

The Balkans Scientific Center
of the Russian Academy of Natural Sciences

3rd
International
Symposium

Modern Trends in Agricultural Production Rural Development and Environmental Protection

P R O C E E D I N G S



1 - 3 July 2021, Vrnjačka Banja, Serbia

**The Balkans Scientific Center of the
Russian Academy of Natural Sciences**



3rd International Symposium:

**Modern Trends in Agricultural
Production Rural Development and
Environmental Protection**

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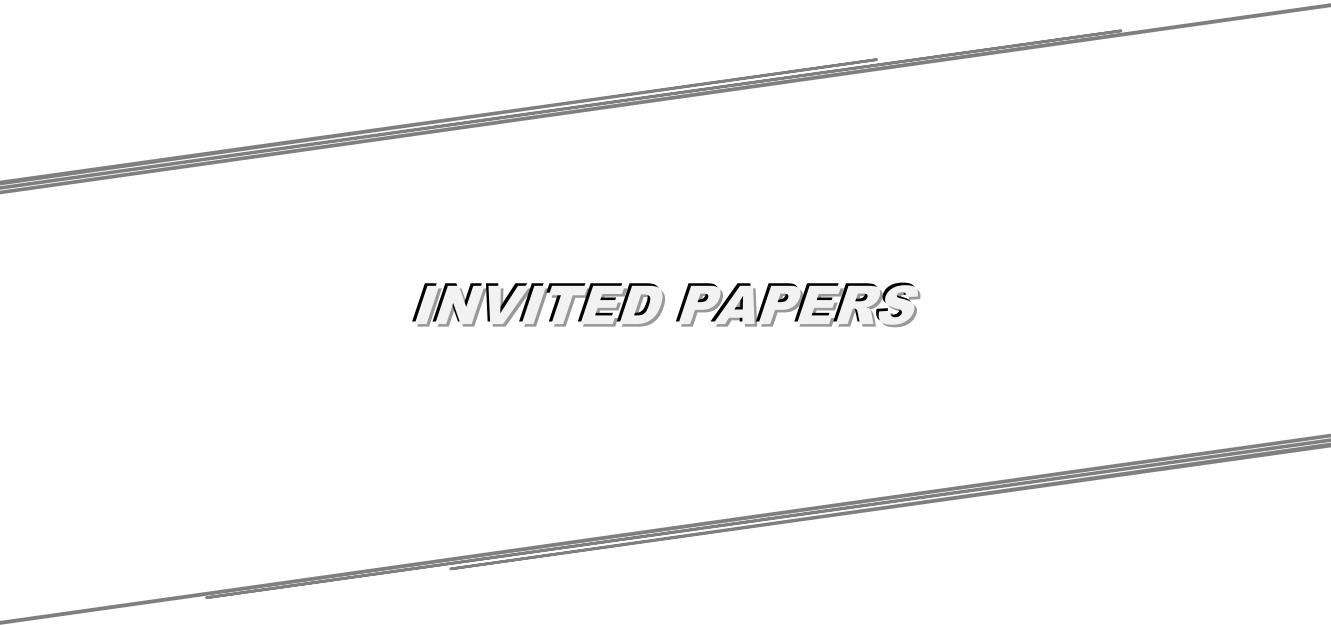
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INVITED PAPERS

**SOME NEW DATA OF AMPHIPODA SENTICAUDATA
(CRUSTACEA) FROM BALKAN PENINSULA
(CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA
321)**

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Abstract: *New distribution data of several members of Amphipoda Senticaudata (Crustacea) from the subterranean waters of Balkan peninsula are presented and some remarks on its taxonomical characteristics are given: Niphargus serbicus S. Karaman 1960, N. hrabei S. Karaman 1932, N. pannonicus S. Karaman 1950B and N. alatus G. Karaman 1973. New localities of Synurella ambulans (F. Muller, 1846) in Serbia are mentioned and reduction of eyes are discussed. N. pannonicus is a new species for fauna of Serbia.*

Keywords *taxonomy, distribution, Amphipoda, subterranean waters, Balkan, Serbia, Niphargus, Synurella,*

INTRODUCTION

The subterranean fauna of Amphipoda on Balkan peninsula are intensively studied during last hundred years and numerous new and endemic taxa have been discovered and described by numerous authors (Schâferna, S. Karaman, Sket, Schellenberg, G. Karaman, Fišer, Mehely, Dobreanu, Manolache, etc.). Nevertheless, the subterranean fauna of Amphipoda and its distribution of this region are only partially known.

During our recent investigations of material collected by me and other collectors, new data of some subterranean Amphipoda species and its areal of distribution are established.

MATERIAL AND METHODS

The samples were preserved in 70% ethanol. The specimens were examined and dissected in the mixture of glycerin and water, using a Wild M 20 stereomicroscope. Later, the dissected specimens were transferred onto slides with Faure liquid used for final preservation. The advantage of Faure liquid is that it is possible to dissolve the liquid on slides using normal water, and remove the dissected pieces for further studies. The body parts were drawn

using a camera lucida attachment and manually inked. Some morphological terminology and setae formulae follow G. Karaman's terminology (Karaman, G. 1969; 2012). All studies in this work are based on the classic morphological, ecological and zoogeographical studies. Terms "setae" and "spines" are used based on its shape, not origin, to avoid the confusion.

TAXONOMICAL PART

Family NIPHARGIDAE

NIPHARGUS SERBICUS S. Karaman 1960

Figs. 1-2

Niphargus (Jovaniphargus) jovanovici serbicus S. Karaman 1960: 75, figs. 1-4, 6;

Niphargus jovanovici serbicus G. Karaman 1972: 5;

Niphargus serbicus Sket 1972: 101, figs. 1-24, 106; G. Karaman 1974C: 24; G. Karaman 1980: 19; G. Karaman 1983: 67, figs. I-III; Barnard & Barnard 1983: 695; G. Karaman & Ruffo 1986: 531; G. Karaman 1988: 25; G. Karaman 1995: 324; G. Karaman 1997A: 47, fig. II, 4-7; G. Karaman 1997B: 350; G. Karaman 2011: 151; Kralj 2001: 168;

Niphargus jovanovici jovanovici (nec S. Kar.) Dobreanu, Manolache & Puscariu 1951: 579, figs. 1-2;

Niphargus jovanovici (part.) Carausu, Dobreanu & Manolache 1955: 262, figs. 240-242;

MATERIAL EXAMINED: Serbia:

S-7399= Paragovo near Sremska Kamenica, Fruška Gora Mt. (Novi Sad reg.), torrent in the forest, pump, December 2020, one exp. mixed with other *Niphargus* species (leg. Raško Karaman & Ivo Karaman).

REMARKS.

The single specimen (male 3.5 mm) from Paragovo mainly agree with description and figures of this species given by previous authors, considering smaller size of the specimen. Epimeral plate 3 strongly angular, with 4 setae along posterior margin and 2 subventral spines (fig. 1G). Urosomal segments 1-2 with one dorsolateral spine on each side. Urosomal segment 1 on each ventroposterior margin with one strong spine near basis of uropod 1-peduncle (fig. 1 I).

Propodus of gnathopod 1 almost ovoid, longer than broad (ratio: 77:56), along posterior margin with 4 transverse rows of setae (fig. 1A); palm inclined half of

propodus-length, defined on outer face by 3 unequal corner S-spines, on inner face by one subcorner R-spine; L-spines and M-setae absent (fig. 1B); dactylus with one median seta at outer margin and 4 setae at inner margin (fig. 1A).

Gnathopod 2 propodus rather larger than that of gnathopod 1, ovoid, along posterior margin with 4 transverse rows of setae (fig. 1C); palm inclined over 2/3 of propodus-length, defined on outer face by 3 unequal corner S-spines, on inner face by one subcorner R-spine; L-spines and M-setae absent (fig. 1D). Dactylus along outer margin with one median seta, along inner margin with 4 setae (fig. 1D). Dactylus of pereopods 3-7 strong, with strong short nail much shorter than pedestal (ratio: 18:57 (fig. 1F). This shape of dactylus is often present in taxa of *jovanovici*-group (or subgenus).

Basipodit of pereopod 7 is longer than broad (ratio: 83:50), along posterior margin with 6-7 setae, ventroposterior lobe not distinctly developed (fig. 1E). Pleopods 1-3 with 2 retinacula; peduncle of pleopods 1-2 with one short anterodistal marginal seta; peduncle of pleopod 3 along posterior margin with 2-3 median short setae.

Uropod 1 peduncle with dorsointernal and dorsoexternal row of spines; rami subequal, with short lateral and distal spines (fig. 1 I). Uropod 2: inner ramus only slightly longer than outer one.

Uropod 3 short and strong, inner ramus scale-like, short (fig. 1K), outer ramus 2-articulated: first article along outer margin with 4 groups of spines, along outer margin with 4 groups of spines mixed with single long plumose setae; second article very short, with one very short subdistal simple seta.

Telson longer than broad (ratio: 70:48), each lobe with 2 short distal spines accompanied by 3 very long plumose setae (fig. 1K). On ventral face of last mesosomal segment appear 2 mediolateral tubercles, characteristic for males (fig. 1H).

LOCUS TYPICUS: well in Ćuprija, Serbia.

LOCALITIES CITED:

AUSTRIA: Thalerhof airport, Graz ; Kasten, Gradenfeld near Graz (G. Karaman, 1997A).

SLOVENIA: Ljubljana (G. Karaman 1983; 1997A); Kozarje; Murska Sobota; Gornja Radgona; village Beričevo near Ljubljana; Ptuj; Gmajna near Slovenj Gradec (Sket 1972).

CROATIA: Opatovina near Zagreb (G. Karaman 1997A); Kutjevo; village Majerje near Varaždin; Koprivnica (Sket 1972).

BOSNIA & HERZEGOVINA: Srednja Slatina village near Bosanski Šamac (G. Karaman 1997A); Čehotina River near Foča (G. Karaman 1983; 1997A); Drina River near Brod na Drini (G. Karaman 1983; 1997A); Torrent Zujevna

near Blažuj (Sarajevo reg.) (G. Karaman 1997A); Sarajevo; Plandište near Ilidža; Kiseljak and Radanovići village NW of Sarajevo; vicinity of Kiseljak; Nova Bila village near Travnik; well between Dolac and Travnik; Dobrovo village near Višegrad; Donji Vakuf (Sket 1972).

SERBIA: Čuprija, wells, (paratypes) (S. Karaman 1960; G. Karaman 1983; 1997A); Trgoviški Timok River near village Trgovište (Knjaževac reg.) (G. Karaman 1983; 1997A); Pek River on road Golubac-Požarevac (G. Karaman 1983; 1997A); Valjevo; village Donja Gorevica near Čačak (Sket 1972); Paragovo near Sremska Kamenica, Fruška Gora Mt (present work).

CRNA GORA (Montenegro): Lim River near village Novšići (output of Plav Lake) (G. Karaman 1988; 1997A).

ROMANIA: Clinceanca (S. of Prahova, Ploesti region) (Dobreanu, Manolache, Puscariu 1951; Carausu, Dobreanu & Manolache 1955).

DISTRIBUTION: Austria, Slovenia, Croatia. Bosnia, Serbia, Cma Gora, Romania (fig. 2).

VARIABILITY. Rather variable species, variability of this species was already discussed by Sket (1972) and G. Karaman (1983, 1997A).

NIPHARGUS PANNONICUS S. Karaman 1950B

Niphargus ilidzensis pannonicus G. Karaman 1972: 5; G. Karaman 1974A: 55; G. Karaman 1974C: 18;

Niphargus (Supraniphargus) ilidzensis pannonicus S. Karaman 1950B: 55, fig. 7-14; S.Karaman 1960: 83; G. Karaman 1962: 44;

Niphargus illidzensis pannonicus Barnard & Barnard 1983: 692; G. Karaman & Ruffo 1986: 526; G. Karaman 2011: 150; Petrescu 2013: 25, figs. 1-13;

Niphargus puteanus pannnicus Zincenco, D. 1968: 376, Bleahu et al.. 1976: 120.

