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A CRITICAL REVIEW OF THE CURRENT CHECKLIST OF BUTTERFLIES OF SERBIA

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ABSTRACT

The latest checklist of butterflies of Serbia (Papilionoidea Latreille, 1802) by Popović & Verovnik (2018) is examined. In consistencies with regard to the cited literature and applied systematics are pointed out. The validity of the viewpoints of the above mentioned authors on the species *Leptidea juvernica* Williams, 1946, *Pyrgus trebevicensis* (Warren, 1926), *Erebia manto* (Denis Schiffermüller, 1775) and *Melitaea ornata* Christoph, 1893 is discussed. It is shown that by imposing new Serbian names to butterflies disregards national and scientific heritage.

Keywords: Butterflies, Papilionoidea, Republic of Serbia.

INTRODUCTION

In the Republic of Serbia, two checklists of butterflies of Serbia have been published in which, besides their scientific names, their local vernacular names are given (Jakšić & Đurić, 2008; Jakšić et al., 2013). Recently, a third checklist was published (Popović & Verovnik, 2018). Thanks to these texts, the knowledge about the fauna of this group of insects in Serbia is gradually being completed. We can safely claim that new species of butterflies in Serbia will be determined. Firstly, there will be those that are present in neighboring countries, and then the invasive species that are spreading from the south towards the north. Here in, we want to look at the above mentioned text of Popović & Verovnik (2018).

A CRITICAL REVIEW OF THE CHECKLIST OF POPOVIĆ AND VEROVNIK (2018)

In their introduction, the authors emphasize that during the compilation of the checklist, 166 references with data on the fauna of the butterflies of Serbia were used, starting from the first work of Frivaldszky (1877). The authors are unaware of earlier works, such as Marsili (1726), who provided the first notes on the butterflies of Vojvodina. After Marsili, and before Frivaldszky (1877), two papers on the butterflies of Serbia were published too. The aforementioned 166 works are given at the end in two lists (with many spelling mistakes), but it is not clear on what principle: what is the difference between the "References" list and the "Supplementary" list when some of the works appear on both lists? Most probably the second list should have been titled "Bibliography of Serbian Butterflies"? In fact, according to our data, there are 410 published works on the butterflies of Serbia, with an additional 20 graduate and master's theses.

The systematic list of butterflies applied by the authors poses a dilemma. Nieukerken et al. (2011), as well as subsequent relevant authors (De Prins, 2016; Aarvik et al., 2017; Leveque et al., 2017), in the superfamily Papilionoidea Latreille, 1802 in first place give the family Papilionidae Latreille, 1802, and then the family Hesperiidae Latreille, 1809. In their paper, the authors have switched the places of these families. Similarly, Popović and Verovnik place the family Riodinidae Grote, 1895 after the family Lycaenidae Leach, 1885, while in Nieukerken et al. (2011) the order was the reverse. It is not known from whom this systematics was taken, since in the introductory part the authors only cite the literature sources for taxonomy and nomenclature, referring to the informal site of Fauna Europaea (Karsholt van Nieukerken, 2013).

Attention is given to the species *Leptidea morsei* (Fenton, 1882). This species has not been found in Serbia for almost 80 years (Miloš Rogulja found 5♂4♀in the period 1920-1939). Rogulja found the specimens on the so-called "Futoški drum"–a 7 km-long road that linked Novi Sad and Futog. Since then, this habitat has disappeared due to urbanization; however, corresponding habitats still exist in the immediate vicinity of the Fruška Gora slopes. Lorković (1993) pointed out that the habitat of L. *morsei* in Croatia is defined by the community of *Lathyreto-quercetum petraeae* HR-T 1957; an equivalent community of *Quercetum confertae-cerris* Rudski 1939 is represented on Fruška Gora. Plants on which caterpillars feed are present there. Of the 16 species of the genus *Lathyrus* L., 1753 in the flora of Vojvodina, 15 are present in the flora of Fruška Gora (Boza et al., 2003). The species *Lathyrus niger* (L.) Bernth.1800 is present at 14 localities, including localities on Fruška Gora. The species *Lathyrus vernus* (L.) Bernh.1800 is present at 17 localities in Vojvodina, among them 12 are on Fruška Gora. Both *Lathyrus* species, which are important for the survival of *Leptidea morsei*, are present in the flora of Fruška Gora. The real problem is that after Rogulja, this species of butterflies on Fruška Gora has not been comprehensively studied.

The value of this checklist is to provide a position with regard to several dubious taxa. Summarizing their results, we can

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say that there is little probability of the presence in Serbia of the species *Colias chrysotheme* (Esper, 1781), *Polyommatus escheri* (Hübner, 1823), *Hipparchia fatua* Freyer, 1843 and *Melanargia russiae* (Esper, 1783).The survival of these and some other species in Serbia is conditioned by a combination of ecological factors in the relevant habitats. By examining the material that was handled and published by Csipe (2006), Popović & Verovnik (2018) correctly concluded that this is a wrong determination, that in fact it is the species C. *erate* (Esper, 1805). However, we believe that the possibility of the presence of this species in Serbia should not be completely ruled out. Kovács (1956) gave a detailed account of its distribution. The author states that in the Collection of the Hungarian Natural History Museum he found a pair of this species from Zavidovići (Bosnia and Herzegovina). It is probable that this species can be found in Serbia in favorable years. As a food plant for caterpillars, *Astragalus austriacus* Jacq.1762 is present at several localities in Vojvodina and around Belgrade (Diklić et al., 1972; Knežević et al., 2012). In this case as well, the problem is that no one has specifically focused on this species, which is very similar to C. *crocea*.

There is less likelihood of finding *Polyommatus escheri* (Hübner, 1823). Monophagous caterpillars seek the species *Astragalus monspessulanus*, represented in Serbia by the taxon *A. monspessulanus* L. subsp.*illyricus* (Bernh.) Chater. Fragmented subpopulations were found at Sukovo sites: Vera (Jerma River gorge) and Petačinci (Tomović, 2009). *P. escheri* (Hübner, 1823) is present in neighboring countries Montenegro, FYR Macedonia and Bulgaria (Kudrna et al., 2015).

A group of dubious taxa with insufficiently defined status is particularly interesting. This group consists of species that are characterized by various terms: bad species (Descimon & Mallet, 2009), sibling species (Mayer, 1942); cryptic species complex (Dincã et al., 2013). In Serbia, several pairs of this group are important. The question of the differentiation of *Leptidea sinapis* and *L. juvernica* has been open for a long time. Descimon & Mallet (2009) treat them as sibling species. In practice, the separation is based on differences in the length of the aedeagus and saccus. However, Neumayer Segerer (1995) and later on Cupedo & Wim Hoen (2006) concluded that there is continuity in the statistical sample of the analyzed parameters. Coutsis (2013) also provided confirmation of continuity. Analysis of the number of chromosomes showed the existence of polymorphism. Undoubtedly, the species *L. sinapis* (n = 29), *L. morsei* (n = 54), *L. amurensis* (n = 61) and *L. duponcheli* (n = 104) constitute an aneuploid array (Federley, 1938; Lorković, 1949, 1990; Maeki, 1958).On the other hand, an analysis of the number of chromosomes in the Palearctic area, from Spain towards Kazakhstan in the case of *L. sinapis*, has shown that this number gradually decreases, from 2n = 106 in Spain to 2n = 56-64 in Kazakhstan.

In the absence of a better method, separation is routinely based on these parameters, with the addition of the appropriate wing characteristics (Cuvelier & Maertens, 2017).Thanks to the work of Hauser (1997), for the determination of our material we have adopted a table of borderline values, as shown here in (Table 1, Fig. 1, Fig. 2).

Table 1. Relation between aedeagus and saccus length in *L. sinapis* and *L. reali* (according to Hauser, 1997).

	Aedeagus (mm)	Saccus (mm)
L. sinapis	1.65 – 1.90	0.80 – 0.90
L. reali	1.70 – 2.10	0.70 – 0.85

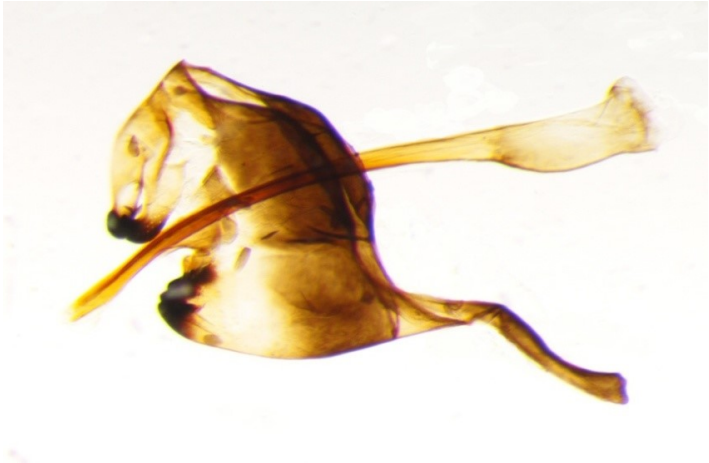

Figure 1. *Leptidea juvernica* Williams, 1946. Male genitalia. Serbia, Rudnik Mt., 600 m, 44° 08’ 26” N; 20° 30’ 00” E, 28. April 1985., Jakšić P. leg et coll.

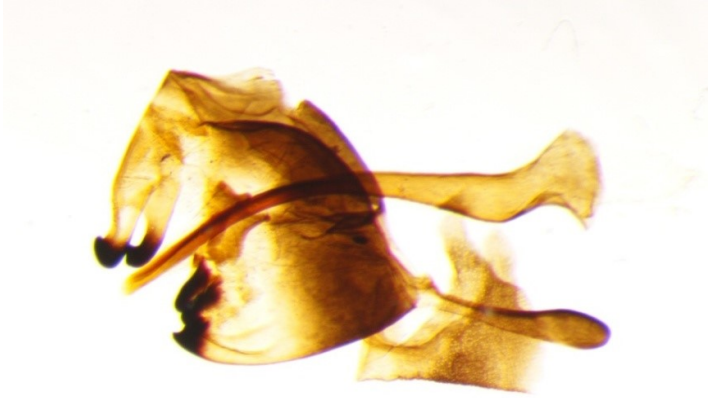

Figure 2. *Leptidea sinapis* Linnaeus, 1758. Male genitalia. Serbia, Vidlič; Mt., Crni Vrh, 1046 m, 43° 10’ 51” N; 22° 38’ 52” E, 29. May 2015., Nahirnić A. leg et coll.

At the time, no difference had been established between *L. reali* and *L. juvernica*, but we can now use these values for the taxon *L. juvernica*. Of around 200 samples of the *L. sinapis* group in our collection, we have made 163 preparations of genital armatures. In this material, there were only 17 specimens (10%) belonging to the species *L. juvernica* from the territory of Serbia, as shown in Table 2.

Table 2. Results of biometric analysis of saccus (SAC) and aedeagus (AED) in *L. sinapis* and *L. juvernica*.

Leptidea juvernica Williams, 1946		
SLIDE No. & PLACE	SAC	AED
	(mm)	(mm)
SR-2711: Jadovnik Mt., Milošev Do	0.8	1.8
SR-2330: Tara Mt., Beli Rzav	0.85	1.8
SR-2306: Kosmaj	0.7	1.75
SR-2102: Paštrik Mt., Gorožup	0.6	1.8
SR-2314: Tutin, Crkvine	0.75	1.7
SR-5740: Priština, Grmija Mt.	0.65	1.7
SR-2123: Bor, Savača	0.7	1.7
SR-2338: Zlatibor Mt., Mokra Gora	0.8	2
SR-2102: Šar-Planina Mt., Izgorenica	0.7	1.7
SR-2048: Bor, Mali Krivelj	0.8	1.75
SR-2683: Jadovnik Mt., Kašanj	0.7	1.6
SR-2125: Bor, Mali Krivelj	0.75	1.9
SR-2328: Tara Mt., Rača River canyon	0.8	1.85
SR-2337: Tara Mt., Kaluđerske Bare	0.8	1.9
SR-2336: Užice, Đetinja River gorge	0.85	1.9
SR-2312: Divčibare	0.75	1.8
SR-1786: Rudnik Mt.	0.75	1.95
Leptidea sinapis (Linnaeus, 1758)		
SR-6763: Peć, Rugovska klisura gorge	0.6	1.4
SR-2313: Tutin, Crkvine	0.65	1.65
SR-2677: Prijepolje, Mileševka river gorge	0.55	1.75
SR-2493: Niš, Jelašnička klisura gorge	0.55	1.5
SR-2406: Zasavica	0.55	1.5
SR-2619: Divčibare	0.6	1.5
SR-2618: Divčibare	0.65	1.5
SR-2600: Stara Planina, Babin Zub	0.6	1.55
SR-2599: Prijepolje, Mileševka river gorge	0.55	1.55
SR-2598: Jadovnik Mt., Sopotnica	0.65	1.6
SR-2304: Kosmaj Mt.	0.6	1.7
SR-2311: Divčibare	0.65	1.65
SR-2307: Ritopek	0.65	1.6
SR-2299: Negotin	0.65	1.65
SR-2303: Bor, Dubašnica	0.6	1.5
SR-2305: Tutin, Crkvine	0.6	1.55
SR-2302: Negotin, Dupljane	0.65	1.55
SR-2384: Fruška Gora Mt., Grabovo	0.6	1.6
SR-2076: Šar-Planina Mt., Rečane	0.65	1.6
SR-2405: Fruška Gora Mt., Beočin	0.6	1.55
SR-2072: Mokra Gora Mt., Istok	0.65	1.5
SR-2046: Bor, Savača	0.65	1.5
SR-2301: Negotin	0.55	1.6
SR-2716: Vidlič Mt., Crni Vrh	0.6	1.6

There is continuity in the size of both analyzed parameters, as pointed out by the abovementioned authors (Table 2).To distinguish species, characters that can be reliably determined are considered, regardless of whether they are morphological, physiological/biochemical, ecological or otherwise. This is the practical aspect of making concrete differentiations among related species. Lorković (1928) proved that the species are complexly differentiated. Consequently, in closely related species it is not enough to rely solely on one parameter. In practice, the desired goal is most often achieved using two or more parameters. In our case, the differentiation between *L. sinapis* and *L. juvernica* can be illustrated using two analyzed parameters (Table 2). If the position of the saccus and aedeagus of each individual specimen is given in a graph in the form of a point, we obtain the result shown in Fig. 3.

Using this method, we can determine the largest number of samples. But statistically, there will always be some that cannot be separated in this way; new parameters are then used. In our case, this could be the color of the apex of the fore-wings.Namely, by examining about 200 samples in our collection, we noticed that the color in L. *juvernica* is gray-black, while in L. *sinapis* it is black-black. The difference is insignificant, but constant and easily observed.

The taxonomic status of the species *Pyrgus trebevicensis* (Warren, 1926) is still uncertain. Depending on the viewpoint of individual authors, it is considered either to be a species or a subspecies. Jakšić (2011) treats this taxon as a species. His argument is in the clearly distant appearance of the valves in three related taxons: *armoricanus*, *alveus* and *trebevicensis* (Jakšić, 2011, Fig. 1). The application of molecular/genetic methods will contribute to a clarification of the status. Many taxa were long treated as subspecies, only to be proven at some point to be species.

It is surprising that the authors, on no scientific basis, excluded the species *Erebia manto* (Denis Schiffermüller, 1775) from the fauna of Serbia, arguing that the locality where it was found is not in Serbia, but in Montenegro. They offer no evidence for this claim. Jakšić & Pešić (1995) provided convincing information about its presence. The species was found on the Serbian side of the mountain pass, Čakor (Fig. 4). Not long ago it was found on the Montenegrin side of the Čakor Pass (Franeta, 2018).

The history of nomenclature changes in the *Melitaea phoebe*-species group is a turbulent one. Jakšić (2011) described it as a new one for Serbia under the then valid name, *Melitaea telona* (Fruhstorfer, 1908). Later, Russell & Tennent (2016) described all the nomenclature ambiguities within this species group. Accordingly, the species *M. ornata* Christoph, 1893 replaced the aforementioned *M. telona* species in Serbia. At the time, the authors expressed doubts about Jakšić's (2011) data on the presence of *M. ornata* Christoph, 1893 in Serbia. In that time, according to Tóth and Varga (2010), main identification parameter wa the depth of the central notch of the saccus. Jakšić determine *M. telona* on the basis of this parameter. Later, Tóth (2012) pointed that discriminative characters are the lunules in the marginal region of underside of hind-wings and ring form of the club of the antennae. Therefore, herein we provide additional arguments confirming the presence of the species in Serbia (Figs. 5, 6 and 7).

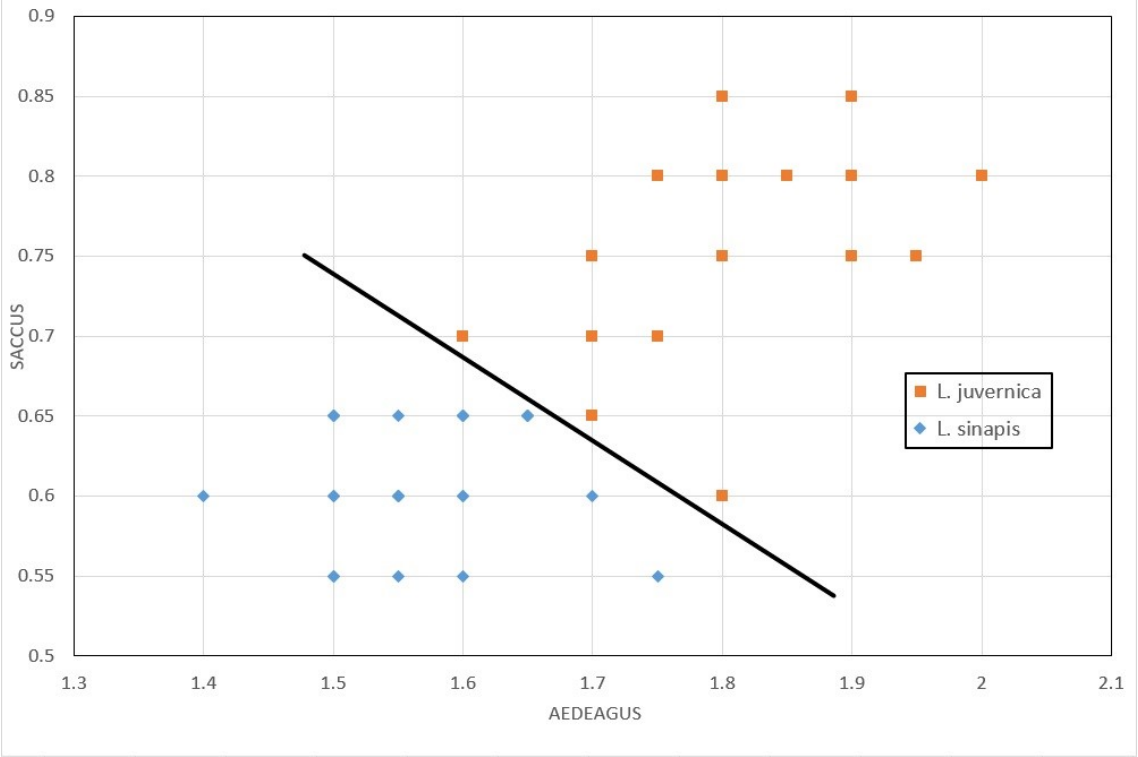

Figure 3. Degree of differentiation between *L. sinapis* and *L. juvernica* on the base on saccus and aedeagus

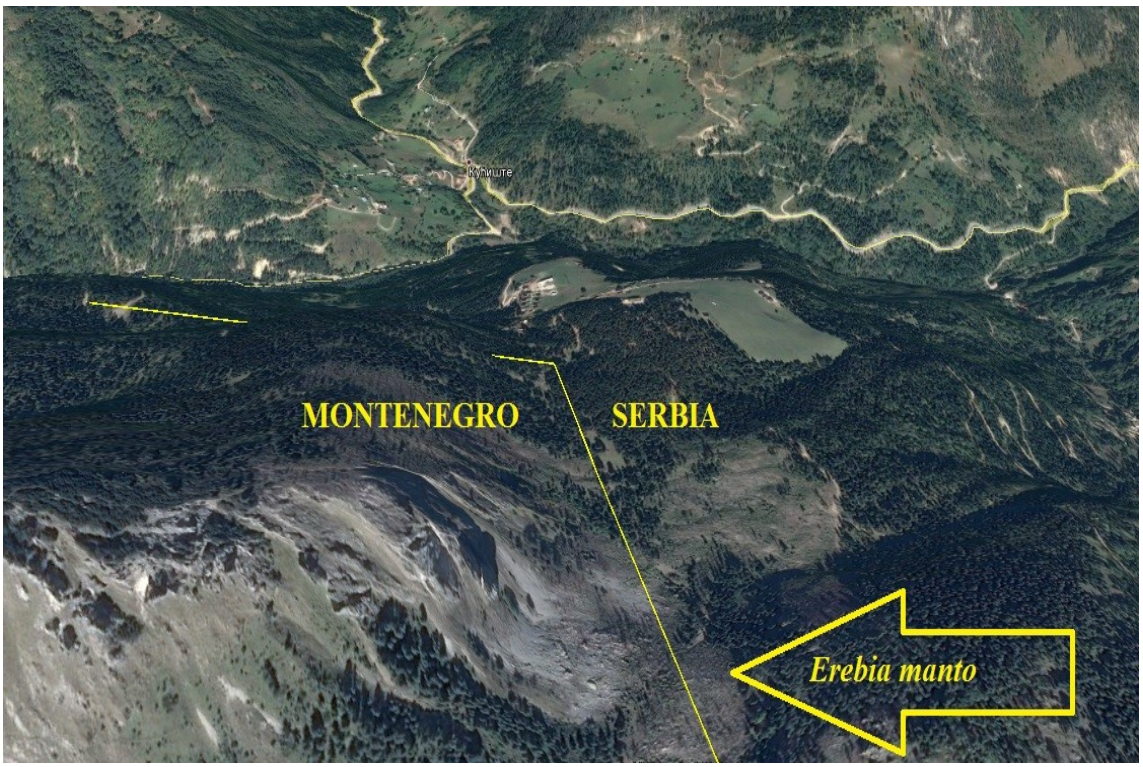

Figure 4. Collecting place of *E. manto* in Serbia on Čakor Pass, 1794 m; 42° 40' 18" N; 20° 05' 15" E. (map source: Google Earth)

The attached Serbian names of butterflies deserve special attention. Language is certainly one of the foundations of every nation. National identity is first reflected in language. Language builds on the culture of a people, preserving its national character, its history.It is a tradition of western European nations to carefully nurture their national terminology for plants, fungi and animals. For example, in English terminology, the vernacular names used by Lewin (1795) Tortoiseshell (*Nymphalis polychloros*), Peacock (*Inachis io*), Chalkhill Blue (*Lysandra coridon*), and many others, are unchanged and in still use after more than two centuries.

Figure 5. *Melitaea ornata* Christopg, 1893. Serbia, Kačanik (Đeneral Janković), 490 m; 42° 14' 26" N; 21° 14' 28" E, 18. May 1979., Jakšić P. leg et coll.

Figure 6. The same specimen, detail of hind-wing: triangle like lunules as a discriminative characters.

Figure 7. The same specimen, the club of the antennae, ring form as a discriminative characters.

Carl Linnaeus' assigning of scientific names was a wise move. He incorporated already existing names from his predecessor. For butterflies, for example, he took over many of the names used by Aldrovandi (1602): P*apilio, minimus, Pyrausta, Pyrallis, Geometra, Bombyce, pityocampa* and many others. Thanks to this, the continuity of names was maintained for four centuries, which is of invaluable educational value.

The tradition of using Serbian names for butterflies is more than two centuries old. It dates from the translation of the notable work Naturgeschichte für Kinder (Raff, Georg Christian, 1783) (Vujić, 1809). In his translation, Vujić gave Serbian names to species. Consequently, this work is part of national heritage, and it is protected by the Constitution of the Republic of Serbia, Article 89: "Everyone is obliged to protect natural rarities and scientific, cultural and historical heritage, as well as goods of public interest in accordance with the Law".

Just before his death, Josif Pančić left the following request to the Serbian Royal Academy, that… "in the records of our Academy, the purity of our beautiful language be preserved, just as the people have given us and as our great teachers V. Karađić and Đ .Daničić prescribed" (Novaković, 2011).

The first list of Serbian names for butterflies was published by Jakšić & Đurić (2008) and included the 193 species known at the time. On the identification of new species in the fauna of Serbia and discovering old works from the period 1809-1941, another list was published (Jakšić et al., 2013). These lists supplemented the first list, without changing the names. An analytical review of the introduction of Serbian names, with the inventory up to the period before the Second World War, was presented by Jakšić (2017).The above works are known to Popović and Verovnik, but they have completely ignored them and introduced new vernacular names. It should be highlighted that this presents a third variant of the names, with numerous differences compared to the previous two (Popović & Đurić, 2011, 2014), and it creates complete confusion: not only are the scientific and cultural heritage ignored, but their own previously assigned names are refused. This cannot be considered valid scientific language.

CONCLUSIONS

Traditional entomological methods have established a solid knowledge of species in the fauna of Serbian butterflies. We have confirmed the presence of the species *Melitaea ornata* Christoph, 1893 in Serbia. We have pointed out that the species *Erebia manto* (Denis Schiffermüller, 1775) is also a member of the fauna of Serbia. Questions regarding dubious taxa from the group of bad species remain unanswered. For the most part, these questions can be resolved by using modern molecular/genetic methods, such as barcoding, etc. Additionally, it is possible to expect the finding of some rare species that exist in Serbia's neighboring countries. Likewise, several invasive species, also present in the countries neighboring Serbia, can be expected to be found. Thus, we believe that the checklist of butterflies of Serbia will soon number over 200 species.

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REFERENCES

Aldrovandi, U. 1602. De animalibvs insectis libri septem: cum singulorum iconibus ad viuum expressis. In Bonon [Bologna] apud Ioan Bapt Bellagambam.

Boza, P., Igić, S. R., Krstić, B., et al. 2003. Distribution of the *Lathyrus* L. 1753 (Fabales, Fabaceae) species in the Vojvodina Province. Zbornik Matice srpske za prirodne nauke, 104, pp. 61-81. doi:10.2298/zmspn0304061b.

Coutsis, G. J. 2013. *Leptidea sinapis* and *Leptidea reali* (Lepidoptera: Pieridae):at what point does the first one of the two end, and the other one begin. Phegea, 41(1), pp. 19–20, 1 tab. with 18 figs.

Csipe, T. 2006. The Insect Collection of the Municipial Museum in Subotica. Museion, 5, pp. 229-272, 2 tabs, 23 figs. [In Serbian, Hungaryan and English summary].

Cupedo, F. & Wim Hoen, F. 2006. *Leptidea sinapis* and *Leptidea reali* (Lepidoptera: Pieridae) in The Netherlands. Entomologische Berichten 66(4): 118–123, 4 tabs, 7 figs.

Cuvelier, S. & Maertens, D. 2017. Trial test of external morphology-based identification of *Leptidea sinapis*, *L. reali* and *L. juvernica* (Lepidoptera: Pieridae) provides opportunity for an online identification platform. Phegea 45(2): 41–45, 4 figs.

Descimon, H. & Mallet, J. 2009. Bad species. In J. Settele, M. Konvicka, T. Shreeve, R. Dennis, D. H. Van Eds., Ecology of Butterflies in Europe. Cambridge University Press. pp. 219-249.

Diklić, N., Cincović, T., & Kojić, M. 1972. Red Fabales. In M. Josifović Ed., Flora SR Srbije. Beograd: SANU. IV, pp. 254–558, figs. [In Serbian].

Dincã, V., Wiklund, C., Lukhtanov, V., et al. 2013. Reproductive isolation and patterns of genetic differentiation in a cryptic butterfly species complex. Journal of Evolutionary Biology, 26(10), pp. 2095-2106. doi:10.1111/jeb.12211.

Federley, H. 1938. Chromosomenzahlen finnlän-discher lepidopteren. Herediтas, 24(4), pp. 397-464. doi:10.1111/j.1601-5223.1938.tb03219.x.

Franeta, F. 2018. Checklist of the butterflies (Lepidoptera: Papilionoidea) of Montenegro. Zootaxa, 4392(1), p. 128. doi:10.11646/zootaxa.4392.1.6.

Hauser, E. 1997. *Leptidea sinapis* (Lennaeus, 1758) und *Leptidea reali* Reissinger 1989: zwei verschieden Arten? (Lepidoptera, Pieridae). Beitr. Naturk. Oberösterreichs, 5, pp. 65–755 figs.

Jakšić, P. 2011. Butterfly species (Lepidoptera; Hesperioidea and Papilionoidea) new to the Serbian fauna. Biologia Nyssana, 2, pp. 45-50.

Jakšić, P., Petrović, S., & Nahirnić, A. 2013. Compendium of Serbian butterflies with vernacular names. Bulletin of the Natural History Museum, 6, pp. 75-88. doi:10.5937/bnhmb1306075j.

Jakšić, P. & Pešić, B. 1995. The distribution of certain Erebia species in Serbia (Lepidoptera, Satyridae) / Rasprostranjenje nekih Erebia vrsta u Srbiji (Lepidoptera, Satyridae). Univerzitetska misao, Prirodne nauke: Priština, 2(1), pp. 23-26, 1996.

Jakšić, P. & Đurić, M. 2008. Srpski nazivi dnevnih leptira (Lepidoptera: Hesperioidea i Papilionoidea). (Serbian Names for Butterflies). In Proceeding of the 9th Symposium of flora of Southeastern Serbia and Neighbouring Regions. Niš. pp. 231-237.

Knežević, A., Džigurski, D., Ljavnaić-Mašić, B., & Čupina, B. 2012. Plant cover of pasture ecosystem located in the vicinity of Muzlja, Serbia. Ratarstvo i povrtarstvo, 49(1), pp. 39-45. doi:10.5937/ratpov49-1204.

Kovács, L. 1956. Some Data Concerning the Subspecific Distribution of Colias chrysotheme Esp. (Lepidoptera). Annals HNHM, Budapest, 48, pp. 425-434.

Kudrna, O., Pennerstorfer, J., & Lux, K. 2015. Distribution Atlas of European butterflies and skippers.Verlag Peks / K., Schwanfeld.

Lewin, W. 1795. The papilios of Great Britain : Systematically arranged, accurately engraved, and painted from nature with the natural history of each species ... /.London: Smithsonian Institution. doi:10.5962/bhl.title.48880.

Lorković, Z. 1928. Analiza pojma i varijabiliteta vrste na osnovi ispitivanja nekih Lepidoptera. Glasnik hrvatskoga prirodoslovnoga društva, Zagreb, XXXIX I XL, pp. 1–64.

Lorković, Z. 1949. Chromosomenzahlen-Vervielfachung bei Schmetterlingen und einr Fall fün$_a$*cherZah.RevueS uissedezoologie*, 56(4), *pp*.243˘249, 4*figs*.

Lorković, Z. 1990. The butterfly chromosomes and their application in systematic and phylogeny. In Kudrna Ed., Butterflies of Europe. 2. Introduction to Lepidopterology. Wiesbaden: AULA Verlag. pp. 332-396.

Lorković, Z. 1993. Ecological association of *Leptidea morsei major* Grund 1905 (Lepidoptera, Pieridae) with the oak forest *Lathyreto-quercetum petraeae* HR–T 1957 in Croatia. Periodicum Biologorum 95(4): 455–457.

Maeki, K. 1958. On the cytotaxonomical relationship in Leptidea (Lepidoptera – Rhopalocera). The Japanese journal of genetics, 33(9), pp. 283-285. doi:10.1266/jjg.33.283.

Mayer, E. 1942. Systematics and the origin of species. New York: Columbia University Press.

Novaković, S. 2011. . Negovanje jezika Srpskog, poslanica Akademiji nauka filosofskih. Srpska Kraljevska Akademija. Glas, X, pp. 1–88. Beograd. [In Serbian].

Popović, M. & Verovnik, R. 2018. Revised checklist of the butterflies of Serbia (Lepidoptera: Papilionoidea). Zootaxa, 4438(3), p. 501. doi:10.11646/zootaxa.4438.3.5.

Popović, M. & Đurić, M. 2011. Dnevni leptiri Srbije. Priručnik. Beograd: HabiProt. 198 pp.

Popović, M. & Đurić, M. 2014. Dnevni leptiri Stare planine (Lepidoptera: Papilionoidea) / Butterflies of Stara Planina (Lepidoptera: Papilionoidea). Beograd: Srbijašume; Beograd: HabiProt. 208 pp.

Russell, P. & Tennent, W. J. 2016. A synonymic list of names associated with western Palaearctic *Melitaea phoebe* (Denis amp; Schiffermüller, 1775) species group taxa (*M. phoebe*; *M. punica* Oberthür, 1876; *M. ornata* Christoph, 1893) (Lepidoptera, Nymphalidae). Nota Lepidopterologica, 39(1), pp. 27-56. doi:10.3897/nl.39.5929.

Tomović, G. 2009. Threat status revision of some taxa from "The Red Data Book of Flora of Serbia 1". Botanica Serbica, 33(1), pp. 33–43, 6 figs.

Tóth, P. J. 2012. Taxonomy, distribution and ecology of Melitaea ornata (Lepidoptera: Nymphalidae).Debrecen: Debreceni Egyetem. pp. 1-117, Dissertation.

Vujić, J. 1809. Jestestvoslovije v polzu najpače junosti. Budim: Pečatano pri Slaveno-Serbskoi Pečatni Kral. Vseučilišta Vengerskago. (translation from German, original: Raff, Georg Christian, 1783. Naturgeschichte für Kinder. Göttingen).

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BACTERIAL LOAD IN RELATION TO PHYSICO-CHEMICAL PARAMETERS AND FAECAL INDICATORS OF DRINKING WATER SOURCE

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ABSTRACT

The aim of this study was to examine the relationship between bacterioplankton and physico-chemical parameters of water that serves as a source of drinking water as well as the relationship between bacterioplankton and the indicators of faecal contamination of water. Bacterioplankton abundance was determined by the cultivation method which included the inoculation of water samples on PCA and R2A media and incubation at 20 °C for 7 days. Total bacterial count was determined by epifluorescence microscopy, and three bacterial morphotypes were distinguished: rods, cocci and curved forms. Bacterial biomass was also determined. Eleven physico-chemical parameters of water were determined: temperature, pH, turbidity, conductivity, UV extinction, $KMnO_4$ consumption, total phosphorus, orthophosphates, ammonium-, nitrite- and nitrate nitrogen. As indicators of faecal contamination, the counts of total coliforms, faecal coliforms, faecal streptococci and sulphite-reducing clostridia were determined. Although there were significantly higher number of bacteria on R2A medium in relation to PCA medium, both media showed significant negative correlation of aerobic mesophilic bacteria with water temperature, in all seasons, and significant positive correlation with UV extinction, turbidity, $KMnO_4$ consumption, total phosphorus, orthophosphates, ammonium nitrogen and nitrate nitrogen. Also, these bacteria showed significant positive correlation with total bacterial count and bacterial biomass, as well as bacterial indicators of faecal contamination.

Keywords: Drinking water, Physico-chemical parameters, Aerobic mesophilic bacteria, Faecal indicators.

INTRODUCTION

The primary point in water supply is the source of drinking water. The quality of the water source is the basic guidance for the selection of processing technology, and the change in this quality is one of the main causes of the deterioration of the quality of water that reaching consumers. In order to respond in a timely manner, in terms of preventing the potential negative impacts of water of inadequate quality on consumers' health, constant monitoring of the basic indicators of the water source status is necessary. The quality of water is defined in terms of its physical, chemical and biological (bacteriological) parameters, and ascertaining its quality is important before use for various intended purposes such as potable, agricultural, recreational and industrial water usages, etc. (Sargaonkar & Deshpande, 2003).

The bacteriological quality of water has traditionally been assessed by monitoring the counts of total coliforms, faecal coliforms and faecal streptococci. Sulphite-reducing clostridia (such as *Clostridium perfringens*) has been suggested as an alternative bacterial indicator of faecal pollution because it is consistently associated with human wastes (Bezirtzoglou et al., 1997).

Bacteria comprise a significant part of planktonic biomass and are responsible for contributing productivity and nutrient cycling in aquatic systems (Muylaert et al., 2002). They have fast growth rates and respond to low levels of pollutants as well as other physical, chemical and biological environmental changes. From detection and effect perspectives, they provide sensitive, meaningful and quantifiable indications of ecological change (Paerl et al, 2003; Freese et al., 2006). Particularly in polluted waters rich in nutrients, bacterial biomass and production are high (Chróst & Siuda, 2006) and heterotrophic bacteria numerically dominate in the bacterioplankton (Szeląg-Wasielewska & Stachnik, 2010). The parameter "heterotrophic bacteria", in our country is called "aerobic mesophilic bacteria", which is more appropriate, because the term "heterotrophic bacteria" refers to all bacteria requiring organic matter for their growth, while all cultivation methods for determining the total count of bacteria detect only a part or subpopulation of heterotrophic bacteria in water or another environment. The monitoring of water supplies for the count of aerobic mesophilic bacteria is useful for monitoring trends or detecting sudden changes in water quality (EA UK, 2002).

According to WHO (2002), heterotrophic plate counts alone cannot indicate a health risk and additional studies on the presence of *E. coli* or other faecal specific indicator microorganisms need to be conducted to establish the potential health risk of the analysed water. Aerobic mesophilic bacteria are

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not indicators of potential risk for human health but some of them are opportunists and can cause various human infections (Bertram et al., 2003). In recent years, a great attention has been paid to non-faecal opportunistic pathogens (Proctor & Hammes, 2015), which mainly belong to the population of aerobic mesophilic bacteria.

Some studies emphasized that appearance and growth of microbiological populations in water can be associated with elevated values of some physico-chemical parameters (Liguori et al., 2010; Poma et al., 2012).

The purpose of this study was to define physico-chemical and bacteriological characteristics of Ćelije Reservoir and the raw water, and to investigate the relationships among the tested parameters, especially the relationships between the count of aerobic mesophilic bacteria and physico-chemical parameters as well as bacterial indicators of the faecal contamination.

EXPERIMENTAL

Study area

Ćelije Reservoir (43°23'22" N; 21°09'48" E; Fig. 1) is located on Rasina River, at 23rd km on the road from Kruševac to Brus. The reservoir was named after the village where the river was dammed. It extends in the direction north-south. Going downstream, the first lake basin, located in the village of Zlatari, belongs to the municipality of Brus, and the remaining two basins, located in the village of Ćelije, belong to the municipality of Kruševac. Ćelije is a typical hilly-mountain reservoir with complex morphometry. It is elongated in shape, about 10 km long and consists of three basins: Zlatari, Vasići and Water-catching basin. Basins have successively increasing depth (Zlatari 6-10 m, Vasići 22-24 m, Water-catching basin about 42 m max), and thus a volume, so the last basin contains more than a half of the lake water.

Sampling

During one year, once a month, the sampling of the Ćelije Reservoir water was carried out in all three basins - Zlatari (two sampling sites - S1 and S2), Vasići (one sampling site - S3) and Water-catching basin (two sampling sites - S4 and S5; Fig. 1). Sampling was carried out at different depths in relation to the temperature distribution. During the circulation periods (January, February, March, November and December), the sampling was carried out at the following depths: S1 - 0.5 m, 1 m, 2-4 m; S2 - 0.5 m, 1 m, 3 m, 6 m, 12 m; S3 - 0.5 m, 1 m, 3 m, 6 m, 13 m, 20 m; S4 - 0.5 m, 1 m, 3 m, 6 m, 14 m, 22 m, 30 m; S5 - 0.5 m, 1 m, 3 m, 6 m, 14 m, 22 m, 35 m. In the periods of stagnation (April, May, June, July, August, September and October), the sampling was carried out at the following depths: S1 - as in the previous period; S2 - 0.5 m, 1 m, 3 m, 5 m, 7 m (thermocline), 12 m; S3 - 0.5 m, 1 m, 3 m, 5 m, 7 m, 13 m, 21 m; S4 - 0.5 m, 1 m, 3 m, 5 m, 7 m, 14 m, 22 m, 30 m; S5 - 0.5 m, 1 m, 3 m, 5 m, 7 m, 14 m, 22 m, 35 m.

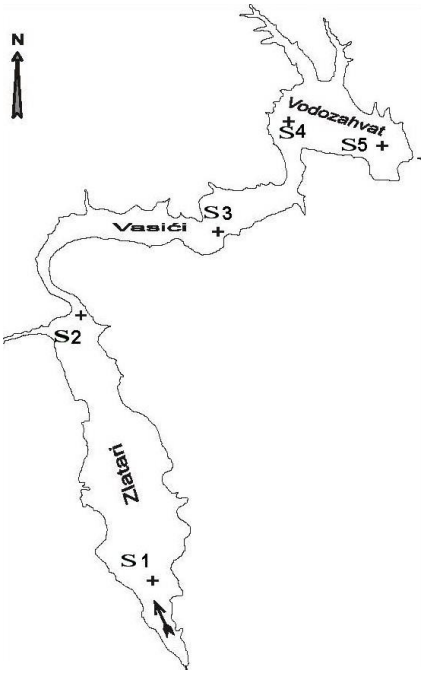

Figure 1. Ćelije Reservoir. S1, S2, S3, S4, S5 - sampling sites (Vodozahvat - Water-catching basin).

During the same year, the sampling of raw water was done at the site of the water intake, once a week.

The samples were taken with a Van Dorn bottle and immediately, in refrigerators, transported to the laboratories of the Drinking Water Plant in Majdevo, where they were processed.

Materials and methods

The water temperature was measured *in situ* by a digital thermometer. Other physico-chemical parameters (pH, turbidity, conductivity, UV extinction, $KMnO_4$ consumption, total phosphorus, orthophosphates, ammonium-, nitrite- and nitrate nitrogen) were determined by standard methods according to APHA (2012). The pH value was determined by the pH-meter. Turbidity of water was determined nefelometrically according to the standard formazine polymer and expressed in nefelometric turbidity units (NTU). The conductivity was determined by the conductometer. UV-extinction was measured at 254 nm using Shimadzu UV-2401 PC ultraviolet/visible spectrophotometer. The $KMnO_4$ consumption was determined by the oxidation of organic matter with potassium permanganate in the acidic environment (H_2SO_4). The total phosphorus was determined spectrophotometrically, by measuring the color intensity of the complex that was built between orthophosphates, ammonium molybdate and antimonyl potassium tartarate, at 630 nm. The concentrations of orthophosphates were determined by ammonium heptamolybdate technique. The ammonia concentrations were determined spectrophotometrically, with

Nesler's reagent. The content of nitrite was determined by sulfanilic acid, spectrophotometrically. The content of nitrate was determined spectrophotometrically with hydrochloric acid.

The total count of aerobic mesophilic bacteria was determined by parallel inoculation of samples on a standard Plate Count Agar (PCA) and on the Reasoner's 2 Agar (R2A). PCA content is as follows: Tryptone (5.0 $g \cdot l^{-1}$), yeast extract (2.5 $g \cdot l^{-1}$), glucose (1.0 $g \cdot l^{-1}$) and agar (15.0 $g \cdot l^{-1}$). R2A content is as follows: Meat peptone (0.5 $g \cdot l^{-1}$), yeast extract (0.5 $g \cdot l^{-1}$), casein hydrolyzate (0.5 $g \cdot l^{-1}$), glucose (0.5 $g \cdot l^{-1}$), starch (0.5 $g \cdot l^{-1}$), K_2HPO_4 (0.3 $g \cdot l^{-1}$), $MgSO_4x7H_2O$ (0.05 $g \cdot l^{-1}$), Na-pyruvate (0.3 $g \cdot l^{-1}$) and agar (15.0 $g \cdot l^{-1}$). The three dilutions: 10^{-1}, 10^{-2} and 10^{-3} of each sample were inoculated in the duplicate for both media. The inoculation was carried out using pour plate technique, and the inoculated plates were incubated at room temperature (20-22 °C) for 7 days. The colonies were counted using the BZG-30 colonies counter (Windaus), through the lens with a magnification of 2x. The count of bacteria was carried out on plates with the smallest dilution that could be read. The obtained number was calculated for 1 ml of the sample, and presented as colony forming units per milliliter of water sample (cfu/ml).

Total bacterial count (direct count) - TBC, was determined by epifluorescence microscopy, after the dying of the water samples with acridine orange, as described by Ćirić (2009). Three morphotypes of bacteria were distinguished: rods, cocci and curved forms.

