *Phanerochaete* [Cerniglia and Sutherland 2001; Kuhad and Gupta 2009]. It is well known that *Pseudomonas* is among the bacteria with high remediation potential of different types of hydrocarbons [Hong *et al*., 2005].

Although there are a number of publications on positive effects of bioremediation, this technique, in some cases, has proved to be unsuccessful [Thompson 2005; Fantroussi and Agathos 2005]. The research indicates that shortly after the application of exogenous microorganisms, the number of these bacteria significantly reduces. Reasons for this can be numerous: competition between added and naturally occurring microorganisms, antagonism or predation (protozoa, bacteriophages), fluctuations in temperature, content of water, pH and availability of the contaminant and nutrient substances. So, it is more practical to use microorganisms isolated from the soil that needs to be decontaminated [Horakova and Nemec 2000]. This technique seems to be more effective because the indigenous bacteria are likely to be better adapted to the soil [Rahman *et al*., 2003].

Because of the importance of bioremediation of oil-polluted soils, the aim of this research was to investigate the potential of five strains of *Pseudomonas* sp. as possible bioremediation agents.

MATERIALS AND METHODS

Strains of *Pseudomonas* sp. denoted by PS4, PS V1, Q16, PS2 and PS D1 are from the Collection of the Faculty of Agriculture, Novi Sad. Strains were cultivated in King's B liquid medium (tripton 10 g l^{-1}; pepton 10 g l^{-1}; $MgSO_4$ 1.5 g l^{-1}; K_2HPO_4 1.5 g l^{-1}; glycerol 10 ml; pH 7). Incubation of the bacterial strains was carried out on a rotary shaker-incubator (BIOSAN ES 20/60), RPM 120, at 28 ^oC. Starter cultures were obtained after 24h (10^8 CFU/ml).

To determine the impact of petroleum products, the 24h old cultures of tested strains were used. 450 µl of petroleum derivates was added to each

bacterial strain. Control was a pure bacterial culture. The following petroleum products were used as tretmants: 1- NK/89 (naphthenic acid, 10^{-5} mol/cm^3), 2- NK/89 (naphthenic acid, 10^{-6} mol/cm^3), 3- NK-ol (alcohols of petroleum acid, 10^{-5} mol/cm^3), 4- NK-ol (alcohols of petroleum acid, 10^{-6} mol/cm^3), 5- NK-CH_3 (methyl esters of petroleum acid, 10^{-5} mol/cm^3), 6- NK-CH_3 (methyl esters of petroleum acid, 10^{-6} mol/cm^3), 7- NK-Aph (secondary amide, 10^{-5} mol/cm^3), 8- NK-Aph (secondary amide, 10^{-6} mol/cm^3), 9- NK-A (primary amide, 10^{-5} mol/cm^3), and 10- NK-A (primary amide, 10^{-6} mol/cm^3).

The growth of the bacterial strains was determined as optical density by a spectrophotometer (Unic SP600) at OD600 after 24 h and 48 h.

RESULTS AND DISCUSSION

In this research, petroleum derivatives influenced the number of tested strains.

Treatments had inhibitory effect on the number of most strains after 24 hours (Table 1). On average, strains Q16 and PS2 were the most sensitive to the influence of petroleum products. Decrease in the number of bacteria was recorded in all variants after 24h. On the other hand, application of treatments 3, 5 and 9 had a positive effect on the increase in the number of three bacterial strains: PS D1 (4.5%), PS V1 (5.43%) and PS4 (12.2%).

Table 1. Influence of petroleum derivates on the number of tested strains of *Pseudomonas* (x10^8 CFU/ml)

Strains	PS4		PS V1		Q16		PS2		PS D1	
Treatments	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Control	1.80	2.24	2.58	1.86	2.48	3.04	2.53	2.56	2.22	2.58
1	1.50	1.38	2.05	*2.96*	1.50	2.96	1.81	*3.04*	1.98	2.40
2	0.28	1.47	2.16	*2.72*	1.60	2.88	1.87	*2.80*	1.86	*2.96*
3	0.80	1.30	2.32	*2.64*	1.56	2.16	1.41	*2.72*	*2.32*	*3.04*
4	0.73	1.25	2.56	*2.96*	1.66	2.42	1.52	*3.20*	2.16	*2.64*
5	1.31	*2.64*	*2.72*	*3.20*	1.71	2.08	1.71	*2.86*	2.08	2.42
6	0.76	2.08	2.58	*2.88*	1.88	2.64	1.5	*3.20*	1.68	*3.20*
7	0.69	1.74	2.70	*3.20*	1.63	2.18	1.28	*3.20*	2.02	2.56
8	1.08	1.92	2.56	*2.80*	1.50	3.04	1.68	*3.20*	1.57	2.26
9	*2.02*[*]	*2.80*	*2.69*	*3.20*	1.80	*3.06*	1.55	*2.72*	1.89	2.24
10	0.69	*2.42*	2.26	*3.12*	1.26	2.88	1.50	*2.86*	2.00	*2.90*

Treatments: 1- NK/89 10^{-5}, 2- NK/89 10^{-6}, 3- NK ol 10^{-5}, 4- NK ol 10^{-6}, 5- NK CH_3 10^{-5}, 6- NK CH_3 10^{-6}, 7- NK Aph 10^{-5}, 8- NK Aph 10^{-6}, 9- NK A 10^{-5}, 10- NK A 10^{-6} mol/cm^3

[*] *Significant p-values, $p < 0.05$ are in italics, according to Fisher's test*

After 48 hours, the use of treatments had a good effect on the bacterial number in PS V1, PS D1 and PS2 strains, concerning the fact that they use C from the petroleum derivates (Table 1).

Application of treatments 5, 7 and 9 mostly affected the number of PS V1 (72%), while the application of treatments 4, 6, 7 and 8 led to the increase in the number of strain PS2 for 25% compared to the control. Also, the number of PS D1 in variants with treatments 2, 3, 4, 6 and 10 was higher than in the control. The results suggest that these three strains use the petroleum derivates as a source of energy, carbon or nitrogen, emphasising their potential to degrade petroleum products.

Emtiazi *et al*. [2005] in a study assessed the utilization of petroleum hydrocarbons by *Pseudomonas* sp. They monitored the change of bacterial growth turbidity (OD600nm) for nine days of incubation in liquid media. *Pseudomonas* sp. was able to use different hydrocarbons as sources of carbon and energy. Utilization of petroleum hydrocarbons by *P. fluorescens* isolated from a petroleum contaminated soil was reported by Bharathi and Vasudevan [2001]. Leahy and Colwell [1990] have reported biodegradation of petroleum oil by *Achromobacter*, *Arthrobacter, Acinetobacter, Alcaligenes, Bacillus, Flavobacterium, Nocardia, Pseudomonas* and *Rhodococcus*. Furthermore, studies of Nasrollahzadeh *et al.* [2007], Shafiee *et al.* [2006], and Mesdaghinia *et al.* [2005] reported biodegradation of phenanthrene by isolated bacteria. Isolation of 12 different bacterial species from polluted marine sites was reported by Kayode-Isola *et al.* [2008]. They found that *Alcaligenes paradoxus, Aeromonas* sp, *Bacillus licheniformis* and *Pseudomonas fluorescens* were efficient in biodegradation of diesel oil. Ting *et al.* [2009] in an experiment using *Pseudomonas lundensis* UTAR FPE2 found that utilization of paraffin and mineral oil is easier in comparison to naphthalene.

According to the results of this study, it can be concluded that *Pseudomonas* strains denoted as PS V1, PS2 and PS D1 showed the potential for bioremediation.

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СОЈЕВИ *PSEUDOMONAS* SP. КАО ПОТЕНЦИЈАЛНИ БИОРЕМЕДИЈАТОРИ

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РЕЗИМЕ: Примена микроорганизама као биоремедијатора једна је од могућности побољшања односно интензивирања разградње загађујућих супстанци које доспевају у земљиште. Циљ ових истраживања био је да се испита ефективност пет сојева *Pseudomonas* sp. као могућих биоремедијационих агенаса. Сојеви су из Колекције Одељења за микробиологију Пољопривредног факултета у Новом Саду. Бактеријски сојеви гајени су у течној King B хранљивој подлози и инкубирани на орбиталној мешалици. Стартер културе добијене су после 24 часа, CFU 10^8. Ове културе коришћене су за анализу утицаја пет различитих природних нафтенских киселина. Раст бактеријских култура праћен је спектрофотометријски мерењем оптичке густине после 24 и 48 часова. Два соја (PS V1 и PS2) имала су бољи раст после 48 часова указујући на чињеницу да користе угљеник (C) из нафтних деривата. Раст ових сојева био је повећан за 72% и 25% при конц. 10^{-5} mol/cm^3 односно 10^{-6} mol/cm^3 деривата. Резултати ових истраживања указују на могућност коришћења одређених бактеријских сојева у својству биоремедијатора.

КЉУЧНЕ РЕЧИ: биоремедијација, нафтенске киселине, *Pseudomonas* sp.

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METABOLIC ACTIVITY OF GUT MICROBIOTA AND XENOBIOTICS

ABSTRACT: The intestine habitat is the natural collection of symbiotic microorganisms. The bacterial population enables many permanent metabolic activities in this environment. Inside the intestine of mammals there are an extended genome of millions of bacterial genes named microbiome. In recent years, there has been an increased interest of scientists to discover the place and the role of bio-ecological content and modulation of gut microbiota in a host organism using prebiotics, probiotics and synbiotics, which may have a great benefit for human health.

KEYWORDS: gut flora, metabolism, xenobiotics

INTRODUCTION

Metabolism is a general term used for chemical transformation of xenobiotics and endogenous nutrients (e.g., proteins, carbohydrates and fats) inside or outside the host. Xenobiotics are all classified as chemical substances that are foreign to the host. They are not nutrient to the body and they enter it through ingestion, inhalation or dermal exposure (drugs, industrial chemicals, pesticides, pollutants, plant and animal toxins, etc.) [Mariat *et al*., 2009; Furet *et al*., 2010; Ley 2010]. The liver is built up of the endoplasmic reticulum (predominantly smooth endoplasmic reticulum) and other tissues which contain a

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large complex of enzymes, together called microsomal enzymes (microsomes are small spherical vesicles derived from endoplasmic reticulum after disruption of cells by centrifugation, and the microsomal enzymes are present in microsomes) [Kleessen *et al*., 2005; Sartor, 2004; Jia *et al*., 2008; Palmer *et al*., 2007]. Microsomal enzymes catalyse glucuronide conjugation, a great part of oxidative reactions, and some reductive and hydrolytic reactions. The factors which affect drug metabolism are: a) species differences (procaine, barbiturates, etc.), b) genetic differences (there are variations among species), c) age (faetus, newborn, old), d) sex (as a reflextion of sex hormones), e) nutrition (some kind of starvation and malnutrition), and f) pathological conditions (of liver or kidney). The human intestinal microflora is composed of different microbiota. There are near 10^{12} organisms per gram of gut content. This environmental circle supports activities of the gut flora as a huge metabolic system. The influence of resident gut microbes on xenobiotic metabolism has been investigated at different levels throughout the past five decades [Bojic Milićević *et al*., 1996; Bojić Milićević *et al*., 1995; Bojić Milićević *et al*., 2005; Bojić Milićević *et al*., 1998; Bojić Milićević *et al*., 1996; Bojić Milićević *et al*., 1997]. The investigations confirmed the influence of microbes on xenobiotics, which can have direct metabolic effects on toxins and other xenobiotics by conventional culture-based techniques, and explained the role of community composition on drugs metabolic profiles through DNA sequence-based phylogeny and metagenomics. This modern view of analysis opens new horizons for research how microbiome compositional and functional variations affect drug action, fate, and toxicity (pharmacomicrobiomics) in human intestine. The key role of these researches has been to describe the microbial communities associated with the human gut, to determine whether there is a common gut microbiome profile shared among healthy humans, and investigate the effect of its changes on health [Hamer 2008]. The explanation of pathways how the microbiome interacts with human metabolic enzymes in the liver and intestine is of the highest interest. The evolution of the complex metabolic interaction between intestinal microbiota in the human gut with its host is multidimensional. The composition of the intestinal microbiome is initially determined by genetic and environmental moments. In fact, external influences like host immune response provide the equilibrium in health and disease. The metabolism of drugs by intestinal bacteria and further by enterocytes with absorption into portal system may give a better understanding of pre-systemic drug metabolism, delivery, and toxicity [Salminen *et al*., 1998].

The gut microbiota is the most predominant and most diverse microbial community in human body. There are hundreds of microbial species, about 10 times more than the number of body cells. Human metabolic processes can reach up to 36% of small molecules in human blood as a treasure of the gut microbiome [Wilson *et al*., 2009; Ley *et al*., 2007; Costello *et al*., 2009]. The gut microbiota is involved in drug metabolism and has been explored since the middle of last century. One of the best definitions of the term *microbiome* was first suggested in 2000 by Joshua Lederberg, a Nobel Prize Laureate, to explain the participation of a large number of microbial genomes associated

with the human body. He made an approach to microbiome as a part of the "human extended genome". The term *microbiomics* refers to the functional aspects related to the microbiome. Pharmacogenomics explains the effect of human genome variations on drug disposition and reaction. Gut microbiota has a significant role and very much influence the metabolism of xenobiotics according to response-modifying process, as in several explored mechanisms. Drug metabolism can be changed by gut flora activity directly by producing enzymes that degrade or activate the drug molecules, or in a special game of the competition with drug molecules crossing by the metabolizing enzymes [Manichanh *et al*., 2008; Ley *et al*., 2006]. The gut microbiota also may affect drugs by modulating the activity or exchanging the levels of the host's drug metabolizing enzymes or by a complex process of producing enzyme. As the consequence of it there are brand new metabolites, originally derived from diet in germ free mice. This circle and activity of huge gut microbiota is in correlation with changes in quantity of liver and intestinal metabolic enzymes before their corresponding levels in mice with conventional gut micro flora ecosystem. The conventional gut microflora in human and mice are proved to be associated with a modest game in the levels of drug-metabolizing enzymes, for example sulfotransferase 1 B1 (SULT1B1). Nicholson et al. tried to make postulates of interaction of host and microecosystem in xenobiotic metabolism in the hindgut by making an interesting model, assuming six different kinds of cells in host and microbiome, each kind of which has its own transcriptome and metabolome depending on its place and change thorough special pathways. It was confirmed a common metabolic pathway between host and microbiota [Sartor 2004; Zoetendal *et al*., 1988]. The external component is human metabonome (the sums and interactions of all the cellular metabolomes). The production of indole-3-propionic acid was shown to be completely dependent in the presence of gut microflora, and could be organized by colonization with the bacterium *Clostridium sporogenes.* A lot of organic acids with phenyl groups were also greatly increased in the presence of gut microbes. Different factors as diet, genotype, and microbial interactions contribute to the diversity, but also to the relative balance of the intestinal microbiome. The two bacterial phyla Firmicutes and Bacteroidetes are dominant among the bacterial population of the intestine. The discovery of this diversity has been made possible by using advanced analytical techniques such as 16S rRNA-targeted oligonucleotide fluorescent probes or mass spectrometry and nuclear magnetic resonance methods. The implementation of these methods provides the differentiation among the microbial flora of each individual, reducing the spread of other species and strains [Ley *et al*., 2005; Turnbaugh *et al*., 2009; Paster *et al*., 2001].