MATERIAL EXAMINED:

Sp. 133= Slavonija, leg. Dr. Mikoljčić (holotype? & paratypes), 7 exp. [Croatia];

S-6524= village Mandjaovac near Sremska Mitrovica towards Fruška Gora, Serbia, spring/torrent in Vranješ, church Sv. Kosta & Damjan, 28.6.2001, many exp. (leg. Gordan Karaman & Božana Karaman);

S-6525= fountain/ spring near Šišatovac monastery, on southern slopes of Fruška Gora Mt. (Sremska Mitrovica reg.), Serbia, 28.6.2001, many exp. (leg. Gordan Karaman & Božana Karaman);

S-6534= Ležimir village, spring near cottage of M. Bobić, southern slope of Fruška Gora Mt. (Sremska Mitrovica reg., Serbia), 28.6.2001 (leg. Gordan

Karaman & Božana Karaman).

REMARKS.

S. Karaman (1950B) described this species from one well in Slavonia (NE Croatia). Petrescu (2013) redescribed this species based on specimens from Romania (Gaura cu musca Cave, above road Orsova-Moldova Noia). Our specimens from Serbia mainly agree with paratypes and these described by Petrescu from Romania, and we have given up on the redescription of this species.

LOCUS TYPICUS: Slavonia, wells (between rivers Sava and Danube), Croatia.

LOCALITIES CITED:

CROATIA: locus typicus (S. Karaman 1950B; G. Karaman 1974A).

SERBIA: Mandjaovac; Šišatovac monastery; Ležimir village (present work).

ROMANIA: Gaura cu musca Cave, above road Orsova-Moldova Noia (Zincenco 1968; Bleahu et al., 1976; Petrescu 2013).

DISTRIBUTION: Croatia, Serbia, Romania. This species is new for fauna of Serbia.

Carausu, Dobreanu & Manolache (1955) figured two species with additional spines on dactylus of some pereopods under the name of already known species: (*Niphargus puteanus elegans* Garbini 1894 from Baia Mare (Baile Usturoi), Satu Mare, Timisoara reg., Romania, and *Niphargus puteanus baloghi* Dudich 1940 [loc. typ.: Baia Mare (Baramures-Kereches), NW Carpathes] described by Carausu et al., (1955) from Izei, Romania. It is not clear the real position of these taxa from Romania, although already Zincenco (1968) mentioned the similarity of *N. elegans* described by Carausu et al., (1955) from Romania with *N. pannonicus*.

NIPHARGUS HRABEI S. Karaman 1932
(restricted synonymy):

Niphargus tatrensis hrabei S. Karaman 1932: 194, figs. 7, 8a; Carausu, Dobreanu & Manolache 1955: 294, figs. 273-276;

Niphargus (Phaenogammarus) hrabei Dudich 1941B: 72; S. Karaman 1960A: 83;

Niphargus hrabei S. Karaman 1950A: 13; Sket 1981: 90, fig. 2 (map); Copilaș-Ciocianu et al., 2017: 4, tab 1;

? *N. thermalis* Dudich, 1941A: 165, figs. 1-3.

MATERIAL EXAMINED: Serbia:

Sp. 117/2= Smederevo, May. 1942, one male mixed with *N. valachicus* (leg. S. Karaman);

S-6674= Labine, Zasavica, pump, 13.3.2002 (leg. S. Stanković). 2 exp.;

S-7260= Jovača, Crna Bara, Sremska Mitrovica (Zasavica reg.), 9.8.2014, 1 juv. exp. mixed with *N. valachicus*, (leg. M. Stanković).

REMARKS AND AFFINITIES

The investigated specimens from Serbia mainly agree with existing descriptions and figures of this species.

Sp. 17/2: Smederevo: male 11.2 mm: Antenna 1 main flagellum 24 articles, main flagellum with 12 articles; accessory flagellum 2-articulated, shorter than last peduncular article. Epimeral plates 1-3 very sharply pointed. Urosomal segment 1 on each ventroposterior corner with one very small spine near basis of uropod 1-peduncle.

Gnathopods 1-2 with moderately large propodus trapezoid, hardly broader than long, palm inclined hardly over half of propodus-length, defined on outer face by one S spine, 2 L-spines and 3 M-setae, on inner face by one R-spine, dactylus long, along outer margin with row of several single median setae.

Pereopods 5-7 with narrowed basipodites without lobes. Dactylus of pereopods 3-7 slender, dactyls of pereopods 3-5 with 3 spines at inner margin, dactylus of pereopods 6-7 with one spine. Peduncle of pleopods 1-2 scarcely setose, peduncle of pleopod 3 along external margin with row of plumose setae.

Uropod 1: peduncle with dorsoexternal row of slender spines and dorsointernal row of setae, at distoventral tip with strong scale-like tubercle; inner ramus is longer than outer one.

Uropod 3 elongated, second article only slightly shorter than first one. Telson slightly longer than broad, with 3-4 distal spines and single short facial setae.

Description of *Niphargus thermalis* Dudich 1941A from St-Lukas thermal spa in Budapest mainly correspond with description and our material of *N. hrabei*.

LOC.TYP.: village Párkány-Nána (Nana Parkan, Southern-Slovakia).

LOCALITIES CITED

This species was cited from several countries, but we limited here citations from regions of Ex Yugoslavia only.

CROATIA: Ivanić grad (Croatia) (Sket 1981), Stream near the road W from, Lupoglav, Zagreb, Croatia (Copilaş-Ciocianu et al., 2017);

SERBIA: Lugovo; Šabac (Copilaş-Ciocianu et al., 2017); Dunav vicinity in Serbia [S. Karaman 1950B; S. Karaman 1960A; G. Karaman 2011]; Zasavica, Labine; Crna Bara, Jovača, (Sremska Mitrovica reg.); Smederevo (new).

DISTRIBUTION: This species is distributed in the middle and lower part of Danube River basin, from SE Germany, southern Slovakia, Hungary, Croatia, Serbia till SE Romania.

NIPHARGUS ALATUS G. Karaman 1973

Niphargus boskovici alatus G. Karaman, 1973: 275, figs. 1-3; G. Karaman, 1974C: 16; Barnard & Barnard, 1983: 690; G. Karaman & Ruffo, 1986: 523; G. Karaman, 1995: 323; G. Karaman 2003: 61.

Niphargus alatus G. Karaman 2014: 50.

LOCUS TYPICUS: spring on Veruša, Prokletije Mts (Montenegro), on cca 1500 m asl.

MATERIAL EXAMINED: Crna Gora (Montenegro)

S-7377= Dulo Cave near village Trešnjica, Medjurječje, June 2018, 2 females 14 mm (leg. M. Karaman);

S-5035= Uvac village, 7 km on road Veruša-Mateševo, pump, 14.10.1990, 2 exp. (leg. S. Vešović);

S-4889= Slatina, affluent of Morača River, , 22.2.1973, 2 exp. (leg. G. Karaman).

REMARKS.

The mentioned specimens agree with figures and description of this species from known springs and subterranean waters.

LOCALITIES CITED: Crna Gora (Montenegro)

Spring in Veruša, Prokletije Mts., on 1500 m asl (affluent of Tara River) (G. Karaman 1973: 275); springs on riverbank of Tara River near Kolašin (G. Karaman 1974C; G. Karaman 2003); Bjelasica Mt., Biogradsko jezero Lake, spring near Biogradska rijeka River, intermixed with *Gammarus balcanicus*; Donja Polja, spring near village Lijevak by Mojkovac; Veruša village, spring near the house of S. Vešović (G. Karaman 2003).

DISTRIBUTION: Montenegro, endemic species.

ECOLOGY. *Niphargus alatus* was found in the springs, subterranean waters along banks of the rivers; in the cave, sometimes mixed with *Gammarus balcanicus* Schäferna 1922.

Family CRANGONYCTIDAE

SYNURELLA AMBULANS (F. Müller, 1846)
(restricted synonymy)

Gammarus ambulans F. Müller 1846: 296, figs. A-C;

Synurella ambulans Schäferna 1922: 57, text figs. 26; 27 gn 1f, gn 2f; gn 1m; gn 2m; s`.ur3; md; d.p.; h.p.; t; 28 p 1-4; 29 p.s. 5; pl. 1, fig. 10; pl. 2. figs. 1-4;

Synurella ambulans ambulans G. Karaman 1974B: 86, figs. I, 1-7; II-V; VI, 1-4; VII, 5.

MATERIAL EXAMINED: Serbia:

S-7361= Tara Mt., Šljivovica, Hajdučka česma, 28.6. 2020 many exp. [leg. Ivo Karaman & Raško Karaman];

S-7367= Platičevo, Ruma, Bara Trskovača, june 2020 many exp. accompanied by *Niphargus valachicus* Dobr. Man. 1933 (leg. Ivo Karaman);

S-7396= Tara Mt., Kremna, Kolovrat, Konjska reka River, pump, 20.10.2020 7 exp. mixed with *Niphargus* sp. (leg. Raško Karaman & Ivo Karaman).

REMARKS. All collected specimens in the subterranean waters (pump) of these three localities are with small eyes. In various other localities, we collected populations with or without eyes; rarely we found populations with mixed specimens blind or with eyes (in this case usually eyes were on various degree of reduction). The question: Why in the same population we can find specimens with different degree of eyes reduction?, seems to be unsolved for the moment. We don't know which ecological and genetic factors conduct to this appearance.

The similar appearance was observed in the springs of Tržič/Monfalcone (NE Italy) where S. Karaman (1931) established *Rivulogammarus pulex danubialis* forma *subterranea* for the specimens with smaller eyes and rather elongated extremities.[= *Gammarus fossarum* forma *subterranea* S. Karaman 1931].

Recently similar situation was observed in northern Italy, in a small stream of the sublacustrine Ticino River basin (Lombardia) where blind specimens of *Gammarus roeselii* Gervais 1835 and these with well developed eyes were collected together (Paganelli et al., 2017). I am agree with suggestions of these authors (Paganelli et al., 2017) that *G. roeselii* belongs to stigoxene type of organisms [accidental or occasional presence in subterranean waters], but the absence of eyes usually have significant taxonomic value (not in this case). They mentioned (p. 106) "Overall, the four eyeless specimens collected in the Venara Stream did not show any other specific morphological adaptations to a

subterranean life and this suggests that the absence of eyes should only be considered as a genetic mutation, without an evolutionary significance”.

We don't know which genetic mutation will have evolutionary significance during certain period of time. It is unknown the ecology of these “genetically modified” blind specimens, did they survive longer time, reproductive problems of blind specimens, nutrition; did they prefer dark subterranean environment, the relations between blind and specimens with eyes, etc. We can suppose that just this kind of genetic mutation (losing of eyes) can be one way to colonize subterranean habitats.

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Fig. 1. *Niphargus serbicus* S. Karaman, 1960, male 3.7 mm, Paragovo near Sremska Kamenica, Fruška Gora Mt.: A= gnathopod 1 propodus; B= distal corner of gnathopod 1 propodus, outer face [S= corner S-spines, outer face; R= subcorner R-spine, inner face]; C= gnathopod 2, propodus; D= distal corner of gnathopod 2 propodus, outer face [S= corner S-spines, outer face; R= subcorner R-spine, inner face]; E= basipodit of pereopod 7; F= dactylus of pereopod 7; G= epimeral plate 3; H= sexual ventral tubercles; I= uropod 1; J= uropod 3; K= telson.