Bacterial biomass - BB estimations were made by examining photographs of random fields from selected samples, and then projecting the resulting images so that the minimum projected cell size was ~ 1 mm. Bacteria were sized by an eyepiece micrometer. Cell length and width were measured and converted to biovolume using the formula of Bratbak (1993). A single formula that worked well for rods and cocci was used to calculate the biovolume. Biovolume was converted to biomass with a conversion of 200 fg C μm^3 (Bratbak, 1993).

Total coliforms (TC) count was determined on Eijkman medium (Merck, Germany) at 37 °C after 48 h incubation, as most probable number per 100 ml of water sample - MPN/100 ml.

Faecal coliforms (FC) count was determined on Eijkman medium (Merck, Germany) at 44.5 °C after 24 h incubation, as MPN/100 ml.

Faecal streptococci (FS) count was determined on Slanetz and Bartley medium (Merck, Germany) with sodium azide and crystaline violet at 37 °C after 72 h incubation, as MPN/100 ml.

Sulphite-reducing clostridia (SC) count was determined on Sulphate agar (Torlak, Serbia) at 37 °C after 48 h incubation, as CFU/100 ml.

Statistical analyses

Determination of the correlation coefficients among the investigated physico-chemical and microbiological parameters of water was carried out using statistical software STATISTICA v. 10, StatSoft, Inc.

RESULTS

Average, maximum and minimum values of physico-chemical parameters in Ćelije Reservoir and the raw water are given in Table 1. The highest average temperature was recorded in summer (16.5 °C), and the lowest in winter (5.9 °C). Starting from the shallowest part of the reservoir (S1) to the deepest (S5), the temperature gradually decreased in all seasons, so that, on average, the water-catching basin was colder than the first basin (Zlatari I) by about 4 °C. The fluctuation of the water temperature usually depends on the season, geographic location and sampling time (Venkatesharaju et al., 2010).

Previous studies have shown that Ćelije belongs to the mesotrophic lake ecosystems with the tendency of increasing of the trophic level (Marković et al., 1998). The sanitary protection zones of the reservoir as the water supply source for the town of Kruševac and its surroundings, although marked, are not respected. There is a strong negative anthropogenic impact on the reservoir because of the existence of the settlements on shores, agricultural land and erosion due to degraded forests in the lake basin.

Ćelije Reservoir is heavy modified water body formed on a type 3 water body and its quality is subject to the Rule book on the parameters of the ecological and chemical status of surface waters and the parameters of the chemical and quantitative status of groundwaters (Službeni glasnik RS, 74/11). On the basis of average annual values of physico-chemical parameters, the source water and raw water belong to the second class (according to the pH value, nitrate nitrogen, total and inorganic phosphorus) and the third class (according to the ammonium nitrogen). Based on the obtained results, it can be said that the reservoir has a moderate ecological potential. The ecological potential of a water body represents the degree to which the quality of the water body's aquatic ecosystem approaches the maximum it could achieve. In principle, there are five ecological potential classes: maximum, good, moderate, poor and bad (Water Framework Directive, 2008). However, in some seasons, some parameters exceeded the values prescribed for that class so, in those seasons water was of worse quality than average for one or even two classes. This is very important because the reservoir is used for drinking water supply in those seasons, too. Thus, orthophosphates in the winter and autumn periods exceeded the given values, especially in the water-catching basin, from where the water is taken for processing. According to this parameter, in these seasons the water was of poorer quality for one class, and it was classified in fourth class, which is not recommended for water supply. In the case of ammonium nitrogen, the average value in the autumn season exceeded the value of 1 mg/l (worse than the fifth class), so in this period the water can not be used for any purpose. The concentration of nitrate nitrogen remained

stable throughout the year and none of the samples did not exceed the value prescribed for the second class. Total phosphorus also remained stable throughout the year and throughout the reservoir.

Table 1. Average, maximum and minimum values of physico-chemical parameters in Ćelije Reservoir and raw water.

Parameter	S1	S2	S3	S4	S5	Ćelije	Raw water
Temperature (°C)	16.0 4.3-24.0	16.1 4.0-27.5	14.4 3.2-25.0	13.5 3.8-26.1	13.4 3.6-26.3	14.3 3.2-27.5	10.2 3.6-13.8
pH	8.35 7.92-8.81	8.27 7.66-8.94	8.14 7.62-9.01	8.06 7.56-9.02	8.07 7.46-9.02	8.14 7.46-9.02	7.78 7.58-7.99
Turbidity (NTU)	11.70 3.40-78.00	9.20 1.80-75.00	5.47 1.10-34.00	3.94 0.92-21.00	4.13 0.64-26.00	5.89 0.64-78.00	6.83 1.40-24.00
Conductivity ($\mu S \cdot cm^{-1}$)	331.2 294.6-361.2	324.9 284.4-360.5	315.7 257.3-357.7	313.3 251.3-352.1	313.6 255.3-365.4	317.4 251.3-365.4	314.3 283.8-347.2
UV extinction, 254 nm	10.8 5.5-43.2	9.5 5.2-41.1	10.7 4.6-29.7	9.7 5.1-26.4	10.0 4.7-25.7	10.1 4.6-43.2	11.7 4.9-25.7
$KMnO_4$ consumption ($mg \cdot l^{-1}$)	13.92 10.55-19.90	12.43 9.23-20.81	11.31 7.90-15.91	10.86 7.58-14.22	11.32 7.90-22.12	11.61 7.58-22.12	10.61 7.58-12.72
Total P ($mg \cdot l^{-1}$)	0.080 0.054-0.184	0.069 0.030-0.216	0.054 0.024-0.187	0.051 0.013-0.124	0.065 0.021-0.264	0.061 0.013-0.264	0.074 0.050-0.102
PO_4^{3-} ($mg \cdot l^{-1}$)	0.020 0.000-0.074	0.024 0.000-0.089	0.024 0.000-0.093	0.024 0.000-0.088	0.031 0.000-0.202	0.026 0.000-0.202	0.042 0.025-0.064
$N\text{-}NH_4$ ($mg \cdot l^{-1}$)	0.107 0.000-0.278	0.197 0.000-1.484	0.162 0.000-1.500	0.139 0.019-0.582	0.342 0.013-5.350	0.204 0.000-5.350	0.177 0.017-0.442
$N\text{-}NO_2$ ($mg \cdot l^{-1}$)	0.010 0.000-0.027	0.013 0.000-0.055	0.019 0.000-0.128	0.012 0.000-0.308	0.015 0.000-0.212	0.016 0.000-0.308	0.009 0.000-0.025
$N\text{-}NO_3$ ($mg \cdot l^{-1}$)	0.481 0.051-1.778	0.409 0.000-1.788	0.547 0.020-1.764	0.642 0.000-1.616	0.586 0.034-1.570	0.540 0.000-1.788	0.916 0.219-1.510

The bacterial abundance (cfu/ml) in the source water ranged between 60 and $3.75x10^4$ (on PCA) and between $2.25x10^2$ and $1.79x10^5$ (on R2A; Tab. 2). The earlier studies have shown that a significantly higher number of aerobic mesophilic bacteria has been detected on the low-nutrient R2A medium in relation to high-nutrient PCA medium, and after seven days of incubation, the number of bacteria on R2A was higher than PCA for 74% (Ćirić & Petrović, 2009).

Table 2. Average, maximum and minimum values of bacterioplankton in Ćelije Reservoir and raw water (PCA-Plate Count Agar, R2A-Reasoner's 2 Agar, TBC-Total Bacterial Count, BB-Bacterial Biomass)

Bacterioplankton properties	S1	S2	S3	S4	S5	Ćelije	Raw water
PCA ($cfu \cdot ml^{-1}$)	$5.61x10^3$ $5.25x10^2$- $3.75x10^4$	$2.83x10^3$ $2.30x10^2$- $3.14x10^4$	$1.72x10^3$ 60-$1.28x10^4$	$1.36x10^3$ 70-$1.73x10^4$	$1.66x10^3$ 7.20x10- $2.57x10^4$	$2.12x10^3$ 60-$3.75x10^4$	$2.03x10^3$ $3.40x10^2$- $1.48x10^4$
R2A ($cfu \cdot ml^{-1}$)	$2.12x10^4$ $1.52x10^3$- $1.10x10^5$	$9.32x10^3$ $3.80x10^2$- $6.23x10^4$	$7.75x10^3$ $2.25x10^2$- $4.91x10^4$	$7.23x10^3$ $3.00x10^2$- $1.79x10^5$	$4.73x10^3$ $3.60x10^2$- $5.15x10^4$	$8.21x10^3$ $2.25x10^2$- $1.79x10^5$	$5.06x10^3$ $9.65x10^2$- $3.48x10^4$
TBC ($no \cdot ml^{-1}$)	$1.79x10^7$ $1.22x10^7$- $2.65x10^7$	$1.07x10^7$ $7.50x10^6$- $1.56x10^7$	$3.46x10^6$ $2.35 \cdot x10^6$- $4.46x10^6$	$2.34x10^6$ $1.33x10^6$- $4.44x10^6$	$1.49x10^6$ $8.06x10^5$- $2.33x10^6$	$7.19x10^6$ $1.33x10^6$- $2.65x10^7$	$1.17x10^6$ $3.10x10^5$- $1.10x10^7$
BB ($\mu gC \cdot l^{-1}$)	546.85 370.63-807.02	327.70 228.66-475.25	105.57 71.74-136.00	71.40 40.67-135.56	45.52 24.57-71.17	219.41 24.57-807.02	41.01 10.86-384.55
Rods in TBC (%)	58.31 38.71-71.08	51.43 35.37-67.50	62.16 50.00-83.33	53.18 40.00-75.00	52.79 41.38-60.98	56.22 35.37-83.33	52.69 29.39-74.11
Cocci in TBC (%)	31.80 21.57-45.48	39.18 30.00-46.60	28.19 16.67-33.33	37.65 25.00-50.00	30.12 21.95-40.00	33.10 16.67-50.00	42.98 21.23-67.62
Curved bacteria in TBC (%)	9.89 3.01-20.48	9.39 0.00-28.93	9.65 0.00-25.42	9.17 0.00-18.52	17.09 5.66-27.59	10.68 0.00-28.93	4.33 0.56-10.08

Bacterial count on both media showed that, going downstream, from the shallowest to the deepest basin, water quality has improved, both due to the natural process of auto purification, and because of the better protection of the water-catching basin. The highest number of bacterioplankton was recorded in March, and the lowest in August. In the early spring, after snow melting, a large amount of water, carrying various materials from the lake shores was instilled into the reservoir. This led to the significant increase in the bacterial count. After that, the count of bacteria dropped. There were two small

increases in count, in June and September, which were probably the results of the anthropogenic activity. According to Kohl (1975), the reservoir belongs to the second class of water (moderately contaminated water). According to the Rule book on the parameters of the ecological and chemical status of surface waters and the parameters of the chemical and quantitative status of groundwater (Službeni glasnik RS, 74/11), based on the average number of aerobic mesophilic bacteria, the reservoir belongs to the second class (when the cultivation was done on PCA medium) *i.e.* to the third class (when the cultivation was done on R2A medium). This means that the reservoir water can be used for water supply, which is contrary to the also valid Rule book of the hygienic safety of drinking water (Službeni list SRJ, 42/98). Under this Regulation, the water of open source used for water supply should not contain more than 300 aerobic mesophilic bacteria in 1 ml. All examined samples of the water of Ćelije Reservoir, including raw water, contained more bacteria than prescribed.

Based on the direct number of bacteria (Tab. 2), the source water was also classified into the class of moderately contaminated waters, while the raw water was classified even in the class of maximum clean waters (Ambrazene, 1976).

The matrix of production moment correlation among analyzed physico-chemical and bacteriological variables is presented in Table 3. Aerobic mesophilic bacteria (on PCA and R2A) showed a significant positive correlation with UV extinction, turbidity, $KMnO_4$ consumption, total phosphorus, orthophosphates, ammonium nitrogen and nitrate nitrogen. Also, these bacteria showed significant positive correlation with total bacterial count and bacterial biomass. This means that any of these bacteriological parameters will equally valid show changes in the bacteriological status of the aquatic ecosystem. A significant negative correlation of the number of bacteria with water temperature in all seasons suggests that bacteria are well adapted to the ambient temperature. Examining the source water, Donde (2012) also obtained the negative correlation between the bacterial count and water temperature. A significantly higher coefficient of correlation between aerobic mesophilic bacteria and turbidity, compared to the correlation coefficient between these bacteria and the $KMnO_4$ consumption, shows that turbidity is a much better indicator of the organic load of water (Eregno, 2013).

Table 3. Matrix of production moment correlation coefficient (r) among analyzed physico-chemical and bacteriological variables of Ćelije Reservoir (Temp.-Temperature, UV ext.-UV extinction, $KMnO_4$ con.-$KMnO_4$ consumption, Orthoph.-Orthophosphates, Cond.-Conductivity, d.-days,TBC-Total Bacterial Count, BB-Bacterial Biomass)

	Temp.	UV ext.	Turb.	pH	$KMnO_4$ con.	Total P	Orthoph.	NH_4-N	NO_2-N	NO_3-N	Cond.	PCA, 7 d.	R2A, 7 d.	TBC	BB	Rods %	Cocci %	Curved %
Temperature	1	-0.666	-0.582	0.033	0.246	-0.641	-0.819	-0.332	-0.012	-0.677	-0.122	-0.620	-0.390	-0.116	-0.116	-0.151	0.260	-0.067
UV extinction	-0.666	1	0.954	0.070	0.187	0.761	0.660	0.338	0.001	0.792	-0.206	0.921	0.768	0.224	0.224	0.097	-0.314	0.196
Turbidity	-0.582	0.954	1	0.045	0.252	0.817	0.632	0.383	0.092	0.802	-0.201	0.923	0.817	0.188	0.188	0.081	-0.278	0.179
pH	0.0333	0.070	0.045	1	0.174	0.284	0.110	0.170	-0.183	0.032	-0.418	0.055	0.051	0.247	0.247	0.138	-0.018	-0.166
$KMnO_4$ consump.	0.246	0.187	0.252	0.174	1	0.207	-0.072	-0.177	-0.353	-0.084	-0.542	0.296	0.414	0.214	0.214	0.072	-0.162	0.071
Total P	-0.641	0.761	0.817	0.284	0.207	1	0.728	0.511	-0.021	0.621	-0.131	0.808	0.697	0.187	0.187	0.070	-0.177	0.090
Orthophosphates	-0.819	0.660	0.632	0.110	-0.072	0.728	1	0.597	0.097	0.611	-0.154	0.617	0.443	0.022	0.022	0.201	-0.401	0.146
NH_4-N	-0.332	0.338	0.383	0.170	-0.177	0.511	0.597	1	0.450	0.371	-0.132	0.277	0.288	-0.240	-0.240	0.014	-0.306	0.299
NO_2-N	-0.012	0.001	0.092	-0.183	-0.353	-0.021	0.097	0.450	1	0.312	0.196	-0.017	0.161	-0.149	-0.149	0.088	-0.069	-0.047
NO_3-N	-0.677	0.792	0.802	0.032	-0.084	0.621	0.611	0.371	0.312	1	-0.113	0.748	0.651	0.145	0.145	0.245	-0.332	0.015
Conductivity	-0.122	-0.206	-0.201	-0.418	-0.542	-0.131	-0.154	-0.132	0.196	-0.113	1	-0.191	-0.245	-0.219	-0.219	-0.062	0.303	-0.231
PCA, 7 d.	-0.620	0.921	0.923	0.055	0.296	0.808	0.617	0.277	-0.017	0.748	-0.191	1	0.847	0.266	0.266	0.135	-0.247	0.074
R2A, 7 d.	-0.390	0.768	0.817	0.051	0.414	0.697	0.443	0.288	0.161	0.651	-0.245	0.847	1	0.391	0.391	0.169	-0.258	0.041
TBC	-0.117	0.224	0.188	0.247	0.214	0.187	0.022	-0.240	-0.149	0.145	-0.219	0.266	0.391	1	1.000	-0.017	0.124	-0.105
BB	-0.117	0.224	0.188	0.247	0.214	0.187	0.022	-0.240	-0.149	0.145	-0.219	0.266	0.391	1.000	1	-0.017	0.124	-0.105
Rods %	-0.151	0.097	0.081	0.138	0.072	0.070	0.201	0.014	0.088	0.245	-0.062	0.135	0.169	-0.017	-0.017	1	-0.674	-0.643
Cocci %	0.260	-0.314	-0.278	-0.018	-0.162	-0.177	-0.401	-0.306	-0.069	-0.332	0.303	-0.247	-0.258	0.124	0.124	-0.674	1	-0.132
Curved %	-0.067	0.196	0.179	-0.166	0.071	0.090	0.146	0.299	-0.047	0.015	-0.231	0.074	0.041	-0.105	-0.105	-0.643	-0.132	1

Red marked correlations are significant at $p < 0.05$ level. N = 55.

Ghorbani et al. (2013) also showed a significantly high positive correlation coeficient (r=0.758) between the count of heterotrophic bacteria and water turbidity and no relationships between the count of bacteria and pH value of water. According to Albaggar (2014), total phosphorus in water was one of the most significant parameters correlated positively with total bacterial numbers, which is in line with the results in this paper. But, contrary to the presented results, this author found strongly negatively correlation of total culturable bacteria with direct bacterial count in freshwater ecosystem. Lemke & Leff (2006)

found a strong positive relationship between total bacterial abundance and total culturable bacteria, which is in accordance to this work.

The high negative correlation between the coccoid form of the bacteria and the elements of trophicity (turbidity, UV extinction, total and inorganic phosphorus, nitrates) confirms the findings that these forms of bacteria dominate in clean waters, since such a shape enables them a more efficient use of a small amount of available nutrients (Jochem, 2001). A limiting-nutrient situation for bacteria is mostly accompanied with changes in cell morphology from rod-shaped cells to more coccoid forms (Velimirov et al., 2011). In this study, we could indeed find significant trends for the longitudinal development of the different morphotypes fitting to this postulation. For vibrio-shaped cells, a significant reduction in cell volume was observed. The percentage of rods (total and free-living cells) significantly decreased towards the Delta, while the percentage of cocci significantly increased following concomitant changes of inorganic nutrients (nitrate and ammonium). This is also shown by the high negative correlation between the coccoid and the rod forms which is dominated in the waters rich in nutrients. The curved form of bacteria also showed a high negative correlation with the rods.

Among the physico-chemical parameters, water temperature was significantly negatively correlated with UV extinction, turbidity, phosphates, nitrates and bacterial load. This shows that the reservoir is a thermally very stable system.

To detect changes in the quality of water ecosystem, it was shown that, among physico-chemical parameters, turbidity was an excellent parameter. Turbidity showed a highly positive correlation with the pollution indicators (total phosphorus, orthophosphates, all forms of the mineral nitrogen and bacterial abundance). Among the bacteriological parameters, the best indicators of system changes are aerobic mesophilic bacteria.

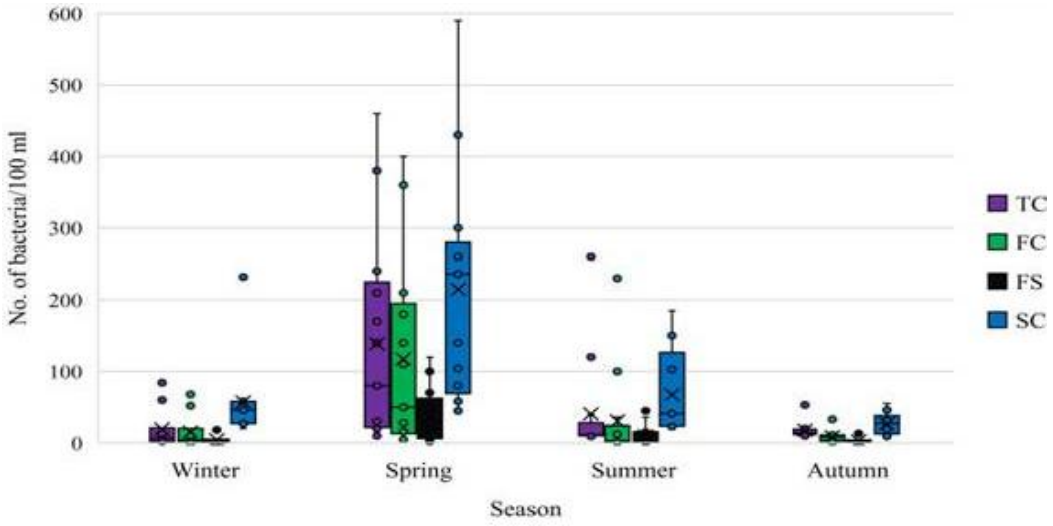

Figure 2. Seasonal variations of faecal indicator bacteria in raw water (TC-Total coliforms, FC-Faecal Coliforms, FS-Faecal Streptococci, SC-Sulphite-reducing Clostridia)

Average values of faecal indicator bacteria in raw water are showed in Fig. 2. All of the bacteriological indicators of faecal contamination had the highest values in 100 ml of a water sample in the spring (TC - 149, FC - 126, FS - 39 and SC - 227), and the lowest in the autumn (TC - 17, FC - 8, FS - 2 and SC - 27). According to Kavka (1994), over than 80% of the samples belong to slightly or less polluted waters. However, since it is an open source of drinking water, which should not contain faecal coliforms and faecal streptococci at all, and for the total coliforms and sulphite-reducing clostridia there is a maximum permissible values of 100 MPN/100 ml and 10 bacteria/100 ml, respectively (Službeni list SRJ, 42/98), all of the raw water samples exceeded these values making this water unfit for the processing into drinking water.

Table 4. Matrix of production moment correlation coefficient (r) between analyzed physico-chemical parameters and faecal indicator bacteria in raw water (TC-Total Coliforms, FC-Faecal Coliforms, FS-Faecal Streptococci, SC-Sulphite-reducing Clstridia)

	Temp.	UV ext.	Turb.	pH	$KMnO_4$ consump.	Total P	PO_4^{3-}	NH_4-N	NO_2-N	NO_3-N	Cond.
TC	-0.344	0.461	0.902	0.369	0.326	0.334	0.168	0.321	0.155	0.421	-0.350
FC	-0.343	0.457	0.913	0.379	0.316	0.339	0.171	0.340	0.155	0.409	-0.340
FS	-0.390	0.614	0.909	0.403	0.382	0.465	0.271	0.416	0.220	0.443	-0.276
SC	-0.528	0.705	0.842	0.485	0.373	0.530	0.368	0.416	0.170	0.590	-0.248

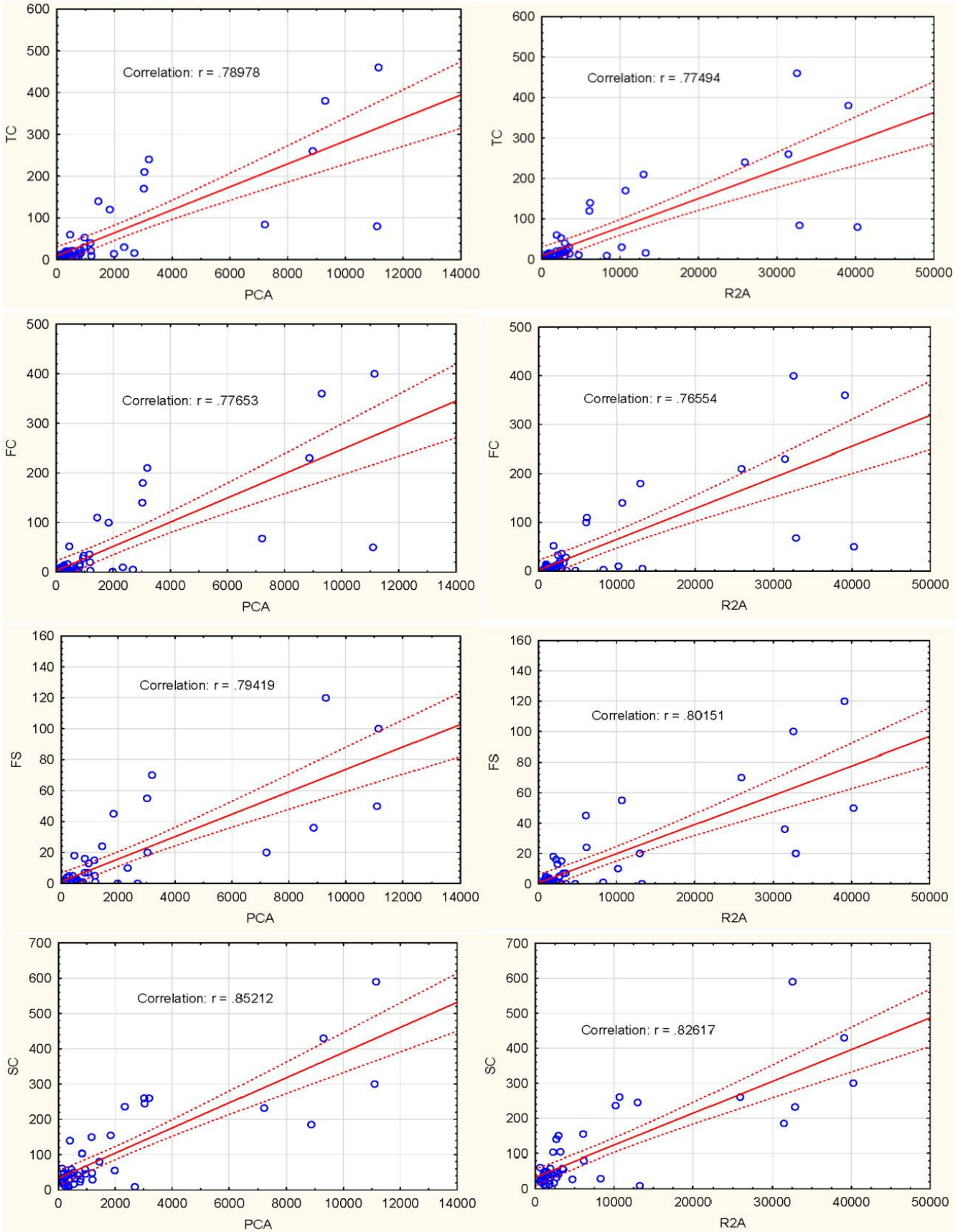

Figure 3. Relationships between aerobic mesophilic bacteria (PCA and R2A) and faecal indicator bacteria in raw water (TC-Total Coliforms, FC-Faecal Coliforms, FS-Faecal Streptococci, SC-Sulphite-reducing Clostridia)

The matrix of production moment correlation between physico-chemical parameters and faecal indicator bacteria in raw water is presented in Table 4. All of the indicators of faecal contamination showed significant negative correlation with temperature, while the coliforms (total and faecal) showed the negative correlation with conductivity, too. The high positive correlation these bacteria showed with the UV extinction, turbidity, pH, $KMnO_4$ consumption, total phosphorus, ammonium and nitrate nitrogen, as well as the absence of the correlation with orthophosphates (except SC) and nitrites.

Relationships between faecal indicator bacteria and aerobic mesophilic bacteria in raw water are shown in Fig. 3. The correlation of faecal bacteria with aerobic mesophiles was determined from the results of cultivation, but not with direct (total) bacterial count, since faecal bacteria and aerobic mesophiles were determined by the same method - cultivation.

Although the differences in the number of bacteria obtained on PCA and R2A media were highly significant, both media showed a very high and very similar positive coefficient of correlation with all of the investigated indicators of the faecal pollution. This is in contrast to the findings of most authors, but there are also those who have obtained similar results. Donde (2012) showed a significantly high positive correlation between heterotrophic bacteria count and some faecal indicators (*Escherichia coli*, intestinal enterococci and *Clostridium perfringens*). In drinking water, Amanidaz et al. (2015) found that relationship between heterotrophic bacteria, coliforms and faecal streptococci was highly significant ($p < 0.05$), but they explained this by the presence of high concentrations of organic carbon, biofilms and nutrients, which are necessary for growth and survival of all microorganisms.

CONCLUSION

Based on the examined physico-chemical parameters, Ćelije Reservoir in some seasons did not correspond to the quality of the drinking water source. These seasons were winter and autumn, when there was a significant increase in the content of orthophosphates and ammonium nitrogen.

Based on the average number of aerobic mesophilic bacteria, the reservoir belonged to the second class (when the cultivation was done on PCA medium) or the third class (when the cultivation was done on R2A medium), but in each sample this number exceeded the upper limit prescribed by the Regulation of the hygienic correctness of drinking water. Based on these results, which are in accordance with the results of many authors, the change of the current regulative, in terms of the introduction of R2A medium for determining the count of aerobic mesophilic bacteria in waters instead of standard high-nutrient medium, can be recommended.

Aerobic mesophilic bacteria (on both media) showed a significant positive correlations with UV extinction, turbidity, $KMnO_4$, total P, orthophosphates, ammonium- and nitrate nitrogen, TBC and BB. A significant negative correlation these bacteria showed with water temperature.

Among the faecal indicator bacteria, aerobic mesophilic bacteria showed high positive correlation with all tested faecal bacteria.

REFERENCES

Albaggar, A. K. A. 2014. Investigation of bacterial community composition and abundance in a lowland arable catchment.UK: University of East Anglia, School of Environmental Sciences. PhD Thesis.

Amanidaz, N., Zafarzadeh, A., & Mahvi, A. H. 2015. The interaction between heterotrophic bacteria and coliform, fecal coliform, fecal streptococci bacteria in water supply networks. Iranian Journal of Public Health, 44(12), pp. 1685-1692.

Ambrazene, Z. P. 1976. Quantitative relationships between microorganisms and their use for evaluation of contamination in river waters. Zhurnal Obshtei Biologii, 37(3), pp. 416-425, In Russian.

-APHA. 2012. Standard methods for the examination of water and wastewater.American Public Health Association, American Water Works Association, Water Environment Federation. 22nd Edition.

Bertram, J., Cotruovo, J., Exner, M., Fricker, C., & Glasmacher, A. 2003. Heterotrophic plate counts and drinking-water safety: The significance of HPCs for water quality and human health. IWA Publishing. pp. 7-16.

Bezirtzoglou, E., Panagiou, A., Savvaidis, I., & Maipa, V. 1997. Distribution of Clostridium perfringensin Polluted Lake Environments. Anaerobe, 3(2-3), pp. 169-172. doi:10.1006/anae.1997.0101

Bratbak, G. 1993. Microscope methods for measuring bacterial biovolume: epifluorescence microscopy, scanning electron microscopy, and transmission electron microscopy. In P.F. Kemp, B. F. Sherr, E. B. Sherr, & J. J. Cole Eds., Handbook of Methods in Aquatic Microbial Ecology. Boca Raton, FL: Lewis Publishers. pp. 309-319.

Chróst, R. J., & Siuda, W. 2006. Microbial production, utilization, and enzymatic degradation of organic matter in the upper trophogenic layer in the pelagial zone of lakes along a eutrophication gradient. Limnology and Oceanography, 51(1part2), pp. 749-762. doi:10.4319/lo.2006.51.1_part_2.0749

Ćirić, S. 2009. Aerobic mesophilic bacteria and low-nutrient R2A medium in monitoring of drinking water quality. Novi Sad: University of Novi Sad, Faculty of Science. PhD Dissertation, in Serbian.

Ćirić, S., & Petrović, O. 2009. Comparison of cultural methods on bacterial recovery in source water for water supply. Fresenius Environmental Bulletin, 18(11a), pp. 2203-2209.

Donde, O. O. 2012. Bacteriological analysis of faecal pollution and solar radiation disinfection of domestic water sources within lake Naivasha Basin, Kenya. Kenya: Egerton University. Master of Science Thesis.

-Environment Agency of United Kingdom (EA UK). 2002. The microbiology of drinking water - Part I - Water quality and

public health. http://www.environment-agency.gov.uk/aboutus/default.aspx.

Freese, H. M., Karsten, U., & Schumann, R. 2006. Bacterial Abundance, Activity, and Viability in the Eutrophic River Warnow, Northeast Germany. Microbial Ecology, 51(1), pp. 117-127. doi:10.1007/s00248-005-0091-5

Ghorbani, J., Moradianfard, S., Reisi, P., & Gheysari, M. S. 2013. Survey of heterotrophic bacteria population changes in Kerman drinking water distribution system and GIS zoning. European Journal of Experimental Biology, 3(2), pp. 476-483.

Jochem, F. J. 2001. Morphology and DNA content of bacterioplankton in the northern Gulf of Mexico: analysis by epifluorescence microscopy and flow cytometry. Aquatic Microbial Ecology, 25, pp. 179-194. doi:10.3354/ame025179

Kavka, G. G. 1994. Erfassung und Bewertung der bakteriologischen Beschaffenheit der Donau im Jahre 1993. Vergleich der Grenzprofile Deutschland-Österreich und Ústerreich-Slowakei. In 30. Arbeitstagung der IAD, Wissenschafftliche Kurzreferate.

Kohl, W. 1975. Uber die Bedeutung Bakteriologischer Untersuchungen fur die Beuerteilung von Fleissgewasser, Dargestellt am Beispiel der osterreich Donau. Archiv fur Hydrobiologie, 44(4), pp. 392-461.

Lemke, M. J., & Leff, L. G. 2006. Culturability of Stream Bacteria Assessed at the Assemblage and Population Levels. Microbial Ecology, 51(3), pp. 365-374. doi:10.1007/s00248-006-9026-z

Liguori, G., Cavallotti, I., Arnese, A., Amiranda, C., Anastasi, D., & Angelillo, I. F. 2010. Microbiological quality of drinking water from dispensers in Italy. BMC Microbiology, 10(1), p. 19. doi:10.1186/1471-2180-10-19

Marković, G., Veljović, P., & Simović, S. 1998. Biološki monitoring većih akumulacija u slivu reke Zapadne Morave. Kruševac: Konferencija "Vodni resursi sliva Velike Morave i njihovo korišćenje". pp. 356-360.

Muylaert, K., van der Gucht, K., Vloemans, N., de Meester, L., Gillis, M., & Vyverman, W. 2002. Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. Applied and Environmental Microbiology, 68(10), pp. 4740-4750.

Paerl, H. W., Dyble, J., & Moisander, P. H. 2003. Microbial indicators of aquatic ecosystem change: current applications to eutrophication studies. FEMS Microbiology Ecology, 46, pp. 233-246.

Poma, H. R., Gutiérrez, D. C., Garcé, B., Gonzo, E. E., & Rajal, V. B. 2012. Towards a rational strategy for monitoring of microbiological quality of ambient waters. Science of Total Environment, 433, pp. 98-109.

Proctor, C. R., & Hammes, F. 2015. Drinking water microbiology - from measurement to management. Current Opinion in Biotechnology, 33, pp. 87-94.

Sargaonkar, A., & Deshpande, V. 2003. Development of an overall ondex of pollution for surface water based on a general classification scheme in Indian context. Environmental Monitoring and Assessment, 89(1), pp. 43-67.

Službeni glasnik RS 2011. Pravilnik o parametrima ekološkog i hemijskog statusa površinskih voda i parametrima hemijskog i kvantitativnog statusa podzemnih voda. Službeni glasnik RS, br. 74.

Službeni list SRJ 1998. Pravilnik o higijenskoj ispravnosti vode za piće. Službeni list SRJ, br. 42.

Szeląg-Wasielewska, E., & Stachnik, W. 2010. Auto and heterotrophic picoplankton in a lowland river (Warta river, Poland). Oceanological and Hydrobiological Studies, 39(1), pp. 137-146.

Venkatesharaju, K., Ravikumar, P., Somashekar, R. K., & Prakash, K. L. 2010. Physico-chemical and bacteriological investigation on the river Cauvery of kollegal strech in Karnataka. Kathmandu University Journal of Science, Engineering and Technology, 6(1), pp. 50-59.

-WHO. 2002. Reducing risks, promoting healthy life. World Health Report 2002.Geneva, Switzerland: World Health Organization.

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Original Scientific Paper

MEDICINAL FLORA OF THE VIDLIČ MOUNTAIN IN SERBIA

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ABSTRACT

A study of medicinal flora of the Vidlič mountain situated in southeastern Serbia was conducted. The presence of 264 plant species, that are considered official or used in folk medicine is recorded. An overview of medicinal plants is given in a systematic order. For each herb species, main medicinal substances which enter in its chemical composition are listed. An overview of plant parts which are curative and which families contain the highest numbers of medicinal species, are presented. A special review of species that contain toxic substances, as well as species that have become so rare in their natural habitats by irrational exploitation in nature that they have been declared protected and strictly protected taxa in the Republic of Serbia is given.

Keywords: Mt. Vidlič, Medicinal plants, Active ingredients, Protected species.

INTRODUCTION

Vidlič mountain, which is situated in the Pirot's county, according with its position, exerts characteristics of the central Balkan area (Ćirić, 1971).

Within our homeland, Vidlić is peripheral mountain, crossed by Serbian-Bulgarian border. Its long and characteristic ridge, beginning above Pirot city, situated in NW-SE direction till the border with Bulgaria, but there doesn't end, continuing to the Bulgarian territory, all along Sofia (Martinović, 1979-1980).

The mountain Vidlič is a continuous belt of about 13 km long and of different widths from 250 to 1500 m (Marinkov, 1999). Its total length is 40 km according to Anđelković & Nikolić (1958).

The westernmost point of the study area on the river Temštica has coordinates: 43°12'2'' north geographical latitude and 22°33'4'' east geographical longitude, and the easternmost point close to the border with Bulgaria at village Vlkovija has coordinates: 43°5'5'' north geographical latitude and 22°55'1'' east geographical longitude (according to Greenwich).

Vidlič Mountain is a branch of Stara planina Mountains according to one group of authors (Mišić et al., 1978). Second group of authors (Vidanović, 1955; Martinović, 1979-1980) things that Vidlič has a separate orographic and tectonic whole, which is completely different from Stara Planina Mountains and Visok.

In geological term the mountain is almost completely built of limestone formations, with the best represented cretaceous and triassic layers.

The prevailing soil types are rendzina and calcomelanosol, which achieve different evolutionary forming phases (Antonović & Mrvić, 2008).

The climate is temperate continental, with hot summers and prominent drought periods, while the upper part of the mountain has features of a mountain climate (Ćirić, 1989).

Deciduous forest of hungarian and turkish oak (*Quercetum frainetto-cerris*) at low altitudes 500-600m, as one of the most widespreaded, considered to be climax in the region (Mišić et al., 1978). Different degradation stages of this community and its derivates with oriental hornbeam (*Carpinus orientalis*) are well represented, in a larger extent on the mountain. Transitional belt between the oak (*Quercetum montanum*) and beech forests was noticed at upper altitudes 700-900m. The upper region of the mountain is covered by moesian beech forests (*Fagetum montanum moesiacum*), considered to be climate-regional community, over 1000m above sea level, to the highest altitude of the mountain, which is 1413 m (Marković et al., 2018). After the clear-cutting, and period of subsequent grazing, many types of meadows, xeric pastures and schrub-like vegetation of the rocky slopes were formed over the vast area of eroded slopes.

THEORETICAL PART

The medicinal flora of the Vidlič Mountain has not been recorded and systematized yet.

The area of Pirot county was visited by our famous botanist Josif Pančić at the end of the XIX century. The first data of Vidlič flora originated since then and refer on the species which Pančić mentioned for Vidlič in the papers: "Flora Kneževine Srbije" and "Dodatak Flori Kneževine Srbije" (Marković, 2013). More intense study of plants of this region was performed by Lujo Adamović (Marković, 2013), in whose papers Vidlič is often mentioned.

Ranđelović et al. (1991) were studied the medicinal plants of Pirot caunty, and they were recorded the part of the medicinal plant from the Vidlič Mountain. In the monography „Lekovite biljke SR Srbije" Sarić, ed. (1989) mentioned which plant species were found on Vidlič Mt.

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Aromatic flora of the Vidlilč Mountain was especially systematized, where it was noticed 60 species with essential oil content (Marković, 2006; Marković et al,, 2009). During the research of the resources of medicinal plants in the Pirot county, Marković et al. (2010a, 2010b) were noticed data of medicinal plants which are recorded on the Vidlič Mountain.

EXPERIMENTAL

Materials and methods

Field studies of medicinal flora of the Vidlič Mountain were carried out in the period 2002-2014. The list of medicinal flora was made according to the systematic order of the species according to Josifović (1970-1977). Only native representatives of the flora were included in the list of medicinal plants (Table1). Some additional allochthonous (introduced, invasive and grown in the gardens) representatives of the flora were not included.

A detailed investigation of the medicinal flora included the area of mountain Vidlič in southeastern Serbia, south of the river Visočica and north of the river Nišava, east of the river Temštica and west of the border with Bulgaria. The result of field research was the plant material that was collected, herbarized, labeled and deposited in the "Herbarium Moesiacum Niš", Faculty of Science and Mathematics, University of Niš (HMN).

Identification of the collected plant material was performed according to the "Flora of SR Serbia" (Josifović, 1970-1977). The nomenclature is adjusted according to the "Flora Europaea" (Tutin, 1964-1980; 1993). The belonging to the group of medicinal plants was determined according to Sarić, ed. (1989).

Table 2. The list of medicinal plants of the Vidlič Mountain.