MICROBIOME – HOST INTERACTIONS

The understanding that the intestinal microbiome interacts with the host has been recognized in different areas. There are considered symbiotic interaction for optimal food processing and local environment for optimal growth

conditions of dominant species, as well as immune interactions that benefit the host and keep a healthy mucosal barrier between intestinal bacteria and the host. The symbiotic interaction between host and microbiome for food processing and digestion is involved in bile acid metabolism and enterohepatic recycling of drugs and some nutrients like supplements of food. Those symbiotic interactions start very soon after birth and remain in the healthy population although an acute disease or disorder of bacterial equilibrium as a consequence of antibiotic therapy may produce chronic disorders that can constantly affect the ability of the host to process food optimally. Some bacterial strains play a very important role in the metabolism of nutrients such as choline or taurine and are essential for the absorption of fatty acids by the host [Pei *et al*., 2004]. While gut bacteria mainly use sugars for energy production leading to the formation of short-chain fatty acids such as acetate, propionate, and butyrate, the host utilizes these metabolic products for its energy consumption – muscles, heart, and brain utilize acetate while butyrate is important for enterocytes. Bacteria in the gut provide the host with essential amino acids and form a large number of vitamins like A, K and biotin. They also take part in biotransformation of bile. It is confirmed that the host genotype contributes to the diversity and specific composition of some bacterial species. Environmental factors such as diet and initial colonization after birth have a stronger influence.

ENTEROCYTE METABOLISM

It is proved that enterocytes are expressed in CYP 450 enzymes, especially CYP 3A4 and CYP 2C family members as well as phase II metabolizing enzymes like UDP-glucosyltransferase (UGT) and sulfotransferase. In fact, phase II conjugation enzymes and sulfotransferase activities are nearly 250–300% higher in the jejunum compared to the liver. The absorption as well gut wall metabolism of drugs depend on the mechanisms belonging to the hepatic metabolism. A lot of drugs first have to come inside the enterocytes in the beginning of metabolism game, mainly CYP 3A4 substrates. Permeability of drug compound is additional factor which plays an important role in drug metabolism [Bik *et al*., 2006; Eckburg *et al*., 2005]. Lipophilic compounds are readily absorbed in the gut and they are ready to be involved into metabolism. In that way, hydrophilic substances may require special active transport systems that limit their absorption. The influence of microbiota on metabolic processes has been known for decades, but the interest in pharmacokinetic and toxicokinetic influence on drug metabolism has grown recently. There are a lot of examples where absorption and metabolism are influenced by sometimes even crucial components of biodegradation of drugs [Eckburg *et al*., 2005]. The bioactivation is the reductive metabolism of sulphasalazine usually used in the treatment of ulcerative colitis which is classified as chronic disease. Also, prontosil and neoprontosil, pro-drugs of sulfanilamide, are metabolized in the large intestines to release the active sulfanilamide drug. This kind of metabolism was later proved for aminosalicylic acid pro-drugs like olsalazine

which results in anti-inflammatory effects in the gut as well as absorption and excretion of the corresponding coupling agent and thesalicylic acid derivative [Hooper *et al.*, 2001; Tlaskova-Hodgenova *et al.*, 2004; Round *et al.*, 2009]. Bioactivation is the example of plenty beneficial effects of microbial metabolism. In some cases metabolic activity of gut microbiota may result in forming of toxic metabolites with local and systemic effects [Haverson *et al.*, 2007; Stappenbeck *et al.*, 2002; Lefebvre *et al.*, 2009; Wong *et al.*, 2006]. The reduction of nitrazepam by gut flora is proved both in rat and human intestinal tract by 7-aminonitrazepam. It is then further metabolized in the liver after absorption of 7-acetylaminonitrazepam, which is known as teratogenic metabolite. Notable toxicity due to bacterial metabolism in the intestine is related to the bone marrow aplasia of a metabolite of the antibiotic chloramphenicol, which can happen in 1% of patients after oral consumption of chloramphenicol and also refers to gut microbiota. In such cases in small intestine there are a high percentage of coliform bacteria that are able to transform chloramphenicol to a toxic metabolite p-aminophenyl-2-amin-1,3-propanediol. Particularly common metabolic reaction that has an important role in fecal drug excretion and enterohepatic recirculation is a deconjugation reaction occurring in the intestinal tract as in the case of paracetamol [Hapfelmeier *et al.*, 2010; Atarashi *et al.*, 2011; Gibson *et al.* 2010; Cani *et al.*, 2009]. A great number of conjugated drugs like glucocorticoids, morphine, indomethacin and sex hormones are excreted via bile as glucuronic or sulfate acid metabolites. Bacterial metabolism of these conjugates consequently form aglycones or desulfated compounds that can be reabsorbed in order to prolong their biological half-life [Larsen *et al.*, 2010; Ley *et al.*, 2010]. There is a number of drugs that are mostly metabolized through phase II sulfation pathways like tamoxifen and apomorphine, among others [Roberfroid *et al.*, 1995; Elmer *et al.*, 1996; Sadler *et al.*, 1998; Neut *et al.*, 1980; Woese 1987]. This activities of gut flora metabolism show an important impact of drug metabolism and toxicity. Metabonomics and gene sequencing can overcome the obstacles and allow for a more comprehensive picture of both intestinal drug metabolism and mechanisms of toxicity [Stahl *et al.*, 1988; Rama *et al.*, 2012; Nicholson *et al.*, 2003].

CONCLUSION

Physiological state of a host depends on the intestinal microbiota. Gut microflora has a huge influence on many pathways of metabolic activities and pathways of drugs and xenobiotics. The habitat of intestine provides survival of beneficial bacteria, which contribute to biotransformation of consumed xenobiotics to therapeutic and toxic products that could be released and reabsorbed by entehepatic recirculation. The future can bring new knowledge about further gut flora role in almost any metabolic activity of the compounds and modern methods of the determination of bacterial genome as the key point in interaction between gut flora and host.

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МЕТАБОЛИЧКА АКТИВНОСТ ЦРЕВНОГ МИКРОБИОМА И КСЕНОБИОТИЦИ

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САЖЕТАК: Цревни тракт је природно станиште симбиотичних микроорганизама. Бактеријска популација омогућава перманентно одвијање многих метаболичких активности у овом еколошком окружењу. Сисари су наоружани обимним геномом милиона бактеријских гена унутар црева познатих под називом микробиом. Последњих година порастао је интерес научника за откривање места и улоге биоеколошког садржаја и модулације цревне флоре у организму домаћина применом пробиотика, пребиотика и синбиотика, који могу корисно да утичу на људско здравље.

КЉУЧНЕ РЕЧИ: цревна флора, метаболизам, ксенобиотици

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FLAVONOIDS AND POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY OF *FICUS CARICA* L. EXTRACTS FROM ROMANIA

ABSTRACT: The objective of this study is to determine flavonoids and polyphenols content and antioxidant activity of extracts of figs growing in Romania. The content of flavonoids and polyphenolic compounds was determined according to the Romanian Pharmacopoeia, the 10th edition, using the standard rutin for flavonoids, catechol for polyphenols and HPLC for flavonoids quantification. Determination of antioxidant activity was done by DPPH scavenging method and at cellular level by attenuation of oxidative damage in human erythrocytes. The experimental results reveal that *Ficus carica* extracts may be a potential source of natural antioxidants.

KEYWORDS: *Ficus carica*, flavonoids, rutin, antioxidant activity, DPPH (Diphenylpicrylhydrazyl)

INTRODUCTION

It is known that many natural compounds have antioxidant activity. Flavonoids belong to a special class of natural compounds being the main active substance in many medicinal herbs. Flavonoids are used in the treatment of many diseases to inhibit specific enzymes, hormones and to stimulate and reduce free radical activity.

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The antioxidant activity of flavonoids is due to a variable number of phenolic groups contained in their chemical structure and their property to form chelates with iron and other transitional metals.

The antioxidant activity is important for the human body because it protects the cells against free radicals, formed as a result in many processes that use oxygen as energy source, and plays an essential role in protection against oxidative degradation [Havsten 2002].

Flavonoids behave as antioxidants in a variety of ways, including direct trapping of the oxygen species, chelation of transition metals involved in the process radicals formation and prevention of the peroxidation process by reducing alkoxyl and peroxyl radicals [Heim *et al.*, 2002]. Also, they are able to modify the synthesis of eicosanoids, to prevent platelets aggregation and to protect lipoproteins against oxidation.

Although some studies indicate that flavonoids have peroxidation action, but only at high doses, they also have anti-inflammatory, antiviral, anti-allergic and protection role in various pathologies [Andersen and Markham 2006].

Ficus carica L. (Moraceae) is a shrub native of South West Asia cultivated since antiquity; originally from Persia and Syria, spread later in Europe and America.

A research report on this species indicates the presence of flavonoids, coumarins, sterols, triterpenoids, and anthocyanins in different parts of the plant. The leaves contain bergapten, quercetin, luteolin, and 4 ‘,5’-dihydropsoralen, the fruits contain cyanide-3-O-glucoside, cyanidin-3-O-rhamnoglucoside, rutin, gallic acid, catechin, epicatechin, saturated fat, cholesterol, sodium, protein, vitamin A, vitamin C, calcium and iron, and the root contains psoralen and bergapten [Anshul *et al.*, 2012].

The leaves and fruits of *Ficus carica* are traditionally used as laxative, stimulant against throat diseases, cough suppressant, emollient, emmenagogue and solvent [Bellakhadar *et al.*, 1991; Guarrera 2003]. Fig has been traditionally used for its medicinal benefits in metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory therapy. The root is used in leucoderma and herpes. The fruits are antipyretic, tonic, purgative, useful in inflammation, faintness, paralysis, hepatic and spleen diseases, angina, and also as hair growth stimulators. The milky juice of fig is expectorant and diuretic, but represents a high risk in contact with eyes.

The decoction of fig leaves can be used in hemorrhoids therapy, while fruits infusion can safely be used as a laxative for children. Fresh leaves were dabbed in wars [Baytop 1984].

The effect of fig leaves decoction on diabetes management has been studied, by maintaining a normal hypoglycemia in the short term [Serraclara *et al.*, 1998]. An aqueous extract of *Ficus carica* leaves may induce a significant hypoglycemic effect in rats, but the mechanism involved in such an effect was not elucidated [Perez *et al.*, 1996]. *Ficus carica* methanolic extract has potent anti-inflammatory activities at the level of cell migration and angiogenesis and may be correlated with its antioxidant potential [Eteraf-Oskouei *et al.*, 2015].

Rutin is the major flavonoid glycoside found in fig. It is the rhamno-glucoside of the flavonoid quercetin and vitamin P. Quercetin is the major flavonoid present in fig leaves, along with luteolin, kaemferol and rutin [Vaya 2006].

Many studies focus on the antioxidant activity of *Ficus carica*. Solomon *et al.* reported that fresh fruits had an antioxidant activity, and Konyalioğlu *et al.* showed that the antioxidant capacity of leaves extract had an antioxidant activity assessed only by the phosphomolibdenum spectrophotometric method.

This study focuses on the antioxidant activity by different methods and on determination of flavonoids and polyphenols content in fig (*Ficus carica*) leaves and fruits growing in Romania.

MATERIALS AND METHODS

Plant material

The plant material (leaves/fruits of *Ficus carica*) was collected from Arad (Romania) and dried at room temperature in a dark place.

Solvents and reagents

Rutin and catechol were purchased from Sigma–Aldrich. The other chemicals of analytical grade were purchased from Chimopar, Bucharest.

Preparation of extracts

The dried and finely ground samples of leaves and fruits (20 g each) were extracted with 250 mL ethanol 70% for 24h at room temperature. The extract was filtered and evaporated under vacuum to about 20 ml.

The extract was stored at 4 ºC to prevent any further degradation [Cacig 2007; Trifunschi and Ardelean 2013].

Determination of polyphenol content

Determination of polyphenol content was made according to Romanian Pharmacopoeia, the 10th edition. Catechol was used as standard. 5 mL of plant extract was mixed with 5 mL of phosphotungstic acid, and 5 mL of this mixture was diluted to 10 mL with sodium carbonate (200 g/L). The absorbance was measured at 430 nm (Metertech SP-8001 UV/Visible spectrophotometer) one minute later. The polyphenol concentration was expressed as the equivalent concentration of catechol. All determinations were performed in triplicate. The calibration curve was prepared with catechol solution ranging from 0.005–0.5 mg/mL.

Determination of flavonoids content

Flavonoids content was determined according to Romanian Pharmacopoeia, the 10th edition. Rutin was used as standard. 5 mL of plant extract was mixed with 5 mL of sodium acetate (100 g/L), 3 ml $AlCl_3$ (25 g/L) and diluted to 25 mL with methanol. The absorbance was measured at 430 nm (Metertech SP-8001 UV/Visible spectrophotometer). The flavonoid concentration was expressed as the equivalent concentration of rutin. All determinations were performed in triplicate. The calibration curve was prepared with rutin solution ranging from 0.5–5 mg/mL.

HPLC analysis of flavonoids

The HPLC analysis was performed using the HPLC YL 900 series instrument coupled with a binary pump, a diode-array detector (DAD), an auto sampler, and a column compartment. Flavonoids from extracts were separated on a Polaris C18 column (5µm, 4 x 125 mm) with a sample injection volume of 20 µl. A gradient elution was used with mobile phase consisting of (A) distilled water : phosphoric acid = 98 : 2 (pH=2.5) and (B) acetonitrile: 50% A and 50% B at the beginning; 80% B (after 12 min), and 50% B (15–20 min). The flow rate was 1.5 ml/min. The capillary temperature was set at 30 °C and λ = 380 nm.