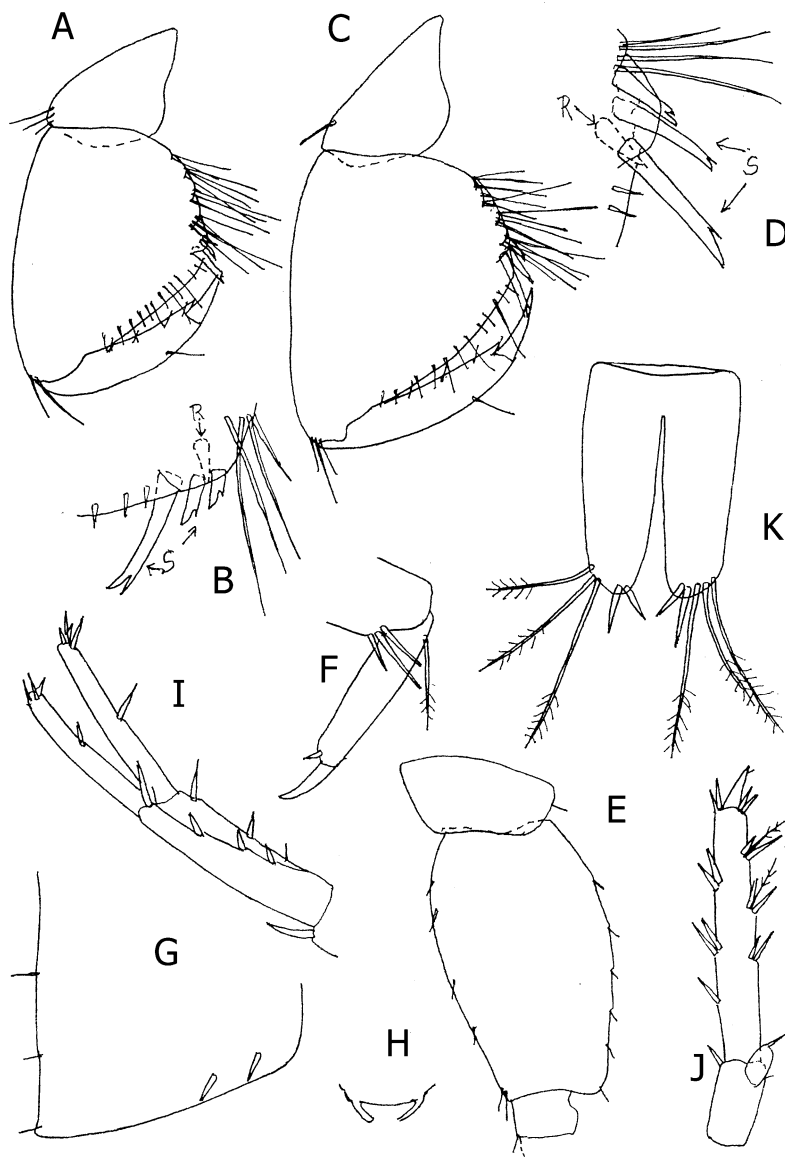
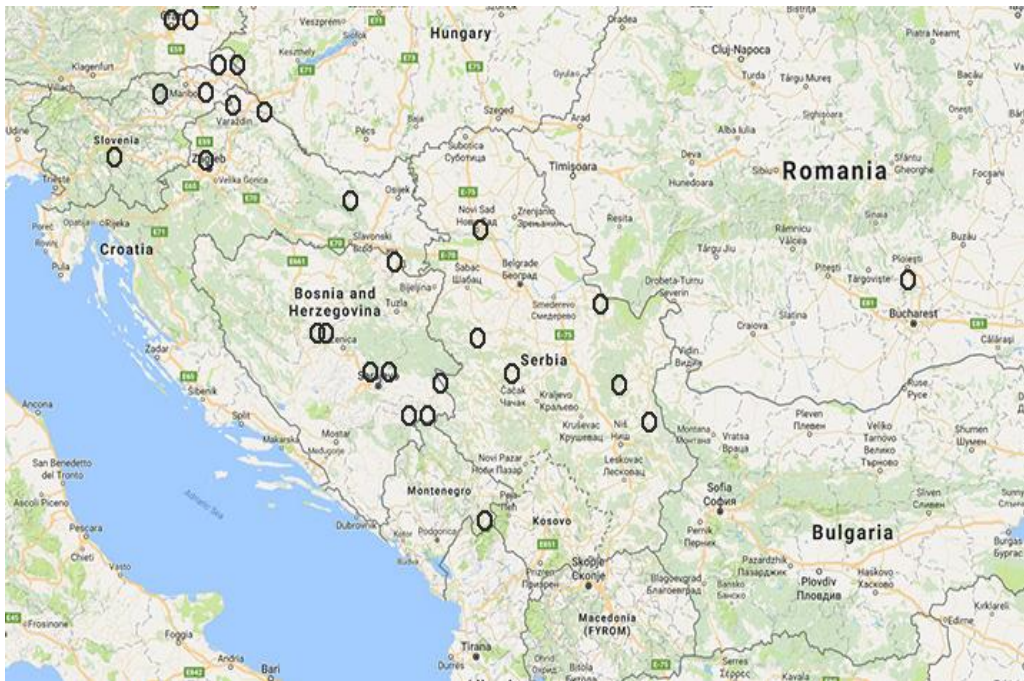


Fig. 2. Distribution of *Niphargus serbicus* S. Karaman, 1960 (circles)



COOPERATIVES IN SERBIA AS THE ROLE MODELS FOR THOSE IN REPUBLIKA SRPSKA

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Abstract: *In this paper, the author point to the fact that cooperatives have been returning in different fields of life, while in modern farming they have become the precondition for the sustainable growth of small farms. There is a large number of such farms in Serbia and joining forces for them means buying the raw material at cheaper price, as well as a facilitated and faster placement of their products on market. An initiative called "500 cooperatives in 500 villages" was launched in 2017 and to this date this number has been exceeded and new cooperatives are being opened on daily basis. The latest data show that today in Serbia there are more than 600 new cooperatives which has corrected old historic mistake when they were abolished by passing the Law on associated labor in 1976. Fifteen years later, since 1989, a few laws have been passed foreseeing the revitalization of cooperatives, the latest one in 2015.*

Finally, after many years of misconceptions and disrespect of original, generally accepted principles of cooperative business, it has come to light that cooperatives represent "economic basis for the survival of villages". Therefore, one of the most important issues today is horizontal and vertical joining of farmers in Serbia into cooperatives, shareholding companies and similar associations. This project has been supported by top political bodies in Serbia, and thanks to subsidy and other forms of aid, it is expected that the number of cooperatives will continue to grow. Since this form of projects does not recognize borders, Serbia will continue to cooperate with other countries in the region, EU and worldwide in sharing the best practice and experience in this field. Namely, cooperatives have been a success story in many countries since the middle of the 19th century. Given the fact that Republika Srpska is in many ways the entity with which Serbia has the closest collaboration, in this paper the author propose further opportunities for cooperation with Republika Srpska that have been discussed by corresponding ministries and their representatives. It is certain that their cooperation, projects and strategies in relation to cooperatives will contribute to improving the living conditions and standards of farmers in rural areas. The existing models of cooperatives work, that has been designed in association with Serbian Academy of Science, is also acceptable and can be applied in Republika Srpska with minor adaptation to specific nature of this entity.

Keywords: *Republic of Serbia, Republika Srpska, cooperatives, borders, collaboration, projects, strategies, village, subsidy, ministries, legislation, law on cooperatives.*

INTRODUCTION

Cooperatives have always had a great importance for the overall social and economic life, not only in our country, but in the whole world. This is particularly true for our villages, which have a great potential. Unfortunately, in the last few decades, in the Republic of Serbia (as well as in many countries around us, especially in some former Yugoslav republics), something quite the opposite has been happening. Villages have become more and more empty; they are dying out and disappearing. The reason for this is that living in rural areas is becoming very difficult and young people do not want to stay in their homes, even though they have better living and working conditions today than their ancestors did. Searching for a safer job and a better living, they leave their homeland and go to numerous countries around the world. Unfortunately, most often with a "one-way ticket".

That is why the fact that cooperatives in Serbia have a very long tradition, longer than 170 years¹, must not be neglected under any circumstances. This is the reason why Serbian Government has passed numerous decisions and laws in recent years in order to encourage and help the renewal of and strengthen the spirit of cooperatives². The main purpose of adopting these acts is the intention of the most important political institutions of Serbia to encourage cooperatives, so that they can become the economic, cultural and educational center of rural development again. It is very important that rural areas- modern villages, that have the appropriate infrastructure, cooperative homes, an ambulance and the appropriate medical staff for the treatment of the population, etc., become a "magnet" for attracting young married couples, who see their future in such environments. Construction and renovation of cooperative homes, opening of post offices in large villages, providing services to agricultural producers by cooperatives (supplying them with seed for sowing, guaranteeing product placement, mediation in obtaining appropriate subventions, etc.), would certainly convince young people to stay in their homes and not to go to other countries, in search of their existence.

COOPERATIVES IN SERBIA

After decades of neglect of cooperatives, significant funds have been invested in the reconstruction and development of the cooperative system in recent years. Our country, like most European and world countries, has finally realized that it must encourage its citizens to unite. Its role in this, at least for

¹ The first Serbian cooperative was founded in 1846 in Backi Petrovac, while the first cooperative in the world, which experienced its strength and prosperity only in the second half of the 19th century, was founded in 1844 in the small English town of Rogel.

² Nowadays, more than 800 million people are involved in cooperatives worldwide.

the time being, is invaluable, because it is expected to directly help those who need it, such as small farms. This means that it is necessary that, as soon as possible and as many people as possible, get involved in the process of revitalization of cooperatives in Serbia, because the existence of cooperatives (in various areas of social life) has undoubtedly given very positive results so far. It is certain that the realization of that process will not be easy at all because farmers, in the past few decades, have stopped trusting the spirit of cooperatives due to many failures, uniting suppression and strengthening of cooperatives on their own.

That is why, in the upcoming period, we must not repeat the past mistakes, but we should create new cooperatives based on the idea of unity, and above all, by gathering honest and hard-working Serbian hosts. Of course, it is very important to emphasize that this is not an easy job, considering that cooperatives, in the post-war period³, went through numerous changes, which were a consequence of socio-political transformations which affected the second and the third Yugoslav state. The "result" of all these changes was the closure of a large number of cooperatives, the fall of some of them into debt or their loss of properties. There are also positive examples (although not so numerous), which indicate that several cooperatives survived and overcome everything that affected them in the past few decades.

Cooperatives are something positive we should strive for in the future. When this term is mentioned, it usually means agricultural cooperatives. This is not true because there should be cooperatives in almost all the areas of social life. Namely, the very word *cooperative* tells us a lot about its essence and significance for the preservation of Serbian tradition, customs, culture and everything that represents the identity of Serbia and its people. If we go back to the past asking when the interest in the concept and structure of the family cooperative arose, as well as its adaptation to changed social, political and historical conditions, and its gradual disappearance, then, we will find the answer in the first half of the 19th century. Vuk Stefanovic Karadzic published *Serbian Dictionary* in 1818. There, he stated that Serbs have a so-called *cooperative*, which was popularly referred to as the *big house*⁴ and which was based on love, cooperation, helping and, above all, respect for the elderly by the younger, parents by the children, the husband by the wife and, especially, the head of the family cooperative (the oldest male member) by all the others. When the ties in the family cooperative weakened, when its gradual disappearance and the transformation of that *big family* into a small one, the so-called *nuclear family*, composed only of parents and their children (whose

³ It refers to the period after the World War II.

⁴ Besides this term, the names: "strong house", "big family" etc. were also used. For further information see: Novakovic, N., *Theories on the origin and structure of family cooperatives*, Population 1-4/2005, p. 105-106.

number is, unfortunately, decreasing from year to year), the biggest problem facing Serbia and its people - the *birth dearth* appeared. The same happened with the creation, development and disappearance of cooperatives.

The fact is that Serbia, just like the rest of the modern world, cannot be imagined without cooperatives, unions, mutual trust, respect and assistance, i.e., without the existence of cooperatives in different areas of life. The word itself originates from Ancient Rome, from the Latin expression "*Plures familiae in eadem domo (more Serbico)*" - "Many families in the same house (more Serbs)", and Vuk Karadzic wrote in his works that this community primarily refers, to the South Slavs, and that it was specific for Serbs at the time when he was born and when he lived and created. One does not have to be too intelligent to conclude that a larger group of people (more numerous) is stronger and more successful than an individual or a group with few members. That is how things used to work and will continue to work in the future, despite the numerous theories that claim that small communities function better or that small businesses (firms) have a better chance of success. On the contrary, when individuals are united in one collective, based on: sensitivity, trust, helping and readiness to solve problems (of any kind) together, then the chances of success are much higher. The situation is the same with companies, various economic organizations, firms, etc., because the rich (big) become even richer and bigger, and the small ones rarely have a chance for that. That is why they must unite in order to: reduce production costs, increase their competitiveness in the market and increase their earnings by selling their own products and services. This is particularly characteristic of the modern system of functioning, in which, as economists usually like to say: "Big fish eat small fish". This leads to small and weak companies disappearing over time, i.e., becoming an integral part of large companies, because they cannot cope with the growing competition in the market. An example of such a large company is General Motors Corporation, a concern that manufactures vehicles in 30 US states and 32 countries around the world and employs approximately 340,000 people nowadays. In addition, we should mention the large banking system "Raiffeisen", which owes its creation to the so-called societies that help the poor. These societies were founded in Germany in the second half of the 19th century, on the initiative of the man this system was named after- Friedrich William Raiffeisen. Later, they later grew into the first credit agricultural cooperatives.