Family	Taxon	Compounds	Herbal drug
Equisetaceae	*Equisetum arvense* L.	mineral substances, flavonoids	Herba
Aspidiaceae	*Dryopteris filix-mas* (L.) Schott **!**	floroglucyne derivatives, tannins, fatty oil	Rhizome
Polypodiaceae	*Polypodium vulgare* L.	tannins, resin, fatty oil, saponins, sugars	Rhizome
Pinaceae	*Abies alba* Miller	essential oil	acicula, strobuli, cortex
	Pinus nigra Arnold	turpentine	Turio
Cupressaceae	*Juniperus communis* L. *	essential oil, tannins, flavonoids	fructus, lignum
Aristolochiaceae	*Asarum europaeum* L. ***!**	essential oil, tannins, glycosides, alkaloids	herba cum radicibus
Ranunculaceae	*Helleborus odorus* Waldst. & Kit. **!**	cardiotonic heterosides, saponins	rhizoma et radix
	Nigella arvensis L.	essential oil, saponins, pfatty oil	Semen
	Nigella damascena L.	essential oil, saponins, fatty oil	Semen
	Aquilegia vulgaris L.	cyanogenetic heterosides, vitamin C	semen, folium, flos
	Hepatica nobilis Schreber ***!**	tannins, saponins	herba, folium
	Pulsatilla montana (Hoppe) Reichenb. subsp. *bulgarica* Rummelsp. ****!**	saponins, tannins, resin	herba recens
	Clematis vitalba L.	glycosides, saponins	Herba
	Clematis recta L.	glycosides, saponins	Herba
	Ranunculus ficaria L.	mucus, essential oil, saponins	Tuber
	Ranunculus repens L. **!**	anemone-camphor, saponins	Herba
	Ranunculus acris L. **!**	anemone-camphor, saponins	Herba
	Ranunculus bulbosus L. **!**	anemone-camphor, saponins	Herba
	Adonis vernalis L. ****!**	cardiotonic glycosides	Herba
Berberidaceae	*Berberis vulgaris* L.	alkaloids, tannins, vitamin C	cortex, radix, folium, fructus
Papaveraceae	*Papaver dubium* L. **!**	alkaloids	Flos
	Papaver rhoeas L. **!**	alkaloids, anthocyanins, mucus	Flos
	Chelidonium majus L.	alkaloids, flavonoids, saponins	radix, herba
	Corydalis cava (L.) Schweigger & Körte subsp. *marschalliana* (Willd.) Hayek **!**	alkaloids	Tuber
	Corydalis solida (L.) Clairv. **!**	alkaloids	Tuber
	Fumaria officinalis L. **!**	alkaloids	Herba
Ulmaceae	*Ulmus glabra* Hudson	tannins, mucus, bitter substance	Cortex

Family	Taxon	Compounds	Herbal drug
Moraceae	*Morus alba* L.	vitamins, pectins, rubbers	folium, fructus recens
Cannabaceae	*Humulus lupulus* L.	resin, tannins, essential oil, flavonoids	Strobuli
Urticaceae	*Urtica dioica* L.	vitamins, tannins	folium, radix, semen
Fagaceae	*Fagus moesiaca* (K.Maly) Czech.	phenolic derivatives, hydrocarbons, organic acids, resin	cortex
	Quercus cerris L.	tannins, catechin, flavonoids	cortex
	Quercus petraea (Mattuschka) Liebl.	tannins, catechin, flavonoids	cortex
	Quercus frainetto Ten.	tannins, catechin, flavonoids	cortex
	Quercus pubescens Willd.	tannins, catechin, flavonoids	cortex
Betulaceae	*Betula pendula* Roth. *	flavonoids, tannins, resin, saponins, essential oil	gemmae, folium
Corylaceae	*Corylus colurna* L.*	tannins, flavonoids, fatty oil	cortex, folium, semen
	Corylus avelana L.	tannins, flavonoids, fatty oil	cortex, folium, semen
Juglandaceae	*Juglans regia* L.	tannins, nafttchynone derivatives, flavonoids, vitamin C, essential oil	folium, fructus
Caryophyllaceae	*Herniaria glabra* L. *	saponins, flavonoids, coumarins, tannins	herba
	Herniaria hirsuta L. *	saponins, flavonoids, coumarins, tannins	herba
	Saponaria officinalis L.	saponins, sugars	radix, herba
Polygonaceae	*Polygonum aviculare* L.	flavonoids, tannins, mucus	herba
	Persicaria hydropiper (L.) Delarbre	flavonoids, tannins, organic acids	herba
	Rumex crispus L.	calcium oxalate	radix, folium
	Rumex sanguineus L.	calcium oxalate	radix, folium,
	Rumex acetosa L.	calcium oxalate	radix, folium
Paeoniaceae	*Paeonia tenuifolia* L. **!	alkaloids, tannins	flos, radix
	Paeonia peregrina Miller **!	alkaloids, tannins	flos, radix
Hypericaceae	*Hypericum hirsutum* L.	flavonoids, tannins, essential oil	herba
	Hypericum perforatum L. *	flavonoids, tannins, essential oil	herba
	Hypericum barbatum Jacq. *	flavonoids, tannins, essential oil	herba
Vioaceae	*Viola odorata* L. *	saponins, bitter substance, mucus	folium, flos, radix
	Viola tricolor L.	mucus, tannins, flavonoids	herba
Brassicaceae	*Sisymbrium officinale* (L.) Scop.	sulfuric glycosides, vitamin C	herba recens, folium recens
	Nasturtium officinale R. Br.	sulfuric heterosides, vitamins	herba recens, folium recens
	Cardamine pratensis L.	sulfuricglycosides, vitamin C, carotene	folium
	Cardamine impatiens L.	sulfuric glycosides, vitamin C, carotene	stipes, folium
	Cardamine flexuosa With.	sulfuricglycosides, vitamin C, carotene	stipes, folium
	Capsella bursa-pastoris (L.) Medicus	flavonoids, tannins	herba
	Sinapis arvensis L.	fatty oil, sinapin	semen
Resedaceae	*Reseda luteola* L.	flavonoids, sulfuric glycosides	herba
	Reseda lutea L.	flavonoids, sulfuric glycosides	herba
Salicaceae	*Populus tremula* L.	flavonoids, salicin	cortex, gemmae
	Populus nigra L.	resin, flavonoids	cortex, gemmae
	Salix alba L.	glycosides, flavonoids, tannins	cortex
	Salix cinerea L.	glycosides, flavonoids, tannins	cortex
	Salix caprea L.	glycosides, flavonoids, tannins	cortex

Family	Taxon	Compounds	Herbal drug
	Salix purpurea L.	glycosides, flavonoids, tannins	cortex
Primulaceae	*Lysimachia nummularia* L.	bitter substance, tannins, saponins	herba
	Lysimachia punctata L.	tannins, saponins, flavonoids	herba
	Lysimachia vulgaris L.	tannins, saponins, flavonoids	herba
	Anagallis arvensis L. !	saponins, tannins	herba
	Primula veris L. *	saponins, glycosides, starch, tannins	flos, rhizoma cum radicibus
Tiliaceae	*Tilia tomentosa* Moench. *	mucus, tannins, mannitol	flos cum bracteis
Malvaceae	*Malva sylvestris* L.	mucus, tannins, anthocyanins	folium, flos
	Malva neglecta Wallr.	mucus, tannins, anthocyanins	folium, flos
Euphorbiaceae	*Euphorbia helioscopia* L. !	bitter - hot substances	semen, succus
	Euphorbia cyparissias L. !	bitter - hot substances	semen, succus
	Euphorbia amygdaloides L. !	bitter - hot substances	semen
Thymeleaceae	*Daphne mezereum* L. !	resin, flavonoids, glycosides, coumarins	cortex
Rosaceae	*Filipendula vulgaris* Moench	essential oil, tannins,	herba, folium, rhizoma
	Filipendula ulmaria (L.) Maxim.	essential oil, tannins, glycosides	folium
	Rubus idaeus L.	tannins, organic acids, flavonoids	folium, fructus recens
	Rubus caesius L.	tannins, organic acids, flavonoids	folium
	Rosa canina L. *	vitamins, pectins, tannins, glycosides, organic acids	fructus
	Agrimonia eupatoria Ledeb.	tannins, bitter substance	herba
	Sanguisorba officinalis L.	tannins, starch, saponins	rhizoma cum radicibus
	Sanguisorba minor Scop.	tannins, starch, saponins, sterols	rhizoma cum radicibus, herba
	Geum urbanum L.	tannins, flavonoids	rhizoma cum radicibus
	Potentilla recta L.	tannins, resin	rhizoma
	Potentilla erecta (L.) Räuschel *	tannins, saponins, resin, starch, flobaphens	rhizoma
	Potentilla reptans L.	tannins, resin	rhizoma
	Fragaria vesca L. *	tannins, flavonoids	folium, fructus
	Alchemilla sp. div. *	tannins, bitter substance	herba, folium
	Cydonia oblonga Miller	organic acids, vitamin C, tannins, mucu	folium, fructus, semen
	Malus sylvestris Miller	vitamins, iodine	fructus, semen
	Sorbus aucuparia L.	tannins, vitamins, organic acids	fructus
	Sorbus torminalis (L.) Crantz	tannins, vitamins, organic acids	fructus
	Sorbus aria (L.) Crantz	tannins, vitamins, rubbers	fructus
	Crataegus monogyna Jacq. *	flavonoids	flos, folium, fructus
	Crataegus laevigata (Poiret) DC. *	flavonoids	flos, folium, fructus
	Prunus spinosa L.	flavonoids, glycosides; tannins, anthocyanins, pectins	flos, fructus
	Prunus avium L.	glycosides	fructus, petiolus
Grossulariaceae	*Ribes uva-crispa* L. *	vitamins, carotenes	folium, fructus
Crassulaceae	*Hylotelephium telephium* (L.) Ohba	alkaloids, flavonoids, tannins, organic acids	herba recens
	Sedum acre L.	alkaloids, flavonoids, tannins, organic acids	herba recens
Fabaceae	*Ononis spinosa* L.	glycosides, triperpens, essential oil	radix

Family	Taxon	Compounds	Herbal drug
	Medicago lupulina L.	vitamins	folium
	Medicago falcata L.	vitamins	folium
	Melilotus officinalis (L.) Pallas	coumarins, flavonoids, tannins	herba
	Anthyllis vulneraria L. *	tannins, saponins, flavonoids	flos, herba
	Genista tinctoria L.	alkaloids, flavonoids, essential oil	herba
	Coronilla varia L. **!**	heterosides	herba
	Coronilla scorpioides (L.) Koch **!**	heterosides	herba
Lythraceae	*Lythrum salicaria* L.	glycosides, tannins, flavonoids, mucus	herba
Oenotheraceae	*Epilobium parviflorum* Schreber *	tannins, flavonoids, mucus	herba, folium
	Epilobium angustifolium L.	tannins, flavonoids, mucus	herba, folium
Anacardiaceae	*Cotinus coggygria* Scop. **!**	tannins, flavonoids	folium
Rutaceae	*Dictamnus albus* L.	essential oil, alkaloids, bitter substance	herba, radix
Aceraceae	*Acer tataricum* L.	tannins	cortex, folium
	Acer platanoides L.	glycosides	succus
Zygophyllaceae	*Tribulus terrestris* L.	saponins, flavonoids	herba, fructus
Oxalidaceae	*Oxalis acetosella* L. **!**	flavone heterosides, oxalyc acid	folium
Geraniaceae	*Geranium macrorrhizum* L. *	essential oil, tannins, flavonoids, pectins, rubbers	rhizoma
	Geranium robertianum L. *	tannins, flavonoids, resin, organic acids	herba
	Erodium cicutarium (L.) L'Hér.	tannins, flavonoids	herba
Polygalaceae	*Polygala amara* L.	saponins, bitter substance	herba
	Polygala vulgaris L.	saponins, bitter substance	herba
Cornaceae	*Cornus mas* L. *	tannins, pectins, organic acids	cortex, folium, fructus
	Cornus sanguinea L.	fatty oil	semen
Araliaceae	*Hedera helix* L. **!**	saponins	folium, stipes, cortex, fructus
Apiaceae	*Sanicula europaea* L.	bitter substance, tannins, saponins	radix, herba
	Eryngium campestre L.	saponins, tannins, essential oil	radix
	Carum carvi L.	essential oil, fatty oil, proteins, carbohydrates	fructus
	Pimpinella saxifraga L.	essential oil, coumarins, saponins, tannins	radix
	Aegopodium podagraria L.	essential oil, vitamin C	herba
	Angelica sylvestris L.	essential oil, resin, bitter substance	radix, fructus
	Peucedanum longifolium Waldst. & Kit.	bitter substance, essential oil	herba, radix
	Pastinaca sativa L. ssp. *urens* (Req. ex Godron) Čelak.	essential oil, fatty oil, coumarins, flavonoids	fructus, radix
	Heracleum sphondylium L.	essential oil, arginine	radix, folium, flos
	Daucus carota L.	carotenes, vitamins, pectins; essential oil, fatty oil	radix, fructus
	Conium maculatum L. **!**	alkaloids	folium, herba, fructus
Celastraceae	*Evonymus europaeus* L. **!**	alkaloids	cortex, stipes, folium, fructus
Rhamnaceae	*Rhamnus cathartica* L.	anthraquinones, flavonoids, pectins, glycosides, mucus	fructus
Apocynaceae	*Vinca minor* L.	alkaloids, flavonoids	folium, herba
	Vinca herbacea Waldst. & Kit. **	alkaloids, flavonoids	folium, herba
Asclepiadaceae	*Vincetoxicum hirundinaria* Medicus **!**	toxic compounds (vincetoxin), resin	rhizoma
Gentianaceae	*Centaurium erythraea* Rafin. *	glycosides, flavonoids	herba
	Gentiana cruciata L. *	bitter substance, tannins, glycosides	herba

Family	Taxon	Compounds	Herbal drug
Oleaceae	*Fraxinus ornus* L.	sugars (mannitol), mineral substances, resin	succus (manna)
	Fraxinus excelsior L.	tannins, flavonoids, coumarins, resin	cortex, folium, semen
	Ligustrum vulgare L. **!**	tannins, essential oil	cortex, folium, fructus
Menyanthaceae	*Menyanthes trifoliata* L.****	heterosides, tannins	folium
Rubiaceae	*Galium odoratum* (L.) Scop. *	coumarin heterosides, bitter substance, tannins	herba
	Galium verum L.	glycosides, tannins, saponins	herba
Caprifoliaceae	*Viburnum opulus* L.	saponins, resin, tannins, glycosides	cortex, fructus
	Sambucus ebulus L.	cyanogenetic heterosides, bitter substance	radix, folium, fructus
	Sambucus nigra L.	mucus, flavonoids, essential oil, tannins	flos, fructus
Valerianaceae	*Valeriana officinalis* L.	essential oil, tannins, starch	rhizoma cum radicibus
Convolvulaceae	*Cuscuta europaea* L.	not studied	herba
	Cuscuta epithimum (L.) L.	not studied	herba
Boraginaceae	*Cynoglossum officinale* L.	alcaloids, tannins	herba, radix
	Symphytum officinale L. *	mucus, tannins, starch	radix
	Anchusa officinalis L.	alkaloids, tannins, mucus	herba
	Pulmonaria officinalis L. *	mucus, mineral substances, tannins, flavonoids	herba
Solanaceae	*Atropa bella-donna* L. **!**	alkaloids	radix, folium
	Hyoscyamus niger L. **!**	alkaloids	folium, semen
	Solanum dulcamara L. **!**	alkaloids, glycosides, tannins	stipes
	Solanum nigrum L. **!**	alkaloids, tannins, saponins	herba, fructus
	Datura stramonium L. **!**	alkaloids	folium, semen
Scrophulariaceae	*Verbascum phlomoides* L.	flavonoids, saponins, mucus	flos
	Verbascum phoeniceum L.	flavonoids, saponins	flos
	Linaria vulgaris Miller **!**	alkaloids, glycosides	herba
	Scrophularia nodosa L. **!**	glycosides, tannins	herba
	Veronica officinalis L. *	flavonoids, glycosides, tannins, organic acids	herba
	Veronica beccabunga L.	flavonoids, glycosides	herba recens
	Digitalis ferruginea L. **!**	cardiotonic glycosides, saponins, flavonoids	folium
	Digitalis lanata Ehrh. **!**	cardiotonic glycosides, saponins, flavonoids	folium
	Euphrasia rostkoviana Hayne *	glycosides, tannins, resin	herba
	Euphrasia stricta D. Wolff ex J. F. Lehm. *	glycosides, tannins, resin	herba
Plantaginaceae	*Plantago major* L.	glycosides, tannins, mucus	folium
	Plantago media L.	glycosides, tannins, mucus	folium
	Plantago lanceolata L.	glycosides, tannins, mucus, flavonoids	folium
Verbenaceae	*Verbena officinalis* L.	glycosides, essential oil, mucus	herba
Lamiaceae	*Ajuga reptans* L.	tannins, phenolic acids	herba
	Teucrium chamaedrys L. *	essential oil, tannins, flavonoids	herba
	Teucrium montanum L. *	bitter substance, tannins, mineral substances, essential oil	herba
	Teucrium polium L.	essential oil, tannins	herba
	Marrubium vulgare L. *	lactone marubin, essential oil, tannins	herba
	Nepeta cataria L.	essential oil, tannins, saponins, glycosides	herba

Family	Taxon	Compounds	Herbal drug
	Nepeta nuda L.	essential oil, tannins, saponins, glycosides	herba
	Glechoma hederacea L.	tannins, bitter substance, essential oil	herba
	Glechoma hirsuta Waldst. & Kit.	bitter substance, tannins, essential oil	herba
	Prunella vulgaris L.	tannins, flavonoids, saponins	herba
	Melittis melissophyllum L.	essential oil, coumarins, flavonoids	herba
	Galeopsis pubescens Besser	bitter substance, tannins, glycosides	herba
	Galeopsis speciosa Miller	tannins, saponins	herba
	Lamium purpurerum L.	saponins, mucus, flavonoids, essential oil	herba
	Leonurus cardiaca L. *	glycosides, alkaloids, tannins, saponins	herba
	Ballota nigra L.	bitter substance, tannins, essential oil	herba
	Stachys officinalis (L.) Trevisan	tannins, glycosides, saponins	herba
	Stachys sylvatica L.	tannins, saponins	herba
	Stachys recta L.	tannins, saponins	herba
	Salvia glutinosa L.	essential oil, bitter substance	folium
	Salvia sclarea L.	essential oil, bitter substance	folium
	Salvia pratensis L.	essential oil, bitter substance	folium
	Salvia nemorosa L.	essential oil, bitter substance	folium
	Melissa officinalis L.	essential oil, flavonoids	folium
	Satureja kitaibelii Wierzb. *	essential oil	herba
	Calamintha officinalis Moench	tannins, bitter substance, essential oil	herba
	Calamintha nepeta (L.) Savi	essential oil	herba
	Hyssopus officinalis L.	essential oil, flavonoids, tannins	herba
	Origanum vulgare L. *	essential oil, tannins	herba
	Thymus sp. div.	essential oil, tannins, bitter substance, flavonoids	herba
	Lycopus europaeus L.	bitter substance, tannins	herba
	Mentha piperita L.	essential oil, tannins, flavonoids	folium
	Mentha pulegium L.	essential oil, tannins, bitter substance	herba
Campanulaceae	*Campanula glomerata* L.	not studied	herba
	Campanula rapunculus L.	inulin	herba, radix
	Campanula trachelium L.	not studied	herba
Asteraceae	*Eupatorium cannabinum* L.	essential oil, resin, tannins	radix, herba
	Solidago virgaurea L. * **!**	tannins, flavonoids, saponins, essential oil	herba
	Bellis perennis L.	bitter substance, tannins, saponins	herba, flos, folium
	Telekia speciosa (Schreber) Baumg.	essential oil, bitter compounds, inulin	radix
	Achillea clypeolata Sibth. & Sm. *	tannins	folium, flos
	Achillea crithmifolia Waldst. & Kit.	bitter substance, essential oil	herba, flos, folium
	Achillea millefolium L. *	bitter substance, essential oil	herba, flos, folium
	Matricaria chamomilla L.	essential oil, flavonoids, coumarins, mucus	flos
	Tanacetum vulgare L.	essential oil, flavonoids, tannins, bitter compounds	flos, herba
	Artemisia vulgaris L.	essential oil, bitter substance, tannins	herba
	Artemisia pontica L. *	essential oil, bitter substance	herba
	Artemisia alba Turra	essential oil, flavonoids, bitter substance	herba
	Tussilago farfara L.	mucus, inulin, tannins, flavonoids	flos, folium

Family	Taxon	Compounds	Herbal drug
	Petasites hybridus (L.) P. Gaertn., B. Mey. & Scherb. *	essential oil, flavonoids, mucus, tannins	radix, folium
	Carlina acaulis L. *	inulin, essential oil, tannins, resin	radix
	Arctium tomentosum Miller	inulin, mucus, essential oil, fatty oil, tannins	radix
	Arctium lappa L. *	inulin, mucus, essential oil, fatty oil, tannins	radix
	Centaurea cyanus L.	anthocyanins, glycosides, tannins	flos
	Cichorium intybus L.	inulin, glycosides, tannins, pectins	herba, radix
	Taraxacum officinale Weber	glycosides, resin, mucus, inulin	radix, herba
	Hieracium pilosella L.	tannins, flavonoids, coumarins, resin, mucus	herba cum radicibus
Liliaceae	*Veratrum album* L.	alkaloids	rhizoma
	Veratrum nigrum L. *	alkaloids	rhizoma
	Colchicum autumnale L. * **!**	alkaloids	semen, tuber
	Lilium martagon L. *	mucus, essential oil	folium, flos, bulbus
	Allium ursinum L. *	sulfuric essential oil, vitamins, mineral substances	herba recens, bulbus recens
	Convallaria majalis L. **!**	cardiotonic glycosides, saponins, flavonoids	herba, folium, flos
Amarylidaceae	*Galanthus nivalis* L. * **!**	choline, phytosterol	bulbus, folium, flos
Asparagaceae	*Asparagus officinalis* L.	asparagine, saponins	rhizoma, radix
Iridaceae	*Crocus tommasinianus* Herbert **!**	alkaloids, carotenoids	stigma
	Crocus adamii Gay **!**	alkaloids, carotenoids	stigma
Dioscoreaceae	*Tamus communis* L. **!**	alkaloids, starch, mucus, calcium oxalate, saponins	rhizoma recens
Orchidaceae	*Orchis morio* L. *	mucus	tuber
	Orchis simia Lam. *	mucus, starch	tuber
	Orchis militaris L. **	mucus, starch	tuber
	Orchis laxiflora Lam. **	mucus	tuber
	Anacamptis pyramidalis (L.) L. C. M. Richard *	mucus, starch, proteins	tuber
	Gymnadenia conopsea (L.) R.Br. *	mucus, starch	tuber
	Platanthera bifolia (L.) L. C. M. Richard *	mucus, starch	tuber
	Platanthera chlorantha (Custer) Reichenb. **	mucus, starch	tuber
Poaceae	*Elymus repens* (L.) Gould	carbohydrates, mucus, sugar alcohols, saponins	rhizoma
Araceae	*Arum maculatum* L. **!**	starch	rhizoma

Legend:

* - protected species (Prilog II) according to National legislative "Službeni glasnik Republike Srbije br. 5/2010, 47/2011, 32/2016, 98/2016 ; ** - strictly protected species (Prilog I) according to National legislative Službeni glasnik Republike Srbije br. 5/2010, 47/2011, 32/2016, 98/2016);

! – plant species with content of toxic substances.

NUMERICAL RESULTS

We are noted 264 plants on Vidlič Mt. that are considered medicinal according to Sarić ed. (1989) in our country (tab. 1.). The group of medicinal plants in Serbia includes 420 species (Sarić, 1989). Complete taxonomic analysis of the medicinal flora at Svrljiški Timok gorge in Eastern Serbia was done by Zlatković & Bogosavljević (2014). They found that the flora of medicinal plant at Svrljiški Timok gorge includes 190 taxa.

Families that contain the largest number of plants on Vidlič Mt. are: Lamiaceae (33), Rosaceae (23), Asteraceae (21), Ranunculaceae (13), Apiaceae (11), Scrophulariaceae (10). Similar results about number of medicinal taxa per

families were found Zlatković & Bogosavljević (2014) in the flora of Svrljiški Timok, where the families with the greatest number of medicinal representatives were Lamiaceae (32 taxa), Asteraceae (20 taxa) and Rosaceae (14 taxa). In the flora of Serbia families that contain the largest number of taxa are also Lamiaceae (16,2%), Asteraceae (12%) and Rosaceae (9%) (Sarić, 1989). The results about utilization of medicinal plants from different families were found by Zlatković et al. (2014) at Mt. Rtanj in Eastern Serbia. According to their results the plants from families Lamiaceae (22%), Rosaceae (20%) and Asteraceae (30%) are most comonly used in traditional medicine.

Aerial herbose part (herba) is most commonly used for medicinal purposes (112 plants). Leave (folium) is used from 70 plants, root (radix) from 33 plants, flower or inflorescence (flos) from 29 plants, fruit (fructus) from 28 plants. Review of plant parts that are medicinal with the number of taxa is given in the table 2.

The largest number of medicinal plants contains tannins as active medicinal substances (118 plants), followed by plants with content of flavonoids (80 plants), essential oils (67 plants), saponins (49 plants), glycosides (38 plants), mucus (37 plants), alkaloids (32 plants), bitter substances (32 plants) vitamins (19 plants) (Graph. 1).

Table 2. Review of herbal drugs with the number of taxa recorded on Vidlič Mt.

Medicinal parts of plants	**Number of plants**
herba - aerial herbose part	112
folium – leave	70
radix – root	33
flos – flower or inflorescence	29
fructus – fruit	28
cortex – bark	24
rhizoma – rhizome	20
semen – seed	17
tuber – tuber	11
succus – sap	4
stipes – stem	4
gemma, turio – bud	2
bulbus – bulb	2
stigma – stigma	2
petiolus – stalks	2

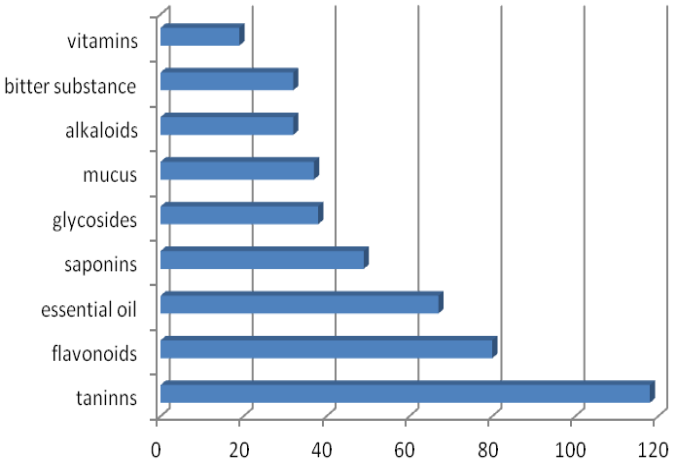

Figure 1. Review of dominant active substances with the number of medicinal plants of the Vidlič Mountain.

Particular attention was given to the plants with content of toxic substances, as well as rare and protected species.

42 plant species (15,91% of the total number of species) contain very toxic substances, so caution is advised in their use.

55 plant species (20,83%) are protected, and 9 species (3,41%) have become so rare with irrational exploatation, and they are strictly protected, with regulation named „Pravilnik o proglašenju i zaštiti strogo zaštićenih i zaštićenih divljih vrsta biljaka, životinja i gljiva“ (Službeni glasnik Republike Srbije br. 5/2010, 47/2011, 32/2016, 98/2016) Strictly protected species recorded on the Vidlič Mt. (*Pulsatilla montana* subsp. *bulgarica*, *Adonis vernalis*, *Paeonia tenuifolia*, *Paeonia peregrina*, *Vinca herbacea*, *Menyanthes trifoliata*, *Orchis militaris*, *Orchis laxiflora* and *Platanthera chlorantha*) are considered as threatended taxons of flora in Serbia, and almost exclusively, cannot be collected and used any purpose (Stevanović ed, 1999).

CONCLUSION

In comparison with several well done, published studies on medicinal flora in surrounding limestone areas of southeastern Serbia, medicinal flora on Vidlič Mt. is very rich and diverse, that includes 264 medicinal plant species. Family Lamiaceae is the richest with medicinal plants. Aerial herbose parts of plants in bloom are produced mostly. The largest number of medicinal plant species of the Vidlič Mt. as a core group of active ingredients containing tannins.

Toxic substances in higher concentrations are registred in 15,91% of analyzed species. Some representatives of the medicinal plants of Vidlič Mt. are strictly protected (3,41%), and 20,83% species are protected.

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REFERENCES

Anđelković, M., & Nikolić, P. 1958. Geološki sastav i tektonika jugozapadnih padina Stare planine. Beograd: SKANj. knj. CCCXVII.

Antonović, G., & Mrvić, V. 2008. Zemljišta sliva Nišave. Beograd, Srbija: Institut za zemljište.

Ćirić, J. 1971. Geografski položaj i granice Pirota i Pirotskog Regiona. Pirotski zbornik, 3, pp. 21-31.

Ćirić, J. 1989. Geografija gornjeg Ponišavlja i Lužnice. In P. Nikolić Ed., Pirotski zbornik.Pirot: Novinsko izdavačka ustanova \"Sloboda\". 16, pp. 9-23.

Josifović, M., Ed. 1970. Flora SR Srbije. Beograd: Srpska Akademija Nauka i Umetnosti. 1970-1986; I-X.

Marinkov, J. 1999. Zabrdje, prirodno-privredni potencijali. Dimitrograd: Narodna biblioteka.

Marković, M. 2006. Prirodni potencijali spontane aromatične lekovite flore planine Vidlič. Univerzitet u Kragujevcu - Prirodno matematički fakultet - Institut za biologiju i ekologiju. magistarska teza.

Marković, M., Pavlović-Muratspahić, D., Matović, M., Markovic, A., & Stankov-Jovanović, V. 2009. Aromatic Flora of the Vidlič Mountain. Biotechnology & Biotechnological Equipment, 23(2), pp. 1225-1229. doi:10.1080/13102818.2009.10817643

Marković, M., Matović, M., Pavlović, D., Zlatković, B., Marković, A., Jotić, B., & Stankov-Jovanović, V. 2010a. Resources of medicinal plants and herbs collector's calendar of Pirot County (Serbia). Biologica nyssana, 1(1-2), pp. 9-21.

Marković, M., Matović, M., Pavlović, D., Zlatković, B., Marković, A., Jotić, B., & Stankov-Jovanović, V. 2010b. Biljarski kalendar subregiona Pirot. In 10th Symposium on the Flora of Southeastern Serbia and Neighbouring regions, Vlasina, 17 to 20 june. Book of abstracts, 103.

Marković, M. 2013. Sukcesije biljnih zajednica na požarištima planine Vidlič, Phd thesis, Institut za biologiju i ekologiju, Prirodno matematički fakultet, Univerzitet u Kragujevcu, 20 june 2010, Book of abstracts, 103.

Marković, S. M., Nikolić, M. B., Zlatković, K. B., Nikolić, S. D., Rakonjac, B. Lj., Stankov-Jovanović, P. V., Djokić, M. M., Ratknić, B. M., Lučić, Ž. A. 2018. Short-term patterns in the post-fire diversity of limestone grasslands and rocky ground vegetation, Applied Ecology and Environmental Research 16(3), pp. 3271-3288. DOI: http://dx.doi.org/10.15666/aeer/1603_3271328

Martinović, Ž. 1979. Vrela i izvori u zabrdskom delu Vidliča - prilog poznavanju podzemnih voda istočne Srbije. Zbornik radova PMF Priština, 1979-1980, VI, pp. 283-300.

Mišić, V., Jovanović-Dunjić, R., Popović, M., Borisavljević, Lj., Antić, M., Danon, J., & Blaženčiž, Ž. 1978. Biljne zajednice i staništa Stare planine. Beograd: Srpska Akademija Nauka i Umetnosti.

Ranđelović, N., Sotirov, S., Stamenković, V., Rexhepi, F., Ranđelović, V., & Zlatković, B. 1991. Lekovito biqe subregiona Pirot. Zbornik radova Tehnološkog fakulteta u Leskovcu, 7/8, pp. 29-33.

Sarić, M. 1989. Lekovite biljke SR Srbije.Beograd: Srpska Akademija Nauka i Umetnosti - Odeljenje prirodno-matematičkih nauka. Posebna izdanja DXCVIII.

Službeni glasnik Republike Srbije 2010. Pravilnik o proglašenju i zaštiti strogo zaštićenih i zaštićenih divljih vrsta biljaka, životinja i gljiva. Službeni glasnik Republike Srbije, . br. 5/10, 47/11 i 32/16.

Stevanović, V., Ed., 1999. Crvena knjiga flore Srbije 1 – iščezli i krajnje ugroženi taksoni.Beograd: Ministarstvo za životnu sredinu Republike Srbije / Univerzitet u Beogradu - Biološki fakultet / Zavod za zaštitu prirode Republike Srbije.

Tutin, T.G., Heywood, V.H. Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M. Webb, D.A., Eds. 1964. Flora Europaea.London: Cambridge University Press. 1964-1980, I-V.

Tutin, T.G., Burges, N.A., Chater, O.A., Edmondson, J.R., Heywood, V.H., Moore, D.M., Valentine, D.H., Walters, S.M., Webb D.A. Eds. 1993. Flora Europaea. London: Cambrige University Press. 1 (2nd Edition).

Vidanović, G. 1955. Visok, privredno geografska ispitivanja.Beograd: Srpska akademija nauka / Geografski institut. Posebna izdanjač knj. 6.

Zlatković, B., Bogosavljević, S., Radivojević, A., Pavlović, M. 2014. Traditional use of the native medicinal plant resource of Mt. Rtanj (Eastern Serbia): ethnobotanical evaluation and comparation., Journal of Etnopharmacology 151(1), pp. 704-713. doi: 10.1016/j.jep.2013.11.037

Zlatković, B., Bogosavljević, S. 2014. Taxonomical and pharmacological valorization of medicinal flora in Timok gorge (Eastern Serbia), Facta Universitatis, Series: Medicine and Biology, 16(2), pp. 76-86.

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CHARACTERIZATION OF NEW SYNTHESIZED Fe_2O_3 NANOPARTICLES AND THEIR APPLICATION AS DETECTION SIGNAL AMPLIFIERS IN HERBICIDE BENTAZONE ELECTROANALYTICAL DETERMINATION

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ABSTRACT

The iron oxide nanoparticles (Fe_2O_3 NPs) were synthesized from two different iron salts by solid-state synthesis method. The synthesized powder of Fe_2O_3 NPs is soluble in water, and the colloidal dispersion was characterized by TEM, FTIR, UV-Vis spectroscopy and zeta potential measurements. Obtained NPs are spherical in shape with narrow particle size distribution and an average diameter of 3 nm. Further, the possible application of Fe_2O_3 NPs was proposed, due to significant electroanalytical signal amplification in the determination of herbicide bentazone in natural river water.

Keywords: Iron oxide, Nanoparticles, Solid-state, Herbicide bentazone, Electroanalysis.

INTRODUCTION

Nanotechnology is the field that has attracted attention over the past decade among many researches, because of multitude possibilities of nanoparticles (NPs) synthesis and/or their surface modification. Because of their small size, NPs have a large surface area-to-volume ratio, and due to that, they have enhanced surface chemistry. There are numerous studies and different methods of inorganic nanoparticles synthesis, both for metal and for metal oxides NPs, with different size, shape and surface coverage (Nguyen et al., 2006; Abdulwahab et al., 2014; Liao et al., 2010; Tang et al., 2006).

Iron NPs, zero-valent, and iron oxide, have gained considerable attention because of their unique physical, especially magnetic, and chemical properties (Sun et al., 2006; Ponder et al., 2001; Hasanzadeh et al., 2015), potential application in medicine (Ling et al., 2015; Zanganeh et al., 2016), and in drinking and/or wastewater treatment (Zhu et al., 2009; Rajput et al., 2016; Li et al., 2017). The iron and iron oxide NPs were previously synthesized using various methods, most often by co-precipitation, sol-gel, chemical reduction, etc. (Huang & Ehrman, 2007; Rani & Varma, 2015; Bashir et al., 2015; Sun et al., 2006; Lu et al., 2007). Further, surface modification of these NPs, in order to prevent their aggregation and/or agglomeration, and also with an aim to improve their surface properties, were reported (Sodipo & Aziz, 2016; Chen et al., 2017). Magnetic iron oxide NPs have been of considerable interest because of their use as sorbents of pesticides from water (Wu et al., 2011). There are two well-known phase of Fe_2O_3 NPs: maghemite, the γ phase obtained at 300 °C, and hematite, the α phase obtained at 600 °C (Karami, 2010).

In this work, we report the synthesis of iron(III) oxide NPs by simple solid-state chemical reaction method, at room temperature, and in the air. The solid-state chemical reaction method presents a mechanochemical process where the chemical reaction occurs during grinding solid precursors. Application of this method provides a high yield of NPs. For Fe_2O_3 NPs synthesis two different iron salts were used as precursors.

As NPs and nanomaterials were recognized as excellent carriers and optical or electrical signal tags in sensing of biomolecules (Lei & Ju, 2012), the main idea of this work, besides obtaining monodisperse colloid dispersion, was also investigation of possibility of improvement in electroanalytical determination of pesticides, as serious environmental pollutants, due to amplification effect of NPs. Hence, previously synthesized Fe_2O_3 NPs obtained from different salts were used to enhance voltammetric response in determination of herbicide bentazone, one of the most frequently used herbicides in Europe, recently studied on boron doped diamond electrode by our research group (Jevtić et al., 2018).

EXPERIMENTAL

Reagents and standard solutions

Iron(II)-sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$), iron(III)-chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), potassium chloride (KCl) and potassium hydroxide (KOH), from Aldrich, were used as received. Stock solution of bentazone, 3-Isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (in further text presents with abbreviation BZ), 1×10^{-3} M was prepared in methanol. This compound was the product of Sigma – Aldrich. Britton Robinson

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buffer (BR) was made from acetic, boric, and phosphoric acid (all 0.2 M) adjusted by sodium hydroxide to pH value 4. Water Purified Millipore Mili-Q with a resistivity of 18 MΩ was used for washing the reaction mixture.

Apparatus and measurements

Transmission electron microscopy (TEM) was used to estimate the shape and the average size of Fe_2O_3 NPs. TEM measurements were carried out using FEI Talos F200X at an operating voltage of 200 kV.

Absorption spectra of iron oxide colloidal dispersion were measured by Perkin Elmer Lambda 35 UV – Vis spectrophotometer using the quartz cuvette with 1cm path length.

FTIR spectra were measured by NICOLET I5 ATR spectrometer.

Zeta potential (ZP) measurements by laser Doppler electrophoresis (LDE) were performed using a Zeta-sizer Nano ZS with 633 nm He-Ne laser (Malvern Instruments, UK). Data were analyzed by the Zetasizer Software Version 6.20 (Malvern Instruments, UK).

Electrochemical measurements were performed at PalmSens 3 potentiostat/galvanostat/impedance analyzer with PSTrace software (PalmSens BV, Netherlands). Three electrode cell was consisted from CH Instruments reference Ag/AgCl electrode and counter Pt electrode, while boron-doped diamond electrode, BDDE, (Windsor Scientific Ltd, Slough, Berkshire, United Kingdom) was used as a working electrode for bentazone determination. DPV parameters were set as in our previous work (Jevtić et al., 2018). Differential pulse voltammetry (DPV) measurements were performed in solutions made as it follows: 2 ml of water from Serbian river Ibar was spiked with 1 ml of a colloidal suspension of synthesized Fe_2O_3 NPs (1.5 mg/10 ml) and proper volume of a standard solution of bentazone, and then fulfilled to 10 ml with BR buffer of pH 4.

RESULTS

Synthesis of iron oxide nanoparticles. Iron oxide NPs can be prepared by the solid-state method, via the chemical reaction between ferrous iron (Fe^{2+}) and/or ferric iron (Fe^{3+}) with the alkaline. In this work, we used two different iron salts, $FeSO_4 \cdot 7H_2O$ and $FeCl_3 \cdot 6H_2O$, in the molar ratio 1 : 2.5 (0.002 mol and 0.005mol). Iron salts were mixed in a mortar and ground for 30 min in air at room temperature; the yellow paste was obtained. The particle size and polydispersity of NPs can be tuned up by various factors (Ali et al., 2016), one of them is an ionic strength, so KCl is added in the reaction mixture to provide small NPs (Li et al., 2002). In yellow paste 0.02 mol of KOH was added to the mortar and grinded for the next 30 min. During grinding, yellow paste became dark brown to black and dry. The reaction mixture then was washed several times with purified water until SO_4^{2-} and Cl^- ions could not be detected. The advantage of this method is its simplicity, low cost, and possibility to be done in every chemistry lab with simple chemical reagents. The chemical reaction goes on by the following equations:

$$FeSO_4 \cdot 7H_2O + 2FeCl_3 \cdot 6H_2O + 8KOH \rightarrow Fe_3O_4 + 6KCl + K_2SO_4 + 17H_2O \quad (1)$$

$$4Fe_3O_4 + O_2 \rightarrow 6Fe_2O_3 \quad (2)$$

In further work NPs were used as a powder or as a colloidal dispersion.

TEM characterization. The morphology and size of Fe_2O_3 NPs were determined from the diluted colloidal dispersion by TEM measurements. TEM micrograph along with particle size distribution are given in Fig. 1.

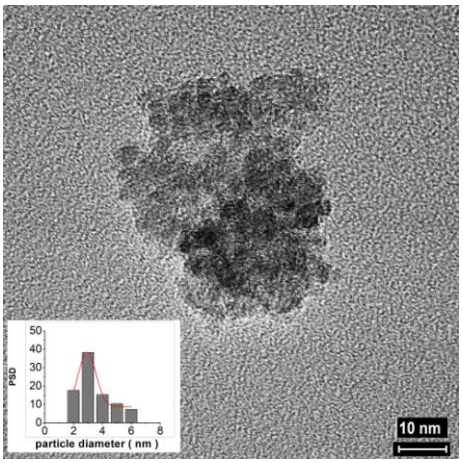

Figure 1. TEM micrograph of iron oxide NPs. Inset: Particle size distribution (PSD) of Fe_2O_3 NPs.

TEM measurements show that Fe_2O_3 NPs are spherical in shape with the average diameter of the particles of 2.9±0.07 nm. The mean particle size was obtained by fitting the TEM data with Gaussian distribution function (Fig. 1, inset).

Spectroscopic study. The absorption spectra of iron oxide colloidal dispersion contain broad absorption band around the area of 300 – 400 nm (Fig. 2.). Obtained data are in agreement with previously reported spectra for iron oxide colloids (Sayed and Polshettiwar, 2015).

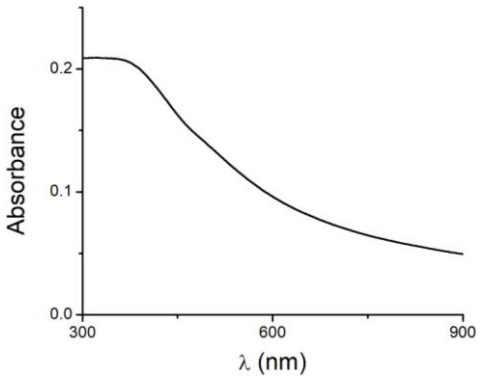

Figure 2. Absorption spectra of iron oxide colloidal dispersion.

Further, colloidal dispersion was left to dry in the air, at room temperature. Obtained powder of Fe_2O_3 NPs was than characterized by FTIR measurements. In FTIR spectrum, Fig. 3, the observed bands at 601 and 888 cm^{-1} are assigned to Fe – O stretching vibrations. The bands at 1640 and 3280 cm^{-1} are from bending and stretching vibrations of bonds in the molecule of water, which is present in a small amount in Fe_2O_3 NPs powder.

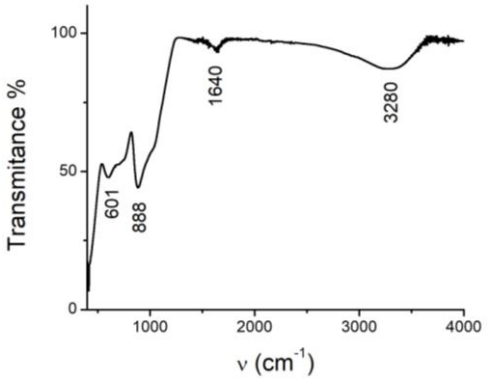

Figure 3. FTIR spectra of iron oxide NPs powder.

Zeta potential measurements. The charge of Fe_2O_3 NPs was determined by zeta potential measurements and shows that the surfaces of NPs are positively charged (+10 mV).

Electrochemistry of bentazone at BDDE in the presence of Fe_2O_3 NPs. Due to previously reported iron nanoparticles application as effective adsorbent material for wastewater and groundwater treatment of toxic contaminants (heavy metals, dyes etc.) (Chen et al., 2008; Saif et al., 2016), river water samples which contain different concentrations of herbicide bentazone were spiked with the synthesized and characterized Fe_2O_3 NPs. The content of BZ in river water samples were examined with previously reported electroanalytical method (Jevtić et al., 2018). Instead of the expected reduction of the voltammetric signal, it was found that the presence of Fe_2O_3 NPs in solution leads to a significant increase of DPV detection signal (Fig. 4). DPV voltammograms of river water samples without Fe_2O_3 NPs were also given in Fig.4 for comparison. As it can be seen, the peak potential for samples with Fe_2O_3 NPs was shifted to higher potentials probably due to interactions of molecules of BZ with the surface of Fe_2O_3 NPs. The main limitation of electroanalytical determination of bentazone represents instability of cation radical of BZ, its dimerization, and adsorption at the electrode (Manuela Garrido et al., 1998; Rahemi et al., 2013). The amplification of the detection signal may occur from the stabilization of cation radicals of bentazone due to interactions with Fe_2O_3 NPs. Nature of those interactions will be a subject of our further research.

Fig. 5 presents DPV voltammograms of BZ in the presence of Fe_2O_3 NPs in solution, recorded in the range of 5 to 90 μM of BZ, under the same experimental conditions and working DPV parameters as in our previous work (Jevtić et al., 2018).

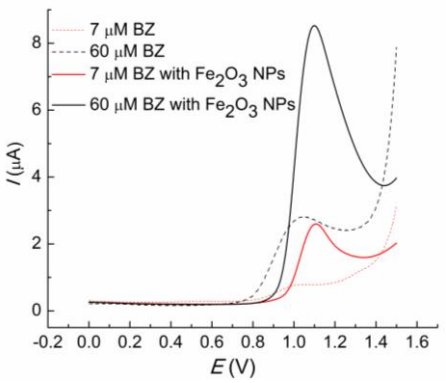

Figure 4. DPV voltammograms of different concentrations of BZ without and with Fe_2O_3 NPs in solution.

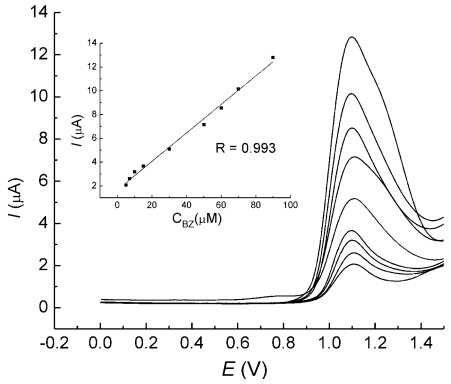

Figure 5. DPV voltammograms of different concentrations of BZ (5, 7, 10, 15, 30, 50, 60, 70, 90 μM) in BR buffer pH 4 at BDDE; Inset: Corresponding calibration curve.

The linear dependence expressed with the regression equation:

$$I\,(\mu A) = 1.660 + 0.119 \times C\,(\mu M); \quad R = 0.993$$

was presented in the inserted graph in the same figure. The amplification of DPV signal of BZ in presence of Fe_2O_3 NPs also resulted in lowering of limit of detection (LOD) to the value of 0.09 μM.