Determination of free radical scavenging activity by DPPH method

DPPH assay is a method for measuring the antioxidant capacity of vegetable products involving the use of free radical 2.2-diphenil-1-picrylhydrazyl. It is used to evaluate the ability of compounds to act as free radical scavengers or hydrogen donors and evaluate the extracts antioxidant activity. The reaction involves color change from violet to yellow which can be monitored to some degree with a spectrophotometer at 517 nm.

It was added in a micro plate 0.25 mL extract and 4 mL DPPH solution (20 mg/dL) and monitored the variation of absorbance at 517 nm for 30 minute at room temperature [Prakash 2001]. Synthetic antioxidants of acid ascorbic and flavonoids standards (quercetin and rutin) were used. The scavenging effect of DPPH was calculated using the equation:

DPPH scavenging effect (%)= $(A_0-A_1)/A_0 \times 100$

All determinations were performed in triplicate.

Antioxidant activity at the cellular level

Attenuation of oxidative stress at the cellular level (leukocytes from healthy blood) was tested for flavonoids and for extracts of *Ficus carica*.

Determination of the antioxidant effect in pretreatment of human leukocytes of flavonoids extracts at different concentrations was based on the "comet tail" that followed the principle of electrophoresis in agarose gel [Lean *et al*., 1999; Norozi *et al*., 1998].

Approximately 100 mL fresh blood collected from a healthy human donor was treated in a polypropylene test tube with 1 ml of RPMI and 10% FCS. The mixture has been left at low temperature for 30 minutes, and then immersed in 1 ml of Ficoll using a micropipette. The obtained sample was centrifuged at 3600 rpm for 15 minutes at 4 °C. The fraction of white blood cells in the form of a pale pink and gray ring was removed after centrifugation by a Pasteur pipette, and immersed again in 1 ml of PBS homogenized using Vortex (solution A).

In six Ependrof labeled tubes have been added 85 ml of solution A (containing between 20,000 to 30,000 leukocytes) to which have been added the following:

-E1 (control) − 100 mL of PBS,

-E2, E3 − 100 mL concentrations of standard solutions of flavonoids (2.7% quercetin and rutin 0.15%) in PBS,

-E4, E5 − 100 mL alcoholic leaves extract and fruits extract of *Ficus carica* L respectively,

-E6 − 100 mL of 0.1% H_2O_2.

Samples E1−E5 were incubated at 37 °C for 30 min.

The sample E6 was kept in the dark at 4 °C during the incubation of the others. After the incubation period, samples E2−E5 were treated with 0.1% H_2O_2 in PBS and stored in the dark for 5 min at low temperature.

The experimental measurements have been made using microscope by direct examination on the lamellas with a wavelength radiation emitted at 520 nm and 620 nm respectively.

The classification of cells was made by a quick visual evaluation, using a scale from 0−4 as follows:

Grade 0 − cells degraded, less than 5% of the total;

Grade 1 − cells poorly degraded, between 5–25% of the total;

Grade 2 − cells with an average of degradation, between 25–45%;

Grade 3 − cells strongly degraded, between 45–70%;

Grade 4 − cells damaged, more than 70%.

* The concentrations of standard solutions of flavonoids have been the same as the ethanolic extract of *Ficus carica* concentration.

Statistical Analysis

The experimental results are expressed as ± means of standard deviation (SD). Data represent the average of three replicates and Microsoft Excel program was used for the statistical analysis.

Table 1. Flavonoid/phenolic contents and antioxidant activity of leaves and fruits of Ficus carica extracts

Ficus carica Extract	Polyphenol content (mg catechol/g)	Flavonoid content (mg rutin/g)	DPPH scavenging effect (%)
Ethanolic extract of leaves	25.25 ±0.001	2.62 ±0.003	44.22
Ethanolic extract of fruits	18.63 ±0,001	1.96 ±0.002	34.23
Quercetin	-	-	57.9
Rutin	-	-	59.6
Ascorbic acid	-	-	53.9

Table 2. HPLC analysis of extracts of Ficus carica

Compound	Ethanolic extract of leaves of *Ficus carica*			Ethanolic extract of fruits of *Ficus carica*		
	t_R [min]	Concentration [%]	Column resolution	t_R [min]	Concentration [%]	Column resolution
Quercetina	10.587	2.5	1.458	10.602	0.08	1.460
Luteolin	12.293	0.07	1.487	12.293	trace	1.485
Kaemferol	13.587	trace	2.073	13.59	trace	2.07
Rutin	14.827	0.2	1.428	14.826	1.8	1.427

Table 3. The effect of Standard solutions of flavonoids on the degradation of leukocytes

The type of treatment used	The percentage of cells with different degrees of damage [%]				
	Gr.0	Gr.1	Gr.2	Gr.3	Gr.4
quercetin (2.7 %) +100µL H_2O_2 0.1%	6.1 ± 0.6	8.22± 0.1	41.5± 2.6	21.3± 4.7	20.4 ± 2.5
rutin (0.15 %) +100µL H_2O_2 0.1%	2.1 ± 0.2	6.4 ± 1.5	31.8 ± 2.1	14.6± 2.1	30.1 ± 1.7
Luteolin (0.07%) +100µL H_2O2 0.1%	16.2± 0.1	21.3± 0.1	32.7± 1.5	21.3± 1.8	7.5± 1.6
kaemferol (0.02%) +100µL H_2O_2 0.1%	4.0± 1.5	10.5± 1.1	33.8± 1.1	24.3± 2.4	24.4± 1.4
The control sample	3.0 ± 0.1	8.4 ± 1.6	36.8 ± 1.4	14.2 ± 2.1	34.4 ± 1.4
cells + solution of H_2O_2 0.1% in PBS (100 µL)	-	1.5 ± 0.1	6.9 ± 0.6	23.4 ± 2.2	55.2 ± 2.1

Table 4. Effect of extracts of leaves and of fruit extracts on degradation of leukocytes

The type of treatment used	The percentage of cells with different degrees of damage [%]				
	Gr.0	Gr.1	Gr.2	Gr.3	Gr.4
Extract of fruits +100 µL H_2O_2 0.1%	6.2 ± 0.1	10.4 ± 2.3	30.8 ± 2.4	12.7± 4.7	21.7 ± 2.6
Extract of leaves +100 µL H_2O_2 0.1%	6.9 ± 0.5	10.7.± 1.4	38.8 ± 1.2	15.6 ± 2.3	16.0 ± 1.5
The control sample	5.0 ± 0.1	8.5 ± 1.1	35.8 ± 2.1	26.2 ± 2.4	18.2 ± 2.4
Cells + solution H_2O_2 0.1% in PBS (100 µL)	18.0 ± 0.2	21.3 ± 0.2	30.6 ± 1.4	19.3 ± 1.2	8.0 ± 0.8

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САДРЖАЈ ФЛАВОНОИДА И ПОЛИФЕНОЛА И ОДРЕЂИВАЊЕ АНТИОКСИДАТИВНЕ АКТИВНОСТИ ЕКСТРАКАТА *FICUS CARICA* L. ИЗ РУМУНИЈЕ

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РЕЗИМЕ: Циљ овог истраживања је утврђивање садржаја флавоноида и полифенола као и антиоксидативне активности екстраката смокве која расте у поднебљу Румуније. Садржај флавоноида и полифенолних једињења одређен је на основу Румунске фармакопеје, 10. издање. Коришћен је стандардни рутин за флавоноиде, катехол за полифеноле и HPLC метода за квантификовање флавоноида. Одређивање антиоксидативне активности урађено је помоћу органског једињења DPPH (2,2-дифенил-1-пикрилхидразил), а на ћелијском нивоу путем слабљења оксидативног оштећења у људским еритроцитима. Експериментални резултати показују да екстракти *Ficus carica* могу бити потенцијални извор природних антиоксиданата.

КЉУЧНЕ РЕЧИ: *Ficus carica*, флавоноиди, рутин, антиоксидативна активност, DPPH (Diphenylpicrylhydrazyl)

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ESSENTIAL OIL CONTENT AND COMPOSITION OF ANISEED

ABSTRACT: The field experiments were carried out during 2011 and 2012 in three localities in Vojvodina (Serbia) with the application of six different fertilizer regimes aimed at determining the content and composition of the aniseed essential oil. It was found that the average essential oil content of aniseed, obtained by hydrodistillation, was 3.72%. The weather conditions during the year and the locality had a statistically significant effect on the essential oil content, while different source of fertilizers was not statistically significant for the essential oil content and its composition. Essential oil composition was determined using GC–MS technique, and a total of 15 compounds were identified. It was found that the major component was *trans*-anethole, 94.78% on the average, and the coefficient of variation was 2%. The second most abundant component was γ-himachalene with 2.53% (CV 28%). All other components were present in less than 1%.

KEYWORDS: fertilization, location, *Pimpinella anisum* L., *trans*-anethole, weather conditions

INTRODUCTION

Aniseed (*Pimpinella anisum* L.) belongs to the *Apiaceae* family. This family is well known for its distinctive flavors, which come from essential oils and are a mix of volatile fragrant compounds that make the essence of the plant. In aniseed there is around 1.5–5.0% of essential oil, and the main component

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of the aniseed essential oil is *trans*-anethole that gives sweet herbaceous odor and taste to this plant [Ullah and Honermeier 2013].

It is known from the literature that aniseed has an effect on the digestive system by increasing secretion of the salivary glands, reducing the acidity of gastric juice. It also has a laxative effect. Apart from this, it has been found that anise has a positive effect on the liver function. Anise has an effect on the nervous system as antiepileptics and analgesics. It also possesses anti-inflammatory and anticancer activity. The effect of anise on the respiratory system is particularly important for the treatment of bronchial asthma. Anise is known for its estrogenic action, thereby its application helps the reduction of bleeding duration and menstrual pains and the reduction of hot flashes in postmenopausal women. Numerous studies have found that anise has an impact on microorganisms that cause many diseases. Aniseed has a wide range of effects on many bacteria, fungi, viruses and amoebas [Shojaii and Fard 2012].

The content and the composition of essential oils of aniseed are affected by ecological conditions, growing techniques and fruit maturity level [Özel 2009]. In case of coriander, which also belongs to Apiaceae family, it was concluded that dependence among temperature, radiance during fruit development and water supply of crops influence essential oil contents and its composition [Gil *et al*., 2002]. The investigation of the composition of essential oil from anise fruits cultivated in different countries show that some significant differences in concentration of some compounds in oil are present [Orav *et al*., 2008]. But in different countries, apart from different climatic and soil characteristics, different ecotypes are cultivated, so differences in essential oil content and composition are huge. Different populations grown in one country also show great variations of chemical compounds in essential oil [Arslan *et al*., 2004; Ipek *et al*., 2004; Naher *et al*., 2012].

For crops such as cereals, pulses, fodder crops, and vegetables, fertilization represents one of the most studied aspects of agronomic techniques, but there are less information about medicinal and aromatic plants [Carrubba 2009]. There are a few papers written about the application of different fertilizers in aniseed crop [Jevđović and Maletić 2006; Darzi *et al*., 2012; Nabizadeh *et al*., 2012; Jevđović *et al*., 2012]. However, there are no information about the influence of fertilizers on chemical composition of essential oil.

The aim of this study was to determine the influence of weather conditions, locations and fertilizers on the composition of anise essential oil. In order to determine this, the field experiments were carried out during 2011 and 2012 in three localities in Serbia with the application of six different fertilizer regimes.

MATERIAL AND METHOD

The field experiments were carried out during 2011 and 2012 in three localities in the Province of Vojvodina: Mošorin, Ostojićevo and Veliki Radinci. The field experiments were set up as a randomized block design with four replications. An experimental plot size was 5 m^2. Sowing of aniseed was carried

out at optimum time for agroecological conditions of Serbia (during April) with a hand seeder. Seeds were sown at row spacing of 0.35 m respecting the density of 200 plants per square meter. Weeds were controlled by hoeing and weeding when needed. The harvest was performed by hand at a full ripening stage.

The influence of six treatments: Slavol, Bactofil B-10, Royal Ofert, vermicompost, NPK (15:15:15), and control (without fertilization) were examined. Slavol and Bactofil B-10 are microbiological fertilizers containing products of bacterial fermentation, natural vitamins, enzymes and growth stimulators. Royal Ofert is a specific poultry manure inoculated with domestic fly larvae, dehydrated and pelleted, whereas vermicompost is cattle manure modified with *Lumbricus terrestris*.

The requested quantities of fertilizers, excluding Slavol, were applied by incorporation to the 5 cm layer of soil before the sowing of seeds. Slavol was applied two times during vegetation period – first time when plants had 3–4 leaves, and second time after 7 days. The dosage of application of these fertilizers is as follows: Slavol (7 l/ha by watering), Bactofil B-10 (1.5 l/ha), Royal Ofert granules (3 t/ha), vermicompost (5 t/ha), and chemical ferilizer NPK (400 kg/ha in formulation 15:15:15, i.e. 60 kg per hectare N, P_2O_5 and K_2O).

Climate in the region of Vojvodina is moderate continental with some continental tendencies. Both experimental years, in the period of active growing season, were warmer in relation to long-term average values and thus were moderately (2011) to severely dry (2012). Chernozem was a type of soil present in all three locations, with neutral reaction to the soil solution and moderate humus content (2.2–2.7%).

Essential oil was obtained from crushed mature fruits by hydrodistillation using Clevenger-type apparatus. The content of essential oil in anise fruits was 3.91% on the average. The chemical composition of essential oil was analyzed using GC/MS (gas chromatography- mass spectrometry) technique. GC-MS analysis was performed using an Agilent 6890 gas chromatograph in conjunction with an Agilent 5973 Network mass selective detector (MSD) in positive ion electron impact (EI) mode. The separation was achieved using Agilent 19091S-433 HP-5MS fused with silica capillary column, 30m × 0.25 mm, i.e. 0.25 μm film thickness. The GC oven temperature was programmed from 60 °C to 285 °C at a rate of 4.3 °C/min. Helium was used as carrier gas; inlet pressure was 25 kPa; linear velocity was 1ml/min at 210 °C. Injector temperature was 250 °C and the injection mode was splitless. MS scan conditions: source temperature – 200 °C; interface temperature – 250 °C; energy – 70 eV; mass scan range was 40–350 amu. Identification of components was done on the basis of retention index and by comparison with reference spectra (Wiley and NIST databases).