Talking about the role of cooperatives and their importance is redundant since the development of cooperative organization has solved many problems. This was, first, realized by the members of the European Union. These problems are, first of all, social and economic, and cooperatives enabled fast employment, with special emphasis on useful experiences in theory and practice, i.e. legal regulations and practical action of states within the large European family (EU). These experiences were, to a large extent, accepted in

our country, so they were very useful in the preparation of the Law on Cooperatives⁵, which is still applicable. Today, when a great economic crisis is on the horizon (both in our country and in the whole world), there is more and more talk that every country should strive for self-sustainability. It should have such economic and social development, which will make it completely independent (very difficult) or as much independent as possible from the rest of the world. Cooperatives can certainly, to a large extent, contribute to such sustainable development, because they have extremely great potential. International experiences so far have clearly shown that cooperative organization has numerous advantages over other forms of organization, i.e., business, and that these advantages are of a very different nature. That is why adaptation of cooperatives to the world economy crisis and to financial uncertainty, as well as their resistance to numerous blows coming from that side⁶ stands out as one of the most important features of cooperative business. What are other social and economic advantages of cooperatives?! The answer is not difficult to give, because cooperative organization is carried out through a democratic model of government - a model, in which all members of the cooperative have equal rights and participate in its management equally. In addition, when people are united, they can withstand competition in the market more easily (cooperatives contribute to encouraging healthy competition), they can provide a better offer of services and can create better products, they have a cheaper access to necessary raw materials and other resources etc. This, certainly, cannot be provided by individuals, which is especially important for agriculture and its revival, revitalization of rural areas and enabling (strengthening) local economic development. It is especially important to emphasize the fact that cooperatives are a direct link between agricultural producers and the market itself and, thus, they allow easier marketing of their products, which, on one hand, reduces their costs and, on the other, increases their income.

That is why it is very important to establish the legal framework of cooperative business and point out the fact that it is very different, even among the member states of the European Union, let alone those states that have not yet become full members of the large European family. Constitutions of European countries regulate this issue differently, so we distinguish three categories of countries: 1) countries whose constitutions recognize the role and value of cooperatives and mention them as such; 2) countries that do not recognize them and do not recognize their existence, i.e., freedom of citizen uniting 3) countries whose constitutions guarantee freedom of citizen uniting, but do not mention cooperatives as institutions in their provisions. When it

⁵ Official Gazette of the Republic of Serbia, no. 112/2015.

⁶ Tomic, D., Gulan, B., Kostov, R., *Guide through Serbian cooperatives*, Institute of Agricultural Economics, Belgrade, 2017, p. 8-9.

comes to the Lisbon Treaty, which is a replacement for the failed Constitution of the European Union, cooperatives are not mentioned, but only the right of citizens to unite is. On the other hand, there are EU members, which in their constitutional text mention cooperatives and recognize their certain social role (Bulgaria, Italy, Spain, Portugal, Hungary, Malta). There are also constitutions of certain countries in which cooperative organization is mentioned but does not explicitly emphasize the existence and activity of cooperatives (Greece, Cyprus, Poland). As for the current Constitution of Serbia from 2006, it does not mention neither the village nor cooperatives and cooperative organization in any single place (except that Article 86, paragraph 1 states that "Private, cooperative and public property are guaranteed "). The situation is somewhat better when we analyze the text of the Constitution of the Republic of Srpska, because, only in one place it says that the Republic regulates and provides: "... development of agriculture and villages" (Article 68, item 8), while in the article 64 it states that "the Republic protects and encourages: ... - cooperatives".

BASIC PRINCIPLES OF COOPERATIVE MANAGEMENT

One of the first principles of cooperatives is their care, both for their members and for their consumers. For the former they should: constantly develop the creative function of business, sales marketing and strengthen competition, and for the latter they should: provide quality products and services, maintain the trust of their customers (users) and always guarantee what they do (provide services or produce). It is, in particular, important to follow innovations in the world, technological progress, and to enable and offer: lower prices, better organization and faster turnover, and, above all, to stay away from the *race for profit*, which is, unfortunately (especially today), ruthless and aims to make as much money as possible at all costs. Preserving the health of consumers and their lives must come first, and, in no case, profit can be ahead of these basic human values. This means that the humanistic approach to business, with its most important postulate *Do unto others as you would have them do unto you*⁷, is crucial. This golden rule is the source of the humanistic nature of cooperatives. Its role is to preserve human health and improve human life, and therefore the marketing system has a significant function of promoting only such services and products that in no way endanger the environment, that do not harm human health and are not dangerous to human life. Therefore, we can say that one of the most important functions of cooperatives is to attract its customers (regardless of whether they are cooperatives or not) with their helpfulness and kindness, honesty, correct

⁷ Flew, A. (ed.), *A Dictionary of Philosophy*, Pan Books in association with The MacMillan Press, London, 1979, p. 134.

attitude and active communication, while respecting their customers' requirements, needs and desires, tastes and attitudes, opportunities and ability to pay, and everything else related to the realization, development and preservation of good business relations. Unfortunately, cooperatives in Serbia are still lagging behind similar organizations in the European Union and advanced countries of the world because they do not apply the adopted, generally accepted, cooperative principles which should be the basis for cooperative organization. Because of that, there is still no necessary trust in cooperatives, primarily agricultural ones. It was lost during the past decades because agricultural producers had been deceived several times and they, most often, got only empty promises. Once lost trust is hard to regain because it was hard to gain.⁸ Therefore, it is necessary to establish this traditional correlation between two important principles, which occupy an important place in the corpus of all other rules on which cooperatives base their organization and business. They are usefulness and morality, and, between them, there must be no conflict, but mutual permeation in the long run. This means that the long-term development strategy is the basis for the existence of modern cooperatives.

Bearing in mind that we have already stated that "for a friend" means: mutual trust, the absence of unfair competition, putting common above individual interests, doing cooperative business in which everyone wins (and no one loses), of course, in proportion to their work and investment, This is the only way for the "ideal of general well-being" to be achieved, while overcoming individualism, selfishness and looking only at one's own interests⁹. This reflects the business spirit of cooperatives, without which there is no success and there are no positive results. In everyday life, it is often said (and it has always been so) that the most important thing is to "be humane", that is that, either as individuals or as members of certain collectives, we must act in accordance with well-known ethical principles, moral decisions, and a code of ethical conduct. It is also said that "it is not difficult to be nice", because, in private or professional life, we can only cheat someone once. Next time, the door will be closed and trust will be lost, and that is catastrophic for business relations¹⁰.

Therefore, every cooperative member should know that such a way of organizing and doing business does not tolerate any fraud or mutual

⁸ The same goes for honor, as many thinkers often state - it is very difficult to gain, but very easy to lose.

⁹ For further information see: *Celebration of the seventieth anniversary of Mihajlo Avramovc*, Cooperative Library of the Association of Procurement Cooperatives of Civil Servants, Belgrade, 1935m p. 176.

¹⁰ Markovic, D., *Cooperative Management*, Association of Scientific Creators and Cooperative Association of Yugoslavia, Belgrade, 2002, p. 48-53.

exploitation, because cooperatives are based on completely different principles, ie. on moral principles. If there is a disturbance of interpersonal relations, disorder of mutual rights, obligations and responsibility for the work, then the functioning of a cooperative and its survival are called into question. It must be governed by understanding and agreement, equal decision-making and mutual assistance, and not arbitrariness and the absence of the rule of law. The principle of democracy is the highest moral principle, and it represents the basis, ie. the foundation of cooperatives, because it does not allow the suppression of freedom of business and of decision-making. On the contrary, it enables complete equality and equivalent distribution of newly created value. However, all the principles of equality and democracy do not allow the so-called: *Equalization*, i.e. distribution of equal parts to everyone, regardless of the contribution to the business. On the contrary, this would violate the principle of a real contribution to the joint business and to the result and it would lead to an unfair redistribution in favor of some and to the detriment of others¹¹.

In order to prevent such mistakes, Serbia passed the Law on Cooperatives in 1996. It was amended and harmonized with modern trends in cooperatives and with the norms valid in the European Union and developed countries, by adopting a new, now valid Law from 2015. It is important to emphasize that the individual share of the cooperative is not independent and unchangeable. On the contrary, it depends on the success of the cooperative's business, and, therefore, it increases or decreases. The distribution of the realized income is done according to the contribution to the joint work, as well as according to the size of the share in the property of the cooperative. Constant control is performed by the cooperative members themselves. In the management of the cooperative itself, one of the basic principles of democracy-*one member, one vote* is applied. According to it, every cooperative member has an equal right to vote. It should also be pointed out (this is often overlooked) that there are numerous cooperatives in different areas of life. People usually think that when the words *cooperative* or *cooperative business* are mentioned, they refer only to agriculture or farming. Quite the opposite, there are many other types of cooperatives that are extremely important. For example: housing, consumer, craft, labor, student, youth, social, health, as well as other types of cooperatives for: production, trade, services and other

¹¹ These principles of "contractual" economy, "equalization", distribution in equal parts (regardless of the contribution to work, all because of maintaining social peace and proving that socialism, as a way to communism, is the best form of distribution), then the principle of social property (everyone's and nobody's), collective (and not personal) responsibilities, etc., were the main reasons for the collapse of such a system. Namely, the contribution to work and quality was not respected, but the "equalization" based on social coercion, in which people were not rewarded according to their work.

activities. Each of them, in its own way, enables easier, faster, cheaper, business of better quality, more efficient and more profitable business of its members. Cooperative unions- independent, interesting, business and professional organizations, are created in order to reduce business costs, to enable easier trade of products, to provide better services, and to generate higher income. Cooperative unions are founded by cooperatives and other cooperative unions, in order to realize, harmonize and improve their business, protect common interests, enable business connections, etc. Such a union has the status of a legal entity, which it gets by registering in the Register, and it takes at least 10 cooperatives for its establishment. A cooperative union has numerous tasks, most important of which are: promoting the above mentioned cooperative principles, encouraging cooperatives, providing professional and other assistance to cooperatives and representing their interests in state bodies, local governments and territorial autonomy bodies, and in other institutions and organizations, as well as representing cooperatives abroad and establishing cooperation with other cooperative associations outside the borders of our country etc. A cooperative union for a certain territory or a certain type of cooperative also organizes and encourages professional trainings, scientific research, informing, publishing and marketing activities, which is of interest for the improvement of cooperatives. It keeps records of cooperatives and cooperative statistics, performs mediation and other affairs in accordance with the Law on cooperatives¹². Having the above in mind, we can freely say that cooperatives have finally returned to Serbia, but also to the Republic of Srpska. In modern agriculture, cooperatives are a prerequisite for (self)sustainability of small farms, given that most such farms are in our country. Uniting enables small producers to purchase raw materials (seeds, fertilizers, planting material, chemicals, fuel, etc.) at a cheaper price, but also to place their goods on the market easier and faster, at pre-known and guaranteed prices. The action *500 cooperatives in 500 villages* is currently going on in our country. But according to the latest reports in the media, based on data obtained from the Ministry in charge of regional development and the Ministry of agriculture, that number has significantly exceeded to more than 600. In order to further strengthen the established cooperatives and to enable the establishment of new ones, the state of Serbia has allocated significant funds from its budget. For example, from 2017 to 2019, only 152 cooperatives were subsidized with 1.7 billion dinars, while in 2020, about 60 more cooperatives will receive such incentives. Competitions for obtaining these funds have been announced and are waiting for new users.