Hence, the next task could be finding optimal experimental conditions and working parameters to achieve more sensitive determination of bentazone in presence of Fe_2O_3 NPs in solution, or even to employ Fe_2O_3 NPs as a modifier on the surface of the electrode.

CONCLUSION

Iron(III) oxide NPs were sucssesfuly synthesized by solid-state chemical reaction method from two different iron salts. The morphology characteristics show fine structured NPs, spherical in shape with a narrow distribution and an average diameter of ~3 nm. In electrochemical studies, Fe_2O_3 NPs show the amplifying effect on the voltammetric determination of herbicide bentazone at BDDE, offering interesting and promising results in

the application of these NPs in sensing of toxic materials and environmental pollutants.

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REFERENCES

Abdulwahab, K. O., Malik, M. A., O'Brien, P., Timco, G. A., Tuna, F., Muryn, C. A., Winpenny, R. E. P., Pattrick, R. A. D., Coker, V. S. , & Arenholz, E. 2014. A One-Pot Synthesis of Monodispersed Iron Cobalt Oxide and Iron Manganese Oxide Nanoparticles from Bimetallic Pivalate Clusters. Chemistry of Materials, 26(2), pp. 999-1013. doi:10.1021/cm403047v

Ali, A., Zafar, H., Zia, M., ul Haq, I., Phull, A. R., Ali, J. S., & Hussain, A. 2016. Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. Nanotechnology, Science and Applications, Volume 9, pp. 49-67. doi:10.2147/nsa.s99986

Bashir, M., Riaz, S., & Naseem, S. 2015. Effect of pH on Ferromagnetic Iron Oxide Nanoparticles. Materials Today: Proceedings, 2(10), pp. 5664-5668. doi:10.1016/j.matpr.2015.11.106

Chen, K., He, J., Li, Y., Cai, X., Zhang, K., Liu, T., Hu, Y., Lin, D., Kong, L. & Liu, J. 2017. Removal of cadmium and lead ions from water by sulfonated magnetic nanoparticle adsorbents. Journal of Colloid and Interface Science, 494, pp. 307-316. doi:10.1016/j.jcis.2017.01.082

Chen, S. Y., Chen, W. H., & Shih, C. J. 2008. Heavy metal removal from wastewater using zero-valent iron nanoparticles. Water Science and Technology, 58(10), pp. 1947-1954. doi:10.2166/wst.2008.556

Garrido, M. E., Lima, C. J. L., Delerue-Matos, M. C. & Brett, M. O. A. 1998. Electrochemical oxidation of bentazon at a glassy carbon electrode: Application to the determination of a commercial herbicide. Talanta, 46, 1131-1135.

Hasanzadeh, M., Shadjou, N., & de la Guardia, M. 2015. Iron and iron-oxide magnetic nanoparticles as signal-amplification elements in electrochemical biosensing. TrAC Trends in Analytical Chemistry, 72, pp. 1-9. doi:10.1016/j.trac.2015.03.016

Huang, K., & Ehrman, S. H. 2007. Synthesis of Iron Nanoparticles via Chemical Reduction with Palladium Ion Seeds. Langmuir, 23(3), pp. 1419-1426. doi:10.1021/la0618364

Jevtić, S., Stefanović, A., Stanković, D. M., Pergal, M. V., Ivanović, A. T., Jokić, A., & Petković, B. B. 2018. Boron-doped diamond electrode — A prestigious unmodified carbon electrode for simple and fast determination of bentazone in river water samples. Diamond and Related Materials, 81, pp. 133-137. doi:10.1016/j.diamond.2017.12.009

Karami, H. 2010. Synthesis and Characterization of Iron Oxide Nanoparticles by Solid State Chemical Reaction Method. Journal of Cluster Science, 21(1), pp. 11-20. doi:10.1007/s10876-009-0278-x

Lei, J., & Ju, H. 2012. Signal amplification using functional nanomaterials for biosensing. Chemical Society Reviews, 41(6), p. 2122. doi:10.1039/c1cs15274b

Li, F., Xu, J., Yu, X., Chen, L., Zhu, J., Yang, Z., & Xin, X. 2002. One-step solid-state reaction synthesis and gas sensing property of tin oxide nanoparticles. Sensors and Actuators B: Chemical, 81(2-3), pp. 165-169. doi:10.1016/s0925-4005(01)00947-9

Li, S., Wang, W., Liang, F., & Zhang, W. 2017. Heavy metal removal using nanoscale zero-valent iron (nZVI): Theory and application. Journal of Hazardous Materials, 322, pp. 163-171. doi:10.1016/j.jhazmat.2016.01.032

Liao, Y., He, L., Huang, J., Zhang, J., Zhuang, L., Shen, H., & Su, C. 2010. Magnetite Nanoparticle-Supported Coordination Polymer Nanofibers: Synthesis and Catalytic Application in Suzuki-Miyaura Coupling. ACS Applied Materials & Interfaces, 2(8), pp. 2333-2338. doi:10.1021/am100354b

Ling, D., Lee, N., & Hyeon, T. 2015. Chemical Synthesis and Assembly of Uniformly Sized Iron Oxide Nanoparticles for Medical Applications. Accounts of Chemical Research, 48(5), pp. 1276-1285. doi:10.1021/acs.accounts.5b00038

Lu, J., Yang, S., Ng, K. M., Su, C., Yeh, C., Wu, Y., & Shieh, D. 2007. Solid-state synthesis of monocrystalline iron oxide nanoparticle based ferrofluid suitable for magnetic resonance imaging contrast application. Nanotechnology, 18(28), pp. 289001-289001. doi:10.1088/0957-4484/18/25/289001

Manuela, G. E., Costa, L. J. L., M. Delerue-Matos, C., & Maria, O. B. A. 1998. Electrochemical oxidation of bentazon at a glassy carbon electrodeApplication to the determination of a commercial herbicide. Talanta, 46(5), pp. 1131-1135. doi:10.1016/s0039-9140(97)00380-9

Nguyen, H. L., Howard, L. E. M., Stinton, G. W., Giblin, S. R., Tanner, B. K., Terry, I., Hughes, A. K., Ross, I. M., Serres, A. & Evans, J. S. O. 2006. Synthesis of Size-Controlled fcc and fct FePt Nanoparticles. Chemistry of Materials, 18(26), pp. 6414-6424. doi:10.1021/cm062127e

Ponder, S. M., Darab, J. G., Bucher, J., Caulder, D., Craig, I., Davis, L., Edelstein, N., Lukens, W., Nitsche, H., Rao, L., Shuh, D. K. & Mallouk, T. E. 2001. Surface Chemistry and Electrochemistry of Supported Zerovalent Iron Nanoparticles in the Remediation of Aqueous Metal Contaminants. Chemistry of Materials, 13(2), pp. 479-486. doi:10.1021/cm000288r

Rahemi, V., Garrido, J. M. P. J., Borges, F., Brett, C. M. A., & Garrido, E. M. P. J. 2013. Electrochemical Determination of the Herbicide Bentazone Using a Carbon Nanotube β-Cyclodextrin Modified Electrode. Electroanalysis, 25, pp. 2360-2366. doi:10.1002/elan.201300230

Rajput, S., Pittman, C. U., & Mohan, D. 2016. Magnetic magnetite (Fe 3 O 4) nanoparticle synthesis and applications for lead (Pb^{2+}) and chromium (Cr^{6+}) removal from water. Journal of Colloid and Interface Science, 468, pp. 334-346. doi:10.1016/j.jcis.2015.12.008

Rani, S., & Varma, G. D. 2015. Superparamagnetism and metamagnetic transition in Fe_3O_4 nanoparticles synthesized via co-precipitation method at different pH. Physica B: Condensed Matter, 472, pp. 66-77. doi:10.1016/j.physb.2015.05.016

Saif, S., Tahir, A., & Chen, Y. 2016. Green Synthesis of Iron Nanoparticles and Their Environmental Applications and

Implications. Nanomaterials, 6(11), p. 209. doi:10.3390/nano6110209

Sayed, F. N., & Polshettiwar, V. 2015. Facile and Sustainable Synthesis of Shaped Iron Oxide Nanoparticles: Effect of Iron Precursor Salts on the Shapes of Iron Oxides. Scientific Reports, 5(1). doi:10.1038/srep09733

Sodipo, B. K., & Aziz, A. A. 2016. Recent advances in synthesis and surface modification of superparamagnetic iron oxide nanoparticles with silica. Journal of Magnetism and Magnetic Materials, 416, pp. 275-291. doi:10.1016/j.jmmm.2016.05.019

Sun, Y., Li, X., Cao, J., Zhang, W., & Wang, H. P. 2006. Characterization of zero-valent iron nanoparticles. Advances in Colloid and Interface Science, 120(1-3), pp. 47-56. doi:10.1016/j.cis.2006.03.001

Tang, B., Wang, G., Zhuo, L., Ge, J., & Cui, L. 2006. Facile Route to α-FeOOH and α-Fe_2O_3 Nanorods and Magnetic Property of α-Fe_2O_3 Nanorods. ChemInform, 37(38). doi:10.1002/chin.200638198

Wu, Q., Zhao, G., Feng, C., Wang, C., & Wang, Z. 2011. Preparation of a graphene-based magnetic nanocomposite for the extraction of carbamate pesticides from environmental water samples. Journal of Chromatography A, 1218(44), pp. 7936-7942. doi:10.1016/j.chroma.2011.09.027

Zanganeh, S., Hutter, G., Spitler, R., Lenkov, O., Mahmoudi, M., Shaw, A., Pajarinen, J. S., Nejadnik, H., Goodman, S., Moseley, M., Coussens, L. M. & Daldrup-Link, H. E. 2016. Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues. Nature Nanotechnology, 11(11), pp. 986-994. doi:10.1038/nnano.2016.168

Zhu, H., Jia, Y., Wu, X., & Wang, H. 2009. Removal of arsenic from water by supported nano zero-valent iron on activated carbon. Journal of Hazardous Materials, 172(2-3), pp. 1591-1596. doi:10.1016/j.jhazmat.2009.08.031

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COMPRESSION SET AND DAMPING PROPERTIES OF OIL-EXTENDED ELASTOMERS FOR SEALANTS PRODUCTION

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ABSTRACT

Damping materials have been widely used in the vibration and noise control to reduce their harmful consequences in different areas such as their application in the drilling equipment, aerospace and naval vessels, transportation vehicles, bridges and high buildings. Because of severe environmental standards, the oil fabrication is developing synthetic environmentally friendly drilling fluids. For a vehicle, applications are produced: the outer and inner beltline seals and the glass run channel. The compression set is the important characteristic of elastomeric sealing materials as spontaneous stress release during application due to the internal pressure or external forces variations. The temperature- and frequency-insensitive damping are of significance for industrial uses. Elastomeric hybrid materials based on terpolymers as network precursor are very common for sealants fabrication. The focus of this work was to prepare oil-extended elastomeric hybrid materials based on ethylene-propylene-diene monomer rubber (EPDM), ground calcium carbonate and furnace nano carbon black. Cure characteristics were carried out on oscillating disc rheometer. The crosslinking of rubber compounds was performed in a molding press. The compression set was assessed using the standard procedure. The morphology of the cryogenically fractured surface of samples with different content of paraffin oil was characterized by scanning electron microscopy (SEM). Mechanical properties and hardness were analyzed before and after the aging of obtained samples. Damping properties of prepared materialswere determined using bending mode. The loss tangent, tan δ, was used as a measure of vibration energy dissipation.

Keywords: Composites, Rubber, Elastomers, Hybrid materials, Filler, Mechanical spectroscopy.

INTRODUCTION

Elastomers are cross-linked polymers with a low modulus of elasticity and the capability to reverse large deformations at their service temperatures. Selecting proper network precursor for elastomers in sealant production requires an assessment of the environment in which it is anticipated to operate. The choice of the basic precursors in combination with adequate fillers, plasticizers, and curing agents are the key criteria for fabrication of the advanced sealant materials. Dynamics or pressure within a system may compel the use of complex sealing profiles or composite seal assemblies, consistedof multiple materials for different operating environments. Elastomers based on ethylene-propylene-diene monomer rubber (EPDM) have good compatibility with fireproof hydraulic fluids, ketones, alkalis, and unsatisfactory compatibility with most oils, gasoline, kerosene, aromatic and aliphatic hydrocarbons halogenated solvents and acids. This material has outstanding weather, heat, steam, ozone resistance and is very often used for microcellular products for example in cold-room doors, in the face seals of industrial respirators and also in automotive paint spray environments.

EPDM based on 5-ethylidene-2-norbornene (ENB) (Figure 1) is very sensitive to oxidation.

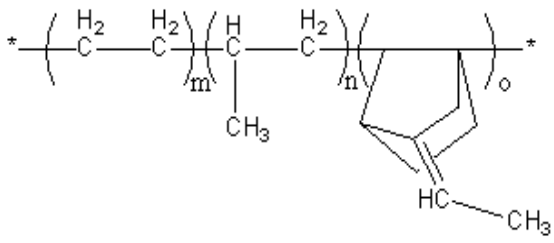

Figure 1. The structure of 5-ethylidene-2-norbornene.

The sensitivity of the ENB is very useful for subsequent crosslinking either by peroxide or sulfur. It is generally accepted that photo- or thermo-oxidation starts on the ENB part. (Jovanović et al., 2013). In the oil and gas industry, these materials are used in a wide range of applications (seals in shafts, pump pistons, flanges, rods, elastomeric belts for power transmission, and flexible hoses). Drilling sector makes an extensive use of elastomeric seals depending on drilling fluids. Because of increasingly severe environmental standards, the industry has developed environmentally friendly, synthetic drilling fluids as alternatives to conventional oil-based. These

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synthetic muds are in some uses incompatible with the conventional rubbers used in equipment. The issuesoccur for the subsea equipment at high pressure and cold temperatures (Akhtar et al., 2018). Rubber gaskets based on EPDM are an important component for a fuel cells fabrication because they work as sealing agent in the stack assembly. They work to provide the correct compression needed and act as a 'barrier' for potential fuel leaks; maximizing the highest possible efficiency. Long-term durability of the fuel cell stacks depends heavily on the functionality of the elastomeric gasket material. The compression set of multilayered elastomeric composites decreases with the increase of the layer number, indicating better sealing capacity and longer working life.

Rubber nanocomposites are heterogeneous systems where the significant volume fraction of macromolecular chains affected and strongly interacted by the nano-sized filler particles. Also, continuous break-up and rearrangement of particles take place due to the dynamic loads applied to the composite materials. It has been recognized that, for a given network precursor and curing system, the surface area and structure of filler, the hydrodynamic effect, and filler–filler and filler-polymer interactions influence the dynamic properties of rubber (Allegra et al., 2008; Leblanc, 2002). In nanostructured materials properties of the interface, effect dominates the volume effect, while in materials with μ-sized fillers the volume effect is dominating (Fröhlich et al., 2005). The rubber-filler interaction has been attributed to chemical bonds formation at the filler surface. (Jovanović et al., 2011)

Physical aging which occurs as the gradual process may greatly affect many properties in a way dependent on the aging temperature. Information about thermal properties is critical for development, processing, and application of elastomeric products. Heat aging stability is a hallmark of EPDM rubber and a requirement for the application in automotive, roofing and other industries. Retention of physical properties after accelerated heat aging is a typical specification for such long-term uses. For applications involving exposure to high temperature steam improved EPDM seals and O-rings are the best solution for submersible pumps. For subsea sealing equipment the main difficulties involves the low temperatures and high pressures. Seawater temperatures when combined with the high pressures could possibly cause the fail of subsurface equipment seals This is attributable to the increase of the elastomer glass transition temperature as extreme pressures decrease its free volume thereby reducing network chains mobility. Elastomeric elements on the rig such as the rotary hose or seals degrade at high temperatures. High-temperature compression set performance is influenced not only by the formation of new crosslinks during heating under compression but also by the inter-conversion of polysulfidic linkages present in the initial vulcanizates. Carbon black improves the aging resistance of elastomers due to its ability to absorb UV light, which otherwise would be absorbed by the polymer and initiate photo-oxidative reactions leading to deterioration. The interactions between rubber and filler particles are obviously initiated in the early stage of mixing but it is well known that the process is not instantaneous. Traditional methods of modification of the Tg value involves the use of copolymers, modified crosslinking agents, plasticizers, and fillers, blending of various polymers, grafting and formation of interpenetrating polymer networks. If elastomeric material is compressed over time, it loses its ability to return to its original thickness. This loss of resiliency may reduce the capability of elastomeric products to perform over a long time period. In rubber industry the compression set values are expressed as a percentage. The lower value is, the better is elastomeric material resistance on permanent deformation under a given temperature and deflection. In this applicative work the goal was to study the influence of active and inactive fillers on the properties of oil-extended elastomeric hybrid materials based on EPDM rubber for sealnt fabrication.

EXPERIMENTAL

Materials and methods

As precursor was used ethylene-propylene diene monomer rubber (based on 5-ethylidene-2-norbornene) Vistalon 9500, produced by Exxon Mobil Chemical, France, 59.8% wt ethylene content and 10.92 % wt diene. Mooney viscosity was determinate under conditions: ML (1 + 4) at (125 °C) was 69.6 Mu. Carbon black was the furnace black type, GPF N-660 Statex, produced by Columbian Chemical Company. The average particle diameter of carbon black was 70 nm. As an inactive filler was used hydrophobic calcium carbonate, Kredafil 150S,. Relative density at 20 °C was 2.7, particle size distribution 1-30 micron. Chalk loading was varied from 0 to 100 phr, carbon black content was 140 phr. Paraffinic oil (Texpar 460. Texaco) was used as extender with an low sulfur, nitrogen and aromatic hydrocarbon content (density at 15°C, kg/l 0.9012 kg/l, Kin viscosity at 40°C, 473.1 mm²/s). It is produced by modern technology for fabrication from the premium grades of mineral oils. Paraffinic process oils with high flash point and high viscosity are better for shelf life of the rubber product. Because paraffinic furfural extract has higher viscosity and paraffinic content are preferred for achieving high retention of properties after thermal ageing in applications like heat resistant sealants, conveyor belts and steam hoses.

Achieving of fast curing reaction was possible using accelerators (thiazoles, mercaptobenzothiazole, dibenzothiazyl disulfide). For compound preparation the rubbers were separately premasticated for about 1 min each, keeping a tight nip gap (0.8 mm), and subsequently blended for 3 minutes. The compounds were prepared on a laboratory electrically heated rubber mill to enable the homogeneous mixture. The mixing time was 20

minutes. After mixing, the Mooney viscosity (ML1+4 at 100 °C) of the compounds was measured by a Mooney viscometer. The sheeted compound was conditioned at 23±2 °C during 24 h prior to cure assessment using the oscillating disc rheometer (Monsanto R-100) at 180 ± 1°C. The compounds were cured by sulfur at 160°C. The curing characteristics: Ml (minimum torque), Mh (maximum torque), t_{c90} (optimum cure time), t_{s2} (scorch time) were registered and curing rate index was calculated (Marković et al., 2009). To perform a systematic study, elastomers without and with active and inactive filler were prepared and crosslinked under similar experimental conditions.

For mechanical properties determination, all test specimens were compression molded at 160 °C during the earlier determined respective optimum cure time (t_{c90}). The dumbbell samples were cut from a 2 mm thick molded sheet. The mechanical properties were obtained from stress/strain measurements by the Tensile tester (Karl Frank, Germany) according to ASTM D412-98a (before and after aging at 70 °C for 46 h in air). The crosshead speed for the tensile test was 500 mm/min. The retained percentage value of tensile strength and elongation at break were calculated. The hardness of the elastomer samples was determined by the standard method using an indentation hardness tester (Shore A type). Samples with flat surface were cut for hardness test. The compression set was assessed using the standard compression set measurement according ASTM D395. The dynamic mechanical spectra of some prepared materials were obtained using a dynamic mechanical thermal analyzer (DMTA 2980, TA Instruments). All samples were analyzed by bending mode over a frequency from 1 to 200 Hz and with a constant strain 0.1%. The measurement was performed under a nitrogen (flow rate 20 ml/min). The maximum in the plot of tan δ versus temperature was taken as the glass to rubber transition, Tg. A JEOL scanning electron microscope SEM was used to perform morphology of oil extended elastomeric materials**.** Each sample was fractured cryogenically and submitted to gold sputtering before analysis.

RESULTS AND DISCUSSION

Curing characteristics

For compounds, type and content of filler affect the cure characteristics. Lots of functional groups such as hydroxyl, carboxyl, lactone, pyrone, ketone, quinone, and phenol exist on the carbon black surface but the amount is small. The effect of chalk loading on cure characteristics for samples with 140 phr carbon black is shown in Figure 2. The correlation curves for cure rate index and filler content are given in the Figure 3. The cure rate of filled compound became faster as the chalk content increased. Retention of physical properties after accelerated heat aging is a typical for the long term use of elastomers.

The used plasticizer for elastomeric hybrid materials based on EPDM as network precursor was parrafinic process oil thus it was very effective because this process oil has the advantage to be compatible with this network precursor (both are non-polar). In practice, this means that there the sweating is prevented even at temperature interval extremes from -40 to +80 °C. In Table 1 are given mechanical properties and harness of prepared hybrid material before aging (BA) and after aging (AA).

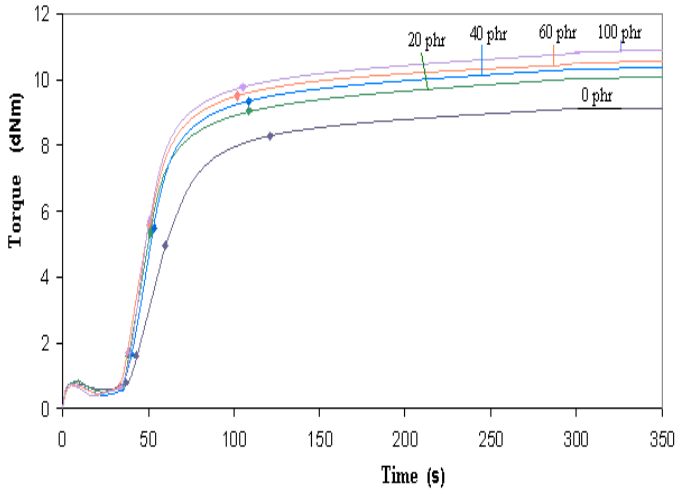

Figure 2. Torque-time dependence for compounds with 140 phr carbon black and different chalk content during curing at 160 °C.

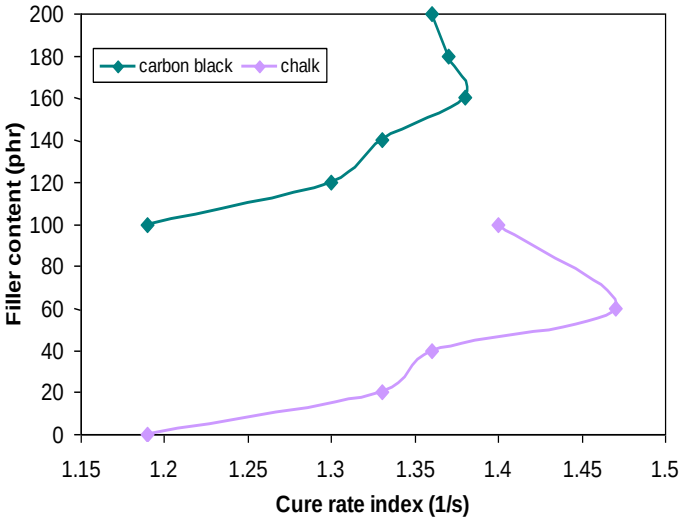

Figure 3. Obtained correlation of curing rate index and filler content for oil-extended elastomeric hybrid materials.

Mechanical properties of hybrid elastomeric materials

It was estimated that for the all samples hardness was increased with the increase of chalk content. As can beobserved, for all samples the hardness was increased with increased filler loadings. The tensile strength decreased with increasing filler loading, whereas elongation at break was increasing at low chalk content, but after what was decreasing. Compression set testing measures the ability of rubber to return to its original thickness after prolonged compressive stresses at a given deflection.

Vibrations often have influence on undesirable motions and dynamic stresses, which could lead to the structure failure and other unwanted consequences. The values of compression set are also given in Table 1. From these results, it can be seen that the compression set values for 25% of deformation are low and adequate for sealant application.

Table 1. Mechanical properties of oil extended elastomer samples based on 140 phr of carbon black and different content of chalk, before (BA) and after aging (AA).

Sample	Hardness (Sh A)		Tensile strength (N/mm^2)		Compression set (%)	
	BA	AA	BA	AA	for 25 %	for 50 %
EPDM/CB140/K0	57.7	58.3	9.64	9.95	9.2	33.8
EPDM/CB140/K20	59.7	59.7	9.05	8.82	6.2	27.7
EPDM/CB140/K40	61.0	61.7	8.25	8.17	9.2	26.2
EPDM/CB140/K60	62.0	63.0	7.36	7.39	9.2	27.7
EPDM/CB140/K100	63.0	63.3	6.43	6.36	9.2	33.8

The dynamic-mechanical behavior of hybrid materials

For the investigation of the low temperature properties, thermal analysis method DMA was applied to assess the region of the glass rubber transition process. The response of elastomers to an applied energy can be energy storage or energy dissipation. For sealing materials, the elastic component is most important. An applied stress induces a strain which forms contact stress or sealing force. As the macromolecular chains rearrange to reduce this stored force, a loss of sealing force occurs. These materials have elastic and damping behaviour because they are viscoelastic materials. Dynamic losses are associated with specific mechanisms of molecular motion in cross-linked materials. The damping (the energy loss per cycle) can be measured from the tangent of the phase angle. The compression set is very important characteristic of elastomeric sealing materials as spontaneous stress release during application due to the internal pressure changes or external forces variations. During heating, the storage modulus shows the typical decrease during glass transition region which is associated with the peaks of loss modulus and loss tangent tan δ.

In this study, the tan δ, was used for assessment of the hybrid materials damping properties. The storage modulus drops by several orders with increasing temperature, while the loss modulus and tan δ show a characteristic peak. The chemical reaction between the filler and elastomer and occlusion of the rubber macromolecules within the pores of the filler particles affect on mechanical response and final morphology under a given set of conditions of temperature and filler loading level. The particles are expected to contribute to the increase of phase angle (tan δ) in the rubbery phase of material beyond the glass transition temperature. The loss tangent (tan δ), defined by the ratio of loss modulus (E″) to storage modulus (E′), can be a measure of the vibration energy dissipation. High-performance damping materials should have a high value of arbitrary defined loss factor (tanδ >0.3) over a broad temperature range. Figure 4 displays the dependence of loss tangent on the temperature at different frequencies for oil-extended sample with 100 phr chalk.

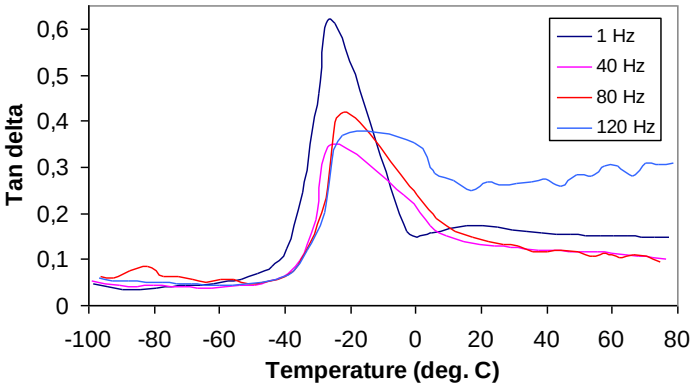

Figure 4. Dependence of loss tangent on the temperature at different frequencies for oil-extended elastomeric hybrid materials with 140 phr furnace carbon black and 100 phr chalk.

It was estimated that chalk addition causes better damping properties under high frequencies in comparison with samples without chalk. The temperature range with tanδ > 0.3 was used to assess the damping capacity for the sample with 140 phr carbon black and different chalk content, as some kind of arbitrary criteria for practical comparison. In the Table 2 are given data for determined Tg values and arbitrary damping capacity.

Table 2. Damping properties of elastomeric materials filled with 140 phr of carbon black and different content of chalk.

Sample	Damping capacity as temperature interval for tanδ > 0.3			Tg (°C)
	1 Hz	40 Hz	80 Hz	
EPDM/CB140/K0	-	-	22.16	-32.50
EPDM/CB140/K40	15.83	8.33	8.33	-33.33
EPDM/CB140/K60	15.83	10.42	10.83	-33.75
EPDM/CB140/K100	24.17	19. 58	15.83	-26.67

The Tg values are determined as the position of tanδ maximum obtained at 1Hz. It was assessed that in glassy region state below about -26 °C, the hybrid materials have a high storage modulus and in the rubberlike state at temperatures above -26 °C the modulus is rather low. The addition of filler particles provides high strength and modulus, whereas the

damping peak height is decreased sharply while the damping peak position is shifted. The expected frequency dependence of the mechanical properties becomes confirmed by the shift of the glass transition region with enlarging the frequency. This is important to know for the dynamic seals devices that may be stressed at high frequencies or during short times during elastomeric material exploitation. Figure 5 displays the correlation of storage modulus assessed at different frequencies of deformation at 25ºC and hardness for prepared elastomeric hybrid materials.

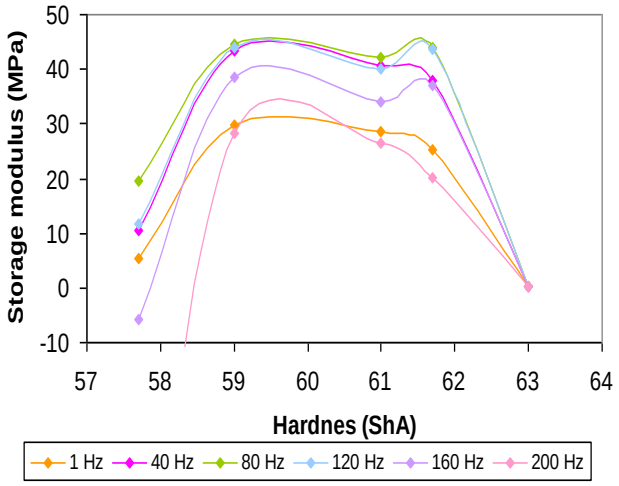

Figure 5. Correlation of storage modulus obtained at different frequencies of dynamic deformation at 25ºC with hardness for prepared elastomeric hybrid materials.

The morphology of oil-extended samples

The morphology of the cryogenically fractured surface of samples with different content of paraffin oil was characterized by scanning electron microscopy. It can be stated that the oil influenced the morphology significant.

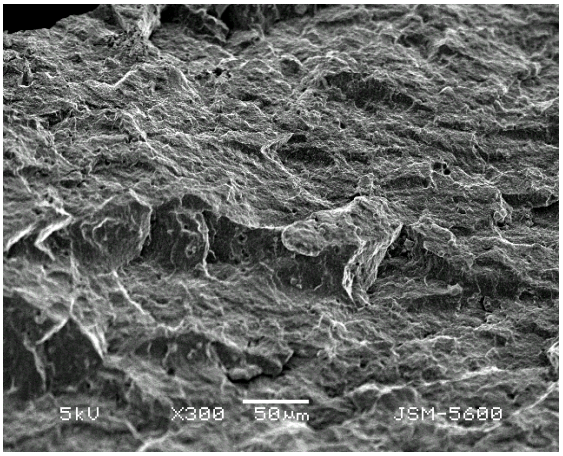

Figure 6. SEM micrograph of elastomers with 140 phr carbon black, 20 phr chalk and 73 phr paraffinic oil (X300).

In the Figures 6-8, are shown SEM micrographs of hybrid materials based on 140 phr carbon black and 20 phr chalk with different content of paraffin oil. As expected, it was noticed, that a higher concentration of paraffinic oil influenced the materials morphology significant. The advantage is to dilute elastomers with less expensive oil and also to improve material processability. This enables the use of higher-molecular-weight network precursors for hybrid materials preparation.

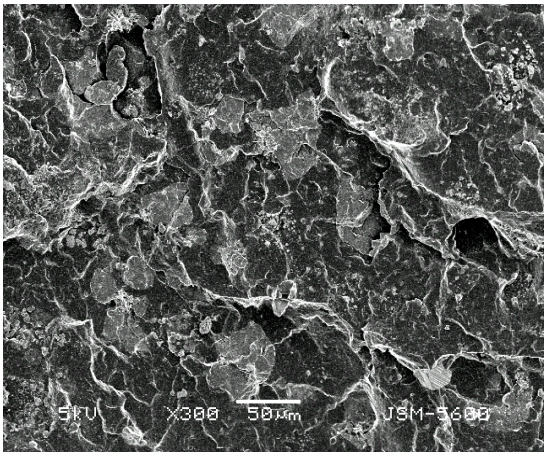

Figure 7. SEM micrograph of elastomers with 140 phr carbon black, 20 phr chalk and 98 phr paraffinic oil (X300).

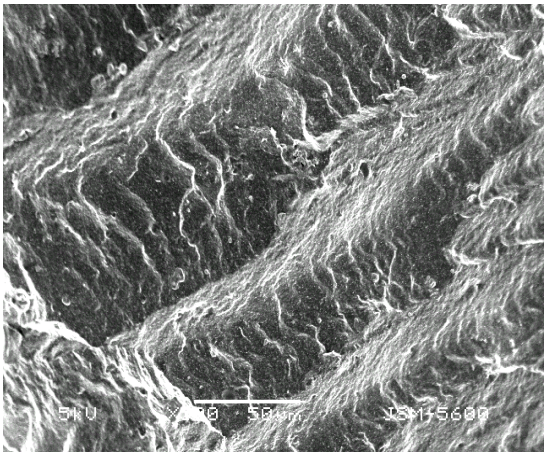

Figure 8. SEM micrograph of elastomers with 140 phr carbon black, 20 phr chalk and 118 phr paraffinic oil (X500).

CONCLUSIONS

The present study was undertaken to give a detailed analysis of the influence of active filler carbon black and inactive ground calcium carbonate on compression set values and dynamical-mechanical properties of elastomeric hybrid materials for sealant fabrication. The used paraffinic process oils with high viscosity were adequate for achieving high properties retention of prepared materials after thermal ageing. The compression set is important as spontaneous stress release of the seal and is simulated what could occur due to the external forces or internal pressure changes during sealant exploitation. It was estimated that values of the compression set obtained for 25% of

deformation are excellent for application. It was assessed that the chalk addition in a small amount, has a positive contribution to modulus and does not change tensile strength. The estimated higher temperature maximum of tan δ is due to the adsorbed hard rubber around the carbon black aggregates. As expected, it was assessed from SEM micrograph that the content of paraffinic oil influenced the hybrid elastomeric materials morphology. The enhanced thermal stability for filled samples is evidenced from the tensile strength however, reductions of some mechanical properties could be noticed .

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REFERENCES

Allegra, G., Raos, G., & Vacatello, M. 2008. Theories and simulations of polymer-based nanocomposites: From chain statistics to reinforcement. Progress in Polymer Science, 33(7), pp. 683-731. doi:10.1016/j.progpolymsci.2008.02.003

Fröhlich, J., Niedermeier, W., & Luginsland, H. 2005. The effect of filler–filler and filler–elastomer interaction on rubber reinforcement. Composites Part A: Applied Science and Manufacturing, 36(4), pp. 449-460. doi:10.1016/j.compositesa.2004.10.004

Jovanović, V., Samaržija-Jovanović, S., Marković, G., Marinović-Cincović, M., & Budinski-Simendić, J. 2011. Mechanical and Morphological Properties Rubber Blends Reinforced with Nanofillers. Kautschuk Gummi Kunststoffe, 9, pp. 52-56.

Jovanović, V., Samaržija-Jovanović, S., Budinski-Simendić, J., Marković, G., & Marinović-Cincović, M. 2013. Composites based on carbon black reinforced NBR/EPDM rubber blends. Composites Part B: Engineering, 45(1), pp. 333-340. doi:10.1016/j.compositesb.2012.05.020

Leblanc, J. 2002. Rubber–filler interactions and rheological properties in filled compounds. Progress in Polymer Science, 27(4), pp. 627-687. doi:10.1016/s0079-6700(01)00040-5

Marković, G., Dević, S., Marinović-Cincović, M., & Budinski-Simendić, J. 2009. Influence of carbon black on reinforcement. Kautschuk Gummi Kunststoffe, 62(6) pp. 299-305.

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NOVEL 4-FERROCENYL-8-(PHENYLTHIO)-1,2,3,4-TETRAHYDROQUINOLINE: DESIGN, SYNTHESIS AND SPECTRAL CHARACTERIZATION

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ABSTRACT

Herein, we report design, synthesis and spectral characterization of novel 4-ferrocenyl-8-(phenylthio)-1,2,3,4-tetrahydroquinoline. Desired synthesis was achieved in three reaction steps, with a good overall yield (67%). First step included aza-Michael addition of 2-(phenylthio)aniline to 1-ferrocenylpropenone, subsequently, the obtained ketone was smoothly reduced to the corresponding 1,3-amino alcohol. The final step was an intramolecular cyclization prompted by acetic acid, proceeding via corresponding α-ferrocenyl carbocation. The synthesized compounds have been isolated pure, and their structure have been undoubtedly confirmed by standard spectral techniques (^{1}H NMR, ^{13}C NMR, IR and elemental analyses).

Keywords: Tetrahydroquinoline ring, Ferrocene, Intramolecular cyclization, α-Ferrocenyl carbocation, Spectral characterization.

INTRODUCTION

As the largest group of organic compounds, heterocycles play an important role in many fields of chemistry (Taylor et al., 2016). They are known to possess a plethora of biological activities which makes them very important substrates in organic synthesis (Taylor et al., 2016). Among the heterocycles, quinolines and their hydrogenated derivatives are of particular importance, since they are known to be biologically active, as well as versatile synthons in organic chemistry (Eicher et al., 2003). A huge number of publications on syntheses and applications of these heterocycles testifies to the great interest of chemists in these compounds (Sridharan et al., 2011).

On the other hand, usage of metals to induce or enhance cytotoxicity of natural compounds or known drugs has increased since the emergence of platinum-based chemotherapy agents in the treatment of cancer (Kelland, 2007). In addition, metallocenes are also known to exhibit a wide range of biological activity. Among them, ferrocene has attracted special attention since it is a neutral, chemically stable and nontoxic molecule (Togni, 1996). The mentioned metallocene has no biological activity, but it can be easily derivatized and functionalized to biological important compounds or oxidized to ferricenium salts (Köpf-Maier et al., 1984; Houlton et al., 1991; Kowalski, 2018). Ferrocene derivatives have a unique structure as well as an excellent redox property, allowing their applications in medicinal chemistry (Dai et al., 2007). These compounds display interesting cytotoxic, antitumor, antimalarial, antifungal and DNA-cleaving activity (Jaouen, 2006). In the context of antimalarial treatments, it is known that metal-containing compounds may possess antiparasitic activity (Gambino & Otero, 2012; Salas et al., 2013; Biot et al., 2012). Ferroquine (FQ), an analogue of chloroquinoline (CQ), has been developed as an important antimalarial drug; it is currently in the Phase II Sanofi portfolio for uncomplicated *P. falciparum* malaria (Malisa et al., 2011; Supan et al., 2012). In terms of developing new drugs based on FQ, this led to the preparation of a numerous analogues (N'Da, & Smith, 2014). It has been observed that the linker between drug hybrids is an important determinant into whether or not the novel compound retains the biological activity of the component parts (Madrid et al., 2006). However, up to date no analogues have been able to compete with FQ in terms of antimalarial activity. Thus, the search for new antimalarials continues and the battle against malaria is far from over.

As a part of a more comprehensive project aimed at the synthesis of new potentially bioactive ferrocene derivatives, we recently reported synthesis of bioactive 2-ferrocenoyl ethyl aryl amines by aza-Michal additions of 1-ferrocenylpropenone on anilines (Damljanović et al., 2011; Pejović et al., 2012). 2-Ferrocenoyl ethyl aryl amines - Mannich bases have been proved to be an excellent starting material for the synthesis of ferrocene derivatives with heterocyclic scaffold (Minić et al., 2015; Minić et al., 2017). Bearing all previously mentioned in mind, we planned to synthesize 1-ferrocenyl-3-((2-(phenylthio)phenyl)amino)-propan-1-one and employ it as starting material for a synthesis of novel ferrocene-containing tetrahydroquinoline derivative. Thus, in this research we put emphasis on the design, synthesis, as well as assignment of ^{1}H

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and ^{13}C NMR spectral data of novel 4-ferrocenyl-8-(phenylthio)-1,2,3,4-tetrahydroquinoline, a promising privilege structure.

EXPERIMENTAL

Materials and measurements

All chemicals were commercially available and used as received, except the solvents, which were purified by distillation. Ultrasonic cleaner Elmasonic S 10 (Elma, Germany), 30W was used for the ultrasonically supported synthesis. Chromatographic separations were carried out using silica gel 60 (Merck, 230–400 mesh ASTM), whereas silica gel 60 on Al plates, (Merck, layer thickness 0.2 mm) was used for TLC. Melting points were determined on a Mel-Temp capillary melting points apparatus, model 1001, and the given values are uncorrected. The ^{1}H and ^{13}C NMR spectra of the samples in $CDCl_3$ were recorded on a Bruker Avance III 400 MHz (^{1}H at 400 MHz, ^{13}C at 101 MHz) NMR spectrometer. Chemical shifts are reported in ppm (δ) values relative to TMS (δ_H 0 ppm) in ^{1}H, ^{13}C and heteronuclear 2D NMR spectra. The coupling constant (*J*) are reported in Hz. Multiplicities of proton resonance are designated as singlet (s), a doublet (d), a doublet of doublets (dd), a triplet (t), a pseudo triplet (pt) doublet of doublets of doublets (ddd), a triplet of triplets (tt), a triplet of doublets of triplets (tdt), a triplet of doublets of doublets (tdd) and multiplets (m). 2D spectra (^{1}H-^{1}H COSY, NOESY, HSQC and HMBC) are performed on the same instrument with a standard pulse sequence. IR measurements were carried out with a Perkin–Elmer FTIR 31725-X spectrophotometer. Microanalyses of carbon, hydrogen and nitrogen were carried out with a Carlo Erba 1106 model microanalyzer; these results agreed favorably with the calculated values.

Synthesis and spectral characterization

*Synthesis of 1-phenyl-3-((2-(phenylthio)phenyl)amino)propan-1-one (**4**)*

A test tube containing a well homogenized mixture of 240 mg (1 mmol) of 1-ferrocenylpropenone, 2 mmol of the 2-(phenylthio)aniline, and 100 mg of montmorillonite K-10 was placed in the ultrasonic cleaner and irradiated for 4 h. Then, CH_2Cl_2 (10 ml) was added to the mixture, and the contents were filtered off. The solid residue was washed with CH_2Cl_2, and the collected organic layers were dried (Na_2SO_4) overnight. After the evaporation of the solvent, the crude mixture was fractioned by flash chromatography on a SiO_2 column. The 2-(phenylthio)aniline has been eluted with toluene, whereas the 1-phenyl-3-((2-(phenylthio)phenyl)amino)propan-1-one has been washed from the column by a mixture of toluene and AcOEt 9 : 1 (v/v). The complete excess of the amines was recovered.

Dark orange solid; mp 86 °C. Rf = 0.7. Yield 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.48 (d, *J* = 7.5 Hz, 1H, Ar), 7.35 (t, *J* = 7.7 Hz, 1H, Ar), 7.19 (t, *J* = 7.5 Hz, 2H, Ar), 7.08 (t, *J* = 8.6 Hz, 3H, Ar), 6.78 (d, *J* = 8.2 Hz, 1H, Ar), 6.70 (t, *J* = 7.4 Hz, 1H, Ar), 5.26 (s, 1H, $COCH_2CH_2N\mathbf{H}$), 4.72 (pt, *J* = 1.9 Hz, 2H, 2×CH, Cp), 4.48 (pt, *J* = 1.9 Hz, 2H, 2×CH, Cp), 4.10 (s, 5H, 5×CH, Cp), 3.58 (d, *J* = 5.1 Hz, 2H, $COCH_2C\mathbf{H_2}NH$), 2.92 (t, *J* = 6.4 Hz, 2H, $COC\mathbf{H_2}CH_2NH$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 202.5 ($\mathbf{C}OCH_2CH_2NH$), 149.1 (C, Ar), 137.9 (C, Ar), 136.9 (C, Ar), 131.9 (C, Ar), 128.9 (C, Ar), 126.5 (C, Ar), 125.3 (C, Ar), 117.1 (C, Ar), 114.5 (C, Ar), 110.5 (C, Ar), 78.8 (C, Cp), 72.4 (C, Cp), 69.8 (C, Cp), 69.2 (C, Cp), 38.5 ($CO\mathbf{C}H_2CH_2NH$), 38.4 ($COCH_2\mathbf{C}H_2NH$). IR (ATR, cm^{-1}): ν = 3361 (N-H) cm^{-1}; ν = 1655 (C=O) cm^{-1}. Anal. Calc. for $C_{25}H_{23}FeNOS$: C, 68.03; H, 5.25; Fe, 12.65; N, 3.17; O, 3.62; S, 7.26. Found: C, 68.08, H, 5.21, N, 3.19 %.