All the data were subjected to statistical analysis (one way ANOVA) using STATISTICA software. Differences between the treatments were performed by LSD range test at 0.05 level. Coefficient of variation (CV) was calculated as standard deviation/mean x 100.

RESULTS

In this study, the average content of essential oil in aniseed obtained by hydrodistillation was 3.91%. It has been found that essential oil content in aniseed depends on weather conditions and locality, while the application of different kinds of fertilizers does not have a significant effect on essential oil content. In the dry year (2012), a significantly lower essential oil amount was noted, and plants grown in Ostojićevo locality contained less essential oil than plants from other two localities.

A total of 15 compounds were identified from aniseed essential oil. It was found that the major component is *trans*-anethole, 94.78% on the average, and coefficient of variation was 2%. This component of aniseed essential oil was influenced only by weather conditions, while the effect of different localities and applied fertilizers was not statistically significant. As it can be seen from Table 1, in the year with average weather conditions (2011), there was less *trans*-anethole in essential oil when compared with dry year (2012).

The second most abundant component of the essential oil is *γ*-himachalene with 2.53% and with coefficient of variation of 28%. The quantity of this component of the essential oil, like in the previous case, was influenced only by weather conditions. In contrast to the *trans*-anethole, the content of this component was lower in the dry year.

All other components of aniseed essential oil were present in less than 1%, and their coefficients of variation were significantly greater. The impact of weather conditions during the year was significant for almost all components, except for *trans*-2-pseudoeugenyl metylbutirate, the presence of which in the essential oil was not affected by any of the factors.

Compounds as *β*-bisabolene, epoxy-2-pseudoeugenyl metylbutirate and *β*-farnesane were present only in the first experimental year, while *cis*-dihydrocarvone and *β*-elemene were present only in the second. These compounds have coefficient of variation above 100, while in the case of *β*-farnesane CV=201, which indicates that this is not a typical component of the aniseed essential oil.

In addition to the weather conditions, locality had a statistically significant impact on the presence of methylchavicol, *α*-zingiberene, *α*-himachalene, *β*-bisabolene, *cis*-anethole, and epoxy-2-pseudoeugenyl metylbutirate.

DISCUSSION

Drought stress significantly affected the essential oil percentage [Aloghareh *et al*., 2013]. Water deficit during stem elongation and umbel appearance reduced oil production in aniseed [Zehtab-Salmasi *et al*., 2001]. Dry conditions during the second experimental year caused significant decrease in essential oil content in our research, similarly to previous studies. Chemical fertilizers were effective in increasing oil percentage compared with unfertilized treatment

Table 1: Content and components of aniseed essential oil (%)

Source of variation		*Essential oil content*	*trans*-anethole	γ-himachalene	*trans*-pseudoisoeugenyl 2-methylbutyrate	methyl chavicol	*trans*-muurola-4(14),5-diene	α-zingiberene	α-himachalene	NI	β-himachalene	*cis*-dihydro carvone	β-bisabolene	*cis*-anethole	epoksy-psdoisoeugenyl 2-metthylbutyrate	β-elemene	β-farnesene
Year	2011	3.93	93,20	3,13	0,95	0,79	0,46	0,36	0,31	0,23	0,19	0,00	0,19	0,08	0,11	0,00	0,02
	2012	3.52	96,35	1,93	0,66	0,19	0,07	0,10	0,12	0,09	0,11	0,28	0,00	0,04	0,00	0,07	0,00
Locality	Mošorin	3.73	94,58	2,59	0,79	0,54	0,25	0,28	0,23	0,16	0,16	0,13	0,13	0,03	0,10	0,03	0,02
	V. Radinci	3.93	95,02	2,44	0,75	0,54	0,24	0,19	0,20	0,16	0,15	0,12	0,07	0,07	0,02	0,03	0,01
	Ostojićevo	3.50	94,73	2,56	0,86	0,38	0,31	0,21	0,22	0,16	0,15	0,16	0,09	0,07	0,05	0,04	0,00
Fertilizer	Control	3.64	94,96	2,35	1,03	0,41	0,23	0,19	0,20	0,15	0,15	0,12	0,08	0,04	0,06	0,02	0,01
	Slavol	3.66	94,35	2,80	0,86	0,51	0,27	0,24	0,24	0,18	0,16	0,18	0,09	0,07	0,05	0,04	0,01
	Bactofil	3.66	94,68	2,60	0,96	0,46	0,24	0,21	0,22	0,16	0,16	0,13	0,06	0,03	0,04	0,04	0,00
	Royal Ofert	3.90	94,89	2,40	0,66	0,53	0,37	0,25	0,21	0,16	0,16	0,15	0,12	0,07	0,07	0,03	0,02
	Vermicompost	3.63	94,98	2,53	0,60	0,51	0,24	0,23	0,22	0,16	0,14	0,13	0,11	0,07	0,06	0,03	0,02
	NPK	3.85	94,80	2,51	0,71	0,50	0,25	0,25	0,21	0,15	0,14	0,13	0,11	0,07	0,07	0,05	0,01
LSD 0.05																	
Year		*	*	*	ns	*	*	*	*	*	*	*	*	*	*	*	*
Locality		*	ns	ns	ns	*	ns	*	*	ns	ns	ns	*	*	*	ns	ns
Fertilizer		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Trial average		**3.72**	94,78	2,53	0,80	0,49	0,27	0,23	0,22	0,16	0,15	0,14	0,09	0,06	0,06	0,03	0,01
CV(%)		9	2	28	63	69	88	70	47	47	34	108	120	75	140	129	201

*significant at level 0.05 according to Fisher's Least Significant Difference Test, ns – not significant, CV – Coefficient of Variation, Ni – Compound is Not Identified

[Yassen *et al*., 2010]. The treatment with phosphate solubilizing microorganisms had a positive effects on essential oil content [Zand *et al*., 2013]. In our experiments, application of different types of fertilizers increased essential oil content of aniseed, but it was not statistically significant.

In a study conducted in Bangladesh [Naher *et al*., 2012] it was concluded that a slight variation of essential oil content and composition of anise depends on several factors such as genotype, stage of maturity, cultivation practices, soil composition and climate differences in various geographical locations. However, there is no data on how weather conditions influence the quality of anise essential oil, but it was known that high temperature and intensive insolation had a strong positive effect on α-bisabolol content, the main component of chamomile essential oil [Seidler-Lozykowska 2010]. This is similar to our investigation, where the content of *trans*-anethole was higher (96.35%) in the year with higher temperatures during vegetation period (2012).

In a study conducted in Turkey, *trans*-anethole ratio varied among localities from 95.57 to 97.24%, and the content of methyl chavicol varied between 1.89 and 3.18% [Tort and Honermeier 2005]. In this study, there was a significantly smaller variation between localities in case of *trans*-anethole (94.58–95.02%), but for methyl chavicol variations between 0.38 (locality Ostojićevo) and 0.54 (localities Mošorin and Veliki Radinci) were registered. Therefore, it can be concluded that in the case of methyl chavicol the influence of locality was significant.

Apart from isomers *trans*-anethole and methyl chavicol, *cis*-anethole was also present in aniseed, but in small proportion (0.06% in trial average). Because *cis*-anethole has toxic properties, international laws limit the concentration of this compound in isolated natural anethole to 0.2% [Orav *et al*., 2008].

Phenylpropanoids such as pseudoeugenyl 2-metylbutirate and *epoxy*-pseudoeugenyl 2-metylbutirate are characteristic of the genus *Pimpinella* and are phytochemical markers for it [Orav *et al*., 2008]. In this study, the pseudoeugenyl 2-metylbutirate was present in all samples, but *epoxy*-pseudoeugenyl 2-metylbutirate was not evident in the dry year (2012). Also, this component was noted in traces in localities Veliki Radinci and Ostojićevo, and in locality Mošorin it was present in the amount of 0.1%.

In essential oils of anise from different European countries [Ullah *et al*., 2013] α, β and γ-himachalene were noted in different concentrations depending on the country origin, as well as α-zingiberene, β-bisabolene and β-elemene. *Cis*–dihydrocarvone in this study was present with 0.26%. This component is usually present in other plants from Apiaceae family like caraway [Raal *et al*., 2012] and dill [Charles *et al*., 1995; Krüger and Hammer 1996]. However, it has not been detected in aniseed essential oil obtained by hydrodistillation. Using supercritical CO_2 extraction [Vîlcu *et al*., 2003], there was obtained 0.16 to 0.24% of this component of anise essential oil, while other authors, using the same method, did not detect this compound [Yamini *et al*., 2008; Rodrigues *et al*., 2003].

CONCLUSION

Essential oil content of aniseed depends on weather conditions and locality, while the application of different types of fertilizers does not have a significant effect on essential oil content and compounds in the oil. Weather conditions during the year had an influence on the content of all compounds in essential oil excluding *trans*-pseudoisoeugenyl 2-methylbutirate. This compound was not influenced by any factor, which asserts the fact that it is a phytochemical marker for genus *Pimpinella*.

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САДРЖАЈ И ХЕМИЈСКИ САСТАВ ЕТАРСКОГ УЉА АНИСА

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РЕЗИМЕ: У раду су приказани резултати испитивања количине и хемијског састава етарског уља аниса гајеног током 2011. и 2012. године на три локалитета у Војводини (Србија), при примени шест различитих врста ђубрива. Утврђено је да је просечна количина етарског уља у плодовима аниса добијена методом дестилације воденом паром 3.72%. Временски услови током године и локалитет имају статистички значајан ефекат на садржај етарског уља и његов хемијски састав, док примена различитих врста ђубрива не утиче на ове параметре. Хемијски састав етарског уља одређен је применом GC–MS анализе, при чему је идентификовано укупно 15 компоненти. Установљено је да је главна компонента *trans*-анетол, се просечно 94,78%, и коефицијентом варијације 2%. Друга најзаступљенија компонента била је γ-химахален са 2,53% (CV 28%). Све остале компоненте биле су присутне са мање од 1%.

КЉУЧНЕ РЕЧИ: ђубрење, локалитет, *Pimpinella anisum* L., *trans*-анетол, услови године

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INFLUENCE OF THE STRUCTURE OF BILE ACIDS ON THEIR PARTITION COEFFICIENT IN DIBUTYL ETHER AND CHLOROFORM

ABSTRACT: Bile acids are well known natural surfactants able to modify the permeability of biological membranes. The logarithm of partition coefficient between, traditionally used, *n*-octanol and water is a measure of lipophilicity as a predictor of solute membrane partitioning.

The aim of this work was to determine partition coefficients of bile acids in a mixture of water and chloroform and dibutyl ether at different pH values and with addition of different concentrations of sodium ions, and to examine the influence of the structure of bile acid nucleus on measured partition coefficients.

Partition coefficients of three bile acid salts were determined using shake-flask method and the concentration of bile acids was determined after twelve hours of shaking at the room temperature in aqueous and organic layer using reversed phase HPLC with DAD detector on 210 nm.

For all three analysed bile acid salts values of logP are lower in dibutyl ether than in chloroform. At certain pH values, curves representing the dependence of partition coefficient on pH value intersect, and these are the pH values for which partition coefficients are the same for both solvents. Increasing the solution ionic strength, this intersection is shifted toward lower pH values. It is found that, for both organic solvents, after the addition of hydroxyl group in the steroid nucleus (i.e. if the bile acid is less hydrophobic) the value of logP falls, especially if more hydroxyl groups are present. With chloroform as a solvent, system quickly comes to excess with electrolyte ions than with dibutyl ether.

KEYWORDS: bile acids, chloroform, dibutyl ether, partition coefficient

INTRODUCTION

Bile acid salts (BA) are amphiphilic steroids with two functionally different molecular surfaces, convex area which is more hydrophobic, and concave surface

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of the steroid core, a less hydrophobic one. BAs are characterized by different number, position and orientation of OH/oxo groups in different C atoms of the steroid skeleton with a consequent influence on their physico-chemical and biological properties [Poša 2011].

In addition to facilitating lipid digestion and absorption, bile acids are critical regulators of many key aspects of intestinal function – cell growth and death, epithelial barrier and transport function, mucus secretion, mucosal immune function, and intestinal motility [Keating 2009]. All these led to a growing interest in their analogues in metabolic disorder therapeutics [Mikov 2007]

Beside that, BAs are used as promoters in transport of some drugs [Mikov 2007; Gordon 1985; Bowe 1997; Poša 2008], and they have been extensively studied as permeability enhancers of various membranes [Yang 2009].

It is shown that electrolyte presence has an important influence on changes in physico-chemical features of bile acids as well as on their modular properties [Yang 2009; Roda 1983].

The logarithm of partition coefficient between *n*-octanol and water (Log P_{oct}) is traditionally used measure of lipophilicity as a predictor of solute membrane partitioning. In many situations, Log *Poct* cannot give a good estimate of the absorption of a drug or its permeation [Roberts 1996; Roberts 1996; Pugh 1996; Raevsky 2000; Wohnsland 2001]. Thus, other solvent systems are needed to yield information that is complementary to Log *Poct* data [Okada 1985]. Four classes of solvent/water systems are suggested to model the partitioning of solutes into membranes [Okada 1985; Leahy 1992a and 1992b]: an amphiprotic solvent such as octanol, a H-bond acceptor solvent such as dibutyl ether, a H-bond donor solvent such as hloroform, and an aprotic inert solvent such as alkane or 1, 2-dichloroethane.

The objective of the investigation reported here was to determine partition coefficients of three bile acids salts (sodium cholate (NaC), sodium deoxycholate (NaD), and sodium litocholate (NaL) (Fig. 1)) between water and chloroform, and dibutyl ether and water at different pH values and with addition of different concentrations of ions (sodium ions), and to examine the influence of the structure of bile acid nucleus on measured partition coefficients.

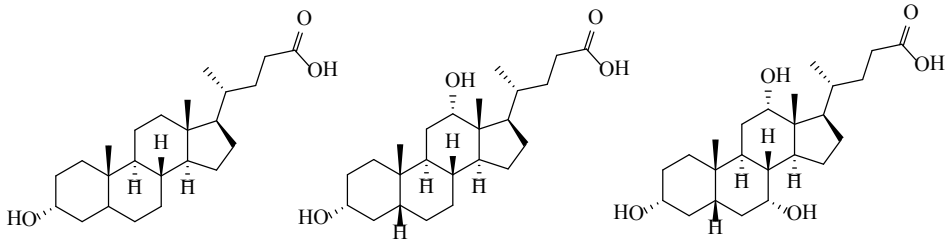

Figure 1.