Given that Serbia and the Republic of Srpska have (and should have) extremely good relations in all areas of social and economic life, this model of

¹² See more about this in: Law on Cooperatives, Article 70.

founding, subsidizing and developing cooperatives has been "accepted" in our environment, above all, in this entity of Bosnia and Herzegovina. That is why, at the end of January this year, in Belgrade, there was a meeting between the representatives of our competent ministries and the Minister of Agriculture, Forestry and Water Management of the Republic of Srpska, Boris Pasalic. On this occasion, Minister Milan Krkobabić stated that: "Cooperatives know no borders", so they talked about cooperation in this area, about current projects between the two countries and about strategies for the upcoming period. The experiences gained in recent years in the Republic of Serbia are certainly of great importance for the acceptance of the existing model of cooperatives in the Republic of Srpska. This model was built in cooperation with Serbian Academy of Sciences and Arts (SASA) and it was estimated that it could be acceptable (applied) in the Republic of Srpska as well. All the specifics of the environment in which this model is accepted should be respected, but the common conclusion is that there is no state renewal without a strong village, which should be urgently revitalized and helped in order to prevent devastation and disappearance of many rural areas. The importance of rural areas was proven during the two-month Covid-19 pandemic, when many people turned to work in their gardens, fields and to cultivating the land. They realized that when they have healthy, organic food, clean air, unpolluted soil and drinking water, then they can solve all troubles and problems much easier. According to official data from June 18th, 2020, there were 20.000 more hectares sown in the Republic of Srpska than usually in that period.

Numerous talks of the representatives of the competent ministries of Serbia and the Republic of Srpska have borne fruit so, the Serbian model of cooperatives will come to life in this entity of Bosnia and Herzegovina, too. Both sides realized that a producer himself, especially the one with a small property (as most of them are), is insufficiently competitive and, almost, invisible on the market. That is why it is necessary for him to unite with others, so that he can survive and sell his products on the market. Of course, without the help of the state, it is difficult to achieve a positive shift, so for 2020 the Government of the Republic of Srpska allocated subsidies (incentives)- 75 million convertible marks (32.5 million euros), which is 4 million marks (2 million euros) more than the previous year. That is still insufficient, but these investments follow the growth of the budget and they increase from year to year¹³. These subsidies are provided for fruit growers, farmers and stockbreeders, as well as for the formation of their cooperatives, because saving the village is a serious issue, which requires immediate measures. Finally, after several decades, it is concluded that cooperatives are the best model for the survival of people in the countryside and that individuals, as well

¹³ According to the calculations done by the authorities, the farmers of Republic of Srpska would need around 200 million convertible marks in relation to the proposed 75 million.

as small farms, cannot survive alone. Only united, with joint efforts, can they make a step forward towards survival and self-sustainability. As noted above, the existing model of cooperatives in the Serbia, conceived in cooperation with SASA, will be applied in the Republic of Srpska, whose representatives hope that in the second half of 2020 this model of renewal of cooperatives will be realized. Serbia will certainly help in every way, as far as it is possible, both in the professional and financial sense, because everyone in both countries has finally realized that, in addition to the fight against the *white plague*, the rescue of villages is the most serious issue, a priority that requires urgent action. That is why the competent ministers of Serbia and the Republic of Srpska agreed on joint action according to the program that will be proposed by the National Team for the Revival of the Villages of Serbia.

When it comes to the Republic of Srpska, in many ways our *closest* and *favorite* entity, it is indisputable that the state of Serbia should help as much as possible and offer its assistance, in accordance with its capacities in all areas of social life. The same goes for cooperatives, since the current Serbian model is acceptable and applicable in the Republic of Srpska (and, certainly, in some other countries around us). It is very important to emphasize here that the mentioned entity of Bosnia and Herzegovina has all state functions and competencies (except for those that are explicitly transferred to the competence of B&H institutions by the Constitution of B&H). Constitution is the highest general legal act and it must be respected in every state, as well as in this former Yugoslav republic. The Constitution of the Republic of Srpska itself ¹⁴ (article 64), says that "the Republic shall protect and encourage: ...; cooperatives and crafts; physical culture and sports ", and the constitutional basis for the Cooperative development program of this entity is contained in Amendment XXXII, paragraph 1, item 8 of the RS Constitution (*Official Gazette of the Republic of Srpska*, No. 28/94). This Amendment stipulates that the Republic of Srpska "regulates and ensures the basic goals and directions of agricultural and rural development."

When it comes to cooperatives in this area, it should be said that the Republic of Srpska has a very long tradition (with agricultural cooperatives in particular) which has gone through various stages of development and work. In that long period, there were both ups and downs. However, despite achieving certain results, cooperatives did not take the place that belongs to them and did not contribute to the development of the economy of the Republic of Srpska as much as they should have and as much as it was expected. The same as for Serbia, for the Republic of Srpska it can be said that the development of the village is only possible if there are agricultural cooperatives, small and medium firms and other forms of business associations. The fate of cooperatives and of

¹⁴ "Official Gazette of the Republic of Srpska", No. 21/92 - refined text, 28/94, 8/96, 13/96, 15/96, 16/96, 21/96, 21/02, 26/02, 30 / 02, 31/02, 69/02, 31/03, 98/03, 115/05, 117/05.

this way of organizing, which in the post-war period (second half of the 20th century) affected Yugoslavia, did not bypass the area currently discussed in this paper - the Republic of Srpska. Cooperatives established after the Second World War, very quickly, turned into their opposite and did not serve the basic purpose for which they were founded. Personal interests prevailed, the essence of cooperative organization was not understood, business organization was poor, cooperatives were (mostly) left to themselves. Control and support mechanisms were not established, cooperative principles were violated. There was no management or, if there was, it was untrained. There were numerous negative (non-cooperative) tendencies, etc. That is why, today, cooperatives need strong state support which will be based on the adopted principles of the International Cooperative Movement because it has become one of the irreplaceable segments of agricultural development and rural survival.

The role of cooperatives is expected to be much greater in the future than at present. We can say that, given that farmers' products are mainly processed for the needs of their own households, while one, much smaller part, is intended for sale on the market, cooperatives in the Republic of Srpska are not at the level they deserve and should be. Cooperatives in this area experienced significant development only in the first decade of the 21st century, so that, at the end of this decade, approximately 400 agricultural cooperatives were registered, with one cooperative consisting, on average, of 18 agricultural cooperatives¹⁵. Despite that statement, it must be said that the stated number of cooperatives, which is much higher today, is not in the function of improving agricultural production, as it was at the beginning of their development, exactly two decades ago. Since most farms are not members of the respective cooperatives, a fundamental reform of the cooperative sector in the Republic of Srpska is necessary. The largest number of active agricultural cooperatives is concentrated in the Banja Luka region - 37%, while the East Sarajevo region participates only with 8%. This situation must be overcome, because cooperatives are extremely important for the development of agriculture and strengthening of the economy (both of developed and underdeveloped countries)¹⁶, where the Republic of Srpska is incomparable with numerous European Union countries.

When it comes to legal regulation of cooperatives, the work of agricultural cooperatives is regulated by the Law, at the highest level. By 2008 the Law on agricultural cooperatives of RS¹⁷ was valid, while the RS Law on

¹⁵ The state of the cooperative sector in RS, Paper presented at the Conference on Agricultural Cooperatives of B&H, Agricultural Cooperative Association of RS, 2009, p. 2.

¹⁶ The Republic of Srpska Development Strategy, Sector Study: Non-Financial Enterprises and Agriculture, RS Institute of Economics and Faculty of Agriculture, University of Banja Luka, 2010

¹⁷ "Official Gazette of RS", no. 18/99.

Agricultural Cooperatives was passed that year¹⁸. Just like in Serbia and other countries of the modern world, cooperatives in the Republic of Srpska are based on generally accepted cooperative values and cooperative principles. Today we can say that the policy of support for the development of agricultural production by the state has changed significantly in recent years and these changes can be felt in all areas of agricultural production. Cooperatives have a special place in such a changed policy of the state, because a lot is being done to build a legislative and strategic framework, to provide subsidies, establish a cooperative audit system, regulate cooperative property rights, conduct a census of cooperative property, establish a register of cooperatives, etc. All these activities can be a very important basis for the modernization of the cooperative sector in the Republic of Srpska and, of course, in strengthening the position of cooperatives, which have, many times, proven to be a very important factor of economic development.

The fact is that there are numerous problems of cooperative organization, legislation and institutional support in the Republic of Srpska, because legislation is still controversial when it comes to the ownership of cooperative property and its registration¹⁹. Also, this regulation is not in line with European norms, there is a lack of understanding and a lack of interest of local governments in supporting cooperatives, there is a noticeable privatization of cooperative property by various subjects, Agricultural development strategy is not implemented in the part related to cooperatives, etc. Therefore, it is necessary to make changes to the Law on Agricultural Cooperatives²⁰, which would enable, as mentioned above, registration of cooperative property and which would eliminate the existing limitations and shortcomings that have been being pointed out for a long time. In the Republic of Srpska, there are so-called active and inactive cooperatives and, therefore, it is necessary to make a "cut", which would carry out the bankruptcy and liquidation of such inactive cooperatives. This would lead to their differentiation and re-registration. It is necessary, just as in the Republic of Serbia, to provide broader support for the development of cooperatives and to give significant incentives to the agricultural sector (such support and incentives existed in the past, but most often they remained just on paper). Finally, cooperation between the two countries should be continued and expanded for mutual assistance, exchange of experiences and for provision of professional services.

In addition to these problems, in the Republic of Srpska, there are numerous unresolved organizational and personnel issues, because the

¹⁸ "Official Gazette of RS", no. 73/08.

¹⁹ Therefore, the conditions under which cooperatives can use cooperative property are also disputable.

²⁰ "Official Gazette of the Republic of Srpska", no. 73/2008, 106/2009 and 78/2011.

planning and coordination of cooperative activities is not at a satisfactory level. The influence of politics (political officials) on the functioning of cooperatives and the implementation of the basic principles on which they operate²¹, represents a significant problem, as well as the non-implementation of one of the basic principles of cooperatives - cooperative education (organized forms of education of its members, as well as the adoption of new knowledge and technologies) which results in insufficient knowledge of agricultural producers, cooperative principles and basic rules, insufficient training of management and insufficient professional training of staff in agricultural cooperatives. All this results in, still, a high degree of distrust of agricultural producers in cooperative organizations, because producers and cooperative members do not participate enough in the management of cooperatives. What contributes to this is a very modest promotion of: cooperatives, basic values of cooperative organizing and of their possibility to significantly contribute to the development of local governments and their communities. Cooperatives must be the main place where direct agricultural producers can get raw materials for their activities and receive incentives for the expansion of production activities. This leads to insufficient activity of cooperatives in the field of rational use and protection of natural resources (land, water, forests, etc.) and their insufficient presence in the field of rural development itself.

In order to solve problems like this, it is necessary (as it was done in Serbia)²² to establish stronger ties between cooperatives and scientific institutions (appropriate Academy of Sciences, institutes, institutes, agencies, etc.) and to strengthen economic activities of cooperatives in their areas. Cooperatives also have a great opportunity to organize certain business centers, markets, stock exchanges and other associations, which are of interest to cooperative members and other producers. And this can encourage the employment of educational staff and other professions in order to improve production activities and management of cooperative organizations. Promotion of cooperatives is insufficient. Therefore, it is necessary to organize it among producers and users of services provided by cooperatives. This can be achieved with well-designed promotional material and by providing basic information about the role of cooperatives in the development of both the local communities and the entire Republic of Srpska.. That is why it is necessary to

²¹ These principles, as we stated in the part related to cooperatives in Serbia, include voluntariness, mutual solidarity, respect for democracy, management of cooperatives under equal conditions, the need for continuous cooperative education, establishing cooperation between cooperatives, etc.