*Synthesis of 1-phenyl-3-((2-(phenylthio)phenyl)amino)propan-1-ol (**5**)*

To a stirred solution of the corresponding 1-ferrocenyl-3-((2-(phenylthio)phenyl)amino)propan-1-one (1 mmol) in MeOH (20 ml) at room temperature, an excess of $NaBH_4$ (5 mmol) was added in several portions (up to 190 mg) and the reaction progress was monitored by TLC. After reduction has been completed (*ca*. 2 h), methanol was distilled off and the residue diluted with water (20 ml). The mixture was extracted with CH_2Cl_2 (two 20 ml portions) and the combined organic layers were washed with water and brine, as well as dried with anh. Na_2SO_4. After filtering off the drying agent and evaporation of the solvent, the crude mixture was purified by column chromatography (SiO_2) affording pure product 1-ferrocenyl-3-((2-(phenylthio)phenyl)amino)propan-1-ol.

Yellow oil. Rf = 0.4 (Hexane/EtOAc, 7:3 (v/v)). Yield 97%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.56 – 7.52 (m, 1H. Ar), 7.36 (ddd, *J* = 8.3, 7.4, 1.6 Hz, 1H, Ar), 7.27 (tt, *J* = 3.5, 1.9 Hz, 2H, Ar), 7.20 – 7.11 (m, 3H, Ar), 6.73 (tdt, *J* = 7.0, 4.4, 2.2 Hz, 2H, Ar), 5.22 (s, 1H, $CHOHCH_2CH_2N\mathbf{H}$), 4.31 (dd, *J* = 8.1, 4.4 Hz, 1H, $C\mathbf{H}OHCH_2CH_2N\mathbf{H}$), 4.17 (d, *J* = 2.5 Hz, 8H, 8×CH, Cp), 4.10 (d, *J* = 1.1 Hz, 1H, CH, Cp), 3.38 – 3.19 (m, 2H, $CHOHCH_2C\mathbf{H_2}NH$), 2.02 – 1.81 (m, 3H, overlapping signals from $CHO\mathbf{H}CH_2CH_2NH$ and $CHOHC\mathbf{H_2}CH_2NH$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 137.8 (C, Ar), 136.0 (C, Ar), 131.5 (C, Ar), 129.0 (C, Ar), 126.3 (C, Ar), 125.9 (C, Ar), 125.3 (C, Ar), 116.7 (C, Ar), 115.4 (C, Ar), 110.5 (C, Ar), 68.7 (C, Cp), 68.4 (C, Cp), 66.9 (C, Cp), 65.4 (C, Cp), 40.5 ($CHOH\mathbf{C}H_2CH_2NH$), 36.9 ($\mathbf{C}HOHCH_2CH_2NH$), 36.8 ($COCH_2\mathbf{C}H_2NH$). IR (ATR, cm^{-1}): ν = 3385 (N-H) cm^{-1}; ν = 3068 (O-H) cm^{-1}. Anal. Calc. for $C_{25}H_{25}FeNOS$: C, 67.72; H, 5.68; Fe, 12.60; N, 3.16; O, 3.61; S, 7.23. Found: C, 67.76, H, 5.66, N, 3.18 %.

*Synthesis of 4-ferrocenyl-8-(phenylthio)-1,2,3,4-tetrahydroquinoline (**6**)*

The mixture of 1-ferrocenyl-3-((2-(phenylthio)phenyl)amino)propan-1-ol (0.5 mmol) and glacial acetic acid (0.5 ml) was ultrasonicated in an ultrasonic cleaner for 2 h. The reaction mixture was neutralized with $NaHCO_3$ (litmus paper) and

extracted with CH_2Cl_2 (two 20 ml portions). The combined organic layers were washed with water and dried overnight (anh. Na_2SO_4). After filtration, the solvent was evaporated, the crude product 4-ferrocenyl-8-(phenylthio)-1,2,3,4-tetrahydro-quinoline was purified by column chromatography (SiO_2).

Yellow solid; mp 133 °C. Rf = 0.6 (Hexane/EtOAc, 7:3 (v/v)). Yield 98%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.32 (dd, J = 7.6, 1.5 Hz, 1H, Ar), 7.27 – 7.18 (m, 3H, Ar), 7.15 (dd, J = 7.5, 1.0 Hz, 1H, Ar), 7.11 – 7.03 (m, 3H, Ar), 6.58 (t, J = 7.6 Hz, 1H, Ar), 5.05 (s, 1H, FcCHCH$_2$CH$_2$N**H**), 4.17 (s, 5H, 5×CH, Cp), 4.14 (s, 1H, FcC**H**CH$_2$CH$_2$NH), 4.12 – 4.08 (m, 2H, 2×CH, Cp), 3.91 – 3.87 (m, 2H, 2×CH, Cp), 3.20 (tdd, J = 11.7, 10.1, 4.4 Hz, 2H, FcCHCH$_2$C**H$_2$**NH), 2.23 – 2.10 (m, 1H, FcCHC**H**$_2$CH$_2$NH), 1.98 – 1.86 (m, 1H, FcCHC**H**$_2$CH$_2$NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 145.2 (C, Ar), 136.8 (C, Ar), 135.4 (C, Ar), 130.8 (C, Ar), 128.4 (C, Ar), 125.4 (C, Ar), 124.5 (C, Ar), 123.5 (C, Ar), 114.9 (C, Ar), 110.9 (C, Ar), 93.2 (C, Cp), 69.2 (C, Cp), 68.2 (C, Cp), 67.3 (C, Cp), 66.2 (C, Cp), 65.3 (C, Cp), 38.5 (FcCH**C**H$_2$CH$_2$NH), 36.3 (Fc**C**HCH$_2$CH$_2$NH), 28.5 (FcCHCH$_2$**C**H$_2$NH). IR (ATR, cm^{-1}): ν = 3345 (N-H) cm^{-1}. Anal. Calc. for $C_{25}H_{23}FeNS$: C, 70.59; H, 5.45; Fe, 13.13; N, 3.29; S, 7.54. Found: C, 70.56, H, 5.46, N, 3.33 %.

RESULTS AND DISCUSSION

Synthesis

First step in target synthesis was finding suitable method for the preparation of 1-ferrocenyl-3-((2-(phenylthio)phenyl)-amino)propan-1-one. Few years ago, we designed and optimized reaction conditions for synthesis of 3-arylamino-1-ferrocenylpropan-1-ones in high yields, which shown to be biological active compounds and excellent starting material (Damljanović et al., 2011; Pejović et al., 2012; Minić et al., 2015; Minić et al., 2017). Thus, we decided to use this protocol. For the test reaction we put 1 mmol of 1-ferrocenylpropenone (synthesized by previously described method (Minić et al., 2017), 2 mmol of 2-(phenylthio)aniline and 100 mg of clay montmorillonite K-10 (see Scheme 1.) and flask has been placed in an ultrasound bath. After 1 hour irradiation and the usual workup, as well as column chromatography (SiO_2), unfortunately, we obtain desired product in only 6% yield, therefore, we decided to increase reaction time on 4 hours. Indeed, when we set reaction under these conditions, to our delight, we successfully synthesized 1-ferrocenyl-3-((2-(phenylthio)phenyl)amino)propan-1-one (**4**) in 70% yield. These results did not require additional screenings, so we accepted them as the optimal ones.

The obtained ketone **4** was then converted into the corresponding 1,3-amino alcohol. For the reduction of **4**, $NaBH_4$ (five equivalents) in MeOH was employed and we efficiently synthesized the 1-ferrocenyl-3-((2-(phenylthio)phenyl)amino)-propan-1-ol (**5**) in high yields (97%), with a short reaction time and absence of sub products (Scheme 1.). Noteworthy, in spite the efficient reduction, synthesized product must be purified by column chromatography. Moreover, γ-amino alcohols are in general versatile synthons in organic chemistry. Therefore, the obtained product **5** beside promising biological activity, represent excellent starting material for further synthesis of ferrocene-containing compounds with an interest from a biological point of view (antibacterial, antimalarial, anti-inflammatory, antitumor etc.) (Pejović et al., 2015; Minić et al., 2018).

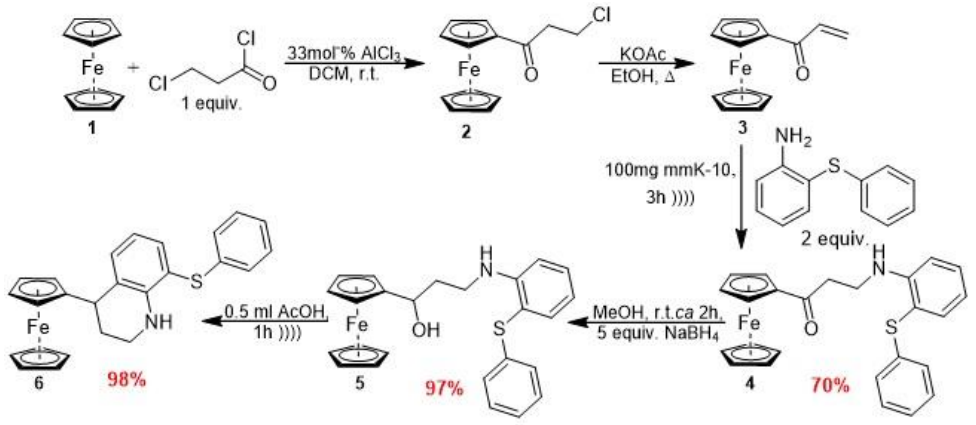
In the last step, ferrocene-containing tetrahydroquinoline compound **6** was premised (Scheme 1). For this synthetic transformation we choose method previously reported by us (Minić et al., 2017), which is efficient and require mild reaction conditions. This investigation started with an ultrasonication of a solution of 0.5 mmol **5** in 0.5 ml of glacial acetic acid. The desired heterocyclic scaffold **6** was isolated after purification by means of column chromatography. It has been shown that 1-ferrocenyl-3-((2-(phenylthio)phenyl)amino)propan-1-ol (**5**) can smoothly undergo an intramolecular Friedel-Crafts-type reaction promoted by acetic acid, giving rise to the corresponding heterocycle in excellent yield (98%). In addition, this ferrocene-containing heterocycle can be regarded as interesting compound with a variety of applications particularly as novel potent antimalarial.

A plausible reaction mechanism of this intramolecular Friedel-Crafts alkylation is illustrated in Scheme 2. Initially, corresponding α-ferrocenyl carbocation **II** has been formed by the protonation of 1,3-amino alcohol **5** (oxonium ion **I**), followed by dehydration. α-Ferrocenyl carbocation **II** is stabilized by the presence of ferrocene core (Bleiholder et al., 2009), moreover, this cation is electrophilic enough to be attacked by the π-electronic system of the benzene ring (considerably activated by the presence of amine nitrogen), making arenium ion **III**. Deprotonation of **III** leads to the final alkylation product **6**.

Spectral characterization

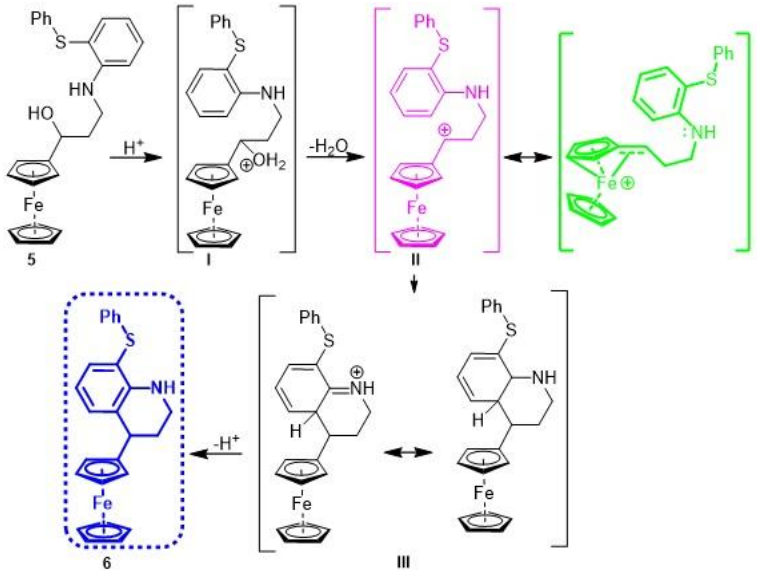
The three newly synthesized compounds, **4**, **5** and **6** described in this work have been fully characterized by standard spectroscopic techniques (IR, ^{1}H and ^{13}C NMR), as well as elemental analyses. All spectral data were fully consistent with the proposed structures.

In the IR spectra of **4**, sharp, medium intensity absorption of NH stretching vibration is observed below 3400 cm^{-1}, indicating that all NH groups are involved in H-bonding interactions. The CO stretching vibration band of the 1'-ferrocene-carbonyl group appear at 1655 cm^{-1}, suggesting the existence of inter- and/or intramolecular H-bonds to the CO functional group. The ^{1}H and ^{13}C NMR spectra of compound **4** display all signals expected for the proposed composition. The ^{1}H NMR data for the newly synthesized compound **4** is typical



Scheme 1. The synthesis of 4-ferrocenyl-8-(phenylthio)-1,2,3,4-tetrahydro-quinoline (**6**) starting from ferrocene (**1**).

for monosubstituted ferrocene (a characteristic intensity pattern of 2 : 2 : 5 for the H-atoms of ferrocene). Two slightly deshielded triplets (or better pseudo triplets) are observed for the cyclopentadienyl (Cp) ring H-atoms at 4.72 ppm (pt, J = 1.9 Hz) and 4.48 ppm (pt, J = 1.9 Hz). These are down fielded to thesinglets assigned to the unsubstituted Cp ring at δ(H) 4.10, which is characteristic for ferrocenes with electron-withdrawing substituents (due to deshielding effect with the increased delocalization of electron density toward the C=O substituent [39]). The first region (0-4 ppm) contains the signals of protons on the aliphatic part of molecules, $COCH_2C\mathbf{H_2}NH$ (δ(H) = 3.58 ppm) and $COC\mathbf{H_2}CH_2NH$ (δ(H) = 2.92 ppm). The signal of proton attributed to amine group was observed at 5.26 ppm. The aromatic region related to the benzene core is located 7.48-6.70 ppm.



Shema 2. Plausible mechanism for the intramolecular Friedel-Crafts alkylation of compound **5.**

The ^{13}C NMR spectra can be analyzed in an analogous manner to the proton spectra, with signals corresponding to the carbonyl group (δ(C) = 202.5 ppm), aromatic core above 110.5 ppm, ferrocene moiety between 69 and 79 ppm and aliphatic carbons at *ca* 38 ppm.

Infrared spectra of the 1-ferrocenyl-3-((2-(phenylthio)phenyl)amino)propan-1-ol (**5**) show characteristic bands associated to N-H stretching vibrations (ν = 3385 cm^{-1}) and C-OH stretching vibrations (ν = 3068 cm^{-1}). Additionally, lack of signal for C=O undoubtedly confirms that the reduction really occurs. The ^{1}H NMR spectra of **5** contain characteristic signals for aliphatic, hydroxy, ferrocene, amino and quinoline protons located in the expected regions. In that context, ferrocene moiety exhibits two signals assigned to hydrogens of the substituted Cp ring and one singlet originated to five protons of the unsubstituted Cp ring. Characteristic signal for H-atom of

amino NH was observed as the singlet in area (5.22 ppm) while aromatic rings show the signals in little bit higher range of 7.56 – 6.73 ppm. Moreover, in the ^{13}C NMR spectra of the **5** signals of the aliphatic, ferrocene and aromatic groups can be recognized in appropriate regions. Additionally, absence of signal attributed to carbonyl group in ^{13}C NMR spectra of compound **5** unambiguously confirmed proposed product structure.

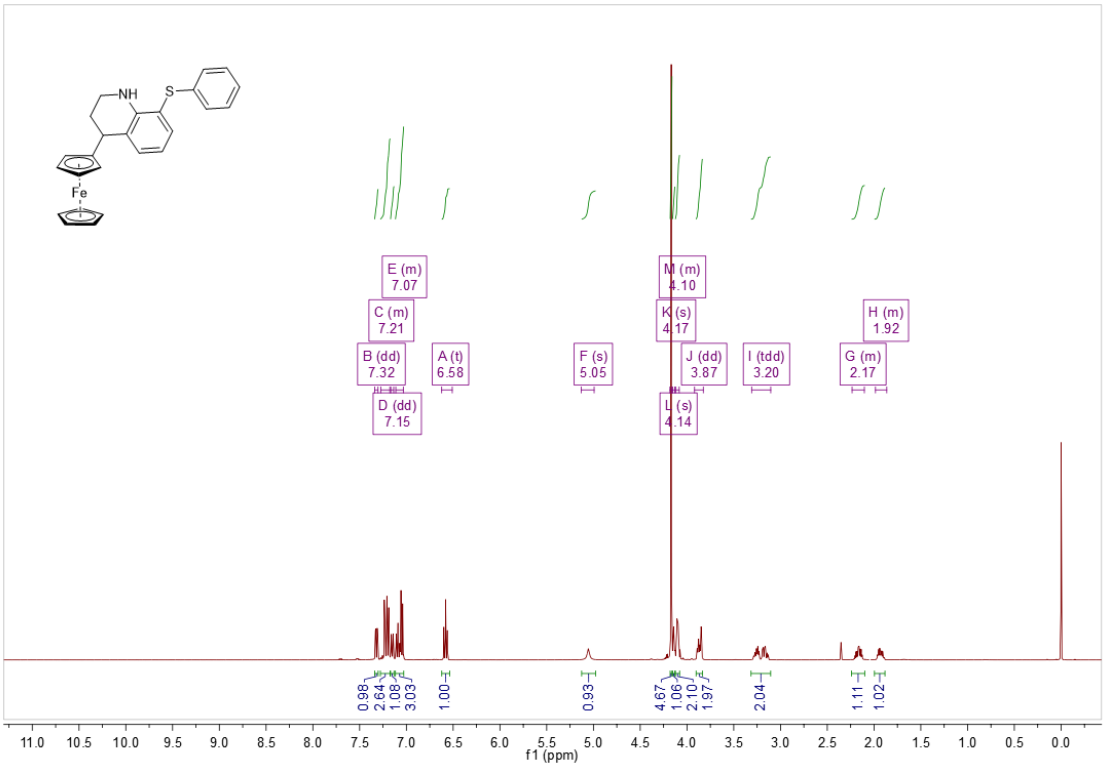


Figure 1. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **6.**

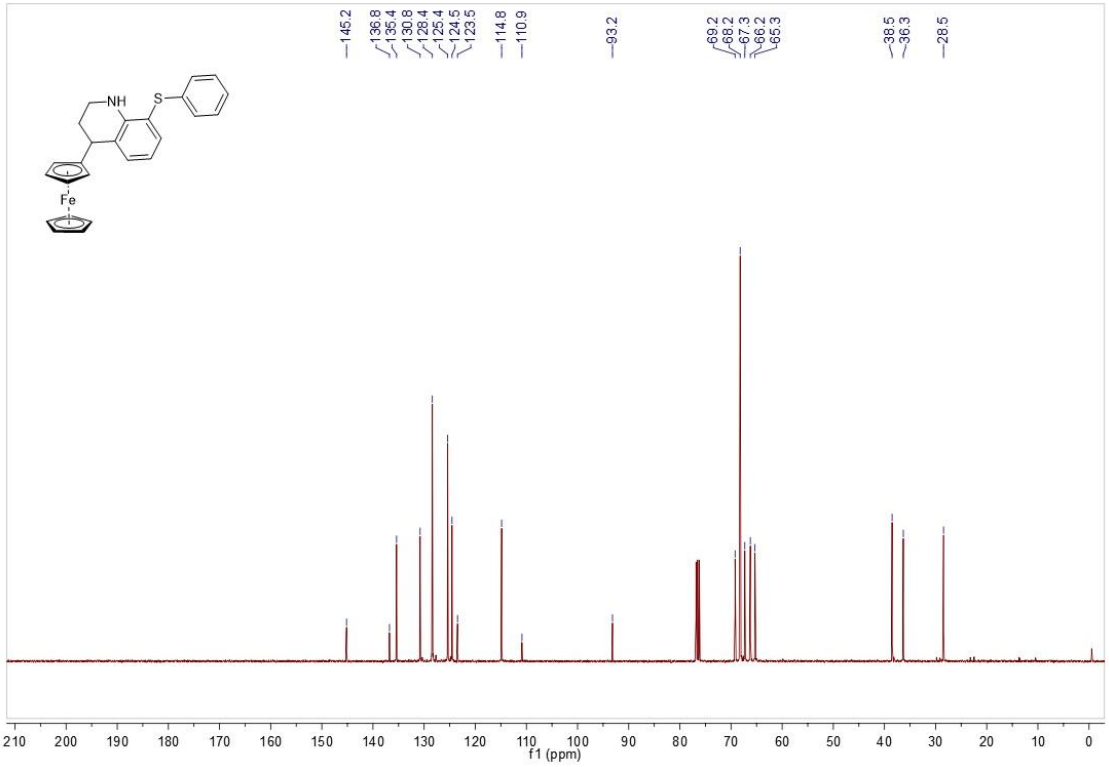


Figure 2. ^{13}C NMR ($CDCl_3$, 101 MHz) spectrum of compound **6.**

The main common feature of the IR spectra of synthesized compound **6** is strong band at 3345 cm^{-1}, which is attributed to the N-H stretching. Noteworthy, in the ^{1}H NMR spectra of product **6** signals of the unsubstituted Cp ring are down fielded to of the singlets assigned to the substituted Cp ring (δ = 4.17 ppm for the unsubstituted, and 4.12 – 3.87 ppm for substituted rings, respectively). Furthermore, in the ^{1}H NMR spectra of compound **6** characteristic signals for aromatic and aliphatic protons appear in the expected regions (see *Experimental part* and Figure 1.).

In the ^{13}C NMR spectra of the 4-ferrocenyl-8-(phenylthio)-1,2,3,4-tetrahydro-quinoline (**6**) signals of the ferrocene and tetrahydroquinoline core can be recognized in appropriate regions. Aromatic and ferrocene carbons show signals in characteristic areas (110–145 and 65–94 ppm, respectively). Aliphatic carbons from tetrahydroquinoline core were relatively non-sensitive to the neighboring NH -group occurring at δ(C) = 38.5 for Fc**C**H**C**H_2CH_2NH δ(C) = 36.3 for Fc**C**HCH_2CH_2NH and δ(C) = 28.5 for FcCHCH_2**C**H_2NH (see *Experimental part* and Figure 2.).

CONCLUSION

Briefly, the intramolecular cyclization of ferrocene-containing 1,3-aminoalcohol via corresponding α-ferrocenyl carbocation have been described. This protocol is practically convenient and proceeds under relatively mild conditions providing easy access to novel 4-ferrocenyl-8-(phenylthio)-1,2,3,4-tetrahydro-quinoline (**6**) in a good overall yield (67%). Structures of novel compounds were undoubtedly confirmed by standard spectroscopic techniques (IR and 1D and 2D NMR), as well as elemental analyses. Eventually, the synthesized compounds could be of interest for the bioactivity studies, therefore, further work to extend this methodology for the synthesis of other nitrogen fused heterocycles is under progress.

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REFERENCES

Biot, C., Castro, W., Botte, C. Y., & Navarro, M. 2012. The therapeutic potential of metal-based antimalarial agents: Implications for the mechanism of action. Dalton Transactions, 41, pp. 6335-6349. doi.10.1039/C2DT12247B

Bleiholder, C., Rominger, F., & Gleiter, R. 2009. α-Metallocenylmethylium Ions and Their Isoelectronic Congeners: A Comparison Based on DFT Calculations. Organometallics, 28, pp. 1014-1017. doi. 10.1021/om800573u

Dai, Z., Ni, J., Huang, X., Lu, G., & Bao, J. 2007. Direct electrochemistry of glucose oxidase immobilized on a hexagonal mesoporous silica-MCM-41 matrix. Bioelectrochemistry, 70, pp 250-256. doi.org/10.1016/j.bioelechem.2006.09.009

Damljanović, I., Stevanović, D., Pejović, A., Vukićević, M., Novaković, S. B., Bogdanović, G. A., Mihajilov-Krstev, M. T., Radulović, N. & Vukićević, R. D. 2011. Antibacterial 3-(arylamino)-1-ferrocenylpropan-1-ones: Synthesis, spectral, electrochemical and structural characterization. Journal of Organometallic Chemistry, 696, pp. 3703-3713. doi.org/10.1016/j.jorganchem.2011.08.016

Eicher, T., Hauptmann, S., & Speicher, A. 2003. The Chemistry of Heterocycles: Structure, Reactions, Syntheses, and Applications. Wiiey-VCH Veriag. 2nd ed., doi.10.1002/352760183X

Gambino, D., & Otero, L. 2012. Perspectives on what ruthenium-based compounds could offer in the development of potential antiparasitic drugs. Inorganica Chimica Acta, 393, pp. 103–114. doi.10.1016/j.ica.2012.05.028

Houlton, A., Roberts, R. M. G., & Silver, J. 1991. Studies on the anti-tumour activity of some iron sandwich compounds. Journal of Organometallic Chemistry, 418, pp. 107-112. doi.org/10.1016/0022-328X(91)86350-Y

Jaouen, G. 2006. Bioorganometallics: Biomolecules, Labeling, Medicine. Weinheim, FRG: John Wiley. doi:10.1002/3527607692

Kelland, L. 2007. The resurgence of platinum-based cancer chemotherapy. Nature Reviews Cancer, 7(8), pp. 573-584. doi:10.1038/nrc2167

Kowalski, K. 2018. Recent developments in the chemistry of ferrocenyl secondary natural product conjugates. Coordination Chemistry Reviews, 366, pp. 91-108. doi:10.1016/j.ccr.2018.04.008

Köpf-Maier, P., Köpf, H., & Neuse, E. W. 1984. Ferrocenium Salts-The First Antineoplastic Iron Compounds. Angewandte Chemie International Edition, in English, 23, pp. 456-457. doi.org/10.1002/anie.198404561

Madrid, P. B., Liou, A. P., Derisi, J. L., & Guy, R. K. 2006. Incorporation of an Intramolecular Hydrogen-Bonding Motif in the Side Chain of 4-Aminoquinolines Enhances Activity against Drug-Resistant P. Falciparum. Journal of Medicinal Chemistry, 49, pp. 4535-4543. doi.10.1021/jm0600951

Malisa, A. L., Pearce, R. J., Mutayoba, B. M., Abdullah, S., Mshinda, H., Kachur, P. S., Bloland, P. & Roper, C. 2011. The evolution of pyrimethamine resistant dhfr in Plasmodium falciparum of south-eastern Tanzania: comparing selection under SP alone vs SP+artesunate combination. Malaria Journal, 10(317), pages 13. doi.10.1186/1475-2875-10-317

Minić, A., Bugarinović, J., Ilić-Komatina, D., Bogdanović, G. A., Damljanović, I., & Stevanović, D. 2018. Synthesis of novel ferrocene-containing 1, 3-thiazinan-2-imines: One-pot reaction promoted by ultrasound irradiation. Tetrahedron Letters, 59, pp. 3499-3502. doi. 10.1016/j.tetlet.2018.08.029

Minić, A., Stevanović, D., Damljanović, I., Pejović, A., Vukićević, M., Bogdanović, G. A., Radulović, N. & Vukićević, R. D. 2015. Synthesis of ferrocene-containing six-

membered cyclic ureas via α-ferrocenyl carbocations. RSC Advances, 5, pp. 24915-24919. doi. 10.1039/C5RA01383F

Minić, A., Stevanović, D., Vukićević, M., Bogdanović, G. A., D'hooghe, M., Radulović, N., & Vukićević, R. D. 2017. Synthesis of novel 4-ferrocenyl-1, 2, 3, 4-tetrahydroquinolines and 4-ferrocenylquinolines via α-ferrocenyl carbenium ions as key intermediates. Tetrahedron, 73, pp 6268-6274. doi. 10.1016/j.tet.2017.09.014

N'da, D., & Smith, P. 2014. Synthesis, in vitro antiplasmodial and antiproliferative activities of a series of quinoline-ferrocene hybrids. Medicinal Chemistry Research, 23, pp. 1214-1224. doi.org/10.1007/s00044-013-0748-4

Pejović, A., Stevanović, D., Damljanović, I., Vukićević, M., Novaković, S. B., Bogdanović, G. A., Mihajilov-Krstev, M. T., Radulović, N. & Vukićević, R. D. 2012. Ultrasound-assisted synthesis of 3-(arylamino)-1-ferrocenylpropan-1-ones. Helvetica Chimica Acta, 95, pp. 1425-1441. doi.org/10.1002/hlca.201200009

Pejović, A., Danneels, B., Desmet, T., Cham, B. T., Nguyen, T., Radulović, N. S., Vukićević, R. D., & D'hooghe, M. 2015. Synthesis and Antimicrobial/Cytotoxic Assessment of Ferrocenyl Oxazinanes, Oxazinan-2-ones, and Tetrahydropyrimidin-2-ones. Synlett, p. 1195-1200. doi. /s-0034-1380348

Salas, P. F., Herrmann, C., & Orvig, C. 2013. Metalloantimalarials. Chemical Reviews, 113, pp. 3450-3492. doi.10.1021/cr3001252

Supan, C., Mombo-Ngoma, G., Dal-Bianco, M. P., Salazar, C. L. O., Issifou, S., Mazuir, F., Filali-Ansary, A., Biot, C., Ter-Minassian, D., Ramharter, M., Kremsner, P. G. & Lell, B. 2012. Pharmacokinetics of Ferroquine, a Novel 4-Aminoquinoline. In Asymptomatic Carriers of Plasmodium falciparum Infections. Antimicrobial Agents and Chemotherapy. 56, pp. 3165–3173. doi. 10.1128/AAC.05359-11

Sridharan, V., Suryavanshi, P. A., & Menéndez, J. C. 2011. Advances in the Chemistry of Tetrahydroquinolines. Chemical Reviews, 111(11) p.p. 7157-7259. doi.10.1021/cr100307m

Taylor, A. P., Robinson, R. P., Fobian, Y. M., Blakemore, D.C., Jones, L. H., & Fadeyi, O. 2016. Modern advances in heterocyclic chemistry in drug discovery. Organic and Biomolecular Chemistry, 14, pp. 6611-6637. doi.10.1039/C6OB00936K

Togni, A. 1996. Planar-Chiral Ferrocenes: Synthetic Methods and Applications. Angewandte Chemie International Edition in English, 35(1314), pp. 1475-1477. doi:10.1002/anie.19961475

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ELEMENTS OF BIOCLIMATOLOGICAL CHARACTERISTICS OF VRANJSKA SPA

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ABSTRACT

Bio-climatological analysis of Vranjska spa, based upon the equivalent temperatures (Teq) and Charles method (vapor weight), along with anthropo-climatic classification of Kruger, updated and adjusted, served as means of determining bio-climatological characteristics. Equivalent temperatures have been calculated for the period between 1985 and 2005, for the meteorological station Vranje, placed into six bio-climatological classes and three weather types. Representative weather types are cold (5.0 °C < Teq < 22.0 °C), agreeable (22.0 °C < Teq < 50.0 °C) and overheated (50.0 °C < Teq < 70.0 °C) with following classes: cold, algid, cool, agreeable, warm, humid and sultry.

Keywords: Equivalent temperature, Vapor weight, Bio-climatology, Vranjska spa, Anthropo-climatic classification, Weather types and classes.

INTRODUCTION

Bio-climatological influence is of great significance, regarded from the medical aspect of multiple effects the climate has on health and the choice of spa. According to Pecelj (2007), mission of ecological paradigm represents the establishing of harmony between a human being and nature through radical change of prevalent system of values and reforming of anthropocentric consciousness and ethic into eco-centric forms and contents of bio-climatological prospecting in eco tourism, thus representing a great opportunity.

When it comes to eco climatology (a symbiosis of climatology and ecology), we start from the scientific definition which further develops to interdisciplinarity, being a ground for understanding the functioning of the earth regions in climate system. All the changes in ecosystems are a significant feedback for climate system. The flow of energy and cycling of substance, water, chemical elements and gasses in ecosystems make climate determinants (Blazejczyk, 2004). Therefore, eco-climatological researches are conducted more often. Eco-climatology, as a scientific discipline, developed out of common interests of scientific researches of appearances and processes related to the atmosphere and biosphere.

According to Bonan (2002) eco-climatology is a combination of physical climatology, micro-meteorology, hydrology, pedology, plants physiology, biochemistry and biogeography of vegetation, made for the purpose of understanding the physicochemical and biological processes which influence climate, and *vice versa*. The importance of knowledge related to climatic and bioclimatic references of climate changes with which humanity faces today, is immeasurable (Pecelj, 2004). It is particularly important to point out the effect of global climate change to eco-climatic and bioclimatic values of spas, being important therapeutic, recreational and ecological destinations (Pecelj et al., 2010). Touristic marketing would be more complete, if it contained bio-climatological analyses and bio-prognosis (Matzarakis, 2006). Since tourism is one of the most perspective economic branches and climatic places ecologically most preserved in ecumene (Blanc, 1975), it is necessary to conduct climatological and bio-climatological analyses (Conrad, 1944). Accordingly, eco-climatological analysis of Vranjska spa has been conducted. Vranjska spa is 12 km away from Vranje, in the direction of north-east, and 5 km away from the international highway E75. It is located in the east part of the basin of Vranje, in the valley of the river Banjštica, the right affluent of the South Morava. Vranjska Spa is at the elevation of 380 m, surrounded with mountains Besna Kobila (1.922 m), Srpska Cuka (1.415 m), Veliki Pester (1.946 m) and Patarica (1.806 m), and therefore protected from strong winds, with temperate continental climate (Kostić, 1965). Vranjska Spa is famous for health tourism, due to the presence of thermal springs. The temperature of the water is 96 ºC in old boreholes, whereas in new, under pressure the temperature is 110 ºC. It is one of the hottest springs in Europe.

Bio-climatological observation of Vranjska spa is based on the combination of temperature and vapor pressure of water (Et=T+2e) which represents the base for determining the physiological feeling of warmth and weather types according to Kruger anthropo-climatic classification. Such a combination also includes, beside the temperature and vapor pressure of water, streaming of the air and air pressure, and it is known as the equivalent temperature. In bioclimatology, the importance of equivalent temperatures (temperature, water vapor pressure, air streaming and air pressure) is reflected through different feelings of warmth in healthy and sick people. In relation to equivalent temperatures there are three weather types (cold, agreeable and overheated) and six physiological feelings of warmth (from the feeling of cold, to the feeling of humidity).

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For the purpose of completing bio-climatological characteristics, beside equivalent temperatures, we further use the vapor weight (Charles method), based on the combination of temperature and relative humidity. The basis of this method is that heat is being transferred more easily in case of dry and windy weather, and more difficult in case of wet weather, without wind. Such unbearable heat is referred to as sultriness in climatology. Wind is also an important bioclimatic agent, since it influences on temperature characteristics, which further influences on bioclimatic effects on humans (Matzarakis, 2002). In dependence of air humidity and air pressure, people experience different feelings of warmth, although at the same temperature (Dukić, 1981).

Equivalent air temperature is practically the temperature of dry air, which would be a result of wet air water vapor condensation under constant pressure, the process which would release the heat spent during the previous process of evaporation. Latent heat, which is released during the processes of condensation and sublimation, is of high importance for equivalent temperatures. The transition of water into different states of aggregation is followed by the release or consumption of energy, thus provoking gigantic processes in geographical belt. To have 1 gram of water turned into gaseous state, it takes the energy of $2.533*10^3$ J (Milosavljević, 1985).

This bio-climatological analysis is based on the data from meteorological station in Vranje, for the period from 1981 to 2010 (Republički hidrometeorološki zavod, meteorološki godišnjaci). In reference to the connection of temperature, water vapor pressure and relative humidity, weather types and physiological feelings of warmth (equivalent temperatures) have been obtained and vapor weight (Table1, 2 and 3, Figure 1 and 2).

Table 1. Classification of physiological feelings of warmth and weather types.

Teq (°C)	Physiological feeling of warmth	Weather type
< 5	Very cold	Cold type
15-18	Cold	
18-22	Algid	
22 – 30	Cool	Agreeable type
30 – 40	Agreeable	
40 – 50	Warm	
50 – 58	Humid	Overheated type
58 – 70	Sultry	
> 70	Very sultry	

PSYSIOLOGICAL FEELING OF WARMTH AND WEATHER TYPES IN VRANJSKA SPA

Cold weather type (5.0 °C < Teq < 22.0 °C) is present in January, February and March. Physiological feeling of warmth classified as cold (Teq = 5.0-18.0 °C) occurs in January (Teq = 9.6 °C) and in February (Teq = 11.9 °C). Physiological feeling of warmth for the algid weather type (Teq = 18.0 - 22.0 °C) is characteristic of March (Teq = 18.3 °C). Physiological feeling of warmth for the type very cold (Teq < 5.0 °C) is not present. Winter is the season with the lowest level of accumulated latent heat. January, February as well as the most of winter, are characterized by the dominant influence of middle-European anticyclone which brings dry and cloudy weather. It is the period of inverse manifestations of primarily radiation type, which is the consequence of both weather and local conditions.

Agreeable weather type (22.0 °C < Teq < 50.0 °C) occurs in April, May, June, September, October, November and December. Physiological feeling of warmth which is referred to as cool, according to the classification (Teq = 22.0 – 30.0 °C) is present in April (Teq = 28.1 °C) and December (Teq = 22.5 °C). The class agreeable (Teq = 30.0 – 40.0 °C) is present in May (Teq = 39.8 °C), October (Teq = 32.9 °C) and in November (Teq = 32.2 °C). The class warm (Teq = 40.0 – 50.0 °C) is present in June (Teq=49.1 °C) and in September (Teq = 43.3 °C). It is the period of the effects of anticyclone activities, and favorable bio-climatological characteristics. The level of accumulated latent heat is then the highest, which is the consequence of thermal characteristics, whose annual cycle follows the water vapor pressure. Overheated weather type (50.0 °C < Teq < 70.0 °C) occurs in July (Teq = 52.9 °C) and in August (Teq = 52.0 °C) within the class humid (Teq = 50.0 - 58.0 °C). The reason lies in antropogeographic influence and in relief which limits the intense streaming of air. There is no occurrence of the classes sultry and very sultry.

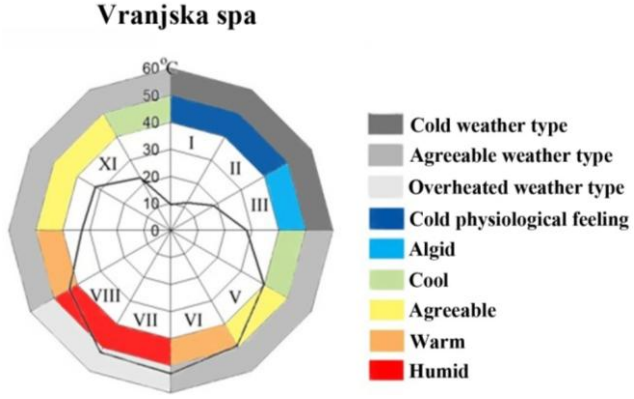

Figure 1. Average monthly equivalent temperatures in Vranjska spa.

ANALYSIS OF THE DEGREE OF AGREEABILITY BY CHARLES' METHOD

German climatologist Charles in his work: „Humidity comfort zone and limiting humidity zones", published in 1950 (Charles, 1950), experimentally defined boundary values of temperature in the function of relative humidity in the absence of

wind, based on which, the degree of physiological disagreeability in hotter and colder part of the year can be presented.

Table 2. Annual cycle of equivalent temperatures (Vranjska spa 1981-2010) in °C.

Teq (°C)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Vranjska Spa	9.6	11.9	18.3	28.1	39.8	49.1	52.9	52.0	43.3	32.9	32.2	22.5

Table 3. Boundary values of temperature and relative humidity for the determining the degree of disagreeability according to Charles.

Upper boundary values of temperature															
f (%)	100	95	90	85	80	75	70	65	60	55	50	45	40	35	30
Tb2 (°C)	15.5	17.3	18.2	19.1	20.1	21.1	22.2	23.4	24.8	25.2	28	30.1	32.2	34.8	35.5
Lower boundary values of temperature															
f (%)		95	90	85	80	75	70	65	60	55	50	45	40	35	
Tb1 (°C)		3.8	3.5	2.8	2.2	1.8	1.5	0.5	0	-0.3	-0.5	-1.5	-2.5	-2.8	

Two formulas have been presented for the calculating of boundary values of temperature for colder (Tb1) and hotter part of the year (Tb2), respectively:

$Tb1 = (-0.0003 \cdot f^2) + (0.1497 \cdot f) - 7.7133,$

$Tb2 = (-17.089 \cdot \ln(f)) + 94.979,$

where Tb is boundary value of temperature (°C) and f is relative humidity (%).

These boundary values of temperature in dependence of relative humidity represent the value based on which we determine bio-climatological agreeability or disagreeability. In Table 3 (lower boundary value of temperature) for each value of relative humidity, there is a boundary value of air temperature, under which human body feels physiological disagreeability, in the absence of wind. Index of winter disagreeability according to Charles refers only to values of relative humidity above 40% and it is sensitive to the temperature range between -6°C and 5 °C. Beyond the range, index shows extreme values of classification, representing physiological comfort at the temperatures above 5°C and extremely cold disagreeability at the temperatures below -6°C.

In Table 3 (upper boundary value of temperature) for each value of relative humidity, there is a boundary value of air temperature, above which human body feels disagreeable in the absence of wind. Index of winter disagreeability, according to Charles, refers only to the values of relative humidity above 30% and it is sensitive to the temperature range between 17°C and 39 °C. Beyond the range, index shows extreme values of classification, representing physiological comfort at the temperatures below 17°C and extremely hot disagreeability at the temperatures above 39°C.

By analyzing the average monthly values of temperature and relative humidity for the period from 1981-2010, it is perceived that the degree of disagreeability in the spa occurs only in the colder part of the year, in January and February, whereas in hotter part of the year there is no disagreeability, except in the case of sultriness in July and August.

Charles' method was represented by the formula, with the help of which boundary values of temperature are determined, in the function of relative humidity, without the influence of wind. The degree of physiological disagreeability is shown for colder (Table 1) and hotter part of the year (Table 2), pointing to a different bioclimatic situation in comparison with former simplified graphs we were using. According to Charlaues' method, the degree of disagreeability occurs in colder part of the year (January and February), whereas there is no disagreeability in hotter part of the year, except in case of sultriness in July and August.

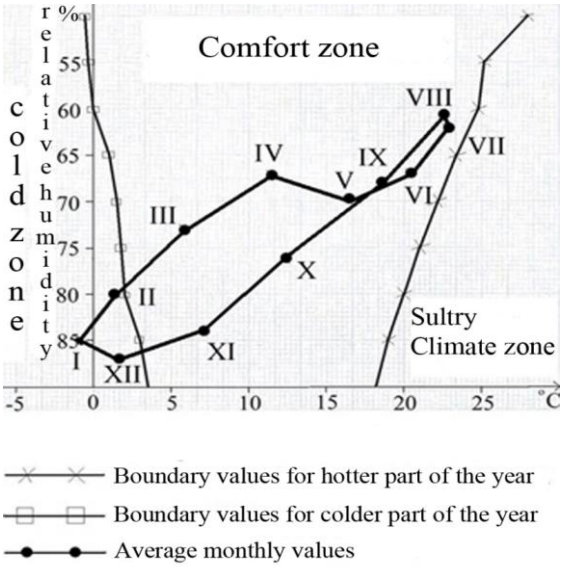

Figure 2. The average occurrence of sultriness in Vranjska spa.

CONCLUSION

Agreeable weather type is predominant and it lasts for seven months (April, May, June, September, October, November and December). The class *Agreeable* dominates in the middle of spring in May (Teq = 39.8 °C), in October (Teq = 32.9 °C) and in November (Teq = 32.2 °C). Cold weather type lasts for three month with dominant classes of *cold and algid*. The class which

is referred to as *humid* during July and August implicates the presence of Overheated weather type.