MATERIALS AND METHODS

Cholic, deoxycholic and lithocholic acid (Sigma, New Zealand, 98%) were transformed to their sodium salts by a known procedure [Roda 1983]. Methanol, HPLC grade, was obtained from Carlo Erba reagenti, Italy, and KH_2PO_4 and Na_2PO_4 from Lachner, Czech Republic. Chloroform (HPLC grade) was purchased from Alfa Aesar and NaCl pro analysis from Merck, Germany.

Partition coefficients were determined using shake-flask method. Solutions of bile acids (NaC, NaD and NaL) were prepared in 0.01 M phosphate buffers at different pH values (pH 5.8, pH 6.2, pH 6.6, pH 7, pH 7.4, pH 7.8, pH 8), of different ionic strength adjusted with sodium chloride (0, 0.05M NaCl, 0.1M NaCl, 0.15M NaCl and 0.2M NaCl), and mixed with the same volume of non-polar phases chloroform and dibutyl ether respectively. Concentration of each bile acid was 4mmol/L in the whole volume. Solutions were mixed for 12 hours using magnetic stirrer. Concentrations od bile acids were determined in both phases using validated HPLC method.

The HPLC system Agilent 1100 Series, equipped with degasser, binary pump, automatic injector and DAD detector with software system for data processing AgilentChemStation was used and the analyses were performed on a reversed-phase C-18 column: Eclipse Plus C18 (250 mm x 3 mm, 5 μm, 250 A°) column (Zorbax SD). The mobile phase was 0.01M phosphate buffer: methanol = 70: 130 v/v maintained at pH 7 and the injection loop was 10 μL. All separations were performed isocratically at a flow rate of 1 ml/min and a column temperature changing of 20. The detection was performed at 210 nm [Roda 1990].

RESULTS AND DISSCUSION

It is important to know partition coefficients (of ionised and unionised form) of bile acids in different organic solvents, since certain types of solution more or less model some organs or anatomic units in biological systems. For example, dibutyl ether models very well the transferring of substances over blood brain barrier.

For all three analysed bile acid salts values of logP are lower in dibutyl ether than in chloroform. There are no data on bile acid partition coefficients in chloroform and dibutyl ether. Literature data on logP values of cholic acid and deoxycholic acid in 1-octanol are higher than in chloroform ($logP_{oct} > logP_{chloroform} > logP_{dibutyl\ ether}$) [Roda 1990]. We suggest that the highest values of partition coefficient of $logP_{oct}$ resulted from the fact that 1-octanol have OH group which is proton acceptor and proton donor at the same time so it has higher ability to form hydrogen bond with OH groups of the α side of the steroid skeleton than in case of chloroform which is proton donor and dibutyl ether, a proton acceptor (Fig. 2).

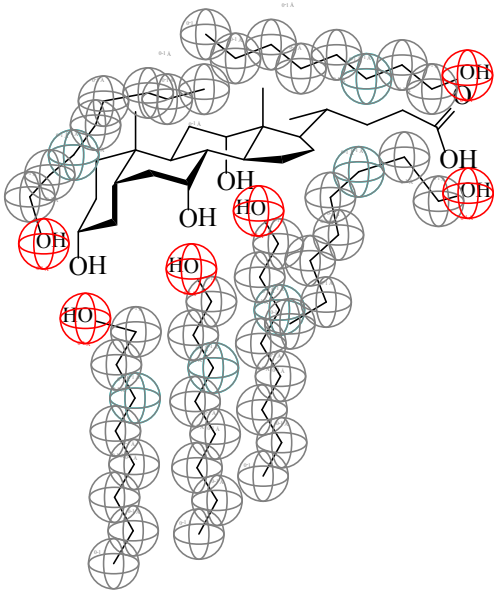

Figure 2.

Also, geometry of 1-octanol molecule makes favourable conditions for solvation of steroid skeleton comparing to chloroform and dibutyl ether. Namely, in 1-octanol polar OH group and hydrophobic tail are completely separated, and if we take into account a high conformational flexibility of octile tail during building of solvation shell, its translation entropy changes a bit compared to chloroform and dibutyl ether (1-octanol participates less in solvation of steroid skeleton than chloroform and dibutyl ether, which is a result of molecular flexibility of 1-octanol which adapt to geometrical shape of the steroid skeleton). The lowest value of partition coefficient for dibutyl ether is probably because two hydrophobic units of this molecule are separated with oxygen bridge which lowers hydrophobic solvation of this molecule.

At certain pH values, curves representing the dependence of partition coefficient on a pH value intersect. These are the pH values for which partition coefficients are the same for both solvents. Increasing the solution ionic strength, this intersection is shifted toward lower pH values (Fig. 3). Solvation effect is more prominent if there is a higher fraction of unionised form of the molecule in equilibrium system (at lower pH values).

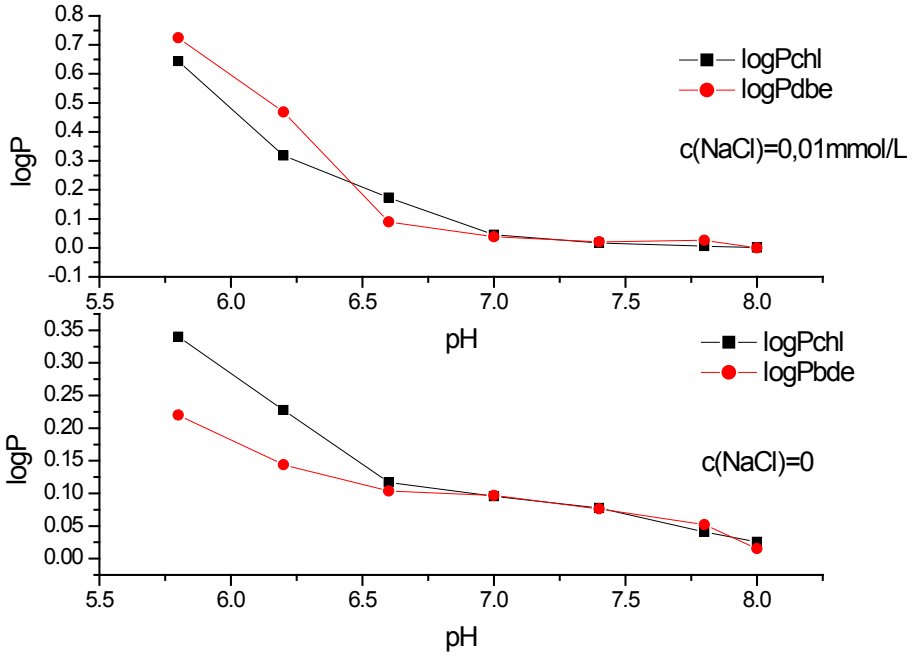

Figure 3.

It is found that, for both organic solvents, after the addition of hydroxyl group in the steroid nucleus (i.e. if the bile acid is less hydrophobic) the value of logP decreases BA(*p*) especially if more hydroxyl groups are present (Table 1). This can be explained as bile acid molecules, with more hydroxyl groups, are more stabilised in an aqueous than in organic solvent.

Table 1. LogP values between chloroform and water for NaC, NaD and NaL at different pH values

	logP		
pH	NaC	NaD	NaL
5.8	0.3402	0.9271	7.6570
6.2	0.2276	0.8810	5.0181
6.6	0.1168	0.7063	3.6124
7	0.0955	0.5329	1.6188
7.4	0.0775	0.3589	0.6672
7.8	0.0408	0.0700	0.4985
8	0.0255	0.1295	0.5974

Namely, the distribution process of bile acids can be presented in two phases. In the first phase, water molecules hydrogen bonded to the bile acids from solvation sheath are liberated, then the bile acid can move to nonpolar solvent. This can be represented with two equations:

$$BA[W]_n \rightleftarrows BA_{(p)} + nW \quad (1)$$

$$BA_{(p)} \rightleftarrows BA_{(u)} \quad (2)$$

where W stands for water molecules, $BA_{(p)}$, $BA_{(u)}$, are bile acids present in polar phase (water) and nonpolar phases (chloroform or dibutyl ether).

Along with the increase of ionic strength, during the whole analysed range of pH values for sodium cholate, we can see the similar behaviour of the curves. The highest jump of the partition coefficient value for chloroform is when the first jump of the concentration of sodium salts (concentration of 0.05 M NaCl) occurs, while for the next jump of NaCl concentration, partition coefficient have the lower value for each examined pH values (Fig. 4). The situation is similar when logP value is measured between dibutyl ether and water but the highest jump of logP value occurs with the second jump of the concentration of sodium chloride (concentration is 0.1 M NaCl). With further increase in amount of sodium ions partition coefficient decreases (Fig. 5).

According to this we can conclude that chloroform/water system faster saturates with electrolyte ions than with dibutyl ether/water system.

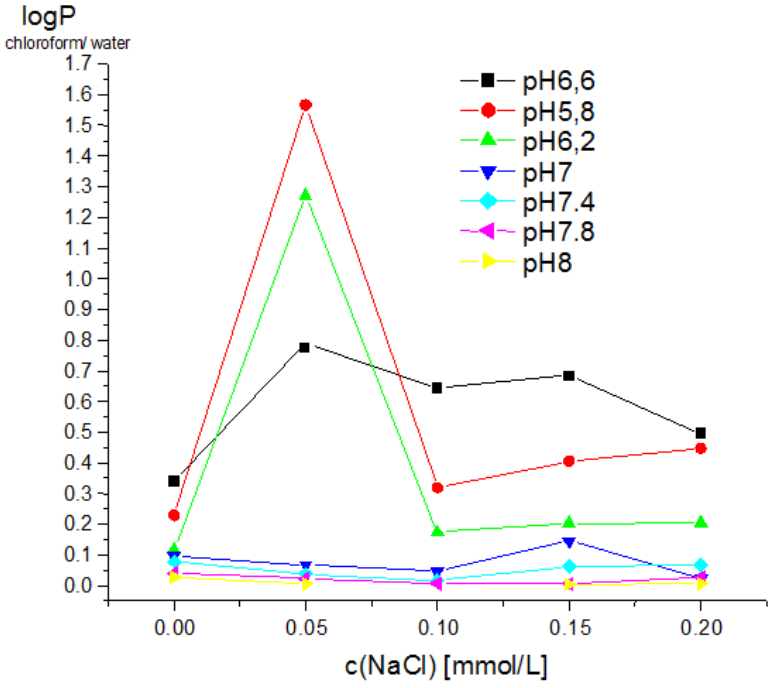

Figure 4.

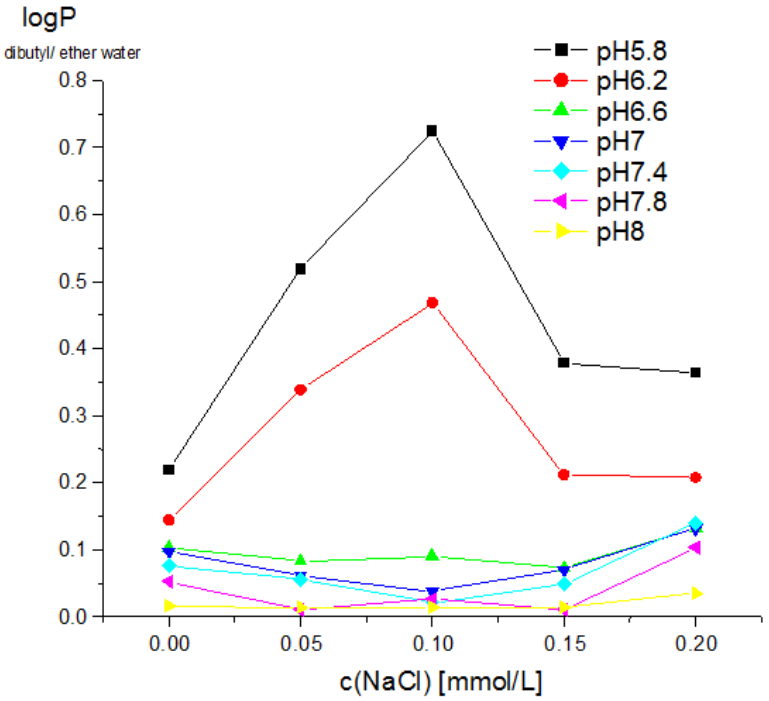

Figure 5.

CONCLUSION

For all three analysed bile acid salts values of logP are lower in dibutyl ether than in chloroform. At certain pH values, curves representing the dependence of partition coefficient on pH value intersect, and these are the pH values for which partition coefficients are the same for both solvents. Increasing the solution ionic strength, this intersection is shifted toward lower pH values. It is found that, for both organic solvents, after the addition of hydroxyl group in the steroid nucleus (i.e. if the bile acid is less hydrophobic) the value of logP decreases, especially if more hydroxyl groups are present. Chloroform/water system faster saturates with electrolyte ions than with dibutyl ether/water system.

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УТИЦАЈ СТРУКТУРЕ ЖУЧНИХ КИСЕЛИНА НА ЊИХОВ ПАРТИЦИОНИ КОЕФИЦИЈЕНТ У ДИБУТИЛ ЕТРУ И ХЛОРОФОРМУ

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РЕЗИМЕ: Жучне киселине добро су познати природни сурфактанти који могу да модификују пропустљивост биолошких мембрана. Логаритам партиционог коефицијента између традиционално коришћенох N-октанола и воде је мера липофилности као предиктор расподеле растворка кроз мембране. Циљ рада је да се одреде партициони коефицијенти жучних киселина између воде и хлороформа и воде и дибутил етра на различитим pH-вредностима и са додатком различитих концентрација натријумових јона, као и да се испита утицај структуре језгра жучних киселина на измерени партициони коефицијент. Партициони коефицијенти три соли жучних киселина одређивани су користећи методу мућкања док су концентрације жучних киселина одређиване након дванаест сати мешања на собној температури у воденом и органском слоју користећи реверзну фазну HPLC методу са DAD детектором на 210 nm. За све три анализиране жучне киселине вредности *logP* су мање у дибутил етру него у хлороформу. На одређеним pH-вредностима криве које представљају зависност партиционог коефицијента од pH-вредности секу се и то су pH-вредности на којима су коефицијенти расподеле исти за оба растварача. Са повећањем јонске јачине раствора тачка пресека помера се ка нижим pH-вредностима. Пронађено је да за оба растварача након додатка хидроксилне групе у стероидно језгро (односно што је жучна киселина мање хидрофобна) вредности *logP* опадају посебно ако је присутно више хидроксилних група. Са хлороформом као растварачем систем се брже засити јонима електролита него са дибутил етром.