²² It should be emphasized here that the situation in Serbia is not ideal either and that the development of cooperatives, ie. its restoration, is only at the very beginning. So far, a lot has been done to solve some of the most important problems related to the development of cooperatives and their greater role in the economic development of the country, but that is far from what it should be and what is expected from their activities.

constantly point out the importance and necessity of cooperative organization, scientific gatherings and round tables and to participate in projects dedicated to cooperatives. This would enable the creation of the necessary database (inventory of cooperatives), which would indicate which cooperatives are active and which are inactive, which ones should be liquidated, and which ones should not. A great contribution to this aim would certainly be the opening of high schools and colleges, as well as faculties for cooperatives (basic academic studies and appropriate modules in master's studies). All this already exists in developed European countries, as well as in some other countries of the world, we just need to apply other people's experiences and adapt them to our own needs, conditions, perceptions and the time in which we live. Serbia and its people began to slowly "melt", risking their own survival when family cooperatives were destroyed. This led to the creation of the so-called "nuclear families", composed only of spouses and one or two children (rarely three). The situation is the same in the Republic of Srpska, so it is necessary to revitalize and support the organization of family cooperatives, the creation of family farms and the return of basic values on which marriage and family, the basic cells of every society, rest. Strategic plan for integral rural development of the Republic of Srpska 2016-2020. submitted by the Government of the Republic of Srpska in 2015, is very important for the future development of this entity of B&H, but its implementation should involve cooperatives to a much greater extent.

A special kind of problems appear in the realization of the program of development of agricultural cooperatives in the Republic of Srpska. They regard financing, complicated procedures for getting loans and high interest rates (the situation is no better in Serbia either). Borrowing from banks makes it very difficult to secure a mortgage, as a guarantee for the return of borrowed funds. Then, illiquidity increased due to the lack of working capital, the small amount of incentive funds from the budget and the burden of cooperatives with debts, which date back to the war period (1990s). What are the solutions to these problems? First, it is necessary to provide more favorable credit funds for the purchase of raw materials, equipment and machinery, to exercise the rights to faster and easier incentives, to consider the possibility of establishing savings and loan cooperatives, to provide support from the budget of local governments and the entire Republic of Srpska in order to continuously increase cooperative property and the participation of cooperatives in the use of incentives and more.

Finally, one of the significant problems in relation to the previous ones is the problem of market and marketing, given that the market of agricultural products in the Republic of Srpska is disorganized and there is unfair

competition, as well as weak protection of domestic production.²³ For agricultural producers, cattle breeders and all other individuals or collectives who create a new product, the guarantee of safe placement and purchase of their products at a guaranteed price is of crucial importance. Unfortunately, this is neither the case in the Republic of Serbia, nor in the Republic of Srpska²⁴ (this is, above all, related to the problem of charging retail chains for delivered products). Normal functioning of cooperatives is burdened by their insufficient technical equipment, outdated machinery and equipment, fragmented possessions of cooperative members, high prices of oil, derivatives and raw materials, poor economic environment in the Republic of Srpska and unfavorable position of agriculture in the primary distribution. The competent institutions and their representatives offer a number of solutions to these problems. These are: introducing certain protective prices for some basic agricultural products, protecting domestic production, strengthening commodity reserves, increasing product quality, institutional market organization, establishing long-term business relationships. with sales chains etc.

CONCLUSION

Based on the above information, we can conclude that cooperatives were formed in difficult economic and social circumstances of the 20th century, when farmers realized that they could improve their market position only by joint efforts, mutual solidarity and mutual assistance, investing their own property in cooperatives. That is why cooperatives in all the European Union countries, as well as the ones in most of other European countries, are one of the main links in the entire food chain. They adapt the products of their members to market requirements while improving their market position and guaranteeing the quality of the products. This way, they significantly contribute to the economic vitality of rural areas, because they represent a significant source of employment. They greatly contribute to the economic growth and development of each country individually. With this aim, at the end of the 20th century, International Cooperative Alliance (ICA) was founded. It includes cooperative organizations in all the sectors of life, and its task is to solve numerous problems that cooperatives face. This Alliance has its own European regional organization, as well as other, less important committees and organizations representing cooperatives on the European continent. Therefore, Serbia and the Republic of Srpska, while respecting the principles on which

²³This also happens in Serbia, where foreign agricultural products- meat and meat products, of poorer quality and at a higher price, are imported from the surrounding countries, other European countries and from around the world.

²⁴ Appropriate business relationships between primary production, processing and marketing are not established.

cooperatives are based, as well as the experiences gained in the work of these international and European alliances and organizations, should continue to revitalize cooperatives in all areas of social life, especially in rural areas in order to help direct producers in the procurement of everything they need to smoothly organize their production activities, and to sell their finished products at guaranteed prices, which can enable them sustainable development and further investment in the production process. What is very important for the smooth functioning of cooperatives and their survival, development and sustainability, is the fact that cooperative activities should lead to the benefits of all members, in accordance with their participation and contribution to the work of cooperatives. Only in this way will cooperatives in the Republic of Serbia and the Republic of Srpska become a significant socio-economic process and an important segment of their economies.

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SUCCESS AND TASKS IN SOIL ECOSYSTEM HEALTH RESEARCH IN THE FIRST QUARTER OF THE 21ST CENTURY

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Introduction

"There is nothing more practical than a good theory" is a well-known expression with which it is difficult to disagree. A good theory means correct, bringing practical results, fruits. The concept of soil fertility - "the birth of a fruit" is still a defining characteristic for the soil, this main source of the living world. Modern requirements in food production oblige to preserve the environment. The famous phrase of the Russian nugget I.V. Michurin "not to expect favors from nature. To take them from her is our task," is recalled as a historical incident. The environment is an ecosystem in which soil is the basis of everything that makes up an ecosystem. Modern definition of the soil it is soil ecosystem (SE) representing an organic–mineral natural product formed under certain natural and climatic conditions; it is maintained by continuous microbial–plant interactions in the initially quantitatively predominant inorganic substance. This product includes the biota, mortmass, and metabolites affected by continuous enzymatic and chemical transformations; it also accumulates biophilic elements. Important biological and physicochemical processes are ongoing in it, including biogeochemical elemental cycles and microbial cycles. The product features a significant buffer capacity against various stress factors, provides nutritional substances for plants and heterotrophic biota, and acts as a source and discharge of biophile elements and biodiversity (Kupriyanov et al., 2010; Semenov, Sokolov, 2016).

Comprehension and understanding that the soil is not a "bioinert body", but a biological, ecological system and, therefore, like all biological objects, has health, can be ill and needs treatment, arose at the very end of the twentieth century (Doran et al., 1996).

There was a fairly quick understanding of the importance and depth of the content of this concept. (Van Bruggen, Semenov, 2000). There was a need to develop methods for the quantitative determination of the essentially immense concept - soil health. "Soil health" is a biological category that reflects the state of activity dynamics of the biotic component in the organic-

mineral complex of the soil; this biological category is characterized by adequate activity of biotic processes (synthesis and hydrolysis) that corresponds to the natural climatic zone, their resistance to disturbing influences (biotic and abiotic stressors), and the “closedness (self-sufficiency)” of biophilic element and microorganism cycles. The healthy soil of agroecosystems is also characterized by the conformity of its quality to standard indicators and adequate fertility (for a specific climatic zone). This definition of soil health is applicable to any soil (with the exception of anomalous soil) and does not contradict the substantive essence of traditional characteristics, but rather integrates their content, since the indicators of the dynamics of the biotic component’s activity are interrelated with both the physicochemical parameters of the soil and the actual soil fertility. (Semenov et al., 2011; Semenov and Semenova, 2018).

Let us consider briefly, summarizing the main successes in the development of knowledge of soil health (SH) - soil ecosystem (SE), development and knowledge of soil health, achieved mainly in Russia.

Soil health (SH) - soil ecosystem (SE) and some parameters

At the very beginning of the XXI, a method was developed and patented, named as a parameter, for the quantitative determination of the health of the soil (SH) - the soil ecosystem. The method is based on the idea of quantitative comparison of substrate-induced respiration (SIR) of two or more soil samples after initiation of respiratory activity of the soil microbial community (MS) heterotrophic substrate (glucose).

The method is published in Russian and English publications (Semenov 2011a; Semenov, Sokolov, 2016; Semenov, Djukich, 2017; Semenov, Semenova, 2018; Spiridonov et al., 2019).

It should be noted that the development of a method for quantitative determination of SP is based on the fundamental ecological concept “disturbing effects and undulating development of microbial populations and microbial communities (Semenov, 2001; Semenov, 2011; Van Bruggen et al., 2006). Thus, a method for the quantitative determination of the heterotrophic (since glucose initiates the heterotrophic component of the soil MS) SH parameter has been developed and is available for use.

Like everything dialectical, the topic and method for determining the health of a soil ecosystem needs to be supplemented and developed. It is well known that providing the soil ecosystem (SE) with N and P compounds is key for the functioning of any pedocenosis, and for organic farming it is critical. The need arose for a method for determining the SH, taking into account the influence of key biophilic elements (N, P) in the soil ecosystem (SE). In this case, a method is needed that would make it possible to determine the ability of

SE to self-sufficiency in such biophilic elements - N, P of the soil ecosystem. In other words, a method was needed to determine the "nitrogen / phosphorus" parameter of the soil health.

Determination of SH taking into account the ability of SE to self-sufficiency in biophilic elements; nitrogen/phosphorus parameter SH

Based on elementary logic, to assess the SH, knowledge is required not only about the activity of the most general biotic processes, which reflects the soil SIR indicator, but also knowledge about the "closedness" of the cycles of biophilic elements in SE, or the ability of SE to self-sufficiency in biophilic elements. By "closedness" is meant the ability of a healthy soil ecosystem to local, cyclical self-provision with biophilic elements, primarily nitrogen (N) and phosphorus (P) compounds (Semenov, Sokolov, 2016; Semenov, Semenova, 2018).

It is generally known that the deficiency of N and P compounds in PE is a key limiting factor in the functioning of any natural pedocenosis, this is especially significant for consumers of agrophytocenosis, and becomes critical for organic farming (Semenov et al., 2016). Our experiments have studied the dynamics of nitrogen fixation and ammonification (as the input of N into SE) and nitrification and denitrification (removal of N from SE) (Emer et al., 2014; Semenov et al., 2019). These dynamic experiments demonstrated that the processes, especially nitrogen fixation, but also ammonification in SE, have serious limitations, which do not allow us to consider them as indicators of the "closedness" or "openness" of the processes of N entry into SE.

It was proposed to "judge" the state of the cycles of "biophilic elements" by the activity of the microbial community (MC) after the disturbing effect - the enrichment of the soil with mineral compounds of these elements. To do this, it was proposed to enrich soil samples by analogy, to identify the heterotrophic parameter of SH, simultaneously with carbon and nitrogen substrates, and, if necessary, with phosphorus. To measure the soil SIR, the rate (V) of CO₂ emission induced by these biophilic elements, in dynamics (by analogy with the determination of the heterotrophic parameter of the SH. Samples of the compared soils (investigated and "conditionally healthy" - reference) are incubated under optimal conditions of humidity and temperature. The SIR inductors are aqueous solutions of glucose, ammonium nitrate, or (if necessary!) Disubstituted sodium phosphate. Determination of SIRs is carried out daily for at least 5 days. The SIR indicator in this case acts as an induced parameter of the health of the soil, soil ecosystem (SE), characterizing its carbon, nitrogen, and even phosphorus exchange of this SE. The calculation of the "nitrogen" and "phosphorus" parameters of the SH is performed in the same

way as when calculating the heterotrophic parameter of the SH (Semenov et al., 2011; Semenov, Semenova, 2018).