By using Charles' method for the determining of sultriness, through combination of relative humidity and temperature, there is a visible curve in vapor weight, being pretty distant from the boundary between the sultry climate zone and comfort zone (agreeable feelings). Thus, there is no real sultriness, even beside high summer temperatures, the reason being in low relative humidity during the summer months. Of course, based on average monthly temperatures, sultry days are not excluded (which is regarded relatively within the very procedure, i.e. the method).

From the point of bioclimatology, in respect to equivalent temperatures and vapor weight which influence the physiological effects of climate, Vranjska spa and its surrounding area is considered to be a beneficial area, which can be further improved in accordance with physico-geographical and anthropo-geographical predispositions, and valorized adequately. It is a matter of serious considerations, belonging to the area of medical policy, touristic plans and interests, as well as contemporary ecological demands.

REFERENCES

Blanc de, J. 1975. Man in the cold. Ch.C. Thomas Publ., Springfield.

Blazejczyk, K. 2004. Assessment of radiation balance in man in various meteorological and geographical conditions, Geographia Polonica, 77(1), pp. 63-76.

Bonan, G. 2002. Ecological Climatology, Concepts and applications. Cambridge: University Press.

Charles, K. 1950. Humidity comfort zone and limiting humidity zones. Erdkunde, v.4, pp. 188-201.

Conrad, V. 1944. Methods in Climatology, Harvard University Press, Cambridge, Massachusetts, p.228.

Dukić, D. 1981. Klimatologija. Beograd: Naučna knjiga.

Kostić, M. 1965. Vranjska Banja. Leskovački zbornik, Knj. V, str. 85-109; Narodni muzej Leskovac, Leskovac.

Matzarakis, A. 2006. Weather- and climate-related information for tourism. Tourism and Hospitality Planning and Development, 3(2), pp. 99-115. doi:10.1080/14790530600938279

Matzarakis, A. 2002. Radiation and Termal Comfort, Proceedings of the 6ht Hellenic Conference in Meteorology, Climatology and Atmospheric Physics, Ioannina, Greece, pp. 739-744.

Meteorološki godišnjaci, 1981-2010. Republičkiv hidrometeorološki zavod, Beograd.

Milosavljević, M. 1985. Klimatologija. Beograd: Naučna knjiga.

Pecelj R. M., Pecelj M., Mandic D., Pecelj J., Vujadinović S., Secerov V., Sabić D., Gajić M., & Milinčić M. 2010. Bioclimatic Assessment of Weather Condition for Recreation in Health Resorts, Proceedings book of 8th WSEAS International Conference on Enviroment, Ecosystems and Development, Athens, Greece, pp. 211-214.

Pecelj, M., Milincic, M., & Pecelj, M. 2007. Bioclimatology and ecoclimatology prospecting: Right out development. Glasnik srpskog geografskog društva, 87(2), pp. 199-210. doi:10.2298/gsgd0702199p

Pecelj, R. M. 2004. Bioklimatska istraživanja, metodologija i multidisciplinarnost, Radovi, Filozofski fakultet, Istočno Sarajevo.

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Original Scientific Paper

CAUSES AND CONSEQUENCES OF DEPOPULATION IN THE MUNICIPALITY OF KURŠUMLIJA

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ABSTRACT

The municipality of Kuršumlija is located in the South of Serbia, encompassing the area of 952 km². According to the census from 2011, it is inhabited by 19,213 people. During the last decades, the municipality is facing the rapid decrease in the number of inhabitants, caused by natural and social factors. The manuscript analyzes the demographic changes reflected in the migration and structure of population in the period after 1948, along with the beginning of the industrial development, to 2011, when the municipality experienced the crash of economy. The analysis established that the municipality of Kuršumlija became emigrational area, especially after the closure of large industrial facilities which were the pillars of development. The young ones and those able for work were forced to leave their hearths, in search for employment and survival. All the settlements of Kuršumlija were included in the negative demographic processes reflected in the constant decrease of population. Big problem was also the intensity of inhabitants' ageing, and lessening of the percentage of young ones, which points out that the municipality is in the phase of the deepest demographic age. The worrying fact is that the tendency of the population rate decrease in the settlements of Kuršumlija 'municipality is prolonged.

Keywords: Demographic changes, Depopulation, Migration, Population, Households.

INTRODUCTION

Migrations represent voluntary or enforced leaving of the place of permanent residence for a certain period of time (Bakić, 1977). They can be of permanent or temporary character (Vojković, 2007). One of the roughest division is on legal and illegal, i.e. voluntary and enforced (Bakić, 1977). Migrations of population may have positive and negative effects. The positive ones are provision of better life conditions for migrants and their families, whereas the negative ones are total or partial voidance of certain areas and depopulation of the same (Blagojević, 2016). In the last fifty years the number of migrants has been very big and it is constantly increasing. The inhabitants of those parts of the world endangered by war are those who are migrating most often, especially from the area of Middle East, Israel and Syria (Bobić et al., 2014). Europe has been always attracting migrants, thus constantly facing the large inflow of population. According to the United Nations' records, more than 50 million people migrated to Europe in the second half of the 20th, and the beginning of the 21st century (Morokvasić, 2013). Those parts of the world to which people mostly migrate suffer enormous demographic changes, which could be both positive and negative. When it comes to its structure, Europe is becoming more and more diverse due to the large inflow of young people. One of the negative consequences of migration is that the population moving in is at the lower level of culture, therefore these differences may cause the crash of a state and its welfare. Member countries of EU are most often those chosen for immigration because of the high life standard and possibility of employment.

In the countries which people emigrated from, negative demographic trends led to the decrease of birth rate, higher mortality rate and the intense ageing of population (Heleniak, 2015).

In the past, Serbia was always an emigrational area with long history and wide territorial dispersion of emigration (Grćić, 2010). The second half of the 20th century was marked by migration and transitory movements on the territory of Serbia as well as in the Balkans in general. In the first half of the 20th century, there were certain demographic changes being caused by the late and intense modernization and urbanization (Rudić, 1978). They brought about the intense migration of the work force on the relation between a country and a city (Maksimović, 1996). The last decade of the 20th century is characterized by economic issues which encompassed all the regions in Serbia, and contributed to the emigration mostly of those able for work, as well as highly qualified people to the countries of Western Europe (Marinović, 1999). According to the Statistical office of the Republic of Serbia, there are 313,411 people currently living abroad for the purpose of temporary work (2011), mostly in Austria and then in Germany and Switzerland. Migrations caused by the political situation occurred at the end of the 20th and the beginning of the 21st century, with the escalation of conflict between Serbians and Albanians in Kosovo and Metohija, which ended with the air attack of NATO against Serbia in March, 1999 (Marinović, 1999). Due to endangered safety, a large number of Kosovo' residents, running from persecution and war, inhabited borderland and central parts of the Republic of Serbia. These internal dispersions have significantly changed the structure of population in the areas they inhabited. One of the negative

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tendencies of migrations in Serbia was the spatial concentration of inhabitants in large urban environments, which caused low fertility rate, thus the intense ageing of the local inhabitants and depopulation (Bobić et al., 2014).

The topic of this research is the analysis of causes and consequences of demographic changes in the settlements of the municipality of Kuršumlija. The task of the research is to point to demographic changes, especially in the structure, which occurred within the period between 1948 and 2011, and which were the result of economic underdevelopment, as well as the analysis of movement and migrations of the overall population, which was conducted based on the statistical data collected from these periods 'census and field research. The results of the research should point to the consequences, which would be the ground for taking the appropriate measures to remove the flaws of SWOT analysis. It is necessary to use the chance given and prevent possible threats.

THEORETICAL PART

Study area

The municipality of Kuršumlija is located in southern Serbia, in the Toplica region. It is spread between 42°52'33" and 43°8'27" of northern latitude and between 19°09'28" and 21°16'4" of eastern longitude (Figure 1).

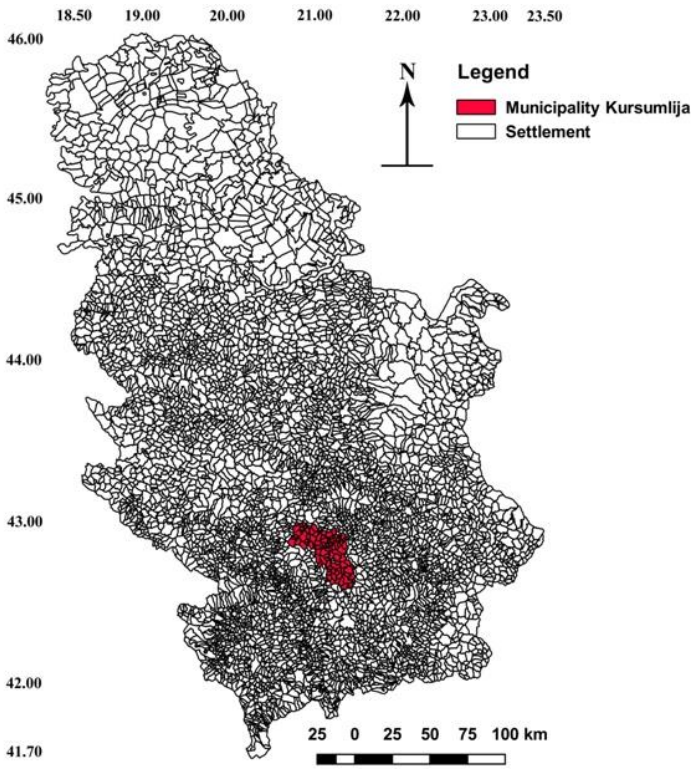

Figure 1. Position of the Municipality of Kuršumlija in the territory of Serbia.

According to its surface (952 km²), the municipality of Kuršumlija belongs to one of the largest municipalities in the Republic of Serbia. Kuršumlija is the largest municipality in the Toplica region, to which its territory belongs. It spreads between Radan mountain in the East, Majdan mountain and mountain pass Prepolac in the South and Kopaonik in the West. It borders with seven municipalities: in the North the municipality of Blace, in the northeast the municipality of Prokuplje, in the South and southeast with the municipalities of Bojnik and Medveđa, in the South the municipality of Podujevo, in the west the municipality of Leposavić, and in the northwest the municipality of Brus. The administrative center is the city of the same name, Kuršumlija, which spreads on the surface of 1, 131 ha of urban territory (Vukoičić, 2014). The municipality of Kuršumlija encompasses 88 settlements according to the records from 2011, with 19,213 inhabitants. The distance of the municipality from Belgrade is 307 km, through Niš, and 266 km through Kruševac. The distance of the municipality from Priština is 66 km through Merdare and 62 km through the mountain pass Prepolac.

EXPERIMENTAL

Materials and methods

In the summer of 2018, field researches were conducted in 20 rural settlements on the territory of the municipality of Kuršumlija. Five out of twenty of them are at the very border with Kosovo and Metohija (Trmka, Dabinovac, Mačja Stena, Rastelica and Mrča) (Vukoičić & Nikolić, 2013). Field researches and questionnaires for 120 inhabitants of different educational level and social status, confirmed the facts stated about the change in the overall number of inhabitants in the settlements of Kuršumlija as well as the prominence of migrations. Based on the same questionnaires, five interviews have been conducted, with people from Kosovo and Metohija. People who finished primary and high school, mostly from the countries, as well as highly educated people working in public facilities, or those currently unemployed, participated in the questioning.

Bad demographic picture is conditioned by the closure of large industrial facilities, which were the pillars of this area's development and the only chance for survival on this territory. In the period between 1948 and 2011, settlements on the territory of the municipality of Kuršumlija are characterized by negative demographic trends, caused by the intense emigration of population from this area (Vukoičić et al., 2011).

The study analyzes the circumstances which cause the massive emigration of population, through questionnaires and statistical analysis. Statistic method processes all the data from the enlisted censuses. Questionnaire data were processed in the statistical programme SPSS 21.

GIS analysis has shown all the settlements in which depopulation is total (with no residents, below 49 residents; from 50 to 199 residents; from 200 to 499; 500 to 999; 1,000 to 1,999 and above 2000 residents).

NUMERICAL RESULTS

The results of the research

In the settlements of the municipality of Kuršumlija there are currently 19,213 inhabitants, with the average age of 35.7 years (34.6 for men and 36.8 for women). There are 7,104 households with 2.7 members in average. The municipality of Kuršumlija is mostly inhabited by Serbians (Statistical office of the Republic of Serbia, 2011). After the World War II, according to the data from the conducted censuses, the number and density of population were growing until 1953. In the first census from 1948 in the settlements of Kuršumlija, there were 37, 284 inhabitants out of whom 2,797 were living in the city and 34,489 in other settlements. In the same year, the density of population was 33.9 residents/km².

In 1953, the number increased to 39,772 inhabitants out of whom 3,134 were living in the city and 34,487 in the other settlements. In the period between the first two censuses, the number of inhabitants increased for 2,448 or 6,6% (Table 1). It was accordingly followed by the increase of density of population, which reached its maximum in the same year, which was 41.7 residents/km². The highest agrarian density of population was also noted in the same year of census 38.9 /km², after which it was constantly decreasing.

Table 1. Movement of the total number of inhabitants in the settlements of the municipality of Kuršumlija between 1948 –2011.

Year	Total		Urban settlement		Rural settlements	
	Number	Growth / Fall Index	Number	Growth / Fall Index	Number	Growth / Fall Index
1948.	37.284	/	2.797	/	34.487	/
1953.	39.772	106,6	3.134	112,0	36.638	106,2
1961.	36.896	92,7	3.848	122,7	33.048	90,2
1971.	31.672	85,8	7.518	195,3	24.154	73,0
1981.	27.629	87,2	10.748	142,9	16.881	69,8
1991.	23.590	85,3	12.710	118,2	10.880	64,4
2002.	21.608	91.5	13.790	108,4	7.818	71,8
2011.	19.213	88,9	13.306	96,4	5.907	75,5

Source: Census of Population, Households and Flats in 2011, comparative overview, book no. 20, Belgrade, 2014

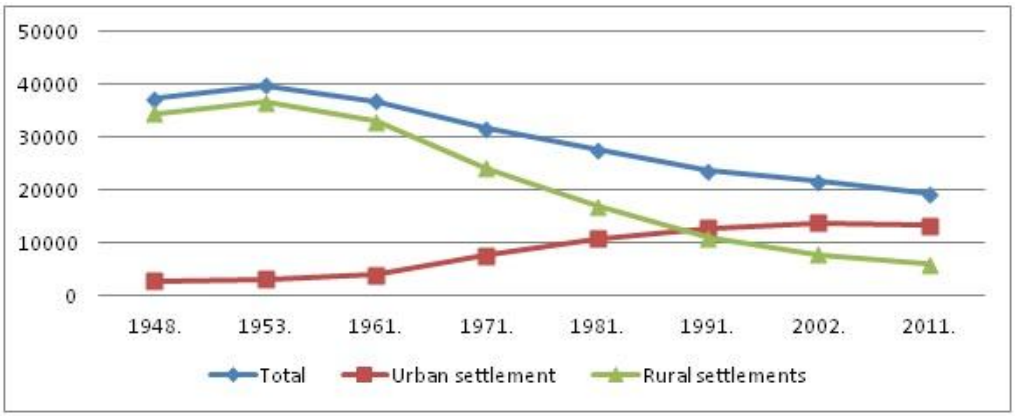

Figure 2. Movement of the total number of inhabitants in the settlements of the municipality of Kuršumlija between 1948 – 2011.

The number of inhabitants in the settlements of the municipality of Kuršumlija has been decreasing constantly in the past decades, which was caused by numerous factors. The only increase was in the period between two censuses, after the World War II, more precisely between 1948 and 1953. Since that period until the census in 2011, the number of inhabitants has decreased for 20,559 residents or 51,65%. According to these data, the settlements from Kuršumlija territory have lost a number of inhabitants greater than the number of those currently living there. The process of depopulation in the municipality of Kuršumlija was not equal in the inter-census periods between 1953 and 2011. In the period between 1953 and 1961 the number decreased for 2,876 people or 7.2%; from 1961 to 1971 for 5,224 or 14.1%; from 1971 to 1981 for 4, 043 (12,7%), from 1981 to 1991 for 4, 039 (14,6%), from 1991 to 2002 for 1, 982 (8,4%), and from 2002 until 2011 the number of residents decreased for 2, 395 or 11,1% (Table 1).

The same situation stands for the population density, therefore since 1953 until the census in 2011, the number is decreased for 48%. The decrease was mostly visible in rural areas, with the rapid fall of the number of inhabitants, the population density fell as well. The highest population density in

other settlements was in 1953-36.6 residents /km² and the lowest in 2011 – 6.2 residents /km² (Table 2). The city area marked the increase until 2002 when it reached 1,220.3 residents /km², which was the consequence of massive migrations between villages and cities (Table 2). According to the data from 2011, the average population density in Serbia was 92 residents/km², in the Toplica region 40,6 residents/km², so that the density in the settlements of the municipality of Kuršumlija was smaller than the average density of the county and the Republic.

Tabel 2. Movement of average population density in settlements in the municipality of Kuršumlija st/km².

Year	1948.	1953.	1961.	1971.	1981.	1991.	2002.	2011.
Total	33,9	41,7	38,7	33,2	29,0	24,7	22,7	20,1
Urban settlement	247,52	277,3	340,5	665,3	951,1	1.124,7	1.220,3	1.177,5
Rural settlement	36,6	38,9	35,1	25.6	17,9	11,5	8,3	6.2

Source: Census of Population, Households and Flats in 2011, comparative overview, book no. 20, Belgrade, 2014

The settlements on the territory of Kuršumlija municipality are mostly of a jagged type, except the city area which is more compact. The settlements mostly belong to the mountain type. Those of a jagged type are located on the slopes of Kopaonik, at the farthest west of the municipality of Kuršumlija, but also on the slopes of Radan mountain, in the east of municipality (Vukoičić et al., 2011). In the period between 1953 and 2011 all the settlements of Kuršumlija municipality underwent the process of redistribution. This led to the constant increase of population in the city, followed by the intense decrease in rural areas. This process also had some positive effects on the unburdening of overpopulated rural areas, especially in the 1960s, because of the high fertility rate. Emigration of inhabitants was specifically from the villages on the slopes of Kopaonik and Radan.

Tabel 3. Number of inhabitants in the settlements of the municipality of Kuršumlija according to the international classification of settlements according to the census data from 1948 and 2011.

Size groups	1948.	2011.
to 49	/	44
50 – 199	20	38
200 – 499	48	4
500 – 999	18	/
1.000 – 1.999	2	/
2.000 and more		

According to the data from the table 3 in the periods of census in 1948 and 2011, the number of inhabitants drastically decreased in other settlements. The data from 1948 stated that only two settlements had below than 100 inhabitants-Žalica (92) and Rastelica (82). There were no settlements in the category below 49 inhabitants.

According to the data from 2011 census, there are 44 settlements below 49 inhabitants. In 1948, there were 20 settlements between 50 and 199 inhabitants, and in 2011 there were 38 (Table 3), (Figure 3). In the years that followed the World War II, two settlements had above 1,000 inhabitants – Rača with 1,143 and Spance, not very far from Kuršumlija, had 1,054 inhabitants in 1948. These two settlements suffered the greatest decrease in population between the first and the last census. Both of them have extremely good traffic position, which has been their pillar of development for a very long period. In Rača, there was also a unit of the textile factory "7 juli" from Kuršumlija which was hiring approximately 800 people from Rača and surrounding villages. Unsuccessful privatization left people unemployed and forced to emigrate to bigger economic centers in search for better life. The demographic image of these settlements changed then, since most of the emigrants were young, which caused the appearance of the ageing households. The city area had the identical situation. As long as the economy was good, and affected the fertility rate, the overall number of inhabitants was increasing. With the crash of large industrial facilities, especially "ŠIK KOPAONIK", which was hiring more than 3,000 people in its best years, intense emigration from this area was noted. The only possibility for the young and educated residents for employment is the existence of the enterprise "Planinka" whose business gives hope for survival and prosperity of the municipality.

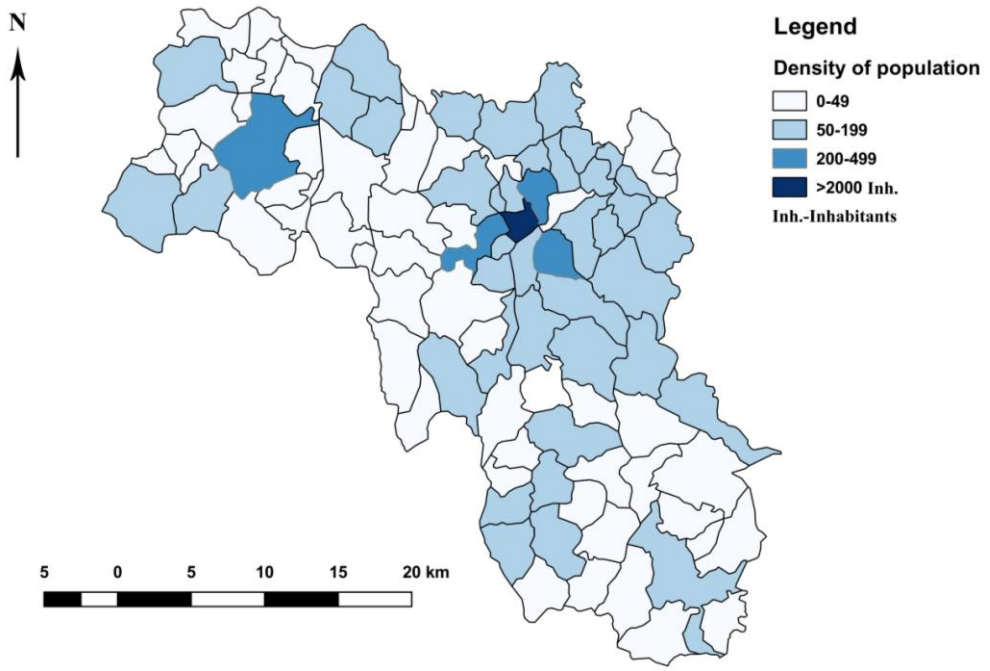

Figure 3. Dispersion and density of population according census of data from 2011.

Results of the questionnaires

51.2% of the overall number of the questioned residents were women. Most of them were at the age between 20 and 39 (51,2%) and most of them were living in a family community. The questionnaire mostly included unemployed people. Out of 127 participants, 11.8% never started working, 21.3% had up to two years of service, and 24.4% had between 2 and 5 years of service, which was at the same time the majority. 7.9 % of the questioned had between 5 and 15 years of service, 21.1% between 15 and 25 years of service, 7.8% between 25 and 30 years of service, and only 5.5% above 30 years. People of different work profiles participated in this questionnaire, from leading positions, highly educated, administrative workers to technical staff and salesmen. Considering the level of their education, 9.4% of them finished primary education, and 52.8% had finished high school which was the majority of the questioned. 0.8% of them were highly qualified, 11.8 % of them higher education, 13.4% with university degrees, and 6.3% of them were still studying (Figure 4).

37.8% of the questioned were without any income. Most of them (21.1%) had monthly income between 15,000 and 25,000 dinars. Almost all the participants stated that it was the first time or the to take part in the questionnaire related to the change of number of municipality residents. Based on the specific questions, 10% of the participants never thought about leaving their place of residence for the sake of employment or for any other reason, 59.8% of them never gave up this idea, while 29% thought about it, but gave up the idea for some other reasons. 51% of the emigrants left their homes for the sake of employment. Bad economic situation in the municipality and impossibility of employment was the main reason of emigration. 23.6% of the questioned stated that they left their homes temporary because of better paid work and for the sake of career advancement. 10 of them noted that their main reason for emigration would be the lack of understanding their environment had shown for their lifestyle.

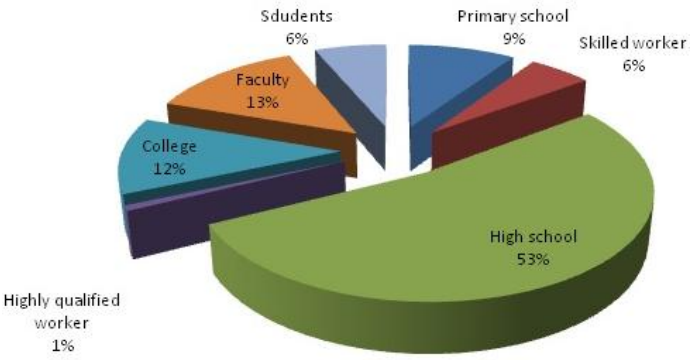

Figure 4. The educational structure of the respondents.

More than half of the questioned (63%) were living in their place of residence since their birth, while 33.9% emigrated from their residence on the territory of the same municipality, mostly villages, during the industrial development of the city. 5.5% of the questioned named education for the reason of their emigration, 7.9% emigrated because of economic reasons and impossibility of employment, 9% emigrated because of the endangered safety, while 15% emigrated for the sake of marriage. One part of the questioned stated that they visit their former residence, but have no intention of returning in any period. 35.4% of the participants stated that emigration is completely influenced by the vicinity of administrative border with Kosovo and Metohija, 101 km long, 46.5% stated that this

reason only partially influenced on emigration, while 15% stated that this reason took no share in the intense emigration from this area. In Kuršumlija, according to the social workers' records, there are 1980 people from Kosovo and Metohija living in the area, 78 of whom are refugees. They have also participated in the questionnaire, and based on the same questions, they confirmed the fact that the main reason for emigration was endangered safety. Figure 5 gives the display of the replies of the participants when asked about satisfaction with the current economic situation in the city. More than a half of them, 53.5% stated that they are very unsatisfied, 37% are unsatisfied, and 4.7% are partially satisfied. 2 of them stated that the economic situation in Kuršumlija is excellent and that they are very satisfied.

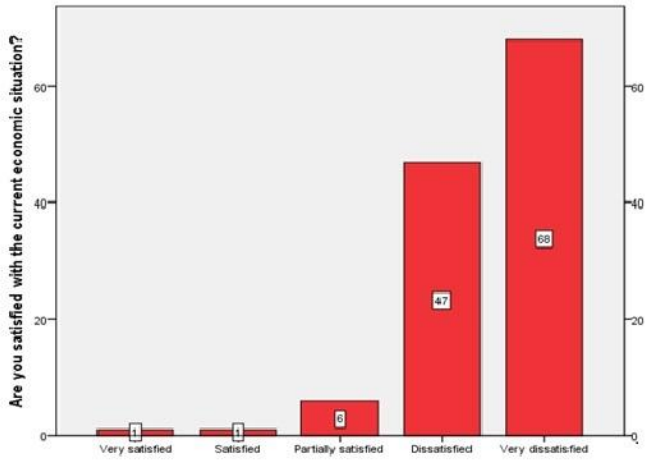

Figure 5.Question from the survey on the current economic situation.

Most of the participants is of opinion that there is a chance for the progress of economy in Kuršumlija, taking into consideration its natural resources and other potentials (Figure 6).

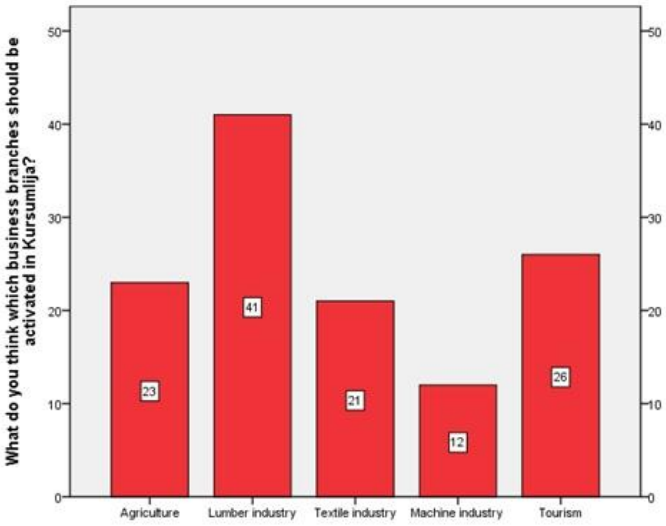

Figure 6. Question from the survey on potential industries to be activated.

Discussion

Kuršumlija is economically pretty underdeveloped area. Possibilities for employment are very little, and during the last few years inconsiderable. This represents the main reason for emigration from this area. Since most of the population emigrating is at its fertile age, it is no wonder that Kuršumlija is being depopulated. Based on the conducted statistical researches and questionnaires, it has been concluded that the number of inhabitants is at continuous decrease. The only increase of the overall population was caused by sequence of factors, firstly by economic prosperity and high fertility rate in the municipality.

The main consequence of depopulation on the territory of Kuršumlija are bad life conditions in mountain villages, reflected in their seclusion and bad traffic connection, as well as the inexistence of electricity, water supply, inexistence of school, health care, post office, supermarket or any other life necessities (Vukoičić et al., 2011).

Searching for work and better life conditions, inhabitants emigrated to bigger cities, where possibilities for employment were bigger. The decrease of fertility rate is one more reason for the depopulation of settlements. Based on the replies of the questionnaire participants, the vicinity of administrative border with Kosovo and Metohija, which is 101 km long, has also influenced the emigration. At the very crossing, depopulation is complete. Vukojevac, which is a settlement on the border, had 223 inhabitants in 1948, and according to the last census in 2011, there were no people. Industrialization brought about daily migrations, from the place of residence, mostly villages, to the place of employment. The number of residents on the territory of Kuršumlija mostly diminished in the period between 1981 and 1991 (Statistical office of the Republic of Serbia, 2014). Bad economy of that period also influenced the decrease. Large number of young and able for work emigrated from the municipality because of better life conditions and in search of employment. Temporary work abroad is one more reason for emigration between these two census periods. Based on the questionnaire, it has been concluded that there were many reasons for depopulation and migrations in Kuršumlija, starting with impossibility for employment, as well as the lack of understanding for lifestyle, which was an additional factor for emigration.

SWOT analysis represents a method of displaying positive and negative factors and gives opportunity for influencing them in time. The analysis helps us establish the advantages or strengths and to determine which municipality resources should be used, as well as the disadvantages or flaws. Furthermore, it determines the opportunities and potential threats for the future development of the municipality of Kuršumlija. Based on the SWOT analysis, it can be concluded that the municipality has significant natural potentials and resources at its disposal, which can contribute significantly to the progress. Good geographical position and the existence of a busy motorway E-80 should be

used properly. The construction of the highway from Niš to Priština is planned on the same direction, which will further promote the existing municipality's potential. Good traffic position can additionally contribute to the attracting of a large number of investors. Healthy environment without big industrial polluters represents a base for the development of ecological branches of economy and new branches of tourism (country, eco, winter, hunting, transit etc.) Special attention should be paid to the usage of renewable energy sources. "The Toplica region gives an excellent opportunity for the construction of solar panels and solar farms. This region as well as the whole southeast Serbia have good chances for new renewable sources of energy" (Valjarević, 2016)

Additional valorization of touristic potentials, as well as the enforcement of Kuršumlijska spa, which has been one of the most famous spas in Serbia for years, is only one of many advantages to be used. Opening of health care units in remote rural areas would stop the drain of population to some extent, and it should be given special attention. Enforcement of country tourism is also one of the advantages which require attention. "Country tourism is a way of revitalizing many rural areas, even those completely depopulated, and it is certainly the way to stop the emigration of young people, because it presupposes the creating of common conditions and higher standards of comfort in rural areas" (Vukoičić et al., 2016).

SWOT - analysis

Strengths	***Flaws***
- Peace in the region - Good geographical position - traffic position on an important motorway E-80, part Niš-Priština, as well as the planned construction of the highway - Existence of three spas (Kuršumlijska, Prolom, Lukovska), - Existence of the natural phenomenon and natural monument "Devil's city", - Good natural conditions (forests, waters, mines, geothermal and mineral waters, flora and fauna), - Possibilities for the application of sustainable energy sources (small hydroelectric power plants and sun collectors) - Rich cultural and historical heritage especially from the medieval period, (monasteries from the period of Nemanjići dinasty, from the 12th century, St. Nicholas and Virgin Mary) - Healthy environment without industrial polluters - Great possibilities for the development of eco-agriculture - Tradition and customs - Hospitality - Development of forestry, hunting industry, and woodworking - Significant possibilities for the development of new branches of tourism (country, eco, winter, hunting, transit and juvenile) - Low prices of products and services	- Political instability - Dormancy of numerous natural advantages (forests, tourism, hunting and fishing possibilities), - Unfavorable demographic status of the municipality, especially in the villages) , - Negative fertility rate and migration of the fertile residents, which leads to depopulation and intense ageing of population , - Bad traffic connection within the municipality, especially between rural areas - Extinction of large number of villages and whole parts of the municipality, especially at the administrative border with Kosovo , - Low industrial activity and extinction of old industrial branches , - Lack of documentation - Bad infrastructural equipment of greater part of municipality, even important villages , - Weak touristic offer, in the area of culture and services - Medieval cultural heritage is not properly included into touristic offer - Underqualified staff, - Low socio-economic status of population - Insecurity of new investments, - Disinterest for the reconstruction of village facilities which could be used for touristic purpose and promotion of country tourism -Vicinity of the administrative border with Kosovo and Metohija
Opportunities	***Threats***
- Recognition of the municipality on the Republican level, especially its touristic destinations - Higher level support (ministries and authorized public organizational, NGOs, foreign organizations for support and help) - Government support and support of the National directorate of employment in a way of providing subvention and non-refundable loans for self-employment , - Branding of agricultural products and their distribution on Serbian and European market - Recognition of spas and spa tourism as important for the development and promotion of Serbia,	- The position of the municipality in South Serbia, as the most underdeveloped and under populated part of the Republic - Politics domination over expertise - Dependence of the future development of the municipality on higher instances (state, region, international funds) , - Bad connection and cooperation with surrounding municipalities in the South, the West and the East - Long administrative border with Kosovo - Unstable political situation in the environment , - Uncontrolled exploitation of forests ,

- the existence of the program of cooperation and support from the state for the municipalities of South Serbia , - Inclusion of spas and locality " Devil's City" into developmental measures of the Republic, and inclusion of the region into appropriate plans and strategies - Possibilities for promotion of cooperation with surrounding municipalities - State highway Niš-Priština as a skeleton of further development of this part of Serbia	- Inability to attract educated and qualified work force from the surrounding area as a way of faster, future development , - More frequent migrations of young, highly educated inhabitants towards bigger economic centers.

CONCLUSION

Based on the conducted statistical and field researches, it has been concluded that the last decades of the 20th and the beginning of the 21st century were marked by prominent demographic changes, manifested through depopulation and intense ageing of the inhabitants. The main reasons for the structural changes and the decrease of overall population and depopulation are prominent emigration, fertility, economic, social and other factors. Taking it all into account, appropriate measures, regarding the population policy, should be taken to diminish the drain of population from this area. The only way to diminish the emigration is to revitalize once large industrial facilities, especially forest industry. Investing in agriculture and processing industry, and development of industrial branches based on existing natural resources and opening small family businesses will greatly decrease the emigration of population. Additional valorization of current touristic potentials, especially investing into rural tourism for whose development there are extraordinary conditions, would also diminish the drain of young and able for work from rural environments and cities. Negative demographic trends have been placing Kuršumlija and the Toplica region into areas with prominent depopulation for years, which had negative consequences for economic and social progress.

REFERENCES

Bakić R. 1977. Opšta demografija. Priština: Prirodno-matematički fakultet u Prištini.

Blagojević, V., & Milosavljević, B. 2016. Političko-pravni okvir savremenih migracija u Jugoistočnoj Evropi. Vojno delo, Institut za strategijska istraživanja, 3/2016, pp. 75-93.

Comparative Review 2014. Census of Population, Households and Flats in 2011. Comparative Review, No. 20, Belgrade.

Grćić, M. 2010. Demografske posledice ratnih gubitaka. Demografija. Geografski fakultet u Beogradu, Beograd.

Heleniak, T. 2015. Changes in the population in the former communist countries of Europe and Asia. In International Encyclopedia of Social and Vedic Science.

Maksimović, M. 1996. Hronika Kosanice. Kuršumlija: Opštinski odbor Kuršumlije.

Official Gazette of the Republic of Serbia 2012. Law on Migration Management. Official Gazette of the Republic of Serbia, No. 107/2012.

Population census, Comparative overview of the number of households, data by settlements 2012. Belgrade. Book 10.

Republic Statistical Office of the Republic of Serbia. Census of Population, Households and Flats in 2011.

Rudić, V. 1978. Stanovništvo Toplice. Beograd: Srpska akademija nauka i umetnosti - Etnografski institut. posebna izdanja, knjiga 17.

Valjarević, A., Đekić, T., Stevanović, V., Ivanović, R., & Jandziković, B. 2018. GIS numerical and remote sensing analyses of forest changes in the Toplica region for the period of 1953–2013. Applied Geography, 92, pp. 131-139. doi:10.1016/j.apgeog.2018.01.016

Valjarević, A. 2016. GIS modelling of solar potential in Toplica region. The University Thought - Publication in Natural Sciences, 6(1), pp. 44-48. doi:10.5937/univtho6-10739

Vukoičić, D., Punišić, M., & Nikolić, M. 2011. Demographic changes and processes in the network of settlements of the municipality of Kuršumlija. Geographical research, No. 3. Faculty of Natural Sciences and Mathematics, Department of Geography, Kosovska Mitrovica.

Vukoičić, D., & Nikolić, M. 2013. Promene broja stanovnika, domaćinstava i gustine naseljenosti u Topličkom okrugu u drugoj polovini XX i početkom XXI veka. Demografija, knj. X, Međunarodni časopis za demografska i ostala društvena istraživanja. Univerzitet u Beogradu, Geografski fakultet – Institut za demografiju, Beograd, ISSN 1820-4244.

Vukoičić, D. 2014. Tourist valorization of urban, spa and rural settlements Gornje Toplice. Kosovska Mitrovica: University of Prishtina with temporary headquarters in Kosovska Mitrovica - Accessory - Faculty of Mathematics.

Vukoičić, D., Nikolić, M., & Raičević, J. 2017. Rural Tourism As The Concept Of Sustainable Economic Development of the Kursumlija Municipalitu. In 4th International Scientific Conference Agribusiness MAK-2017 „EUROPEAN ROAD" IPARD 2015-2020, 2017, Kopaonik, Serbia. pp. 123-134.

Vukoičić, D., Nikolić, M., & Raičević, J. 2016. Contemporary demographic changes in settlements along the administrative line in Serbia towards autonomous province of Kosovo and Metohija. The University Thought - Publication in Natural Sciences, 6(2), pp. 17-26. doi:10.5937/univtho6-12758

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Original Scientific Paper

COMPARATIVE PERFORMANCE ANALYSIS OF SOME ACCELERATED AND HYBRID ACCELERATED GRADIENT MODELS

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ABSTRACT

We analyze a performance profile of several accelerated and hybrid accelerated methods. All comparative methods are at least linearly convergent and have satisfied numerical characteristics regarding tested metrics: number of iterations, CPU time and number of function evaluations. Among the chosen set of methods we numerically show which one is the most efficient and the most effective. Therewith, we derived a conclusion about what type of method is more preferable to use considering analyzed metrics.

Keywords: Gradient descent methods, Line search, Convergence rate.

ACCELERATED FACTOR IN ACCELERATED GRADIENT MODELS

We are analyzing accelerated gradient descent iterations for solving unconstrained optimization problems, mathematically described as:

$$\min f(x),\ x \in \mathbb{R}^n \tag{1}$$

where $f : \mathbb{R}^n \to \mathbb{R}$ is an objective function which we want to minimize. For function f we assume that it is uniformly convex and twice continuously differentiable function. Instead of usual iterative optimization schemes, expressed by:

$$x_{k+1} = x_k + t_k d_k, \tag{2}$$

we focus on *accelerated gradient iterations* given by the following expression

$$x_{k+1} = x_k - \gamma_k^{-1} t_k d_k. \tag{3}$$

In (2) and (3) x_{k+1} stays for the next iterative function value, x_k is the current iterative function value, t_k is the iterative step size value and d_k is the search direction vector. In (3) scalar γ_k presents an iterative approximation parameter. Many authors confirmed, mostly numerically, that this parameter upgrades performance profile of posed optimization method. Nevertheless, in Stanimirović & Miladinović (2010) a class of methods containing acceleration factor is denoted as *the class of accelerated gradient methods*. From the analysis presented in Petrović & Kontrec (2017) we can conclude that one of the most efficient way for calculating acceleration parameter is through the features of the second order Taylor's series taken on the objective accelerated iteration. Although there are some alternative modes for deriving the acceleration parameter, we mention here several highly efficient accelerated models with acceleration parameter obtained by the Taylor's development: Andrei (2006, 2008); Petrović & Stanimirović (2014); Petrović (2015); Stanimirović et al. (2015); Stanimirović & Miladinović (2010). In this regard we display the expressions for acceleration parameters of some above mentioned methods:

$$\theta_k^{AGD} = -\frac{t_k g_k^T g_k}{t_k y_k^T g_k}$$

$$\gamma_{k+1}^{ADD} = 2\frac{f(x_{k+1})-f(x_k)-\alpha_k g_k^T\left(\alpha_k d_k-\gamma_k^{-1}g_k\right)}{\left(\alpha_k d_k-\gamma_k^{-1}g_k\right)^T\left(\alpha_k d_k-\gamma_k^{-1}g_k\right)}$$

$$\gamma_{k+1}^{ADSS} = 2\frac{f(x_{k+1})-f(x_k)+\left(\alpha_k\gamma_k{}^{-1}+\beta_k\right)\|g_k\|^2}{\left(\alpha_k\gamma_k{}^{-1}+\beta_k\right)^2\|g_k\|^2},$$

$$\gamma_{k+1}^{TADSS} = 2\frac{f(x_{k+1})-f(x_k)+\psi_k\|g_k\|^2}{\psi_k^2\|g_k\|^2}$$

$$\gamma_{k+1}^{SM} = 2\gamma_k\frac{\gamma_k\left[f(x_{k+1})-f(x_k)\right]+t_k\|g_k\|^2}{t_k^2\|g_k\|^2}.$$

Regarding the theory of unconstrained optimization methods, we have a unique opinion that there are two crucial elements which defined a relevant iterative optimization scheme. The first one is the vector direction, d_k, which directs the minima search. It is usually required to fulfil the descending condition:

$$g_k^T d_k < 0. \tag{4}$$

The second, equally important, is the value of the iterative step length, t_k. This element is obtained trough the exact or inexact line search procedure. In practical purpose, the inexact algorithms are certainly more preferable choice for obtaining the optimal step size iterative value. With this regard, from all above exposed, we can rightly conclude that besides these two listed elements the value of acceleration parameter, γ_k, is also important and crucial factor for one optimization method.

This paper is organized in the following way. In the second section we give an overview of some important hybrid models and hybridization process applied on accelerated gradient schemes. In the main section 3, we display obtained numerical results of four chosen models, conduct a comparative analysis and bring up a conclusion.

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HYBRID MODELS

Some authors investigate a hybrid iterative systems for solving optimization problems. One of the first in this field was Picard. In his work Picard (1890) presented the following set of two iterations for solving optimization problems:

$$\begin{cases} u_1 = u \in \mathbb{C}, \\ u_{k+1} = Tu_k, \quad k \in \mathbb{N}, \end{cases} \tag{5}$$

Later on, Mann exposed his set of expressions and called it *mean value methods in iterations*

$$\begin{cases} v_1 = v \in \mathbb{C}, \\ v_{k+1} = (1-\alpha_k)v_k + \alpha_k T v_k, \quad k \in \mathbb{N}. \end{cases} \tag{6}$$

Further on, Ishikawa presented a three-term model as next:

$$\begin{cases} z_1 = z \in \mathbb{C}, \\ z_{k+1} = (1-\alpha_k)z_k + \alpha_k T y_k, \\ y_k = (1-\beta_k)z_k + \beta_k T z_k, \qquad k \in \mathbb{N}. \end{cases} \tag{7}$$

In the above displayed schemes v_k, z_k and y_k present the sequences defined by related iterations, parameters $\{\alpha_k\}, \{\beta_k\} \in (0,1)$ and $T : \mathbb{C} \to \mathbb{C}$ is a mapping defined on nonempty convex subset C of a normed space $\mathbb{E}$.

In a recent research Khan (2013), introduced the following set of relations

$$\begin{cases} x_1 = x \in \mathbb{R}, \\ x_{k+1} = Ty_k, \\ y_k = (1-\alpha_k)x_k + \alpha_k T x_k, \quad k \in \mathbb{N}. \end{cases} \tag{8}$$

In the same paper the author shows the advantages of posed process and confirms that so defined model outperforms previous three mentioned methods.