КЉУЧНЕ РЕЧИ: дибутил етар, хлороформ, партициони коефицијент, жучне киселине

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GASEOUS AND PARTICULATE URBAN AIR POLLUTION IN THE REGION OF VOJVODINA (SERBIA)

ABSTRACT: The present study focuses on interpretations of the temporal variations and variations between urban locations of sulfur dioxide (SO_2), nitrogen dioxide (NO_2) and black smoke (BS) during the period 2001–2008 in the Vojvodina Region of Serbia (VR_S). In this study we examined variations of pollutants concentrations during household heating and non-heating seasons and the effect of household heating, traffic, rainfall and wind speed on the air pollution levels of SO_2, NO_2 and BS in eight locations. The analyses showed that the annual limit values of these pollutants as recommended by the Serbian regulations and recommendations were not exceeded, unlike the daily limits. Higher SO_2 concentrations during household heating season in four locations indicate the substantial impact of household heating on air quality. Positive effects of the use of environmentally cleaner fuels were observed in only two locations. The growing impact of traffic on air pollution is shown by the increasing trend of NO_2 during both seasons. Calm wind conditions and an absence of rainfall were found to have incremental effects on pollution levels in most locations.

KEYWORDS: air pollution, black smoke, nitrogen dioxide, sulfur dioxide, Vojvodina

INTRODUCTION

Air quality has become one of the predominant factors affecting quality of life. Atmospheric pollutants are responsible for a variety of respiratory illnesses and are known to increase morbidity and mortality [Brunekreef and Holgate 2002]. Worldwide, the World Health Organization (WHO) estimates that as many as 1.4 billion urban residents breathe in air pollutant concentrations that

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exceed the WHO guideline values [WHO, 2002]. Population growth in cities leads to an increase in pollutant levels as a result of increases in emissions from sources such as traffic. Based on their effects on health and the environment, several air pollutants have been identified as *criteria pollutants* by the U.S. Environmental Protection Agency (EPA): ground-level ozone, carbon monoxide, sulfur dioxide, nitrogen oxides, particulate matter and lead. Controlling atmospheric levels of these pollutants has the potential to provide substantial benefits to both human health and the environment.

According to the World Meteorological Organization (WMO) [2004], the relationship between climate conditions, human activities and quality of life can be analyzed according to the effects of UV-B radiation, thermal environment and air pollution. Because air pollution influences UV-B level and thermal environment, all three factors have a synergetic effect on humans and the environment. Two well-documented papers elaborate on these issues in the Vojvodina Region of Serbia (VR_S) through (i) an analysis of indices related to thermal environment and UV-B radiation together with climate conditions [Malinović-Milićević *et al*., 2013] and (ii) an analysis of UV-B radiation dose complexity using Kolmogorov complexity measures; the decrease in this complexity is strongly related to the increase in air pollution [Mihailović *et al*., 2013]. However, these papers do not comment in detail on air pollution in this region. To complete the triangle of components that have a synergetic effect on humans and the environment, we investigated the air quality in the VR_S through an accurate determination of the levels, variations and sources of the main atmospheric pollutants. The present study focuses on interpretations of the temporal variations and variations between urban locations of three air pollutants – sulfur dioxide (SO_2), nitrogen dioxide (NO_2) and black smoke (BS) – in this region. The objectives are: (i) examination of the differences between pollutants concentrations during household heating and non-heating seasons and (ii) determination of influence of specific pollution sources (heating of households and traffic) and meteorological factors (rainfall and wind speed) on pollution levels.

MATERIALS AND METHODS

Description of the study area

The target area was the Vojvodina Region of Serbia (VR_S), located in the northern part of Serbia, in the southern Pannonian lowland (18°51′–21°33′E, 44°37′–46°11′N; 75–641 m above sea level). This area is mostly flat, with the exception of Fruška Gora Mountain to the south. Vojvodina has a continental climate with elements of a sub-humid and thermal climate [Katić *et al.,* 1979]. The mean annual air temperature is 11 °C, and the mean annual precipitation is 602 mm [Mihailović *et al*., 2004].

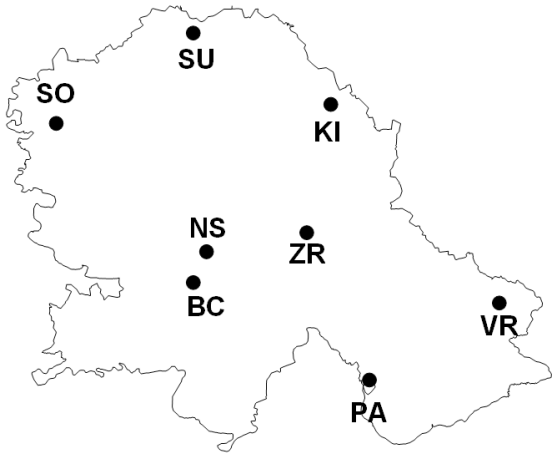

Figure 1. *Vojvodina Region of Serbia (VR_S) showing the sites examined in this study.*

Vojvodina is the most developed and the most urbanized (56.7%) region of the Republic of Serbia. This region is the principal area of food production in Serbia, with a total surface area of 21,506 km^2 (Fig. 1) and a population of two million people. Up to the early 1990s, this area was industrially developed. However, after the events of the 1990s (civil war, military intervention, etc.), industry decreased, leading to major changes in the sources of urban air pollution. Current major urban air pollution sources in the VR_S are substantially increased car traffic and household heating based on coal and natural gas.

Data collection and analyses

To describe the air quality in the VR_S, we used SO_2, NO_2 and *NS* data from the local urban networks for Novi Sad (NS), Subotica (SU), Sombor (SO), Kikinda (KI), Zrenjanin (ZR), Vršac (VR), Pančevo (PA) and Beočin (BC) for the period 2001–2008. According to Serbian regulations and recommendations, the levels of SO_2, NO_2 and *BS* were recorded daily as the concentration determined over a 24-h sample of the air. Although the EPA has recommended measuring particles in terms of PM_{10} and $PM_{2.5}$ since 1978, in the analyzed period, Serbia used *BS* as a surrogate for *PM* concentrations and specifically for concentrations of fine particles. After reviewing the available data, Ostro [1994] concluded that BS is roughly equivalent to PM_{10}. However, there is no precise equivalence for the black smoke measurements with other methods.

Arithmetical mean concentrations of air pollutants were calculated every day for all measuring locations to ensure representativeness for each town. Missing data in the time series at the observation stations were filled using the Catmull-Rom spline and bootstrap statistical methods [Catmull and Rom 1974; Efron and Tibshirani 1993]. The validity of the applied statistical methods was

analyzed by comparing arithmetical mean, minimum, maximum, coefficient of quartile variation and standard deviation between the measured data and the completed time series. The results showed minimal statistical differences (up to 2.7%), confirming that the use of the applied statistical methods was appropriate.

RESULTS AND DISCUSSION

Concentration levels

The distributions of the annual mean pollution levels in the urban environments of the VR_S are presented in Figure 2, and the distributions of SO_2, NO_2 and *BS* concentrations in locations considered in this study are shown in Figure 3. The calculated statistical quantifiers in this figure are the 1st and 3rd quartiles (bottom and top of the box), the median (the line inside the box), the 10th and 90th percentile limits (the whiskers), the mean (circle) and the maximum (cross). The dashed and solid lines represent the Serbian Air Quality Standard for the 24-hour and annual averages, respectively.

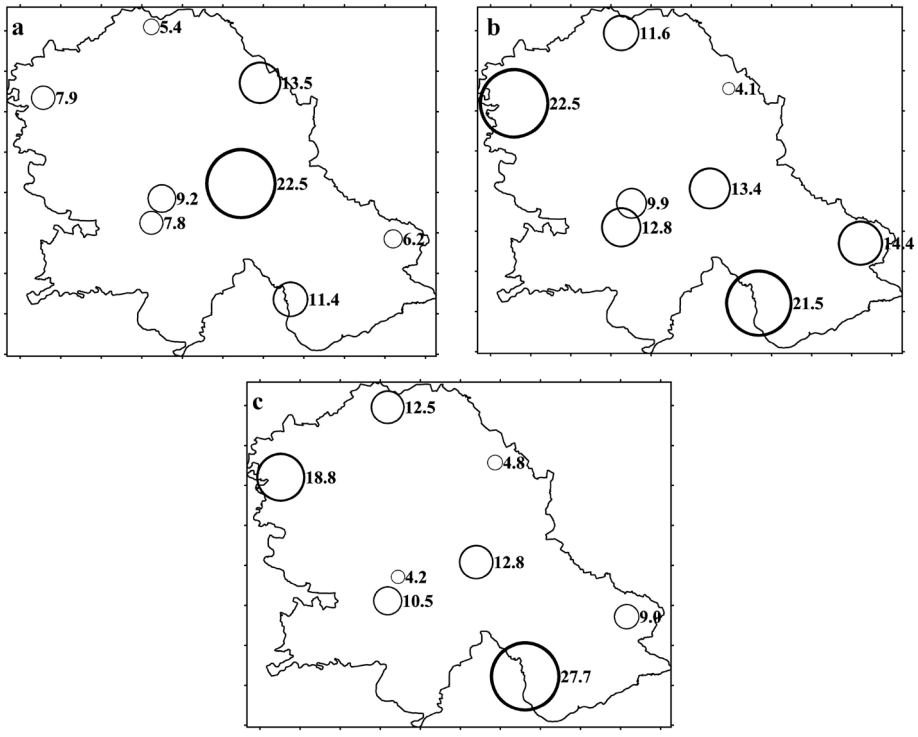

Figure 2. *Distribution of (a) SO_2 (μg m^{-3}), (b) NO_2 (μg m^{-3}) and (c) BS (μg m^{-3}) in the urban locations of the VR_S for the period 2001–2008.*

Figure 3 shows that Serbian annual standards for all three pollutants are not violated in any of the locations. The average annual SO_2 concentrations did not exceed 29 μg m^{-3}. This value is (i) below the level for urban areas in developing countries (40–80 μg m^{-3}) and (ii) at the level recommended for urban areas in the EU countries (6–35 μg m^{-3}), i.e., the levels prescribed by the WHO [2006]. The largest concentration was found in ZR. The average annual concentrations of NO_2 were at the lower limit of typical values for world urban areas (20–90 μg m^{-3}) only in PA and SO, whereas in other locations, the NO_2 concentration were below these values. The panels in Figure 3 show that all average annual concentrations of *BS* are below the limit values, whereas the daily limit values are exceeded in all locations. A comparison of the distributions of NO_2 and *BS* in the urban locations (Fig. 2) reveals a similarity in the patterns of these pollutants that suggests the same type and source of contamination. The maximum values of both NO_2 and *BS* occur in PA and SO.

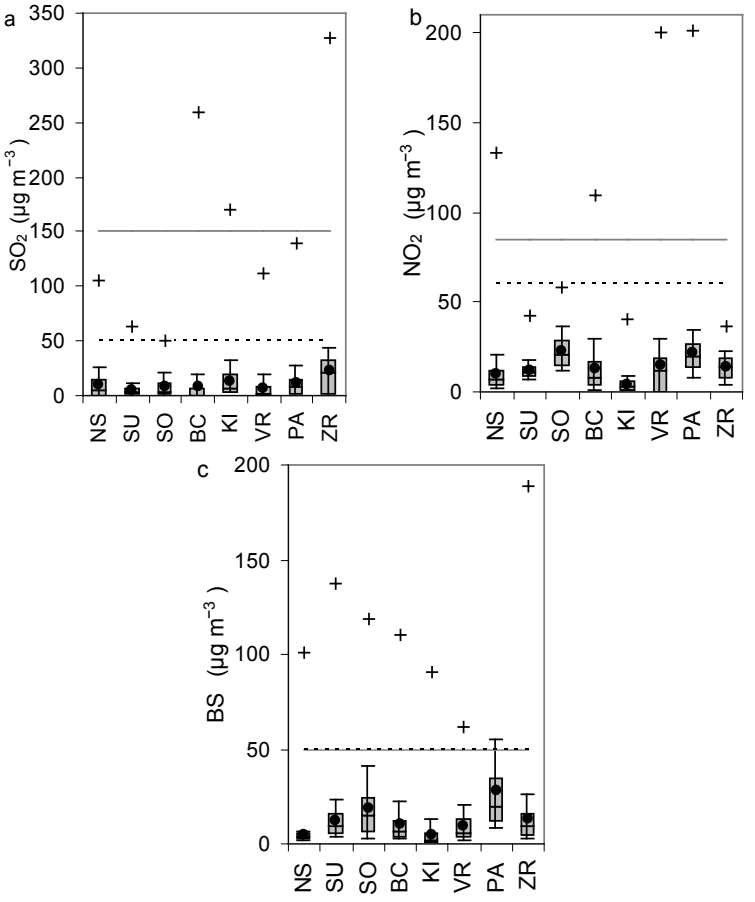

Figure 3. *Box and whisker plots of the distributions of (a) SO_2 (μg m^{-3}), (b) NO_2 (μg m^{-3}) and (c) BS (μg m^{-3}) in the VR_S for the period 2001–2008.*

Seasonal variation

To examine the seasonal variation of pollutants and the seasonal importance of specific pollution sources, the eight-year study period was divided into household heating (H) and non-heating seasons (Nh), ranging between October 15 and April 15 and between April 16 and October 14, respectively. Student's t-test was used to determine the significant differences between pollutant levels for the H and Nh seasons in the VR_ S (Table 1).

Variations of *BS* reach a maximum in the H period in all locations, directly influenced by emissions from household heating and greater atmospheric stability. Significant differences for NO_2 were observed in five locations. In NS, BC and KI, the differences were positive, which indicates the prevalence of natural gas heating in households in these locations. Negative differences in SO and VR indicate that traffic has a substantial impact on the NO_2 levels as well as the more frequent use of coal or heating oil for households heating. Positive significant differences for SO_2 were found in four locations (SU, SO, VR and PA), indicating the considerable influence of household heating on SO_2 levels and the frequent use of coal or heating oil for heating purposes instead of natural gas.