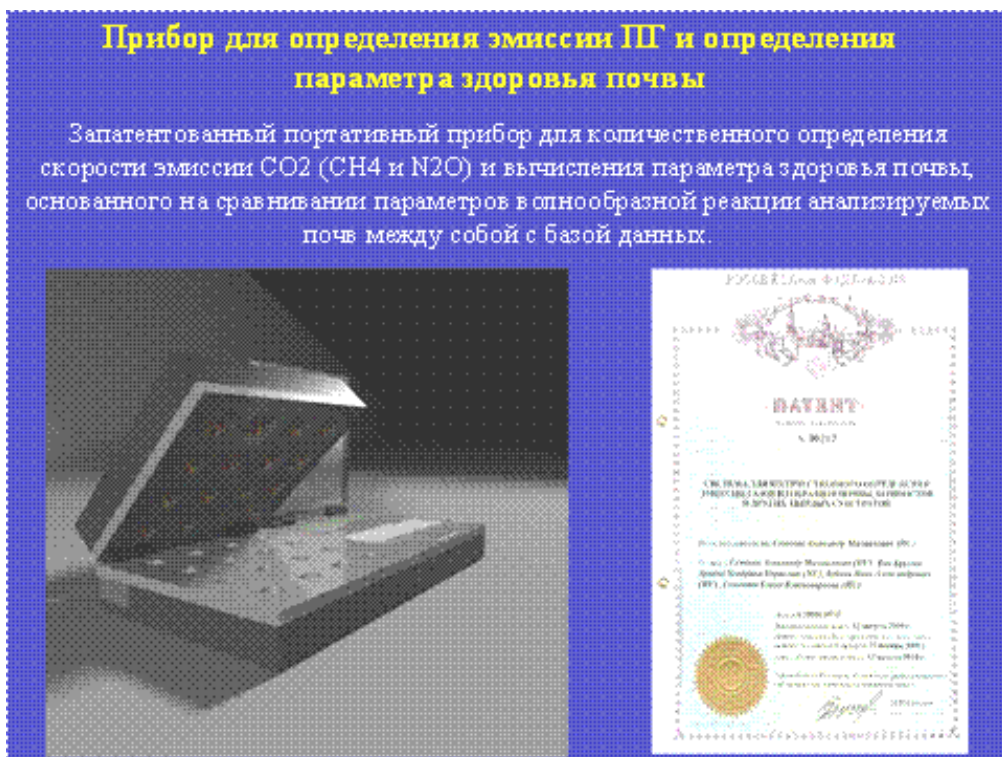
These methods, already widely known, but of course, need to be developed and supplemented. The methods urgently need to be supplemented with several more methods. There is an urgent need for express methods for testing the chemical and biological toxicity of a healthy soil ecosystem. We need not only general methods for determining the SH, we also need specific methods. Methods for determining the specific "bacterial" and "fungal" SH are needed to differentiate the state of the source of health and the sources of the SE disease, for the differential diagnosis of SE. Methods are needed that would reflect the role and contribution of soil mesofauna to the health of the soil ecosystem. It was noticed long ago that in the soil, in common parlance, called rich, fertile, many different soil biota live, in particular, for example, earthworms. In this regard, new, different indicators are needed that would reflect the role and treasure of a very significant component of the soil mesobiota. Soil mesobiota is an incubator and distributor of various microorganisms. To do this, it is necessary to study the influence of the population of, for example, earthworms on the microbial activity of the soil, to determine the contribution of the population of earthworms to the health of the soil ecosystem.

Thus, methods of quantitative determination of SH, general and one specific parameter - nitrogen and phosphorus are considered. The problems of further development of general, specific and special methods for determining the SH are discussed. All such methods require widespread application and accumulation of data bases on SH in different soils and in different conditions.

A device for automation and exclusion of subjectivity in the determination of SH, for the simultaneous quantitative determination of greenhouse gas emissions: CO₂, CH₄, N₂O from soil samples

It is quite obvious that the more data is obtained and, therefore, accumulated on the health parameters of different soils, different parameters and different characteristics that contribute to the knowledge of the soil ecosystem, the more adequately it will be possible to manage both the fertility and quality of soils and the preservation of ecosystems. The proposed methods for determining soil health parameters are relatively easy to use. However, any scientific methods and approaches require knowledge, skills and time, which is most expensive. For the widespread use of the proposed methods for determining the SH, devices are needed that would facilitate, help and save the user's time and results. For this purpose, it was proposed to create an automated, robotic, computerized device. The design and software of such a device would make it possible to determine the current (actual) state of the SH,

compare and save the data, of course, contribute to the protection of the environment and climate, since parting is programmed to determine three greenhouse gases CO₂, CH₄, N₂O.



A description of the proposed device is in the patent, which is publicly available (in web) and of course the author has (Semenov et al., 2009; https://yandex.ru/patents/doc/RU90212U1_20091227). The introduction of such a tool into practice will undoubtedly contribute to the production of products and the protection of the environment.

To the method for determining the SH for cultivated areas of agro-soil adjacent or distant from each other, of the same genesis and landscape, but in the absence of "fallow", i.e. (control) as known to be healthy soil

Soil health can be quantified using substrate-induced respiration samples from two or more soils. According to the developed method, as a comparative, i.e. control ("healthy") soil sample, it is recommended to use a known healthy soil, for example, "fallow", of the same genesis and landscape.

However, to find (select) a sample of "fallow", i.e. knowingly healthy soil is not always possible. A problem arises not only for non-specialists in soil science, but even for specialists in soil sciences. The problem becomes more complicated when it becomes necessary to determine the SH of two plots of field soil of the same genesis and landscape, but distant from each other, but "fallow", i.e. there is no known healthy soil of the same genesis and landscape. If this problem is solved subjectively, then there will be unreliable results in the SH and wrong decisions will be made.

It is proposed to solve this problem by determining the amount, i.e., the concentration of the substance - geosmin (geosmin) in the compared soil samples. Geosmin is a product of the vital activity of soil microorganisms: bacteria of the actinomycete line, the genus streptomycetes, blue-green bacteria (cyanobacteria) and possibly microomycetes. This compound is a gaseous volatile organic substances, i.e., in general, is a product of the soil microbial community. The concentration of this substance is also associated with the "smell" of freshly cultivated soil (plowed, moistened, that is, exposed to the disturbing effect [URL: http://aromatic.ucoz.ru/publ/chem_pakhnet_zemlja/1-1-0-20].

The concentration of this substance, geosmin, can be determined chromatographically by comparing two or more soil samples. Assuming, for example, that "healthier soil has a higher concentration of geosmin." The proposed method, along with the proposed device for measuring greenhouse gas emissions from SE, will allow solving many everyday difficulties in determining the SH.

Molecular biological methods (MBM) in the determination of SH

Before discussing the application of molecular biological methods (MBM) and their capabilities in determining the SH, let us name some of the advantages of MBM for the ecology of soil ecosystems. The advantage of MBM is modernity, attractiveness. Molecular biological methods make it possible to determine the list of bacteria in a soil sample. MBM essentially gives the results of the microbial biodiversity of the soil sample, suggesting the potential activities of the uncultivated microbiome.

The known method for the determination of SH, which in essence can be called traditional, is based on the measurement of substrate-induced respiration (SIR) of soil samples after the initiation of respiration of microorganisms by the substrate (glucose). This approach is based on one of the important population laws of biology (ecology): "only that process is significant and noticeable if this process is carried out by microorganisms, of which there are many and they are active" (Semenov et al., 2019a).

The SIR method for determining health also has several advantages. The main advantage is that the method provides relevant results. The disadvantages of the method are its limitations: only aerobic microorganisms (MO) are "taken into account", only glucose-active microorganisms, the method does not include MOs that are in an inactive state (although their contribution and is not important for actual health). The explanations for the listed restrictions are as follows: most agricultural crops, with the exception of the rice rhizosphere, grow under aerobic conditions; the beginning of the metabolism of aerobic microorganisms - glycolysis, where the initial metabolite is glucose. The cycle of tricarboxylic acids and as the beginning of the respiratory chain - everywhere begins with glucose; inactive cells - do not affect health either positively or negatively.

MBM also has some limitations. MBM studies of SE provide only potential, predictive results. They have many objective difficulties, performance difficulties. MBMs are very expensive, time-consuming, complex, requiring a highly qualified technician and laboratory.

To use the MBM to determine the SH, it is necessary, of course, to compare again, the performance indicators of the test subject and obviously healthy soils, i.e., the principle of comparing dynamics is inevitable. The results of which MBMs used for the analysis of the two compared soils can be applied in determining the SH? For example, the results of the DGGE method or metabarkoding in the form of operational taxonomic units - OTU, i.e., amplicons, (sequences), of the test soil and selected as healthy soil. For example, to compare the dynamics of the number of OTUs for several days after the disturbing impact, as it is in the publication (Semenov et al., 2013). It is quite obvious that in order to transform the results of "comparative bacterial biodiversity" into the results of the SH assessment, either fundamentally new approaches or adaptation of known ones are needed. Of the known, it is possible to try to adapt the approach and calculation of the SH index by analogy with the already approved approach in the method for determining salary based on the dynamics of SIR. So, the use of MBM to determine the SH needs serious development and testing.

Determination and use of the properties of SE suppressive activity, methods of activation (induction) of SE activity suppressiveness and the methods of healing and therapy

The suppressive activity of the soil ecosystem is a well-known and useful property. This property requires further development of assessment methods, its stimulation and management methods. It should be noted that the phenomenon of the suppressive activity of the soil ecosystem is more appropriate to consider and discuss for organic farming soils. In agroecosystems that even use low input technologies, the suppressive activity of SE can be significantly suppressed. Of course, some methods for

determining the suppressiveness of SE activity are already known (Alabouvette et al., 2004; Toropova, Kirichenko. 2015; He et al., 2012).

Methods of activation (induction) of suppressive activity of SE are also known (Toropova et al., 2016; Toropova et al., 2017). However, the above-mentioned methods of detecting, evaluating and using knowledge about soil suppressiveness, with their undoubted necessity and significance, have a complex drawback. This is the tangible complexity and duration of the analysis (time and laboriousness), the need for a microbiological laboratory and a qualified analyst - performer. This limits their availability for the average land user. Therefore, it is necessary to use and improve other fundamental approaches that are proposed for the "treatment" of soils (Semenov, Semeneva, 2018).

And, so, to solve the problems of determining and using the properties of the suppressive activity of PE in everyday practice, for the widespread use of methods of activation (induction) of suppressive activity of PE, further serious developments are needed.

Oligotrophication of terrestrial ecosystems as a methodology for chronic health improvement of soil ecosystems

It is quite obvious that all or almost all of the proposed methods, when applied, can show their significance, efficiency, first of all, in organic agriculture (organic agriculture, organic farming system). Slightly less - with highly intensive agriculture (conventional agriculture). However, the technology of oligotrophication of agroecosystems, as a method of conscious management of agrosystems, is applicable not only to organic farming technologies. This method can (and should!) be used to maintain and health any SE. There is a discussion of approaches to ligotrophication of agroecosystems as a way to improve and maintain soil health (Semenov et al., 2011; Semenov at al., 2017).

It remains only to understand that oligotrophic agroecosystems are not only more environmentally friendly, but also more economically profitable due to the reduction of irreplaceable energy costs for "eutrophication

Conclusion

The proposed methods are undoubtedly significant and their demand is increasing. These methods are not equivalent in their elaboration. Some of the methods need to be improved and calibrated. A broad comparison database is also needed. However, the intensity of the development of the SH problem, progress in the development of methods and their application, depends largely on the reaction of the world, first scientific, and then consumer society. The

discussed methods are mainly developed by the authors of this communication. The answer to a possible question about the contribution of the international scientific community in the development of the SH topic, in the development of methods for determining the SH, given that the SH problem was posed by American scientists (USA), is rather difficult to find. An analysis of the literature leads to an interesting conclusion. In the USA and Western European countries, the scientific community and practitioners focused on the "depths" of the use of organic farming, and the studies of the theory and practice of soil health, the health of the soil ecosystem were postponed for "later" (*www.greencoverseed.com).