Taking good sides of the iteration set (8) the authors in Petrović et al. (2017) applied accelerated gradient descent SM method, presented in Stanimirović & Miladinović (2010), on this three-term relation. As a result a hybrid accelerated scheme is developed. We call this iteration the HSM method and it is defined by the expression:

$$x_{k+1} = x_k - \alpha t_k \gamma_k^{-1} g_k, \tag{9}$$

where parameter $\alpha \in (1,2)$ and $\gamma_k \equiv \gamma_k^{HSM}$ is iterative acceleration parameter which is computed using the second order Taylor's series of the HSM iteration

$$\gamma_{k+1} \equiv \gamma_{k+1}^{HSM} = 2\gamma_k \frac{\gamma_k \left[f(x_{k+1}) - f(x_k)\right] + (\alpha_k + 1)t_k \|g_k\|^2}{(\alpha_k+1)^2 t_k^2 \|g_k\|^2}. \tag{10}$$

In Petrović et al. (2017) the authors proved that the HSM method is at least linearly convergent on the set of uniformly convex and strictly convex quadratic functions. Numerical tests confirm significant benefits when the HSM scheme is used instead of its forerunner, the SM iteration. All these advantages indicate that this new hybridization concept can be applied on some other accelerated model and upgrade its features.

In Panić et al. (2018) some initial improvement is taken on the HSM iteration and the modified version of the HSM scheme is introduced. This model is denoted as the MHSM method. The improvement regarding the HSM iteration consists in reducing the initial step length value of the Backtracking line search algorithm. Numerical experiments show some betterment compared to the starting HSM method.

NUMERICAL COMPUTATIONS AND CONCLUSIONS DRAWN

In this section we expose comparative analysis of performance profile of four chosen methods. First comparative models is the accelerated gradient SM method presented in Stanimirović & Miladinović (2010). Second one is the hybrid accelerated method HSM from Petrović et al. (2017) which presents a hybridization of the SM method. Third model is the MHSM introduced in Panić et al. (2018) and it presents modified version of the HSM, where an initial improvement of starting value in Backtracking line search procedure was taken. Final comparative method is the accelerated gradient TADSS method which is revealed in Stanimirović et al. (2015). We now display algorithms of all listed methods:

Algorithm 0.1 SM-method Stanimirović & Miladinović (2010)

Require: Objective function $f(x)$ and chosen initial point $x_0 \in dom(f)$.

1: Set $k = 0$ and compute $f(x_0)$, $g_0 = \nabla f(x_0)$ and take $\gamma_0 = 1$.
2: If test criteria are fulfilled then stop the iteration; otherwise, go to the next step.
3: (Backtracking) Find the step size $t_k \in (0,1]$ using Backtracking procedure with $d_k = -\gamma_k^{-1} g_k$.
4: Compute $x_{k+1} = x_k - t_k \gamma_k^{-1} g_k$, $f(x_{k+1})$ and $g_{k+1} = \nabla f(x_{k+1})$.
5: Determine the scalar approximation γ_{k+1} of the Hessian of f at the point x_{k+1} using γ_{k+1}^{SM} representation.
6: If $\gamma_{k+1} < 0$, then take $\gamma_{k+1} = 1$.
7: Set $k := k+1$, go to the step 2.
8: Return x_{k+1} and $f(x_{k+1})$.

Algorithm 0.2 HSM-method Petrović et al. (2017)

Require: Function $f(x)$, $\alpha \in (1,2)$, initial point $x_0 \in dom(f)$.

1: Set $k = 0$ and calculate $f(x_0)$, $g_0 = \nabla f(x_0)$, set $\gamma_0 = 1$.
2: Check the test criteria; if stopping criteria are fulfilled then stop the algorithm; otherwise, go to the next step.
3: Applying Backtracking Algorithm: Compute the value of step size $t_k \in (0,1]$ taking $d_k = -\gamma_k^{-1} g_k$.
4: Determine $x_{k+1} = x_k - \alpha t_k \gamma_k^{-1} g_k$, $f(x_{k+1})$ and $g_{k+1} = \nabla f(x_{k+1})$.
5: Compute γ_{k+1}, approximation of the Hessian of function f at the point x_{k+1} using γ_{k+1}^{HSM} representation.
6: If $\gamma_{k+1} < 0$ take $\gamma_{k+1} = 1$.
7: $k := k+1$, go to the step 2.
8: Return x_{k+1} and $f(x_{k+1})$.

Algorithm 0.3 MHSM-method Panić et al. (2018)

Require: Function $f(x)$, $\alpha \in (1,2)$, initial point $x_0 \in dom(f)$.

1: Set $k = 0$ and calculate $f(x_0)$, $g_0 = \nabla f(x_0)$, set $\gamma_0 = 1$.
2: Check the test criteria; if stopping criteria are fulfilled then stop the algorithm; otherwise, go to the next step.
3: Applying Backtracking Algorithm: Compute the value of step size $t_k \in (0, \frac{1}{\alpha}]$ taking $d_k = -\gamma_k^{-1} g_k$.
4: Determine $x_{k+1} = x_k - \alpha t_k \gamma_k^{-1} g_k$, $f(x_{k+1})$ and $g_{k+1} = \nabla f(x_{k+1})$.
5: Compute γ_{k+1}, approximation of the Hessian of function f at the point x_{k+1} using γ_{k+1}^{HSM} representation.
6: If $\gamma_{k+1} < 0$ take $\gamma_{k+1} = 1$.
7: $k := k + 1$, go to the step 2.
8: Return x_{k+1} and $f(x_{k+1})$.

Algorithm 0.4 TADSS-method Stanimirović et al. (2015)

Require: $0 < \rho < 1, \quad 0 < \tau < 1$, x_0, $\gamma_0 = 1$.

1: Set $k = 0$, compute $f(x_0)$, g_0 and take $\gamma_0 = 1$.
2: If $\|g_k\| < \epsilon$, then go to Step 9, else continue by the next step.
3: Find the step size α_k applying Backtracking Algorithm.
4: Compute x_{k+1} using $x_{k+1} = x_k - [\alpha_k(\gamma_k^{-1} - 1) + 1]g_k$.
5: Determine the scalar γ_{k+1} using γ_{k+1}^{TADSS} representation.
6: If $\gamma_{k+1} < 0$ than take $\gamma_{k+1} = 1$.
7: Set $k := k + 1$, go to the step 2.
8: Return x_{k+1} and $f(x_{k+1})$.

We conducted numerical tests, for each comparative model, on 12 functions from Andrei (2008) for ten different number of variables: 100, 500, 1000, 1500, 2000, 3000, 5000, 7000, 8000, 10000. In the next six tables we reveal achieved results of all tested models. In order to simplify the table representations we paired the results of the SM and the MHSM algorithms and there with the results of the HSM and the TADSS. In the first three tables i.e. Tables (1) (2) and (3) we illustrate the results of obtained number of iterations, CPU time and the number of function evaluations, respectively, for the first pair of methods (SM and MHSM).

Table 1. Numerical results of *SM* and *MHSM* methods tested on 12 large scale test functions regarding number of iterations metric.

Test function	No. of iterations	
	SM	MHSM
Extended Penalty	536	449
Perturbed quadratic	41689	9228
Raydan-1	14149	7374
Extended Three Expon...	141	320
Quadratic QF1	45245	6530
Extended Quad. Penalty QP1	225	289
Extended Quad. Penalty QP2	1582	5247
Quadratic QF2	46662	11281
Extended EP1	63	217
Arwhead	228	1312
Almost Perturbed Quadratic	45098	9344
QUARTC Function	10	10

Table 2. Numerical results of *SM* and *MHSM* methods tested on 12 large scale test functions regarding CPU time metric.

Test function	No. of iterations	
	SM	MHSM
Extended Penalty	536	449
Perturbed quadratic	3	5
Raydan-1	60	80
Extended Three Expon...	0	2
Quadratic QF1	365	66
Extended Quad. Penalty QP1	0	3
Extended Quad. Penalty QP2	5	52
Quadratic QF2	504	186
Extended EP1	0	0
Arwhead	6	39
Almost Perturbed Quadratic	501	116
QUARTC Function	0	0

Table 3. Numerical results of *SM* and *MHSM* methods tested on 12 large scale test functions regarding number of function evaluations metric.

Test function	No. of func.evaluations	
	SM	MHSM
Extended Penalty	2851	5487
Perturbed quadratic	233149	74903
Raydan-1	76418	43588
Extended Three Expon...	723	1525
Quadratic QF1	253994	48796
Extended Quad. Penalty QP1	2305	2332
Extended Quad. Penalty QP2	11307	37303
Quadratic QF2	258694	100259
Extended EP1	628	1947
Arwhead	4142	13105
Almost Perturbed Quadratic	250191	76563
QUARTC Function	30	30

Table 4. Numerical results of *TADSS* and *HSM* methods tested on 12 large scale test functions regarding number of iterations metric.

Test function	No. of iterations	
	TADSS	HSM
Extended Penalty	40	400
Perturbed quadratic	11618	17086
Raydan-1	823	8377
Extended Three Expon...	40	413
Quadratic QF1	6191	15826
Extended Quad. Penalty QP1	50	338
Extended Quad. Penalty QP2	86	2704
Quadratic QF2	50	19816
Extended EP1	249	186
Arwhead	50	1023
Almost Perturbed Quadratic	11344	16980
QUARTC Function	10	10

Table 5. Numerical results of *TADSS* and *HSM* methods tested on 12 large scale test functions regarding CPU time metric.

Test function	CPU	
	TADSS	HSM
Extended Penalty	0	0
Perturbed quadratic	0	58
Raydan-1	3	32
Extended Three Expon...	0	0
Quadratic QF1	1	78
Extended Quad. Penalty QP1	0	0
Extended Quad. Penalty QP2	0	10
Quadratic QF2	0	198
Extended EP1	0	0
Arwhead	0	7
Almost Perturbed Quadratic	0	146
QUARTC Function	0	0

Table 6. Numerical results of *TADSS* and *HSM* methods tested on 12 large scale test functions regarding number of function evaluations metric.

Test function	No. of func.evaluations	
	TADSS	HSM
Extended Penalty	1123	4823
Perturbed quadratic	31349	137297
Raydan-1	10369	48952
Extended Three Expon...	400	1835
Quadratic QF1	16976	121539
Extended Quad. Penalty QP1	517	2224
Extended Quad. Penalty QP2	638	21102
Quadratic QF2	532	170950
Extended EP1	767	1514
Arwhead	549	12076
Almost Perturbed Quadratic	30838	139053
QUARTC Function	30	30

In the Tables (4), (5) and (6) we display the numerical outcomes for the second pair of methods (TADSS and HSM). Table (4) contains the number of iterations data, Table (5) contains CPU time data and Table (6) contains the number of function evaluations data, respectively, for both models.

For all tests the usual exit condition was taken:

$$\|g_k\| \leq 10^{-6} \quad \text{and} \quad \frac{|f(x_{k+1}) - f(x_k)|}{1 + |f(x_k)|} \leq 10^{-16}.$$

From last six tables we can count that the TADSS method outperform all others regarding all three analyzed metrics. Considering number of iterations in case of 8 test function TADSS is gives the best results, follows the MHSM with 2 test functions and the SM with 1 test functions. For 1 test function all methods show the same number of iterations, the same number of function evaluations and the same CPU time. Regarding the number of function evaluations, the TADSS upgrades the rest of comparative mathods in 10 test functions, while the SM gives the best results for 1 test function. Considering the CPU time metric, the TADSS shows convincingly best outcomes.

More clearer comparative view can be seen from the next Table (7) where the average values with respect to all three measured characteristics, regarding all four comparative methods, are included.

Table 7. Average numerical outcomes for 12 test functions tried out on 10 numerical experiments in each iteration.

Aver. perf.	HSM	MHSM	SM	TADSS
Num. of iter.	6929.92	4300.08	16302.33	2545.92
CPU time (sec)	44.08	52.42	148.5	0.33
Num. of fun. eval.	55116.75	33819.83	91202.67	7840.67

From the previous Table (7) we can see that the TADSS in a large degree outperforms the other three schemes regarding all three measured metrics. More precise, considering the number of iterations the TADSS shows almost three times better results than the HSM, approximately 1.7 times better than the MHSM and even 6.5 times better results than the SM method. Regrading the number of function evaluations the TADSS obtains about 7 times lower outcomes compered to the HSM method, more then 4 times lower values compared to the MHSM and about 11.5 times lower number compared to the SM method. When the CPU time is is considered, the TADSS shows nearly 133.5, 159, 0.33 and 450 times faster execution time when compared to the HSM, the MHSM and the SM respectively. This fabulous numerical outcomes in favor to the TADSS scheme are not so surprising since in Stanimirović et al. (2015) the advantage of this method in comparatione to the SM scheme is numerically confirmed. Since in Petrović et al. (2017); Panić et al. (2018) betterment of the hybrid and modified hybrid version of the SM method in comparatione to the SM iteration is detected, it is not surprising that the HSM and MHSM in Table (7) give better outcomes than the SM model.

The previous analysis lead us to an interesting question: if we make a hybrid model of the TADSS method would it be more efficient than the very TADSS iteration? The answer on this question will be revealed trough some further investigations.

From presented facts we can conclude that the hybridization process presented in Khan (2013) is a good way for improving a performance profile of the certain optimization method.

REFERENCES

Andrei, N. 2006, An acceleration of gradient descent algorithm with backtracking for unconstrained optimization. Numerical Algorithms, 42(1), pp. 63-73. doi:10.1007/s11075-006-9023-9

Andrei, N. 2008, An unconstrained optimization test functions collection. Advanced Modeling and Optimization, 10 (1), pp. 147-161.

Khan, S. 2013, A Picard-Mann hybrid iterative process. Fixed Point Theory and Applications, 2013(1), p. 69. doi:10.1186/1687-1812-2013-69

Panić, S., Petrović, M., & Carević, M. 2018, Initial Improvement Of The Hybrid Accelerated Gradient Descent Process. Bulletin of the Australian Mathematical Society, 98(02), pp. 331-338. doi:10.1017/s0004972718000552

Petrović, M. 2015, An Accelerated Double Step Size model in unconstrained optimization. Applied Mathematics and Computation, 250, pp. 309-319. doi:10.1016/j.amc.2014.10.104, 2015

Petrović, M. & Kontrec, N. 2017, Determination of accelerated factors in gradient decent iterations based on Taylor's series. University thought. Nat. Sci., 7(1), pp. 41-45. doi:10.5937/univtho7-14337

Petrović, M., Rakočević, V., Kontrec, N., Panić, S., & Ilić, D. 2017, Hybridization of accelearted gradient descent method. Numerical Algorithms, 79(3), pp 769–786, doi:10.1007/s11075-017-0460-4

Petrović, M. & Stanimirović, P. 2014, Accelerated gradient descent methods with line search. Numerical Algorithms, 54(4), pp. 503-520. doi:10.1007/s11075-009-9350-8

Picard, E. 1890, Memoire sur la theorie des equations aux derivees partielles et la methode des approximations successives. J. Math. Pures Appl., 6, pp. 145-210.

Stanimirović, P. S. & Miladinović, M. B. 2010, Accelerated gradient descent methods with line search. Numerical Algorithms, 54(4), pp. 503-520. doi:10.1007/s11075-009-9350-8

Stanimirović, P. S., Milovanović, G. V., Petrović, M., & Kontrec, N. Z. 2015, A Transformation of Accelerated Double Step Size Method for Unconstrained Optimization. Mathematical Problems in Engineering, pp. 1-8. doi:10.1155/2015/283679

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TRANSITION BETWEEN FLAT MAGNITUDE AND FLAT GROUP DELAY LOW PASS RECURSIVE DIGITAL FILTERS

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ABSTRACT

Many a paper has been written on the characteristics of the continuous-time and discrete-time low-pass filters which are designed to have either maximally flat magnitude characteristics or maximally flat group delay responses. The polynomial (all pole) low pass recursive digital filters which are characterised by characterise the transition between a flat magnitude and flat group delay characteristics, named Transitional Butterworth Thiran (TBTh) recursive digital filters, is considered. The characteristics of the resulting filter change gradually from the characteristics of the well known Butterwort's filter to the characteristics of the Thiran's filter with the maximally flat group delay characteristic. Poles of the TBTh filter are obtained by interpolating between poles Butterworth and Thiran's filters by varying a parametar which controls the pole positions that enables a tradeoff between the steepness magnitude characteristic at passband edge and group delay deviation.

Keywords: Transitional filters, IIR filter, Butterworth filter, Thiran filter, Group delay.

INTRODUCTION

In the field of circuits and system theory, transitional filters combine the frequency response of two different filters (typically stopband attenuation and constant group delay types) to achieve a compromise between attenuation and group delay characteristics. There are generally two types of transitional filters: transitional filters combining filter poles and transitional filters combining classical orthogonal polynomials.

Combining filter poles by means of an interpolation formula results in transitional characteristics. In other words, the transitional AB (TAB) filters having characteristics that tranzition smoothly from those of filter A to those of filter B as a design parameter *m* is varied from zero to one. The transition is accomplished using the method which have been described of Peless & Murakami (1957), which have found each pole location of the TBT transfer function in the complex frequency plane. They were to change the transfer function smoothly from that of the Butterworth to those of the Thomson filters by varying a parameter which controls the pole positions. Although a large variety of filter specifications can be satisfied by an appropriate choice of the variabile parametar, this class of filters offers no advantages over Butterworth or Thomson filters in respect of steady-state and transient responses. Other particular method will operate directly on the filter poles (Aiello & Angelo, 1974) is transitional Legendre-Thomson (TLT) whose frequency characteristics can be approximated in a maximum cutoff rate (Papoulis, 1958) (Legendre) or in a maximally flat delay sense (Thomson).

In the paper Johnson et al. (1979) have considered transitional rational filters using the interpolation method of Peless and Murakami to locate both the zeros and poles of the transition filter. The transitional rational AB filters are obtained by finding each pole (zero) of the TRAB filter as an interpolation between a pole (zero) of the A filter and a corresponding pole (zero) of the B filter. In particular example, A filter is well known inverse Chebyshev filter, and B filter is Bessel rational filter (Johnson et al., 1976).

Other allpole monotonic filter functions with a mentioned variable parameter *m* that enables a tradeoff between the maximum permissible overshoot and the rise time in the time domain, so that a large variety of filter specifications can be met in practical design with a single class of filter functions, are transitional Halpern-Thomson filters (Lazović & Radmanović, 1975).

These filters share the fact that the transition operation is performed in the complex s-plane with pole locations bounded by the two filter responses and they are not suitable for mapping into discrete time domain.

The mixture of two classical orthogonal polynomials to generate a new polynomial is an alternative means of generating transitional filters. These comprise the well-known transitional Butterworth– Chebyshev (TBC) filter, a mixture of Butterworth and Chebyshev components (Budak & Aronhime, 1971; Thajchayapong et al., 1978; Roy & Varanasi, 1978). Transitional ultraspherical-ultraspherical (TUU) filters as a generalization of the transitional Butterworth-Chebyshev fites is presented in the paper Attikiouzel & Dang (1978).

In addition to the transient filters with simple poles, transitional filters with multiple poles proposed in the papers Stojanović & Pavlović (1979) and Stojanović & Pavlović (1980). These allpole functions, based on the Butterwortth and Chebyshev filters, have been derived by numerical optimization of the magnitude

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passband response under the constraint of a double or higher-order multiplicity of the dominant pair of poles.

A technique commonly used in the design of recursive digital filters is the use of bilinear transformation of continuous-time filter transfer functions. Moreover, such filters are not the true classical counterparts in the digital domain. A method for direct computing the poles of transitional all-pole Butterworth-Chebyshev recursive digital filters is presented in the papers Nikolić & Stojanović (1996); Stojanović & V. Nikolić (1998); Stojanović & Nikolić (1993). These filters offer a gradual transition between Butterworth and Chebyshev filters. Number of transitional filter is n, where n is the degree of transitional filters.

The main object of this paprr is to introduce a new class of discrete-time allpole filters with a variable parameter that enables a tradeoff between the maximally flat passband attenuation (Butterworth) and the maximally flat group delay (Thiran), so that a large variety of filter specifications can be met in practical design with a single class of filter functions.

These linear time-Invariant digital filters, referred to as transitional Butterworth-Thiran (TBTh) filters, to different from TBT filtes, provide considerably trade off between constant group delay and attenuation characteristic. The transfer function of certain TBTh filters has been computed, tabulated and the figures clearly illystrate the transitional nature of these filters.

SHORT REVIEW OF THE BUTTERWORTH ALLPOLE IIR DIGITAL FILTERS

The general form of the squared magnitude characteristic, both in continuous-time and discrete-time domain, of a lowpass Butterworth filter wityh no transmission zeros (Stojanović et al., 2014) may be expressed as

$$|H_B(x^2)|^2 = \frac{1}{1+\varepsilon^2 x^{2n}}, \tag{1}$$

where n is filter degree, x is frequency variable and ε is is a design parameter that controls the passband loss, known as the passband edge ripple factor, which is related to the maximal passband attenuation a_{max} (in dB), as evidenced by $\varepsilon = \sqrt{10^{0.1a_{max}} - 1}$. Without loss of generality, $\varepsilon^2 = 1$ can be substituted in (1), then the maximum passband attenuation is specified as $a_{max} = 3$ dB.

If frequency variable x is continuous-time angular frequency, $x^2 = -s^2$, then function (1) is the magnitude characteristic of the continuous-time low-pass Butterworth transfer function, because $s = j\omega$ at real frequencies.

On the other hand, the discrete-time polynomial filter is designed from the magnitude function (1) by a technique known as analytic continuation, trough substitution (Stamenković & Stojanović, 2014)

$$x^2 \mapsto \frac{\sin^2\frac{\omega T}{2}}{\sin^2\frac{\omega_c T}{2}} = -\frac{(z-1)^2}{(2\alpha)^2 z}\Big|_{z=e^{j\omega T}}, \tag{2}$$

where ω is continuous-time angular frequency, ω_c is the 3-dB cutoff frequency, $\alpha = \sin(\omega_c T/2)$ and T is sampling period, which shows that this transformation leads to the transfer function with multiple zero at the origin in the z-plane. Substituting (5) into (1) the discrete-time magnitude squared function $H_B(z)H_B(1/z)H_B(z)H_B(1/z)$ of allpole lowpass Butterworth filter is obtained as folloes

$$H_B(z)H_B(\frac{1}{z}) = \frac{z^n}{d_0 + d_1 z + \cdots + d_1 z^{2n-1} + d_0 z^{2n}}, \tag{3}$$

where

$$d_i = \begin{cases} (-1)^{i+n}\varepsilon\binom{2n}{i}\frac{1}{4\alpha^n}, & \text{za } i = 0, 1, \ldots, n-1, \\ \varepsilon\binom{2n}{n}\frac{1}{4\alpha^n} + 1, & \text{za } i = n. \end{cases} \tag{4}$$

are the first $n+1$ coefficients d_i in the closed form of the mirror-image polynomials.

By equating the denominator of (3) with zero, roots will occur in the mirror-image pairs. Poles of the polynomial IIR Butterworth filters $H_B(z)$ are merely roots lying inside the unit circle p_i, hence the resulting filter is stable. Thus

$$H_B(z^{-1}) = \frac{h_0}{z^{-n} + a_2 z^{-n+1} + \cdots + a_n z^{-1} + a_{n+1}}, \tag{5}$$

where $h_0 = \sum_{i=1}^{n+1} a_i$ is a constant that ensures that amplitude $|H_B(\omega)|$ is bounded above by unity.

IIR DIGITAL FILTERS WITH MAXIMALLY FLAT GROUP DELAY

Thiran (1971) developed an analytical method for deriving the all pole transfer function of the IIR digital filter that approximates a constant group delay in the maximally flat sense. Let τ be the prescribed group delay, and the allpole transfer function be chosen in the closed form as

$$H_T(z^{-1}) = \frac{\frac{2n!}{n!}\frac{1}{\prod_{i=n+1}^{2n}(2\tau+i)}}{\sum_{k=0}^{n}(-1)^k\binom{n}{k}\prod_{i=0}^{n}\frac{2\tau+i}{2\tau+k+i}z^{-k}} = \frac{\sum_{i=0}^{n} a_i}{\sum_{i=0}^{n} a_i z^{-i}}, \tag{6}$$

whose gain is adjusted to unity at $z^{-1} = 1$.

The group delay $\tau_T(z^{-1})$ of the low-pass filter $H_T(z^{-1})$ is mirror-image, rational function in z, that can be easily calculated by employing the following formula

$$\begin{aligned} \tau_T(z^{-1}) &= -\frac{1}{2}\Big[\frac{z}{H_T(z^{-1}))}\frac{\dot{H}_T(z^{-1})}{\dot{z}} - \frac{z}{H_T(z)}\frac{\dot{H}_T(z)}{\dot{z}}\Big] \\ &= \frac{1}{2}\Big[\frac{\sum_{i=1}^{n} i a_i z^{-i}}{\sum_{i=0}^{n} a_i z^{-i}} + \frac{\sum_{i=1}^{n} i a_i z^{-n+i}}{\sum_{i=0}^{n} z^{-n+i}}\Big] \end{aligned} \tag{7}$$

For a filter of degree n and with a maximally flat group delay the first $n-1$ derivatives of the group delay with respect to z^2 are zero at $z = 1$.

Using (7), it can be derived an expression for the group delay of transfer function (5) or (6) at $z^{-1} = 1$ ($\omega = 0$) as (Stamenković et al., 2018)

$$\tau_T(1) = -\frac{\sum_{i=0}^{n} i a_i}{\sum_{i=0}^{n} a_i} \tag{8}$$

and at $z^{-1} = -1$ ($\omega = \pi$) as

$$\tau_T(-1) = -\frac{\sum_{i=0}^{n} i(-1)^{i+1} a_i}{\sum_{i=0}^{n} (-1)^{i+1} a_i} \tag{9}$$

Equations (8) and (9) are valid for all polynomial filters.

Thiran has also shown that the above transfer function is stable for all finite positive values of τ. Using the above formula, the coefficients of the denominator polynomial of $H_T(z^{-1})$ for $n = 8$ (and $\tau = 2$) are tabulated in Table 1.

TRANSFER FUNCTION OF THE TRANSITIONAL FILTER

The poles position of the transitional Butterworth-Thiran transfer function is determined by defining a real parameter m that provides a linear variation in the phasorangle of the pole in a relation involving the corresponding Butterworth and Thiran poles

$$s_k = s_{kB}^{1-m} s_{kT}^{m}, \text{ for } 0 \le m \le 1 \tag{10}$$

where s_k is the generical pole of the TBTh transfer function, s_{kB} and s_{kT}, the Butterworth and Thiran poles, respectively. The position of s_k varies smoothly, as m increases, from $s_k = s_{kB}$ for $m = 0$, to $s_k = s_{kT}$ for $m = 1$. The magnitude and the argument of s_k are

$$\begin{aligned} |s_k| &= |s_{kB}|^{1-m} |s_{kT}|^{m} \\ \arg(s_k) &= \arg(s_{kB}) - m \arg(s_{kB}) + m \arg(s_{kT}) \end{aligned} \tag{11}$$

Such relations are used to construct the generical TBTh pole from the corresponding Butterworth and Thiran poles..

TRANSFER FUNCTION OF THE TRANSITIONAL FILTER

The poles position of the transitional Butterworth-Thiran transfer function is determined by defining a real parameter m that provides a linear variation in the phasorangle of the pole in a relation involving the corresponding Butterworth and Thiran poles

$$s_k = s_{kB}^{1-m} s_{kT}^{m}, \text{ for } 0 \le m \le 1 \tag{12}$$

where s_k is the generical pole of the TBTh transfer function, s_{kB} and s_{kT}, the Butterworth and Thiran poles, respectively. The position of s_k varies smoothly, as m increases, from $s_k = s_{kB}$ for $m = 0$, to $s_k = s_{kT}$ for $m = 1$. The magnitude and the argument of s_k are

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Such relations are used to construct the generical TBTh pole from the corresponding Butterworth and Thiran poles.

NUMERICAL RESULTS

The results of the approximation are shown in the two examples with low pass filters of the eighth and tenth degree.

In the first example the normalized value of the group delay at the origin of all eight degree filters is two samples. The transition filter is achieved for $m = 0.6$. In order to get the proposed value of group delay at the origin, the 3 dB bandwidth of the Butterworth filter is adjusted to the value $\omega_c T/\pi = 0.3163$.

For $\tau = 2$, the 3 dB bandwidth of the Thiran filter is $\omega_p T/\pi = 0.2322$, while the bandwidth of transitional filter is slightly wider than the Butterworth filter, and it has a value of $\omega_p T/\pi = 0.2683$. On the other hand, the maximum value of group delay of the Butterworth filter is 5.33 samples, while the maximum value of group delay of the transition filter is reduced to 3.22 samples.

Table 1. The coefficients of the Butterworth's, Thiran's and TBTh filters for $m = 0.6$.

Coef.	Butterworth	TBTh	Thiran
a_1	1.0000000	1.0000000	1.0000000
a_2	–3.3158176	–2.9408615	–2.4615386
a_3	5.4638252	4.3454928	3.0769231
a_4	–5.5777755	–4.0307426	–2.4615386
a_5	3.7806220	2.5096502	1.3461539
a_6	–1.7209603	–1.0589553	–0.5067874
a_7	0.5095335	0.2928378	0.1266968
a_8	–0.0891724	–0.0481745	–0.0190522
a_9	0.0070302	0.0035898	0.0013098

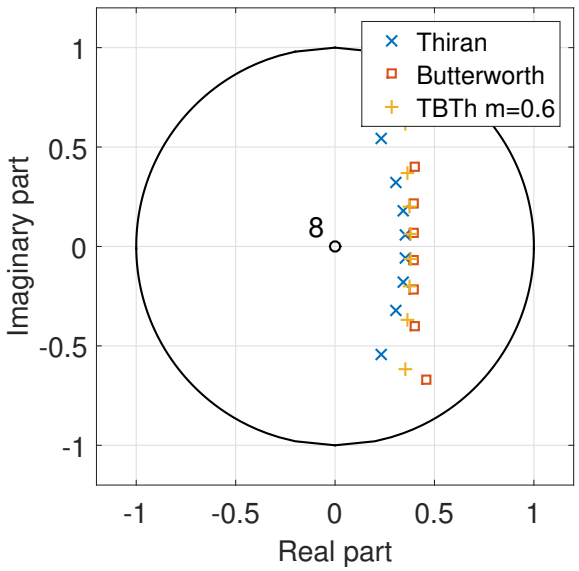

Figure 1. Plot poles and zeros in the z-plane for the 8th degree transfer functions whose coefficients are given in Table 1.

Table 1 displays the coefficients of recursive digital low pass filters of the eighth degree: Butterworth, Thiran and the transitional filter for parameter $m = 0.6$. In order to compare the charac-

teristics of TThB filters with those of other filters, the group delay at $z^{-1} = 1$ is taken to be identical, and gain at $z^{-1} = 1$ is adjusted to unity. The verification of the transfer functions design given in Table 1 can be performed by the Eq. (8)[1]. The plot poles and zeros in the z-plane of the previously mentioned filters is shown on Figure 1, while their frequency responses can be seen on Figure 2. All filters have a zero of the eighth degree at the origin.

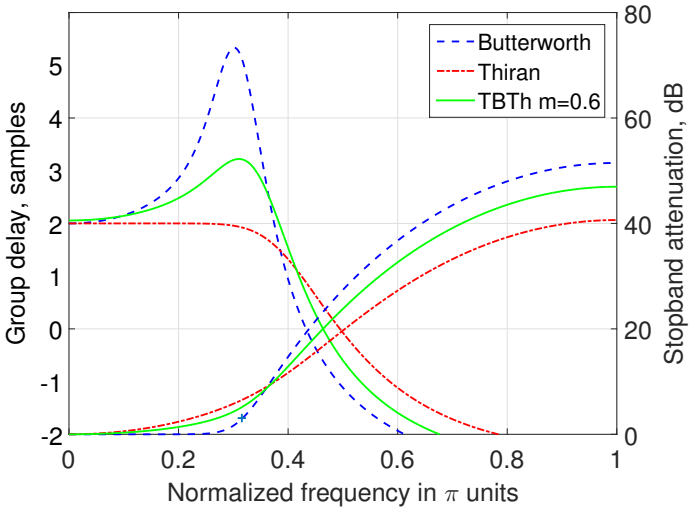

Figure 2. Attenuation and group delay response of the 8th degree filters.

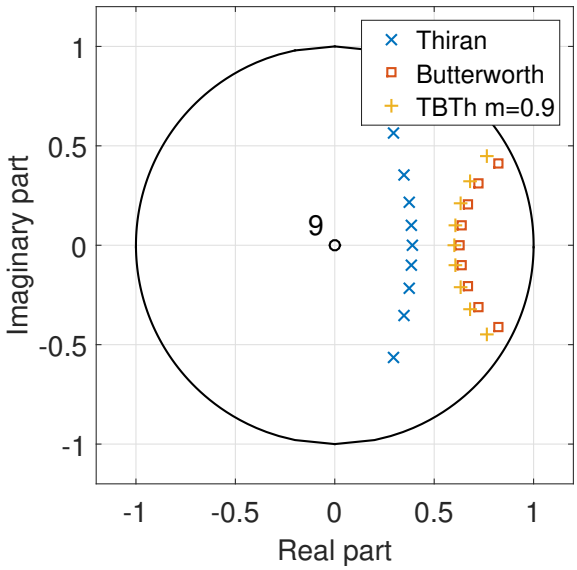

Figure 3. Plot poles and zeros in the z-plane for the 10th degree transfer functions.

In the second example the Butterworth, Chebyshev and transitional filters of the tenth degree, when all the filters have the same 3 dB bandwidth of $\omega_c T = 0.15\pi$, are compared. Figure 3 shows the zero and pole position in the z-plane, and Figure 4 shows the corresponding frequency responses for all three transfer functions.

[1] For all transfer functions given in Table 1 the group delay at origin is $\tau(1) = 2$.

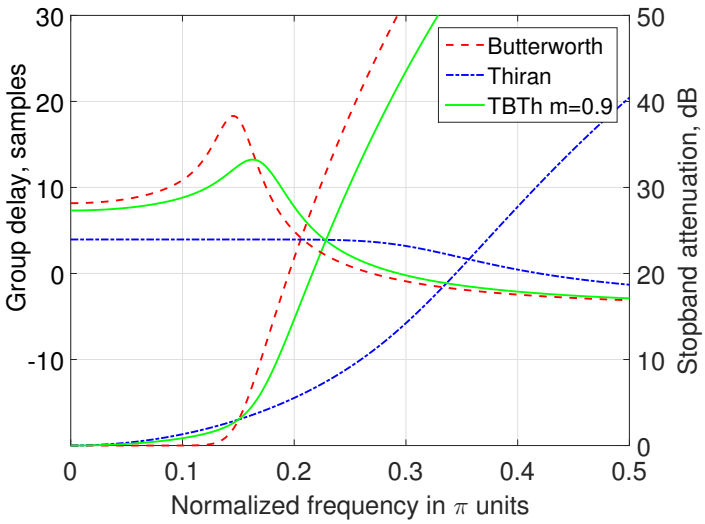

Figure 4. Attenuation and group delay response of the 10th degree filters. The 3-dB cut off frequency is $\omega_c T = 0.15\pi$.

Figure 4 shows that for higher values the parameter m achieves a good compromise between stopband attenuation and group delay distortion. On the normalized frequency $\omega = 0.2\pi$ attenuation of the transition filter rises from 5.49 dB for the TBTh filter, to 11.94 dB. The change in group delay of the transition filter is 5.69 samples, which is considerably lower compared to the changes in group delay of the Butterworth filter which is 11.92 samples.

CONCLUSIONS

In this paper, a simple method of approximation of transitional Butterworth-Thiran (TBTh) recursive digital filters is proposed. By changing one parameter, the characteristics of these filters change continually from the characteristics of a Butterworth which is maximally flat in the passband filter to those of a Thiran filter which have maximally flat group delay. If we allow for slight deviation of the group delay characteristic from the constant value, the filter stopband attenuation can be significantly increased. In this way, a compromise between the phase distortion in the bandwidth and stop band attenuation can been found.

Keeping in mind that the proposed approximation belongs in the class of auto-regressive (AR) filters, further research includes approximation of the ARMA (Auto Regressive Moving Average) recursive digital filters with maximally flat group delay. In other words, Thirans filter can be expanded using an FIR amplitude corrector, with which it will be possible to preserve the maximally flat group delay of Thirans filter, and improve attenuation in both the passband and in stopband.

REFERENCES

Aiello, G. & Angelo, P. 1974. Transitional Legendre Thomson filters. IEEE Transactions on Circuits and Systems, 21(1), pp. 159-162. doi:10.1109/tcs.1974.1083782.

Attikiouzel, J. & Dang, T. P. 1978. On transitional ultraspherical-ultraspherical filters. Proceedings of the IEEE, 66(6), pp. 703-706. doi:10.1109/proc.1978.10996.

Budak, A. & Aronhime, P. 1971. Transitional Butterworth-Chebyshev filters. IEEE Transactions on Circuit Theory, 18(3), pp. 413-415. doi:10.1109/tct.1971.1083276.

Johnson, J., Johnson, D., Boudra, P., & Stokes, V. 1976. Filters using Bessel-type polynomials. IEEE Transactions on Circuits and Systems, 23(2), pp. 96-99. doi:10.1109/tcs.1976.1084174.

Johnson, J., Johnson, D., Perritt, R., & LaCarna, R. 1979. Transitional rational filters. IEEE Transactions on Circuits and Systems, 26(11), pp. 976-979. doi:10.1109/tcs.1979.1084591.

Lazović, S. M. & Radmanović, M. D. J. 1975. Transitional Halpern-Thomson filters. Electronics Letters, 11(11), p. 229. doi:10.1049/el:19750174.

Nikolić, S. V. & Stojanović, V. S. 1996. Transitional Butterworth-Chebyshev recursive digital filters. International Journal of Electronics, 80(1), pp. 13-20. doi:10.1080/002072196137552.

Papoulis, A. 1958. Optimum Filters with Monotonic Response. Proceedings of the IRE, 46(3), pp. 606-609. doi:10.1109/jrproc.1958.286876.

Peless, Y. & Murakami. 1957. Analysis and synthesis of tranzitional Butterworth-Thomson filters and bandpass amplifier. RCA Rev., 18(3), pp. 60-94.

Roy, S. & Varanasi, P. 1978. Transitional Butterworth–Chebyshev filters. Electronics Letters, 14(6), p. 179. doi:10.1049/el:19780119.

Stamenković, N., Stojanović, N., & Perinić, G. 2018. Group Delay Equalization of Polynomial Recursive Digital Filters in Maximal Flat Sense. Journal of Circuits, Systems and Computers, , p. 1950173. doi:10.1142/s0218126619501731.

Stamenković, N. & Stojanović, V. 2014. On the design transitional Legendre–Butterworth filters. International Journal of Electronics Letters, 2(3), pp. 186-195. doi:10.1080/00207217.2014.894138.

Stojanović, N., Stamenković, N., & Stojanović, V. 2014. All-pole recursive digital filters design based on ultraspherical polynomials. Radioengineering, 23(3), pp. 949-953.

Stojanović, V. S. & Nikolić, S. V. 1993. Direct design of transitional Butterworth-Chebyshev recursive digital filters. Electronics Letters, 29(3), p. 286. doi:10.1049/el:19930195.

Stojanović, V. S. & Pavlović, V. D. 1979. Transitional Murromaf–Murroer filters. Electronics Letters, 15(12), p. 356. doi:10.1049/el:19790253.

Stojanović, V. V. S. & V. Nikolić, S. 1998. Direct design of sharp cutoff low-pass recursive digital filters. International Journal of Electronics, 85(5), pp. 589-596. doi:10.1080/002072198133879.

Stojanović, V. S. & Pavlović, V. D. 1980. Transitional multiple-pole transfer functions for active-filter design. Electronics Letters, 16(1), p. 21. doi:10.1049/el:19800018.

Thajchayapong, P., Cheevasuvit, F., & Manapee, S. 1978. Transitional Butterworth-Chebyshev filters. Electronics Letters, 14(20), p. 680. doi:10.1049/el:19780456.

Thiran, J. P. 1971. Recursive digital filters with maximally flat group delay. IEEE Transactions on Circuit Theory, 18(6), pp. 659-664. doi:10.1109/tct.1971.1083363.

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Original Scientific Paper

SOME NEW FIXED POINT RESULTS FOR CONVEX CONTRACTIONS IN B-METRIC SPACES

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ABSTRACT

The purpose of this paper is to consider various results for convex contraction mappings in the context of B-metric spaces. We, among other things, generalize, extend, correct and enrich the recent published results from the context of convex contractions defined on an ordinary metric spaces to the ones on the so–called B-metric spaces. One example shows that this generalization is genuine. Let us note that this paper represents only the beginning of our investigation of the properties of convex contractions observed in any general metric space.
In the papers that are to be published, our considerations are applied to cone metric spaces, partial metric spaces, G-metrics, G_B-metrics, extended B-metric spaces and many others.

Keywords: Convex contraction, Fixed point, B-metric space, B-Cauchy sequence

INTRODUCTION AND PRELIMINARIES

Since S. Banach proved his theorem that every contraction on a complete metric space has a unique fixed point, in his doctoral dissertation in 1922, many mathematicians have tried to generalize the famous result. Hundreds of scientific papers have considered this issue.

The previously mentioned statement, leads in two key directions: either the axioms of metric spaces are affected ($d_Y(u,v) = 0$ if and only if $u = v$; $d_Y(u,v) = d_Y(v,u)$; $d_Y(u,w) \leq B(d_Y(u,v) + d_Y(v,w))$), where d_Y is a function defined on the Cartesian product $Y \times Y$, where Y is a non-empty set with values in $[0,+\infty)$, or it affected the Banach contraction condition ($d_Y(fu,fv) = kd_Y(u,v)$, $\forall u,v \in Y$, where $k \in [0,1)$). More details about Banach generalization could be seen in (Aleksić et. al., 2019a), (Aleksić et. al., 2019b; Aleksić et. al., 2019c; Alghamdi et. al., 2011; Ampadu, 2017a; Ampadu, 2017b; Andras, 2003; Baakhtin, 1989; Collaco & E Silva, 1997; Istratescu, 1981; Istratescu, 1982; Istratescu, 1983; Jeong & Rhoades, 2005; Kirk et. al., 2003; Kirk & Shahzad, 2014; Rhoades, 1977).

Recently, fixed point theory has been one of the most important research fields in nonlinear and functional analysis. It has wide applications in many disciplines like studying the existence of solutions for nonlinear (algebraic, differential and integral) equations, a system of linear (nonlinear) equations and convergence of many computational methods, economics, sports, medical sciences, etc.

Throughout this paper, $\mathbb{N}$ denotes the set of all positive integers. We repeat some definitions and results, which will be needed in the sequel.

Definition 1. Let Y be a (non-empty) set and $B \geq 1$ a given real number. A function $d_Y : Y \times Y \to [0,+\infty)$ is said to be a B-metric on Y if the following conditions are satisfied:

(b_1) $d_Y(u,v) = 0$ if and only if $u = v$;

(b_2) $d_Y(u,v) = d_Y(v,u)$ for all $u,v \in Y$;

(b_3) $d_Y(u,w) \leq B(d_Y(u,v) + d_Y(v,w))$ for all $u,v,w \in Y$.

The triplet (Y,d_Y,B) is called a B-metric space with coefficient B.

For more notions such as B-continuous, B-convergence, B-completeness, B-Cauchy sequence, all in the framework of B-metric spaces, the reader is referred to (Aleksić et. al., 2019a; Aleksić et. al., 2019b; Aleksić et. al., 2019c; Ampadu, 2017a; Baakhtin, 1989; Kirk & Shahzad, 2014).

For example, the sequence $\{u_n\}$ in B-metric space (Y,d_Y) converges to the point $u \in Y$ if for each positive number η there is a natural number k_0 such that when $n \geq k_0$ we have $d_Y(u_n,u) < \eta$. This can be also written as $u_n \to u$ when $n \to \infty$ or even as $\lim_{n\to\infty} d_Y(u_n,u) = 0$.

The sequence in B-metric space (Y,d_Y) is B-Cauchy if the following is satisfied: For each positive number η there is a natural number k_0 such that when $m,n \geq k_0$ we have $d_Y(u_n,u_m) < \eta$. We can also write the last expression in the form $\lim_{m,n\to\infty} d_Y(u_n,u_m) = 0$.

We say that the B-metric space is B-complete if every B-Cauchy sequence in it converges.