Table 1. Statistical analysis of SO_2 (μg m^{-3}), NO_2 (μg m^{-3}) and *BS* (μg m^{-3}) levels in the VR_S for the household heating (H) and non-heating (Nh) seasons during the period 2001–2008.

	SO_2		NO_2		*BS*	
	H-Nh difference (μg m^{-3})	p [a]	H-Nh difference (μg m^{-3})	p	H-Nh difference (μg m^{-3})	p
NS	–[b]	–	2.043	0.157	1.294	0.032
SU	4.028	0.002	–	–	5.300	0.062
SO	8.051	0.000	-4.329	0.003	7.653	0.000
BC	–	–	4.645	0.172	6.301	0.005
KI	–	–	1.589	0.151	4.541	0.000
VR	1.192	0.055	-2.309	0.002	2.975	0.000
PA	8.969	0.007	–	–	20.352	0.000
ZR	–	–	–	–	5.695	0.016

[a] p represents significance level, [b] Non-significant results (p>0.2)

The Mann-Kendall test was used together with Sen's slope estimator for the determination of significant trends and slope magnitudes [Sen 1968; Helsel and Frans 2006]. The Mann-Kendall test was used as suggested by the WMO [1988] to assess trends in environmental data time series. The Mann-Kendall test does not require an assumption of normality and only indicates the direction

but not the magnitude of significant trends [McBean and Motiee 2008]. The magnitudes of the trends were assessed by Sen's slope estimator.

In Table 2, Sen's slope estimator shows the magnitude of the daily concentrations trends of SO_2, NO_2 and *BS* for the H and Nh seasons. The results of the trend analyses show a significant increasing trend in NO_2 levels in five locations (SU, SO, BC, PA, ZR). An increasing trend in both seasons reflects the increased use of vehicles in these urban areas. The three-fold faster increase in NO_2 concentration and the three-fold faster decrease in SO_2 concentration in the H season compared with the Nh season in PA signify a shift from household heating with heating oil and coal to heating with natural gas. Similar but less pronounced NO_2 and SO_2 trends are observed in SU. A negative SO_2 trend and a positive NO_2 trend in the Nh season in PA and SU indicate the growing importance of traffic-related air pollution in these locations. The biggest increase in SO_2 trends in both seasons as well as a significant increase in NO_2 and *BS* trends were observed in ZR location. The biggest increase in *BS* trends for both seasons were observed in SU, which may be interpreted as an enhanced concentration of diesel vehicles due to the proximity of two traffic border crossings.

Table 2. Results of the Mann–Kendall test analyses of the trends and Sen's slope estimates of SO_2 (μg m^{-3}), NO_2 (μg m^{-3}) and *BS* (μg m^{-3}) in the VR_S for the household heating (H) and non-heating (Nh) seasons during the period 2001–2008.

	SO_2		NO_2		*BS*	
	H	Nh	H	Nh	H	Nh
NS	–[a]	–	–	–	-0.002*	-0.002*
SU	-0.002*	-0.001*	0.002*	0.003*	0.009*	0.008*
SO	–	–	0.001***	0.001***	–	–
BC	–	–	0.001**	0.002*	0.001*	0.002*
KI	0.001*	–	–	–	–	–
VR	–	–	–	–	–	–
PA	-0.006*	-0.002*	0.009*	0.003*	–	-0.001**
ZR	0.007*	0.005*	0.004*	0.004*	0.001**	0.003*

* Statistical significance at the 99.9% level, ** Statistical significance at the 95% level, *** Statistical significance at the 90% level, [a] Non-significant results (statistical significance less than 90%)

Correlations between pollutants and selected meteorological variables

The significant Spearman correlation coefficients for the pair-wise comparisons between SO_2, NO_2 and *BS* are displayed in Tab. 3. The correlations between pollutants were weak to moderate (ranging from 0.180–0.665). The

significant impact of traffic-related and household heating emissions on the particulate matter concentrations is indicated by the positive correlation coefficients between *BS* and gaseous pollutants observed in most locations. The negative correlation between *BS* and NO_2 and the positive correlation between *BS* and SO_2 in VR indicate coal and heating oil used for households heating as the main sources of particles. The negative correlation between SO_2 and NO_2 together with the positive correlation between SO_2 and *BS* in SU indicate coal and heating oil used for household heating as the most common sources of SO_2 and *BS*.

Table 3. Spearman correlation coefficients for daily average concentrations of SO_2, NO_2 and *BS*

	SO_2 NO_2	SO_2 BS	NO_2 BS
NS	0.407*	0.219**	–
SU	0.210**	0.421*	0.628*
SO	-0.368*	0.529*	–
BC	–[a]	–	0.395*
KI	–	–	0.363*
VR	–	0.340**	-0.208**
PA	0.237**	0.665*	0.485*
ZR	0.394*	0.180***	0.397*

* Statistical significance at the 99.9% level, ** Statistical significance at the 95% level, *** Statistical significance at the 90% level, [a] Non-significant results (statistical significance less than 90%)

To determine the influence of meteorological factors on the levels of air pollution, the SO_2, NO_2 and *BS* concentrations in NS, SU, SO, KI, VS and ZR were compared with the rainfall and wind speed data. We examined the percentage differences of the average pollutant concentrations under calm (v_c) and windy (v_w) conditions as well as dry (P_d) and rainy days (P_r). The results are given in Tab. 4. Calm wind conditions were defined as the state in which the wind speed was below 1 m s^{-1}. An inspection of Table 4 suggests that the differences in NO_2 and *BS* concentrations are higher (i) during stagnant or weak flow regimes (0.5–17.2% and 3.8–33.5%, respectively) and (ii) during dry days (1.7–31.4% and 0.6–76.5%, respectively). Differences in SO_2 follow this pattern in the majority of locations, except in NS. Considerably higher concentrations of SO_2 in NS during windy days compared to days without wind (17.2%) indicate an unfavorable location of the source of this pollutant in relation to the direction of the wind.

Table 4. Percentage differences of average daily pollutant concentrations under different wind and rainfall conditions. The symbols have the following meanings: calm (v_c), windy (v_w), dry (P_d) and rainy (P_r) conditions.

	SO_2		NO_2		*BS*	
	$v_c - v_w$ (%)	$P_d - P_r$ (%)	$v_c - v_w$ (%)	$P_d - P_r$ (%)	$v_c - v_w$ (%)	$P_d - P_r$ (%)
NS	-17.9	-3.2	0.5	1.7	3.8	5.7
SU	19.6	14.0	17.2	31.4	16.1	76.5
SO	0.4	8.8	5.7	6.3	12.8	12.8
KI	6.3	15.8	12.5	2.9	33.5	25.3
VS	6.0	18.8	8.0	5.0	12.5	0.6
ZR	13.7	8.3	13.1	2.9	10.5	9.9

CONCLUSIONS

In the present study, we analyzed temporal variations and variations between urban locations of sulfur dioxide (SO_2), nitrogen dioxide (NO_2) and black smoke (*BS*) in the Vojvodina Region of Serbia (VR_S) for the period 2001–2008. Specifically, we examined variations of pollutants concentrations during household heating and non-heating seasons and the effect of household heating, traffic, rainfall and wind speed on the air pollution levels of SO_2, NO_2 and *BS* in eight locations. The analyses show that the annual limit values for all three pollutants were not exceeded, whereas the daily limit values were exceeded in all locations for *BS* and SO_2, and in four locations for NO_2. Although fossil fuels have been gradually replaced with cleaner natural gas for the purposes of households heating, the higher SO_2 concentrations during the households heating season in several locations (SU, SO, VR and PA) show a significant impact of this pollutant on air quality. The positive effects of the use of environmentally cleaner fuels are visible in PA and SU, where a decrease in the concentration of SO_2 was observed during the households heating season. The increasing trend of NO_2 in both seasons indicates the growing impact of traffic on air pollution in the VR_S. The examination of the relationship between pollutant concentrations and meteorological elements shows incremental effects of calm and dry conditions on pollutant concentrations in most locations.

The results strongly suggest that pollution levels in urban locations in Vojvodina should be continuously monitored over a wide network of sites to observe the effects of applied environmental measures and to provide recommendations for their improvement.

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ЗАГАЂЕЊЕ ВАЗДУХА ГАСОВИМА И ЧЕСТИЦАМА У РЕГИОНУ ВОЈВОДИНЕ (СРБИЈА)

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РЕЗИМЕ: У раду су анализиране временске и просторне варијације концентрација сумпор-диоксида (SO_2), азот-диоксида (NO_2) и чађи (BS) у ваздуху осам насеља у региону Војводине, у периоду од 2001. до 2008. године. Испитиване су промене концентрација загађујућих материја током грејне и вангрејне сезоне као и утицај грејања домаћинстава, падавина и брзине ветра на концентрације SO_2, NO_2 и BS. Резултати су показали следеће: (1) годишње граничне вредности све три загађујуће материје нису прекорачене нити у једном насељу, (2) дневне граничне вредности за BS и SO_2 прекорачене су у свим насељима, док су дневне граничне вредности за NO_2 прекорачене у четири насеља (Вршац, Панчево, Нови Сад и Беочин), (3) велики утицај употребе фосилних горива за загревање домаћинстава видљив је у четири насеља (Суботица, Сомбор, Вршац и Панчево) у којима је концентрација SO_2 значајно виша током грејне сезоне, (4) позитивни ефекти преласка на еколошки чистија горива виде се у Панчеву и Суботици где је током грејне сезоне забележен силазни тренд концентрација SO_2, (5) узлазни тренд концентрација NO_2 током целе године у већини насеља указује на све већи утицај саобраћаја на квалитет ваздуха у Војводини, и (6) дани без ветра и падавина погодују повећању концентрација загађујућих материја на већини локација.

КЉУЧНЕ РЕЧИ: азот-диоксид, чађ, сумпор-диоксид, Војводина, загађење ваздуха

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MULTI-CRITERIA APPROVAL FOR EVALUATING LANDSCAPE MANAGEMENT STRATEGIES (CASE STUDY: FRUŠKA GORA NATIONAL PARK)

ABSTRACT: This paper deals with the problem of multi-criteria evaluation of four management strategies for Fruška Gora National Park. The criteria set was defined in accordance with the IUCN guidelines for management of national parks. Four strategies were evaluated by testing preference intensities for each alternative with the respect to each criterion. Alternatives with preference above the approval threshold were approved, and a multi-criteria approval matrix was generated. According to the matrix, the most suitable management strategy was identified. It implies an intense protection of natural resources and landscape diversity in the national park by applying bio-engineering measures. This illustrative example proved that multi-criteria approval can be considered as a good decision support tool when there is no need for a deeper insight into cardinal values of criteria weights and alternatives, or if the decision maker has to select few from many of alternatives to reduce the decision problem. For a more precise analysis, it is recommended to combine multi-criteria approval with other decision support tools, and future studies might deal with this problem in order to define an alternative framework for decision making in landscape management.

KEYWORDS: decision making, management strategy, multi-criteria approval, Fruška Gora National Park

INTRODUCTION

National parks are highly valuable from many aspects, especially because they contribute to sustainable regional development, biodiversity preservation, natural disaster prevention, regional welfare effects, environmental education and training, etc. [Mose and Weixlbaumer 2007]. Therefore, the management of national parks should be focused on different objectives such as: support of

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environmental processes, protection of natural biodiversity and its underlying ecological structures, promotion of tourism, education and recreation, etc. [IUCN, 2008]. Management strategies are supposed to maintain or upgrade the main functions of national parks [Palomo *et al.*, 2013]:

- provisioning (water, food, raw material provision, renewable energy, etc.),
- regulating (habitat for species, climate and water regulation, air purification, erosion control, etc.) and
- cultural (scientific knowledge, natural and rural tourism, spiritual and aesthetic values, etc.).

Evaluation of different strategies for national parks management can be done by applying multi-criteria methods and tools [e.g. the Analytic hierarchy process – AHP, Saaty, 1980; PROMETHEE, Brans *et al.*, 1986; ELECTRE, Roy, 1968; Compromise Programming – CP Zeleny, 1973, etc.] and/or voting techniques belonging to the social choice theory [Kangas *et al.*, 2006].

This research demonstrates the application of multi-criteria approval for evaluating several landscape management strategies for Fruška Gora National Park, Serbia. Even though only one expert performed decision making, similar procedure is applicable in a group decision making context [Laukkanen *et al.*, 2005]. Contemporary studies state that inclusion of different stakeholder groups in managing national parks has not become mandatory, but it is highly recommended. It is worthy to mention that in the previous research, reported in [Lakićević 2013], the same management strategies were assessed and evaluated in a group context by authorized representatives of different stakeholder groups. The results obtained in this research are compared with earlier ones and the differences are discussed.

Nowadays, it is also quite common to combine different methods and techniques in making environmental decisions [Kangas and Kangas 2003; Lakićević *et al.*, 2014]. Apart from the already known methods described in [Laukkanen *et al.*, 2005], future research agenda could include linking multi-criteria approval with other decision support tools in order to provide an alternative and upgraded framework for decision-making in landscape planning tasks.

MATERIAL AND METHOD

Fruška Gora is proclaimed a national park in 1960 and is well known for its remarkable cultural and natural values. The total actively protected area occupies 25,393 ha and recent studies identified several problems in the area, such as spread of invasive species along with the suppression of oak trees [Bobinac 2003; Vasić *et al.*, 2012], intensification of erosion processes [Dragićević *et al.*, 2013], etc. Experts suggest that properly defined landscape management strategies could preserve existing park's qualities and ensure the major problems in the park to diminish in the near future [Vlada AP Vojvodine, 2011].

In this paper we consider four management strategies (alternatives) for the national park, aiming at determination of the most suitable one. The strategies were defined in [Lakićević 2013], taking into account the preferences of different

stakeholder groups. In short (for details consult Lakićević 2013), management strategies are as follows:

- (a_1) Maintain the current policy without significant alternations;
- (a_2) Develop eco-tourism and network of tourist and recreation facilities in well-preserved natural areas;
- (a_3) Provide an intense protection of natural resources and landscape diversity by applying bio-engineering measures;
- (a_4) Develop sustainable organic agriculture in order to provide stable incomes to the local residents.

For the purpose of evaluation of these management strategies, the criteria set is defined in accordance with the recommendations stated by the International Union for Conservation of Nature – IUCN [1994, 2008]. The original set [IUCN, 1994] is altered to a small extent and eight criteria are merged into six and then listed by decreasing importance:

- (C_1) Maintenance of environmental services (the most important);
- (C_2) Preservation of biodiversity;
- (C_3) Tourism, recreation and education;
- (C_4) Protection of specific natural/cultural features;
- (C_5) Wilderness protection;
- (C_6) Sustainable use of resources from natural ecosystems (the least important).

Multi-criteria approval

Multi-criteria approval [Fraser and Hauge 1998] begins by determining the set of alternatives under consideration: $A = \{a_1, a_2, ..., a_i, ... a_m\}$. The next step is to identify the set of criteria: $C = \{c_1, c_2, ..., c_j, ... c_n\}$ for comparing the set of alternatives. A decision maker ranks the criteria by the importance, from the most important to the least important, and then evaluates which of the alternatives are above and which are below the average for each criterion being considered. In cases when the utility for a criterion can be treated as *linear transformation of the criterion measures* a precise average for the criterion can be calculated as:

$$\bar{c}_j = \sum_{i=1}^{m} \frac{c_j(a_i)}{m}$$

$$\text{and } a_i \epsilon \begin{cases} P_j \text{ if } c_j(a_i) > \bar{c}_j \\ P'_j \text{ if } c_j(a_i) \leq \bar{c}_j \end{cases}.$$

If $a_i \in P_j$ alternative i is approved for criterion j and if $a_i \in P'_j$ alternative i is not approved for this criterion. In addition, the process of pronouncing the alternatives as approved or disapproved for a certain criterion can also be a result of classifying the alternatives into different categories, such as excellent,

average, undesirable, etc. [Laukkanen *et al*., 2002]. Based on this classification it is possible to define the average value (in this case – category) for each criterion, and therefore to determine their approval/disapproval threshold, as input data for deriving final decision.

In order to get the final results, it is necessary to form a matrix, where columns are criteria ordered by importance, and rows present alternatives. Matrix cells are filled in accordance with the approval/disapproval of an alternative for a certain criterion ('+' denotes approval and '–' disapproval). In the end, the data provided in matrix are supposed to be analyzed, and one of the outcomes shown in Figure 1 may happen.

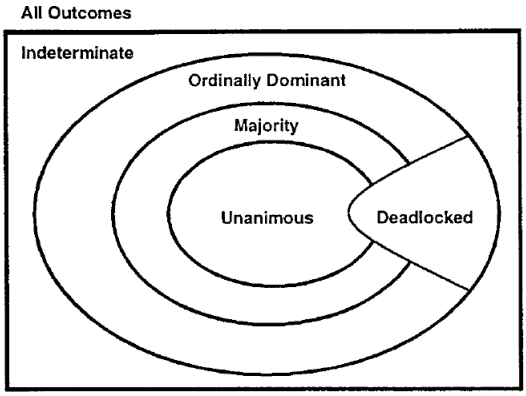

Figure 1. *Multi-criteria approval classifications [Fraser and Hauge 1998].*

A brief explanation of possible outcomes is provided in the following text.

Unanimous – only one alternative is approved upon all criteria, and it is declared a winner.

Majority – only one alternative is approved for the majority of the most important criteria and it is the winner.

Ordinally dominant – one alternative is superior to remaining alternatives based on ordinal ranking of criteria and information regarding criteria approval.

Deadlocked – at least two alternatives are approved and disapproved on exactly the same criteria. In this case there is no single winner.

Indeterminate – one alternative cannot be labeled as superior to the others based on ordinal criteria importance and it is not possible to declare the winner. More information is necessary in order to get the final decision.

RESULTS AND DISCUSSION

The multi-criteria approval evaluation of four management strategies with respect to six relevant criteria was done by one decision maker, a landscape architect. For the analysis that follows, ranking of criteria was not relevant and thus it was not performed.

Evaluation of alternatives was supported by testing preference intensities for each alternative with the respect to each criterion. The preference intensities are marked as follows: undesirable (ud), not good (ng), below average (ba), average (av), good (gd), very good (vg) and excellent (ex), as suggested by [Laukkanen *et al.,* 2002]. The evaluation results are presented in Fig. 2.

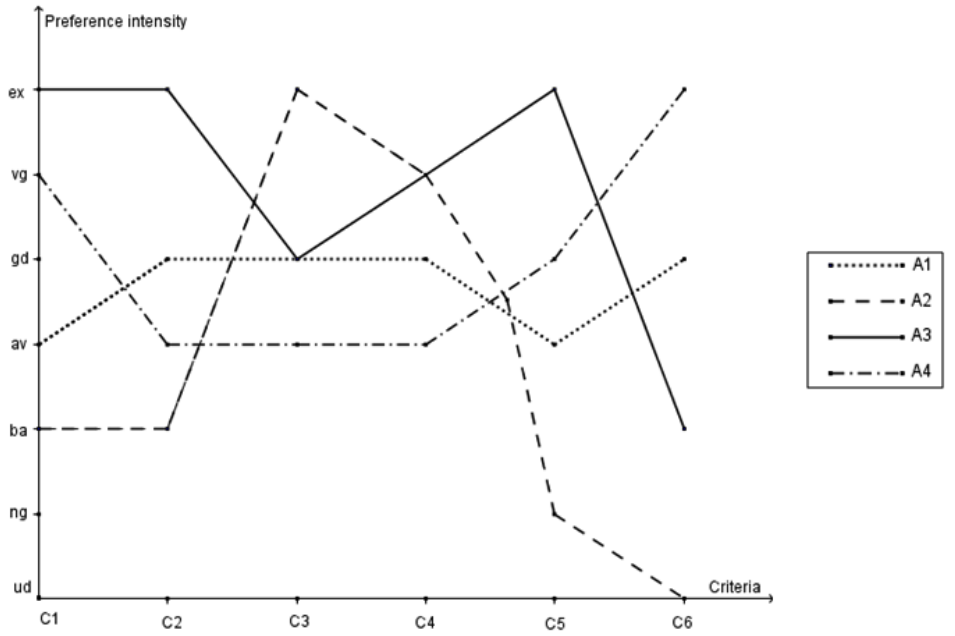

Figure 2. *Evaluation of alternatives with respect to criteria set*

Based on the results of evaluation provided in Figure 2 it was possible to generate the multi-criteria approval matrix (Figure 3) and analyze the final decision.

Alternatives	Criteria					
	C_1	C_2	C_3	C_4	C_5	C_6
a_1	-	+	-	-	-	+
a_2	-	-	+	-	-	-
a_3	+	+	-	+	+	-
a_4	+	-	-	+	+	+

Figure 3. *Multi-criteria approval matrix*

Fig. 3 shows that alternative a_3 can be labeled as the ordinally dominant winner. This management strategy predicts conduction of bio-engineering measures aiming to ensure high protection of natural resources and values in the national park.

It is worthy to mention that the final decision in the previous research [Lakićević 2013] differs, as it recognizes alternative a_4 as the most suitable management strategy. In the precedent study the decision was made in a group context by applying AHP, the technique of cumulative voting and consensus convergence model (CCM). The decision makers in that research were representatives of six stakeholder groups: local authorities, academic experts, members of non-governmental organizations (NGOs), tourists, local residents and business persons (i.e. people who finantially benefit from Fruška Gora National Park). Their group decision derrived by applying above listed methods declared as a winner the management strategy which advocates development of sustainable organic agriculture.

In short, different results are identified in cases of individual and group decision making when evaluating same management strategies for Fruška Gora National Park. The reason for that can be regarded as a consequence of applying different decision support methods. In addition, strategy a_4 aims to provide stable incomes for the local residents and this can be the other important reason why this strategy is preferred over *nature protection strategy* (a_3), when representatives of different stakeholder groups were included in the decision making process.

However, multi-criteria approval can be a good starting point in decision making process, as it can be used for providing a quick acquaintance with the problem as well as for discarding the irrelevant alternatives. Multi-criteria approval requires defining the rank of the criteria and testing the alternatives' performance for each criterion. This can be a good base for performing AHP evaluations in pair-wise manner or the number of alternatives which are supposed to be considered as a final solution can be reduced (those would be the alternatives disapproved for the majority of the most important criteria). Reducing the number of alternatives makes the application of AHP easier and ensures better consistency of evaluation [Bozóki *et al*., 2013].

CONCLUSION

There are different techniques and approaches supporting the process of individual and group decision making in landscape management studies [Srđevic *et al*., 2013). Multi-criteria approval and its upgraded versions are recognized as good support tools for the decision making processes when one or more decision makers have to just mutually compare the criteria and the alternatives regardless of the deeper insight into the real cardinal values of the decision matrix such as, for example, criteria weights and/or alternative real numerical ratings across criteria. The consequence could be the difference in the final results if the decision making approaches for individual/group contexts are different. The proof that this may happen is described in the present study; namely, the most suitable management plan for Fruška Gora National Park selected by method described here is not the same as the one identified as the

best in [Lakićević 2013]. The difference in results is also partly a consequence of different stakeholders involved in these two researches.

To summarize, for a good quality decision making process it is essential to apply appropriate methodology and to include relevant decision makers. Selection of the methods to be applied depends on the decision makers' background, responsibility, personal preferences, etc. The decision making process can be trusted to one person, but in that case s/he should act and decide respecting the needs of all stakeholders that will be affected by the final decision. In the group decision making context, combing different methods has a significant potential as it, for instance, encourages participation of different stakeholders [Lakićević *et al*., 2014] and reduces the disadvantages of the methods when they are applied solely [Kangas and Kangas 2003]. In that regard, future studies might deal with the problem of combining multi-criteria approval with other decision support methods in making environmental decisions. One possible solution could be to start the decision-making process with application of multi-criteria approval and to continue it using AHP. The purpose of the first step would be to analyze the problem at a glance and to possibly reduce the number of alternatives, by discarding the least relevant ones. The next step would imply the application of AHP, in order to obtain more precise and comprehensive results than application of multi-criteria approval could provide itself.

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ВИШЕКРИТЕРИЈУМСКО ОДОБРЕЊЕ У ВРЕДНОВАЊУ СТРАТЕГИЈА УПРАВЉАЊА ПРЕДЕЛИМА (СТУДИЈА СЛУЧАЈА: НАЦИОНАЛНИ ПАРК ФРУШКА ГОРА)

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РЕЗИМЕ: Рад се бави вишекритеријумским вредновањем четири стратегије управљања Националним парком „Фрушка гора“. Скуп критеријума за вредновање је дефинисан у складу са IUCN смерницама за управљање националним парковима, а критеријуми су рангирани према значају. Четири стратегије су вредноване помоћу тестирања интензитета преференци за сваку алтернативу у односу на сваки критеријум. Алтернативе са интензитетом преференци изнад одговарајућег прага су оцењене као одобрене за дати критеријум и формирана је матрица вишекритеријумског одобрења. На основу резултата у матрици идентификована је најпогоднија стратегија управљања која подразумева интензивну заштиту природних ресурса и предеоне разноврсности у националном парку. Овај илустративни пример доказује да вишекритеријумско одобрење може да се сматра као добар алат за подршку одлучивању уколико није неопходно детаљно анализирати кардиналне вредности критеријума и тежине алтернатива или када доносилац одлука треба да изабере неколико од већег броја алтернатива у циљу поједностављења проблема одлучивања. За прецизнију анализу препоручено је да се комбинује примена вишекритеријумског одобрења и других алата за подршку одлучивању (нпр. Аналитичког хијерархијског процеса). Наредна истраживања могла би да се баве овим проблемом, у циљу дефинисања новог приступа за доношење одлука у управљању пределима.

КЉУЧНЕ РЕЧИ: одлучивање, стратегија управљања, вишекритеријумско одобрење, Национални парк „Фрушка гора“

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5. References

5.1. List the References alphabetically. Examples:

(a) Articles from journals: Last name CD, Last name CD (2009): Title of the article. Title of the journal (abbreviated form) 135: 122-129.
(b) Chapters in the book: Last name ED, Last name AS, Last name IP (2011): Title of the pertinent part from the book. In: Last name CA, last name IF (eds.), Title of the book, Vol.4, Publisher, City
(c) Books: Last name VG, Last name CS (2009): Title of the cited book. Publisher, City

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

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

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

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

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

6. Units, names, abbreviations, and formulas

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

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

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

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

7. Figures

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

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

8. Tables

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

8.2. Number the tables using Arabic numerals.

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

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

9. Electronic copy of the article

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

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

УПУТСТВО АУТОРИМА
(www.maticasrpska.org.rs)

1. Опште напомене

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

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

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

2. Припрема рукописа

2.1. Текст рада пише се електронски на страни A4 (21x29,5 cm), с маргинама од 2,5 cm, увлачењем првог реда новог пасуса, и размаком међу редовима 1,5. Текст треба писати у фонту *Times New Roman* словима величине 12 а сажетак, кључне речи, резиме и подножне напомене словима величине 10 pt.

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

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

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

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

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

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

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

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

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

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

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

5. Литература

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

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

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

(в) Књиге: Презиме VG, Презиме CS (2009): Наслов цитиране књиге. Издавач, Град.

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

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

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

5.2. Референце у тексту треба да укључе презиме аутора и годину издања. Ако има два аутора, треба навести обојицу, а у случају три или више аутора треба навести првог аутора и назначити "et al.".
5.3. Ако се наводе два или више радова истог или истих аутора, објављених у истој години, потребно је у тексту и списку литературе ставити а, б, ц, итд. иза године објављивања.
5.4. Имена часописа треба скраћивати према "Bibliographic Guide for Authors and Editors" (BIOSIS, Chemical Abstracts Service and Engineerings Index, Inc.,).
5.5. Референце се не преводе на језик рада. Наслови цитираних домаћих часописа дају се у оригиналном, скраћеном облику. Ако је референца нпр. на српском језику на крају се стави (Sr).

6. Јединице, имена, скраћенице и формуле
6.1. Треба користити SI ознаке за јединице (SI Systeme International d'Un.); изузетно се могу користити и друге званично прихваћене јединице.
6.2. Називе живих организама на латинском треба писати италиком.
6.3. При коришћењу скраћеница у тексту, пун термин треба навести приликом првог спомињања, а скраћеницу додати у загради.
6.4. Хемијске структурне формуле и сложене једначине треба нацртати и припремити за фотографску репродукцију.

7. Илустрације
7.1. За илустрације могу се користити црно беле фотографије и цртежи доброг квалитета.
7.2. Свака илустрација треба да има текст (легенду) који објашњава садржај прилога (испод слике).

8. Табеле

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

8.2. Табеле се означавају арапским бројевима.

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

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

9. Копија рада у електронској форми

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

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

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