Let us note that every B-convergent sequence in the B-metric space (Y,d_Y) is also B-Cauchy. Indeed, this fact follows simply from the next inequality:

$$\frac{1}{B}d_Y(u_n,u_m) \leq d_Y(u_n,u) + d_Y(u,u_m).$$

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It is well-known that in every ordinary metric space (M, d^M) the metric d^M is a continuous function with two variables. This means that $d^M(a_n, b_n) \to d^M(a, b)$ when $n \to \infty$ whenever $a_n \to a$ and $b_n \to b$ in the case when $n \to \infty$.

However, it is not the case in B-metric spaces, and this is one of the most important properties that distinguishes B-metric spaces (if $B > 1$) from metric spaces ($B = 1$).

The following, well-known, example in the literature shows this.

Example 2. Let $Y = \mathbb{N} \cup \{\infty\}$ and $d_Y : Y \times Y \to [0, +\infty)$ be defined in the following way

$$d_Y(p,q) = \begin{cases} 0, & \text{if } p = q, \\ \left|\frac{1}{p} - \frac{1}{q}\right|, & \text{if } p, q \text{ are even, or } pq = \infty, \\ 5, & \text{if } p, q \text{ are odd, and } p \neq q, \\ 2, & \text{in other cases.} \end{cases}$$

It is easy to verify that the triangle relation is fulfilled in the context of the B-metric space for $B = 3$, i.e.,

$$d_Y(p,q) \leq 3\left(d_Y(p,r) + d_Y(r,q)\right),$$

for all $p, q, r \in Y$. The other two axioms are obviously satisfied.

We check that the B-metric d_Y is not B-continuous ($B = 3$).

Indeed, if $a_n = 2n$, for each $n \in \mathbb{N}$, then $d_Y(a_n, \infty) = d_Y(2n, \infty) \to 0$, when $n \to \infty$, i.e., $a_n \to \infty$, but it is obvious that $d_Y(a_n, 1) = 2$ is not $d_Y(\infty, 1)$, when $n \to \infty$.

Note that the previous example shows that there exists a B-metric d_Y which is not a metric.

It is important to know how B-metrics can be obtained from the ordinary metric. In that case, the obtained B-metric is a continuous function with two variables. So we have the following:

Let (M, d^M) be a given metric space and let $s > 1$ be a given real number. Then, with $d_M(u, v) = \left(d^M(u, v)\right)^s$ one B-metric is defined on a non-empty set M, where $B = 2^{s-1}$. Obviously, d_M is a continuous B-metric on the set M.

In order to prove a more general result than the previous one, a well-known property of the real function $x \mapsto x^b$, $x > 0$, $b > 1$, is used and it is given in the form

$$\left(\frac{u+v}{2}\right)^b \leq \frac{u^b + v^b}{2},$$

whenever u and v are positive numbers and $b > 1$.

Now, let's state this well-known result:

Let (M, d^M) be a given metric space and let $b > 1$, $\lambda \geq 0$, $\alpha > 0$, $u, v \in M$. We define the mapping

$$d_M(u, v) = \lambda d^M(u, v) + \alpha\left(d^M(u, v)\right)^b.$$

We have that the function d_M is not the ordinary metric, but (M, d^M) is B- metric space, where coefficient $B = 2^{b-1}$. Indeed, since it is sufficient to check only the triangle relation (the first two axioms are proved trivial) by applying the previous property of the convex function $x \mapsto x^b$, $x > 0$, $b > 1$, we have

$$d_M(u,v) = \lambda d^M(u,v) + \alpha\left(d^M(u,v)\right)^b \leq \lambda\left(d^M(u,w) + d^M(w,v)\right) +$$

$$+\alpha\left(\left(d^M(u,w)\right)^b + \left(d^M(w,v)\right)^b\right) \leq \lambda\left(d^M(u,w) + d^M(w,v)\right) +$$

$$+2^{b-1}\alpha\left(\left(d^M(u,w)\right)^b + \left(d^M(w,v)\right)^b\right) \leq 2^{b-1}\left(d^M(u,w) + d^M(w,v)\right).$$

Example 3. Let $t \in (0, 1)$ and let

$$Y = l_t(\mathbb{R}) := \left\{u = \{u_n\} \subset \mathbb{R} : \sum_{n=1}^{+\infty} |u_n|^t < \infty\right\}.$$

For $u = \{u_n\}, v = \{v_n\} \in Y$, we define

$$d_M(u,v) = \left(\sum_{n=1}^{+\infty} |u_n - v_n|^t\right)^{\frac{1}{t}}.$$

Using the previous property it is easy to show that (Y, d_M) is a B–metric space with coefficient $B = 2^{\frac{1}{t}}$.

At the end of this introductory section, we note the following result that has recently been one of the most popular in the works from the context of the B-metric space:

• If for some sequence $\{u_n\}$ in the B-metric space (Y, d_Y) is satisfied the inequality:

$$d_M(u_{n+1}, u_n) \leq \mu d_M(u_n, u_{n-1}),$$

for each $n \in \mathbb{N}$, where $\mu \in [0, 1)$, then the sequence $\{u_n\}$ is B-Cauchy. Otherwise, in the general case, the following auxiliary result is most often used:

• Let (Y, d_Y) be a given B-metric space with a coefficient $B \geq 1$. Suppose that $\{u_n\}$ and $\{v_n\}$ are given sequences that respectively converge to a points u and v. Then the following applies

$$\frac{1}{B^2} d_Y(u,v) \leq \lim_{n\to\infty} \inf d_Y(u_n, v_n) \leq \lim_{n\to\infty} \sup d_Y(u_n, v_n) \leq B^2 d_Y(u,v).$$

If, in the previous inequality we put that $u = v$, we obtained $\lim_{n\to\infty} d_Y(u_n, v_n) = 0$. Moreover, in that case, for every w we have

$$\frac{1}{B} d_Y(u,w) \leq \lim_{n\to\infty} \inf d_Y(u_n, w) \leq \lim_{n\to\infty} \sup d_Y(u_n, w) \leq B\, d_Y(u,w).$$

MAIN RESULTS

In this note we will consider the next contractive condition defined in the context of so-called B-metric spaces. For B-metric spaces see (Aleksić et. al., 2019a; Ampadu, 2017a; Kirk & Shahzad, 2014). Let Y be a non-empty set and d_Y be a metric. Recall from Istratescu (Istratescu, 1981) that a map $I : Y \to Y$ is called a convex contraction mapping of order 2, if for all $u, v \in Y$, $p, q \geq 0$, $p + q < 1$, it holds that

$$d_Y\left(I^2u, I^2v\right) \leq p\, d_Y(Iu, Iv) + q\, d_Y(u, v). \tag{1}$$

Alternatively, one could say $I : Y \to Y$ is a convex type contraction mapping of order 2, if for all $u, v \in Y$ and $0 \leq k \leq \frac{1}{4}$ it holds that

$$d_Y\left(I^2u, I^2v\right) \leq k\left[d_Y(Iu, Iv) + d_Y(u, v)\right]. \tag{2}$$

In the case that (Y, d_Y) is a B-metric space with the coefficient $B \geq 1$ then $p, q \geq 0$, $p + q < \frac{1}{B^2}$, that is, $p + q < \frac{1}{4B^2}$ if eq. (2) holds.

Our following results improve ones from from (Ampadu, 2017a) and (Ampadu, 2017b).

Theorem 4. Let (Y, d_Y) be a B-complete B-metric space with the coefficient $B \geq 1$ and let $I : Y \to Y$ be a B-continuous mapping such that for all $u, v \in Y$ eq. (1) holds, where $p, q \geq 0$, $p + q < \frac{1}{B^2}$. Then I has a unique fixed point (say $z \in Y$) and for all $u \in Y$ the sequence $\{I^n u\}_{n=1}^{+\infty}$ converges to the fixed point z.

Proof. The uniqueness is clear simple and therefore we prove only the existence.

Let z_0 be an arbitrary but fixed point in Y and consider the orbit of z_0 under I, that is, the set $(I^n(z_0))_0^{+\infty}$, $I^0(z_0) = z_0$. Set $R = \max\left\{d_Y(z_0, I(z_0)), d_Y\left(I(z_0), I^2(z_0)\right)\right\}$. Thus for any $m \geq 2$

$$\begin{aligned} & d_Y\left(I^{2m+1}(z_0), I^{2m}(z_0)\right) \\ & \leq p\, d_Y\left(I^{2m}(z_0), I^{2m-1}(z_0)\right) \\ & + q\, d_Y\left(I^{2m-1}(z_0), I^{2m-2}(z_0)\right) \end{aligned} \tag{3}$$

and

$$\begin{aligned} & d_Y\left(I^{2m-1}(z_0), I^{2m}(z_0)\right) \\ & \leq p\, d_Y\left(I^{2m-2}(z_0), I^{2m-1}(z_0)\right) \\ & + q\, d_Y\left(I^{2m-3}(z_0), I^{2m-2}(z_0)\right). \end{aligned} \tag{4}$$

The condition (3) implies the next relations:

$$\begin{aligned} & d_Y\left(I^3(z_0), I^2(z_0)\right) \leq p\, d_Y\left(I^2(z_0), I^1(z_0)\right) \\ & + q\, d_Y\left(I^1(z_0), z_0\right) \leq R(p+q), \end{aligned}$$

$$\begin{aligned} & d_Y\left(I^4(z_0), I^3(z_0)\right) \leq p d_Y\left(I^3(z_0), I^2(z_0)\right) \\ & + q\, d_Y\left(I^2(z_0), I(z_0)\right) \leq p R(p+q) + q R \leq R(p+q), \end{aligned}$$

$$\begin{aligned} & d_Y\left(I^5(z_0), I^4(z_0)\right) \leq p\, d_Y\left(I^4(z_0), I^3(z_0)\right) \\ & + q\, d_Y\left(I^3(z_0), I^2(z_0)\right) \leq p R(p+q) \\ & + q R(p+q) = R(p+q)^2, \end{aligned}$$

because $p + q < \frac{1}{B^2} \leq 1$.

An induction argument shows that

$$d_Y\left(I^{2m+1}(z_0), I^{2m}(z_0)\right) \leq R(p+q)^m < \frac{R}{B^{2m}} \tag{5}$$

and the similar by eq. (4) that

$$d_Y\left(I^{2m-1}(z_0), I^{2m}(z_0)\right) \leq R(p+q)^m < \frac{R}{B^{2m}}, \tag{6}$$

in the case that $B > 1$. If $B = 1$ the proof follows by (Istratescu, 1981).

Now according (Aleksić et al., 2019a, Remark 2.1.) for $m < n$ follows

$$\begin{aligned} & d_Y(I^m(z_0), I^n(z_0)) \leq B\, d_Y\left(I^m(z_0), I^{m+1}(z_0)\right) \\ & + B^2\, d_Y\left(I^{m+1}(z_0), I^{m+2}(z_0)\right) + \cdots + \\ & + B^{n-m-1}\, d_Y\left(I^{n-2}(z_0), I^{n-1}(z_0)\right) \\ & + B^{n-m-1}\, d_Y\left(I^{n-1}(z_0), I^n(z_0)\right). \end{aligned} \tag{7}$$

Further from (7) we obtain that $I^n(z_0)$ is a B-Cauchy sequence in B-metric space (Y, d_Y) with the coefficient $B > 1$.

Indeed, if $m = 2k$, $n = 2l$ ($m = 2k$, $n = 2l - 1$ or $m = 2k - 1$, $n = 2l$), then by using (5), (6) and (7) the result follows. Since (Y, d_Y) is a B-complete B-metric space, then there exists $z \in Y$ such that $u_n \to z$ as $n \to \infty$, i.e., $Iz = z$ because I is B-continuous mapping. The proof of Theorem 4 is complete.

Let for example $m = 4$, $n = 10$, i.e., $k = 2$, $l = 5$. Then

$$\begin{aligned} & d_Y\left(I^4(z_0), I^{10}(z_0)\right) \leq B\, d_Y\left(I^4(z_0), I^5(z_0)\right) \\ & + B^2\, d_Y\left(I^5(z_0), I^6(z_0)\right) + B^3\, d_Y\left(I^6(z_0), I^7(z_0)\right) \\ & + B^4\, d_Y\left(I^7(z_0), I^8(z_0)\right) + B^5\, d_Y\left(I^8(z_0), I^9(z_0)\right) \\ & + B^5\, d_Y\left(I^9(z_0), I^{10}(z_0)\right) \leq 2B(p+q)^2 \\ & + 2B^3(p+q)^3 + 2B^5(p+q)^4 + 2B^7(p+q)^5 = \\ & 2B(p+q)^2\, \frac{1 - \left[B^2(p+q)\right]^4}{1 - B^2(p+q)} \leq 2B(p+q)^2(1 + \cdots). \end{aligned}$$

Hence, follows the method for general case, for example $m = 2k$, $n = 2l$. ■

Corollary 5. (Istratescu, 1981, Theorem 1.2.) Let (Y, d_Y) be a complete metric space and let $I : Y \to Y$ be a continuous mapping such that for all $u, v \in Y$ eq. (1) holds, where $p, q \geq 0$, $p + q < 1$. Then I has a unique fixed point (say $z \in Y$) and for all $u \in Y$ the sequence $\{I^n u\}_{n=1}^{+\infty}$ converges to the fixed point z.

Remark 6. It is worth to notice that our proof given in Theorem 4 improves the corresponding from (Ampadu, 2017, Theorem 8). This means that our Theorem 4 is a new result for convex contractions in the context of B-metric spaces given first time in existing literature.

The next is a modification of Example 9 from (Ampadu, 2017).

Example 7. Let $Y = [1, 2]$ and $d_Y(u, v) = |u - v|^2$ and define $I : Y \to Y$ by $I(u) = \frac{u+3}{4}$ for all $u \in Y$. Now observe that with $p = q = \frac{1}{272}$ we have that (1) holds, i.e.,

$$d_Y\left(I^2(u), I^2(v)\right) \leq \frac{1}{272}\, d_Y(Iu, Iv) + \frac{1}{272}\, d_Y(u, v),$$

for all $u, v \in Y = [1, 2]$.

Remark 8. In the past year, the new class of the B-metric space called Extended B-metric spaces is becoming actual for research. It is defined by applying

$$(\mathrm{b}_3')\quad d_Y(u, w) \leq \Lambda(u, w)\left(d_Y(u, v) + d_Y(v, w)\right)$$

instead of axiom (b_3) for every $u, v, w \in Y$, where Λ is a function that maps $Y \times Y \to [0, +\infty)$. Obviously, every B-metric space is Extended B-metric.

Let us note that the previous considerations of B-convex contractions can be also treated in other classes of general metric spaces (partial metric spaces, G metric spaces, G_B-metric spaces, B-metric spaces, extended B-metric spaces and many others...).

In fact, even in the context of ordinary metric space, the following important questions have not been considered:

Question 1: What about the existence of the fixed point mapping in the case of complete metric spaces when the right hand side of the expression (1) is:

a) $k \max \left\{ d_Y(u,v), \frac{1}{2}[d_Y(u,Iu) + d_Y(v,Iv)], \frac{1}{2}[d_Y(u,Iv) + d_Y(u,v)\right.$ Ćirić general contraction of I order;

b) $k \max \left\{ d_Y(u,v), d_Y(u,Iu), d_Y(v,Iv), \frac{1}{2}[d_Y(u,Iv) + d_Y(Iu,v)\right.$ Ćirić general contraction of II order;

c) $k \max \{d_Y(u,v), d_Y(u,Iu), d_Y(v,Iv), d_Y(u,Iv), d_Y(Iu,v)\}$ Ćirić quasi-contraction.

Question 2: What about the existence of the fixed point mapping in the case of B-metric spaces when the right hand side of the expression (1) is:

a) $k \max \left\{ d_Y(u,v), \frac{1}{2B}[d_Y(u,Iu) + d_Y(v,Iv)], \frac{1}{2B}[d_Y(u,Iv) + d_Y(u,v)\right.$;

b) $k \max \left\{ d_Y(u,v), d_Y(u,Iu), d_Y(v,Iv), \frac{1}{2B}[d_Y(u,Iv) + d_Y(Iu,v)\right.$;

c) $k \max \{d_Y(u,v), d_Y(u,Iu), d_Y(v,Iv), d_Y(u,Iv), d_Y(Iu,v)\}$.

Question 3: It is interesting to consider and the case if convex contraction I is not continuous and if it is defined on a metric, that is, on a B-metric space.

Question 4: To consider the existence and uniqueness of the fixed point of continuous convex contraction of the third order, defined both on the ordinary metric and on the B-metric space. In this case the contraindicated condition has a form

$$d_Y(I^3u, I^3v) \leq p\, d_Y(I^2u, I^2v) + q\, d_Y(Iu, Iv) + r\, d_Y(u,v),$$

for all $u, v \in Y$, where $p, q, r \in [0, +\infty)$ and $p + q + r < 1$ in the case of the metric space. Find a condition if (Y, d_Y) is a B-metric space with a coefficient $B > 1$.

We conclude our work with another important question for this field.

Question 5: Let M and N be a closed subsets in the complete metric space (Y, d_Y) such that IM is a subset of N and IN is a subset of M. If for all $u \in M$ and for all $v \in N$ it follows that

$$d_Y(I^2u, I^2v) \leq p\, d_Y(Iu, Iv) + q\, d_Y(u,v),$$

then the continuous mapping I has a unique fixed point in cross-section of sets M and N. Prove or disprove this claim by example.

Remark 9. Since the termcyclic type mapping was introduced in (Kirk et al., 2003) and thus generalized the famous Banach theorem, many researchers considered the newly introduced phenomenon and obtained corresponding results in various classes of general metric spaces. As far as we know from literature, this type of mapping has not been considered in the case of convex contractions of Istratescu. Therefore, the previous question is interesting for researchers working in this part of the non-linear analysis. Assuming that $M = N = Y$, a positive answer to the last question gives us as a consequence the main result of Istratescu. In other words, we would get a true generalization of Istratescu's result.

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REFERENCES

Aleksić, S., Došenović, T., Mitrović, Z., & Radenović, S. 2019a. Remarks on common fixed point results for generalized $\alpha_* - \psi$ contraction multivalued mappings in b-metric spaces, Adv. in Fixed Point Theory, 9 (1), pp. 1-16.

Aleksić, S., Kadelburg, Z. T., Mitrović, Z., & Radenović, S. 2019b. A new survey: Cone metric spaces. Journal of the International Mathematical Virtual Institute, 9, pp. 93-121.

Aleksić, S. T., Mitrović, Z., & Radenović, S. 2019c. Picard sequences in b-metric spaces, too appear in Fixed Point Theory 2019-2020.

Alnafei, S. H., Radenović, S., & Shahzad, N. 2011. Fixed point theorems for mappings with convex diminishing diameters on cone metric spaces. Applied Mathematics Letters, 24(12), pp. 2162-2166. doi:10.1016/j.aml.2011.06.019

Ampadu, C. K. 2017. On the analogue of the convex contraction mapping theorem for tri-cyclic convex contraction mappings of order 2 in b-metric space. J. Global Research Math. Archives, 4(6), pp. 1-5.

Ampadu, C. B. 2018. Some Fixed Point Theory Results For Convex Contraction Mapping Of Order 2. JP Journal of Fixed Point Theory and Applications, 12(2-3), pp. 81-130. doi:10.17654/fp0120230081

Andras, Sz. 2003. Fiber Picard operators and convex contractions. Fixed Point Theory, 4, pp. 121-129.

Bakhtin, I. A. 1989. The contraction mapping principle in quasi-metric spaces. Funct. Anal, 30, pp. 26-37.

Collaço, P., & Silva J. C. 1997. . A complete comparison of 25 contraction conditions. Nonlinear Analysis: Theory, Methods and Applications, 30(1), pp. 471-476. doi:10.1016/s0362-546x(97)00353-2

Istratescu, V. I. 1981. Some fixed point theorems for convex contraction mappings and convex non-expansive mapping (I). Libertas Mathematica, 1, pp. 151-163.

Istratescu, V. I. 1982. Some fixed point theorems for convex contraction mappings and mappings with convex diminishing diameters. — I. Annali di Matematica Pura ed Applicata, 130(1), pp. 89-104. doi:10.1007/bf01761490

Istratescu, V. I. 1983. Some fixed point theorems for convex contraction mappings and mappings with convex diminishing diameters, II. Annali di Matematica Pura ed Applicata, 134(1), pp. 327-362. doi:10.1007/bf01773511

Jeong, G. S., & Rhoades, B. E. 2005.. Maps for which F(T)=F(T^n). *FixedPointTheoryAppl*, 6, *pp.*71 – 105.

irk, W. A., Srinivasan, P. S., & Veeramani, P. 2003. Fixed points for mappings satisfying cyclical contractive conditions. Fixed Point Theory, 4, pp. 79-89.

Kirk, W., & Shahzad, N. 2014. Fixed Point Theory in Distance Spaces.Cham: Springer Science and Business Media LLC. doi:10.1007/978-3-319-10927-5

Rhoades, B. E. 1977. A comparison of various definitions of contractive mappings. Transactions of the American Mathematical Society, 226, pp. 257-257. doi:10.1090/s0002-9947-1977-0433430-4

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ASSESSMENT OF RADIATION RISK FROM DRINKING WATER AT PUBLIC FOUNTAINS ON THE WIDER TERRITORY OF KRUŠEVAC

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ABSTRACT

Measurements of radon concentrations in drinking waters conducted on wider territory of the town of Kruševac included 16 public fountains, of which 4 are in the city itself and 12 in the surrounding area. Measurements of radon concentrations in water were carried out using radon detector RAD7 (DURRIDGE Co.). The values of radon concentrations in water are in the range of (5.3 ± 0.8) Bq l^{-1} to (71.0 ± 7.2) Bq l^{-1} and are below the reference level of 100 Bq l^{-1}, recommended by the European Commission. Since radon represents one of the most important causes of malignant diseases, annual effective doses of ingestion and inhalation are determined, whose mean values are in the range of 0.04 mSv y^{-1} to 0.52 mSv y^{-1}, and from 14.84 μSv y^{-1} to 198.8 μSv y^{-1}, respectively. During sampling, water temperatures and their pH values were measured. These results are the first results of measuring radon in water in this area by this method. The aim of this paper is to evaluate whether there is, or not, a radiological risk to people's health due to the use of water primarily for drinking, but also for other purposes.

Keywords: Radon, Water, RAD7, Temperature, pH, Annual effective doses of internal radiation.

INTRODUCTION

Numerous epidemiological studies have shown that the presence of radon in the environment is considered to be the second most important cause of lung malignancy (immediately after long-term exposure to tobacco smoke) (Manzur et al., 2008; Mitev et al., 2012; Aleissa et al., 2013). Therefore, great attention is paid to investigating the concentration of this radionuclide in water, air (especially indoors) and soil. Radon is a gas without color, odor and taste and therefore cannot be detected by human senses.

As a gas it penetrates the walls and the soil and accumulates in indoor spaces, and the organism is first introduced by inhalation. On the other hand, the solubility of radon in the water and the transport to large distances, allows the accumulation of radon in it, especially if water destroys uranium-rich rocks on its path (Appelton, 2013; Jowzaee, 2013). The resulting radon can be inserted into the water by the effect of the rivers and the flow of groundwater and it can be transported to greater distances. Radon is usually present in significant concentrations in those underground waters that are in contact with granite, gneiss, shale, and also sandstone and limestone. The presence of radon in water is influenced by a number of other factors, such as: water circulation pathways, presence of soluble gases, temperature and pressure, so the concentration of radon at relatively close sites can be different (Todorović et al., 2015). According to the European Commission's recommendation, the upper limit for radon concentration in groundwater that can be used for drinking is 100 Bq l^{-1} (WHO, 2004; European Commission, 2013). When radon-rich groundwater reaches the surface (springs, wells, public fountains), the concentration of radon in the water will rapidly decline during its movement and purification. Generally, groundwater is characterized by a higher concentration of radon from surface waters. However, if such water is used for drinking, or for some other purpose directly from the place of origin, as it is usual in the rural environment, there will still be a high concentration of radon, and the time elapsed from collection to consumption will not be enough to potential health risks associated with radon and its short-lived offspring would be prevented (Galan et al., 2004). It is therefore important to control the concentration of radon in water at the sources and public fountains in order to determine whether it is safe from the radiological point of view.

In human body, radon can be introduced by ingestion and inhalation, with an effective dose in the first place depending on its concentration in water, metabolism and kinetics in the body. Since radon is an aqua-phobic, and easily leaves the water, its presence in the water does not pose a direct danger to health (Todorović et al., 2012), but the radon released from such water indoor air will enhance indoor radon. Therefore, great attention is paid to measuring of radon concentration indoor radon atmosphere (Žunić et al., 2006; Nikolov et al., 2012). It was estimated that the inhalation of radon and its offspring, released from drinking water, caused as much as 89% of lung cancer, while the remaining 11% of the carcinoma in the first order of the digestive tract was due to ingestion of water with increased radon concentrations (USEPA, 1999).

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An important parameter of water is relative acidity or alkalinity, which is determined by measurement of pH value. Low pH value is the most important parameter linked to the high radium concentration, because it is readily soluble in low pH water (Sharma & Sharma, 2013; Kasić et al., 2016). Another important parameter that affects the presence of radon in water is temperature. It is noticed that content of radon in water decreases with an increase in temperature (Cothern, 1987).

This study presents the results of measurements of radon concentrations in water sampled on 16 public fountains on wider territory of the city of Kruševac. On the basis of the obtained values, the effective doses of inhalation and ingestion of radon were determined annually and summed up in the total dose of radiation exposed to the population in the area.

STUDY AREA

Kruševac (43 ° 35'N, 21 ° 19'E) is a city located in the central part of Serbia. According to the official census, the city has 64 500 inhabitants, while on the wider territory of the city there are 131 386 inhabitants. Figure 1 shows the map of 16 investigated locations.

Figure 1. Map of investigated area.

When looking at the geological structure of the investigated area, it is noted that the oldest crevices are crystalline shards of high degree of metamorphism, mostly of sedimentary origin. Among them, two complexes are distinguished: (1) ectic, within the Bloc of Big Jastrebac and smaller parties in the Kruševac neogenogen basin, and (2) magmatical, in the Mojsina Mountains with the presence of striated biotic and muscular gneisses.

In the area of Kruševac depression, that is, the largest part of the area investigated, there are the trenches of Drenova, Rasina and Dedina (Geological atlas of Serbia, 1999). Of the 16 explored public fountains 6, there are neogeneous clay, sand and gravel - "Pčela" Šanac, Kapidžija, Gaglovo, Jasika 1 and Jasika 2. On the pleistocene river terraces of the West Morava and Rasina there are Canina fountain, Čitluk 1 and Čitluk 2, fountain near FAM gate, the Lazareva big fountain and the Zulina fountain. Gari and Pakašnica are found on neogeneous clay, sand and gravel. Lazaric fountain is located on holocene alluvium, and the fountain of Sv. Petke in Šanac is located on crystalline shales of high degree of metamorphism.

EXPERIMENTAL

Materials and methods

In this study, radon concentration in water was measured by alpha spectrometer RAD7 (Durrige Co.) in Laboratory for Nuclear Physic at the Faculty of Science, University of Novi Sad. The base of this device is a hemisphere chamber of 0.7 l capacity, which inner side is coated with a material that is a good conductor. In the center of the chamber there is a silicon α-detector. At the inlet to the chamber there is a filter that prevents the entrance of radon progenies in the chamber and thus allows the measurement of only radon concentrations in it. The high voltage electric field (2000-2500 V) inside the detector chamber accelerates and directs to the surface of detector only positive ion of Po-218 originated from radon decay. The deposited Po-218 emits α-particles that, with a probability of 50%, enter the active volume n of detector and produce an electric signal which intensity corresponds to the energy of incident alpha particle.

In order to measure radon concentration in water RAD H_2O accessory to RAD7 detector was used with grab sampling method and two available protocols Wat-40 or Wat-250. It enables the aeration of sampled water are removing the available radon from water into the measurement chamber. The efficiency of extraction of radon from water into the air circulating to the measuring chamber depends slightly on the ambient temperature, but it is always greater than 90%. After 5 minutes of aeration in the measurement system equilibrium is reached, and there is no more radon that could be extracted from the sample. If the radon concentration is lower than 100 Bq l^{-1}, the usage of 250 ml bottles is recommended, with the extraction efficiency 94%. Otherwise, the water should be sampled in 40 ml bottles, where the extraction efficiency is 99% (RAD7 RAD H_2O).

RESULTS AND DISCUSSION

Within this study, waters from public fountains were sampled in 1.5-liter plastic bottles. During the sampling battles must be filled up to the very top and closed under a jet of water

to prevent accumulation of radon under the lid. The results measurements are summarized and presented in Table 1.

Table 1. Results of measurements at 16 investigating locations.

No. Location	T (^oC)	pH	C_o (Bql^{-1})	C_{corr} (Bql^{-1})	E_{ingest} ($mSv\ y^{-1}$)	E_{inhal} ($\mu Sv\ y^{-1}$)
1. Gari	14	7.14	2.4±0.5	6.0±1.2	0.04	16.80
2. Pakašnica	14	7.34	7.7±1.0	19.2±2.5	0.14	53.76
3. Lazaric	12	7.02	10.1±1.7	30.1±5.1	0.21	84.28
4. FAM gate	16	7.75	2.3±0.5	6.7±1.4	0.05	18.76
5. Lazareva	15	6.90	1.8±0.3	5.3±0.8	0.04	14.84
6. Zulina	16	6.94	3.4±0.5	10.0±1.5	0.07	28.00
7. "Pčela" Šanac	13	6.95	4.7±1.0	14.1±3.0	0.10	39.48
8. Pasjak	15	6.93	3.7±0.7	10.7±2.0	0.08	29.96
9. Kapidžija	13	6.99	2.2±1.1	6.5±3.2	0.05	18.20
10. Gaglovo	14	7.19	3.8±0.3	11.4±0.9	0.08	31.92
11. Jasika 1	12	6.91	10.5±2.1	14.9±2.9	0.11	41.72
12. Jasika 2	14	7.06	9.2±0.9	13.1±1.3	0.09	36.68
13. Sv.Petka Šanac	11	6.87	45±4	64.8±5.8	0.47	181.44
14. Čitluk 1	13	5.82	49±5	71.0±7.2	0.52	198.80
15. Canina	12	6.65	6.4±2.0	11.2±3.5	0.08	31.36
16. Čitluk 2	14	5.9	10.8±1.3	19.1±2.3	0.14	53.48

Since the concentration of radon could not be measured at the sampling point, it was very important that the time elapsed from sampling to measurement was reduced to a minimum, due to the decay of radon (half-life is 3.82 days). In addition to the measured radon concentration C_o, its decay correlated value C_{corr} is determined as follows (Todorović et al., 2012):

$$C_{corr}=C_o \cdot e^{(-(\ln 2 \cdot t)/T1/2)} \quad (1)$$

where time t defined the time elapsed from sampling to radon concentration measurement, which was in interval from 2 to 6 days in this study.

The results indicate that the concentration of radon in water ranges from 5.3 Bq l^{-1} – for Lazaric fountain to 71.0 Bq l^{-1} – for Čitluk1, with a mean value of 19.69 Bq l^{-1} and a standard deviation of 19.9 Bq l^{-1}. Slightly elevated radon concentrations were detected in waters from the fountain of Sv. Petka in Šanac– (64.8 Bq l^{-1}) and Čitluk1 (71.0 Bq l^{-1}), which is expected for the geological structure of the location terrain. However, result for Čitluk 2 indicates that although it is located in the same geological area, the difference in radon concentration is evident, which is probably due to various underground flows of these waters.

The radon concentrations present in this study are somewhat higher than, for instance, those measured in other part of Serbia: (4.6±8.5) Bq l^{-1} to (18.6±1.3) Bq l^{-1} (Todorović et al., 2012) and (7±1 Bq l^{-1}) to (21±2 Bq l^{-1}), except 149±12 Bq l^{-1} in Niska Banja (Vučković et al., 2014) and in the region: (4.0 to 17.4) Bq l^{-1} (Radolić et al., 2008) and (214 to 3702) mBq l^{-1} (Kasić et al., 2016), but still below the proposed reference level of the EU Commission Recommendation.

A low pH value of 5.82 measured in Čitluk1 water sample also indicates an increased presence of radon. But it is not possible to speak in general terms about the correlation between radon water concentration and pH value, which can be seen in Figure 2, which is confirmed by the small value Pearson's coeficient r = 0.26.

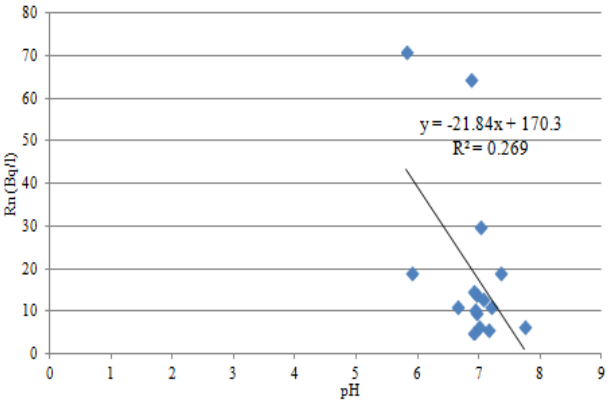

Figure 2. Correlation between radon concentrations and pH values of the water samples.

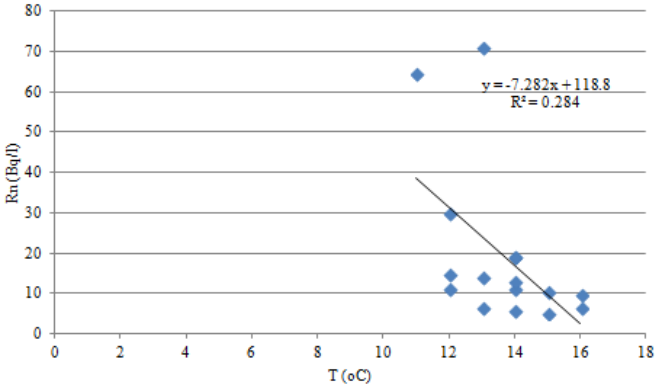

Figure 3. Correlation between radon concentrations and the temperatures of the water samples.

The temperature of the sampled waters is in the range of 11°C – for the fountain of Sv. Petka up to 16°C – for Zulina fountain and fountain near FAM gate and has no effect on the

concentration of radon in water, as indicated by the value of the factor r = 0.28, Figure 3.

Annual effective doses of internal radiation

For the control of the population exposure to natural sources of radiation, the concentration of radon in drinking water is a relevant parameter dose assessment (Kumar et al., 2016). The total effective dose of internal irradiation with radon dissolved in water consists of two components: the first defines the effective dose of ingestion, while the other defines the effective dose of radon inhalation.

Radon-rich water goes directly into the stomach, where radon can diffuse the body through the walls of the stomach to radiosensitive cells in the body. Part of the injected radionuclide can remain in the same sites for a long time, and some may be linked to macrophages and transferred further to the lymphatic cells. These cells can receive radiation doses of α-particles emitted during the decay of radon and its short-lived progeny in the stomach wall (UNSCEAR, 2008). The range of α-particles in the tissues is (40-70) μm, depending on their energy. The effective dose received from the ingestion of radon and its progeny and related to gastric tissue, expressed in mSv y^{-1}, is determined as follows (WHO, 2003):

$$E_{ing} = K \times C_{Rn} \times KM \times t \quad (2)$$

where: K is a conversion factor of 10^{-8} Sv Bq^{-1} for adults, and 2×10^{-8} Sv Bq^{-1} for children (WHO, 2003); C_{Rn} is the concentration of radon in water; KM is a consumption factor (optimal 2 l/d) and t is time of 365 days (USEPA, 1999). Since the concentration of radon in water decreases over time, the effective dose of ingestion refers to the initial concentration of radon in water (European Commission, 1998). The annual effective radiation dose by ingestion of radon inlet is in the interval from 0.04 mSv y^{-1} (Gari and Lazareva fountain) to 0.52 mSv y^{-1} (Čitluk 1), with mean value of 0.14 mSv y^{-1} and standard deviation of 0.14 mSv y^{-1}.

It should be emphasized that the radiation dose in the lungs is mainly derived from radon short-lived daughters in bronchioles and it is less than dose form radon itself. Radon is largely thrown out of the lungs by the exhausted air. Inhalation of radon progeny can lead to inhomogeneous deposition on the human respiratory tract and bronchial epithelium, which increases the risk of lung cancer. The dose received by inhalation of radon depends on the diameter of the inhaled surrounding particles of aerosols which are generally descended by the fetus and can vary by a factor of ~ 2 in normal home conditions. The effective dose of inhalation received by the lung tissue is obtained by multiplying the concentration of radon in water by a conversion factor of 2.8 μSv Bq^{-1} m^3 (WHO, 2003). Based on the present concentration of radon, the annual effective dose of inhalation is in the range of 14.84 μSv y^{-1} for Lazareva fountain to 198.8 μSv y^{-1} Čitluk 1, with mean value of 54.78 μSv y^{-1} and standard deviation of 55.8 μSv y^{-1}.

CONCLUSION

The highest concentrations of radon were measured in the water samples from the Čitluk 1 and the fountain of Sv. Petka and yeald 71.0 Bq l^{-1} and 64.8 Bq l^{-1}, respectively. The lowest concentration was measured in the water sample from the Lazarian fountain and yeald 5.3 Bq l^{-1}. The results of this study indicate that the concentrations of radon in water samples taken from public fountains at 16 different locations with a mean value of 19.72 Bq l^{-1} indicate that the radon concentrations in public drinking water samples are mostly low enough and below the proposed reference level of the EU Commission Recommendation. On that basis, it can be concluded that the water of these public fountains from the radiation aspect can be safely used for drinking, but also for other purposes. Unfortunately, up till now, there is no specific national regulation for the radon content in drinking water in Serbia.

On the variation of radon concentration in water of great influence, the geological structure of the terrain on which the fountain is located, as well as the path of groundwater movement, indicates a significant concentration of radon in a sample of water from Čitluk 1 from 71.0 Bq l^{-1} to which the groundwater comes passing through shards and sands.

The temperature of the sampled waters is in the range of 11°C for the fountain of Sv. Petka up to 16°C for Zulina fountain and fountain near FAM gate with no effect on the concentration of radon in water. The measured pH value is in the range of 5.82 (Čitluk 1) to 7.75 (fountain near FAM gate) and is not accompanied by a fully enhanced presence of radon in water.

The mean values of the effective doses of inhalation and ingestion of radon at the annual level are 0.14 mSv y^{-1} and 55.21 μSv y^{-1}, respectively and indicate that the water of these public fountains from the radiation aspect can be safely used for drinking, but also for other purposes optionally. It should indicate the significant contribution of the manuscript with its applications.

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REFERENCES

Aleissa, K. A., Alghamdi, A. S., Almasoud, F. I., & Islam, M. S. 2013. Measurement of radon levels in groundwater supplies of Riyadh with liquid scintillation counter and the associated radiation dose. Radiation Protection Dosimetry, 154(1), pp. 95-103. doi:10.1093/rpd/ncs140

Appleton, J. D. 2013. Radon in air and water. In O. Selinus Ed., Essentials of Medical Geology: Impacts of the Natural Environment on Public Health. Elsevier Amsterdam, pp. 227-262.

Cothern, C., & Smith, J. E. 1987. Environmental Radon. New York: Plenum Press.

-Durridge Co. RAD7 RAD H2O: Radon in Water Accessory.

-European Commission. 2013. Council Directive 2013/51/ EURATOM of 22 October 2013: Laying down requirements for the protection of the health of the general public with regard to radioactive substances in water intended for human consumption. Off J. Eur. Commun, L296:12-21.

-European Commission. 1998. European drinking water directive 98/83/EC of 3rd November 1998 on the quality of water intended for human consumption. Official J L, pp. 330.

Galán, L. M., Martín, S. A., & Gómez, E. V. 2004. Application of ultra–low level liquid scintillation to the determination of 222Rn in groundwater. Journal of Radioanalytical and Nuclear Chemistry, 261(3), pp. 631-636. doi:10.1023/b:jrnc.0000037106.78880.d0

Jowzaee, S. 2013. Determination of selected natural radionuclide concentrations in southwestern Caspian groundwater using liquid scintillation counting. Radiation Protection Dosimetry, 157(2), pp. 234-241. doi:10.1093/rpd/nct132

Kasić, A., Kasumović, A., Adrović, F., & Hodžić, M. 2016. Radon measurements in well and spring water of the Tuzla area, Bosnia and Herzegovina. Archives of Industrial Hygiene and Toxicology, 67(4), pp. 332-339. doi:10.1515/aiht-2016-67-2788

Kumar, A., Kaur, M., Sharma, S., & Mehra, R. 2016. A study of radon concentration in drinking water samples of Amritsar city of Punjab (India). Radiation Protection and Environment, 39(1), p. 13. doi:10.4103/0972-0464.185155

Manzoor, F., Alaamer, A. S., & Tahir, S. N. A. 2008. Exposures to 222Rn from consumption of underground municipal water supplies in Pakistan. Radiation Protection Dosimetry, 130(3), pp. 392-396. doi:10.1093/rpd/ncn156

-Ministry of Mining and Energy of Serbia. 1999. Geologicam maps: 1:2.000 000. In Geological Atlas of Serbia. Beograd: Geomagnetic Institute; Beograd: Barex. second edition, No. 2, on Serbian.

Mitev, K., Dimitrova, I., Zhivkova, V., Georgiev, S., Gerganov, G., Pressyanov, D., & Boshkova, T. 2012. Measurement of Rn-222 in water by absorption in polycarbonates and liquid scintillation counting. Nucl Instrum Meth, 677, pp. 31-40.

Nikolov, J., Todorović, N., Petrović Pantić, T., Forkapić, S., Mrđa, D., Bikit, I., Krmar, M., & Vesković, M. 2012. Exposure to radon in the radon spa Niška Banja, Serbia. Radiation Measurements, 47(6), pp. 443-450. doi:10.1016/j.radmeas.2012.04.006

Radolić, V., Šarić, I., & Miklavčić, I. 2008. Radon u vodi gradskih vodovoda u naseljima Požeško-slavonske županije [Radon in public waterworks of the cities of Požega-Slavonia County]. In D. Barišić, Ž. Grahek, B. I. Krajcar, & S. Miljanić Eds., Proceedings of the Seventh Symposium of the Croatian Radiation Protection Association, Opatija, Croatia.Zagreb: Croatian Radiation Protection Association. pp. 294-299. May; 29-31, on Croatian.

Sharma, N., & Sharma, R. 2013. Survey of radon concentration in drinking water samples of Hoshiarpur and Ropar districts of Punjab, India. Adv Appl Sci Res, 4, pp. 226-31.

Todorović, N., Nikolov, J., Petrović, P. T., Kovačević, J., Stojković, I., & Krmar, M. 2015. Radon in Water: Hydrogeology and Health Implication. New York: Nova Science Publishers.

Todorovic, N., Nikolov, J., Forkapic, S., Bikit, I., Mrdja, D., Krmar, M., & Veskovic, M. 2012. Public exposure to radon in drinking water in Serbia. Applied Radiation and Isotopes, 70(3), pp. 543-549. doi:10.1016/j.apradiso.2011.11.045

-United Nations Scientific Committee on the effects of Atomic Radiation (UNSCEAR). 2008. Sources and effects of Ionizing Radiation. In Report to the General Assembly with Scientific Annexes. New York: United Nations.

-US Environmental Protection Agency (USEPA). 1999. Radon in drinking water health risk reduction and cost analysis: EPA Federal Register. Washington, DC: Office of Radiation Programs. 64.

Vuckovic, B., Gulan, Lj., Milic. L. G., , Adrovic, F., & Radovanovic, D. 2014. Ovreview of radon concentrations in air and in water in Serbian spas. In Proceedings of Second East Europiean Radon Symposium SEERAS. Niš, Serbia. pp 39-42. May; 27-30.

-World Health Organisation (WHO). 2004. Guidelines for drinking water quality. Geneva. 3rd ed.

-World Health Organization (WHO). 2003. Guidelines for drinking water quality: Health Criteria and Other Information. Geneva.

Žunić, Z. S., Kobal, I., Vaupotič, J., Kozak, K., Mazur, J., Birovljev, A., Janik, M., Čeliković, I., Ujić, P., Demajo, A., Krstić, G., Jakupi, B., Quarto, M., & Bochicchio, F. 2006. High natural radiation exposure in radon spa areas: A detailed field investigation in Niška Banja (Balkan region). Journal of Environmental Radioactivity, 89(3), pp. 249-260. doi:10.1016/j.jenvrad.2006.05.01

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