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BOVINE RESPIRATORY DISEASE COMPLEX (BRDC) - ALTERNATIVES TO COMMON USES OF ANTIBIOTICS

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Abstract: *Bovine respiratory disease complex (BRDC) is the strongest cause of morbidity and mortality in beef cattle, and the prevention or treatment of BRDC without the use of antimicrobial drugs (AMD) is inconceivable. For each assessed BRDC risk category, feedlot veterinarians create an optimal program to reduce the risk of developing BRDC. When appearing BRDC, AMD is widely used in fattening cattle (e.g. metaphylaxis, on the basis of their BRD risk classification). Appropriately implementation of metaphylaxis in case of BRDC has been shown to dramatically reduce the devastating effects of BRDC, improving cattle health and preventing huge economic losses to farmers. Poor result to AMD threatens cattle health may lead to increased antimicrobial use (AMU), higher costs of production and potentially contribution to the dissemination of antimicrobial-resistant genes to other bacteria in cattle and possibly the environment. Antimicrobials of herb origin are of interest to the pharmaceutical industry for the control of pathogen microorganisms. The use of antibiotics has significantly improved the treatment of bacterial infections, the emergence of antibiotic resistant strains of bacteria has led to the continuing search for useful natural antimicrobials.*

Keywords - BRDC, antibiotics alternative

Introduction

The results of numerous studies on the use of antibiotics (ABs) in the prevention and treatment of animals for slaughter prove that it promotes antibiotic resistance (U.S. Department of Health and Human Services, Food and Drug Administration, 2016). If a certain AB is used very often in the treatment of animals, there is a far higher chance that bacteria will develop resistance, through various mechanisms, and that preparation will be less and less effective, over time. If ABs are used for preventive purposes, and the doses used are low, it is realistic to expect the development of resistance to the same

AB when they are used in therapeutic, high doses (World Health Organization - WHO Report, 2016). Frequent use of one AB accelerates the spread of resistance genes to other ABs, even from groups that are not related to the drug used at all (Canton and Ruiz-Garbajosa, 2011). The most important thing is to direct all scientific and professional forces towards responsible management of the use of antibiotics, using them only when the disease is diagnosed, and to change the practice of using antibiotics in the routine prevention of the disease.

Denmark, Sweden and the Netherlands have taken the lead in efforts to reduce the use of ABs in meat-producing animals. Research has shown that reduced use of ABs leads to a reduction in the number of resistant bacteria, with moderate to insignificant economic effects on producers or consumers (Speksnijder et al., 2015).

In the United States, public opinion and popular media coverage of the use of ABs in the treatment of animals for slaughter has led to increased demand and production of meat from animals raised without the use of ABs including meat of organic origin and poultry (Consumer report, 2012; Morris et al., 2012; Perone, 2012).

In order to better understand the potential for replacing ABs with natural antimicrobials (non-antibiotic alternatives), and when it comes to a syndrome such as the BRDC, the following issues are considered:

- BRDC has been identified as a bovine disease that is usually treated with ABS. There is published literature and databases on approved drugs;
- alternative natural products and management strategies that could effectively reduce the preventive use of ABs are identified and
- the success of other countries in reducing their routine use of ABs for the prevention of disease in cattle is investigated and summarized, and described in adequate scientific and professional publications.

BRDC is most often treated with the following ABs: tetracyclines, florfenicol and tulathromycin. As alternatives are proposed: preconditioning programs, dietary manipulations and feed supplements, probiotics and prebiotics, remote early disease identification systems and immunomodulatory medication.

Because multiple factors can cause or contribute to BRDC, there is no single method to prevent its development. As with many diseases occurring in stressed animals, a combination of measures to lower stress and boost immunity is the best means to prevent illness in the first place.

BRDC is one of the most important causes of health disorders of fattening cattle and dairy cows, manifested with extremely high morbidity and high mortality. BRDC incurs extremely high costs in the cattle industry, as a consequence of reduced production, increased labor costs and reduced carcass

value, regardless of the use of modern ones vaccines and antibiotics (Irsik et al., 2006; Gershwin et al., 2015). The development of BRDC is the result of intricate interactions between cattle, environment, and pathogens (Setiyaningsih et al., 2018). BRDC is caused by several viruses (individually, or more often in combination): bovine respiratory syncytial virus (BRSV), bovine herpes type 1 (BoHV-1) and bovine viral diarrhea virus (BVDV). Above mentioned viruses predispose animals to coinfection with one or more bacteria: *Pasteurella multocida*, *Mannheimia haemolytica*, *Mycoplasma bovis* and *Histophilus somni*, indicating a multifactorial process (Taylor et al., 2010a; Gershwin et al., 2015). Viral agents such as BRSV and bovine parainfluenza virus 3 (BPIV-3) are designated as the most common challengers of BRDC. The pathogenesis of BRDC is still largely unknown to researchers, particularly in the advanced stage of chronic disease, in addition to coinfection with other primary bacterial agents like *M. bovis* (Mehinagic et al., 2019). Study conducted by Klima et al. (2020) suggests that between 2011 and 2016 there was a significant increase in the prevalence of MultiDrug Resistance (MDR) isolates of *M. haemolytica* and *P. multocida* associated with BRD. This increase in MDR in the causative agent of BRDC strongly indicates the need to replace antibiotics with new natural antimicrobial substances, mostly of plant origin.

There has been a more recent approach to addressing these gaps and authors identified signatures of tissue-specific transcriptional responses indicative of tropism in the coordination of host's immune tissue responses to infection by viral or bacterial infections, and shows that this tissue tropism in host transcriptional response to BRDC pathogens results in the activation of different networks of response genes. The results of above mentioned research serve as a basis for the development of innovative therapeutic strategies and for the selection of cattle with enhanced resistance to BRDC (Behura et al., 2017). Predisposing factors that facilitate the development of BRDC are numerous, and the most important are: stress, weaning, too high temperature, overcrowding of cattle in stalls in the facility, dust, humidity, transportation and frequent changes in diet composition (Taylor et al., 2010). The scientific public is divided: one part have concluded that the viral pathogens are the main pathogens of BRDC that causing primarily respiratory lesions are Bovine Herpes Virus-1 (BoHV-1), Bovine respiratory Syncytial Virus (BRSV) and Bovine parainfluenza Virus-3 (BPIV-3) (Kirchhoff et al., 2014). Unique viral agents is Bovine Viral Diarrhoea Virus (BVDV), because of intrauterine infection in first trimester can lead in persistently infection (PI) of cattle, chronically ill or dying in feedlots (Kurćubić et al., 2011). Immunosuppressive effect of the BVDV infections have facilitate the occurrence of BRDC, primarily due to the creation of favorable conditions for the development of bacterial agents, the most common of which are: sixteen serotypes of *M.*

haemolytica, *P. multocida*, *Histophilus somni*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Corynebacterium bovis*, *Streptococcus* spp. and *Micrococcus* spp. (Taylor et al., 2010a). Mycoplasmas caused serious cattle disease, which leads to negative economic and welfare impacts (Parker et al., 2018). *M. bovis* is widespread throughout the world and recognized as one of the most important and frequently isolated Mycoplasma species associated like cattle disease (Bednarek et al., 2012; Fox, 2012). Other often isolated mycoplasmas are: *Mycoplasma bovirhinis*, *Mycoplasma dispar*, *Ureaplasma diversum* and even *Mycoplasma canis* (Szymańska et al., 2010).

Calves with BRDC are detected in 91% of cases in the first 27 days after arrival at the pens (Buhman et al., 2000). Morbidity risks of BRDC cases in feedlot cattle are highest within the first 45 days after arrival to the feedlot. Morbidity was highest in weeks 1 to 3, and decreased through the 12-week period (Edwards, 1996). Clinical signs are most often manifested by high fever (40-41.5 °C), depression, decreased appetite, nasal and ocular discharge, coughing and varying degrees of dyspnea. Unpredictable etiology of BRDC is almost always polymicrobial and associated with predisposing risk factors.

The approach to BRDC therapy is based on the use of AMD, primarily and frequently antibacterial agents. Francoz et al. (2012) reported that experimental BRDC models provide useful information necessary for the use of supportive medicaments (nonsteroidal anti-inflammatory drugs, immunomodulators, bronchodilators). The spread of bacteria that infect cattle and lead to BRDC is prevented by the use of AMD, at a time when the number of „high-risk“ calves exceeds the „tolerance level“. Mass medication (metaphylaxis) gives good results in reducing morbidity, using AMD selected on the basis of susceptibility testing, mainly with prolonged action, for the treatment of cattle who are suspected of BRDC or which have been in contact with infected cattle (Edwards, 2010; Urban-Chmiel and Grooms, 2012; Anholt et al., 2017). Extremely good efficiency (70-90%) in the treatment of cattle diseased from BRDC caused by *Pasteurella*'s in period of 1-2 days after treatment showed new macrolide antibiotics, third generation cephalosporins, fluoroquinolones and fenicols. Tulatromycin, florfenicol, ceftiofur are much more effective against most *Pasteurella* strains who are resistant to other antibacterial agents. Its liposolubility allowed that quickly and completely absorbed in the lung parenchyma and there are held for seven days at higher concentrations than the minimal inhibitory concentration (MIC) for most bacteria and mycoplasma. Jezdimirović et al. (2011) has been examined the clinical efficacy of tulathromycin (TU) and florfenicol (FL) in the treatment of bronchopneumonia (BP) caused by *Pasteurella multocida* isolated from diseased six months old Holstein calves. TU proved to be a drug of first choice in the treatment of BP caused by *P. multocida*. Recently findings of Kurćubić et al. (2012) revealed that the most common bacteria in fattening cattle

suffering from BRDC were *P. multocida*, *Aeromonas viridans* and *Corynebacterium bovis*, suggesting on their bigger importance in BRDC in Serbian beef cattle with regard to *M. haemolytica*, predominantly determined worldwide. Susceptibility testing showed that the most efficient ABs against *P. multocida* isolates were enrofloxacin and florfenicol (100% of examined isolates sensitive on both ABs). Bovine isolates of *M. haemolytica*, *P. multocida*, and *H. somni*, collected over the last ten years were tested on the *in vitro* sensitivity of the commonly applied *ad us vet* ABs that are available in the market Portis et al. (2012). Ceftiofur retains its activity against all tested bacterial isolates Penicillin still retains a strong antimicrobial effect on isolates of *P. multocida* and *H. somni*. Enrofloxacin MIC₅₀ values against the three examined pathogens also remaining the same, unlike the MIC₉₀ values, which increased by at least 4-doubling dilutions. Ninety percent or more of *M. haemolytica* and *H. somni* isolates were susceptible to florfenicol, while susceptibility among *P. multocida* was 79% or greater. All three bacteria showed declines in MIC₅₀ and MIC₉₀ values on tilmicosin and tulathromycin.

Tildipirosine (Zuprevo®) has also become one of the AB of choice for metaphylaxis (treatment and control) of bovine respiratory disease (BRD) associated with *M. haemolytica*, *P. multocida*, and *H. somni*, on the Serbian market. This drug has previously shown efficacy when applied to animals meeting the above BRD case definition (Theurer et al., 2018; Nickell et al., 2020).

Prevention of BRDC without routine use of antibiotics

Antimicrobial (antiviral, antibacterial and antifungal) activity of various plant species

The numerous reasons for limitations on the use of antiviral (AV) drugs in human and veterinary medicine in comparison with the use of AMD are a consequence of the occurrence of viral mutation, new viruses, toxic effects, the severity of viral diseases, ability of virus to survive intracellularly, the high costs and the non-availability of specific antiviral chemical agents against veterinary pathogens. Low effectiveness of AV drugs require discoveries a new powerfull AV compounds. Most AV compounds against viruses of veterinary importance are still used as animal models in the development of human antiviral drugs (e.g surrogate model for the hepatitis C virus is BVDV). Plants are a gift of nature and synthesize substances very useful for medical use, and they make it possible production of new, favorable, drugs with significant therapeutic potential. Advancement of analytical chemistry tools, the development of standardization and extraction methods and the standardization of virus testing is imperative today (Pushpa et al., 2013; Zeedan, 2016). Plants

have an abundance of active substances (phytochemicals) with various biological effects including AV effects (e.g. inhibit different parts of the replication cycles of various types of DNA and RNA viruses). This AV action is conditioned by the presence of polyphenols, rosmarinic acid, chlorogenic and caffeic acid (Jassim and Naji, 2003), which interact in holistic manner, increasing the effect of each of the active substances separately - synergy (Gobbo-Neto and Lopes, 2007). There is an urgent need to identify alternatives of AMD, such as natural products, and develop novel and effective treatment protocols with significantly reduced usage, and dosage of AMD in order to minimize the development of antimicrobial resistance (AMR). Both tulathromycin and gallic acid were effective against *M. haemolytica*, *P. multocida*, and their mixed culture. A strong additive or weak synergistic effect was observed between tulathromycin and gallic acid against both bacteria. Pre-exposure to tulathromycin generated bacterial resistance to the antimicrobial in *M. haemolytica* but not in *P. multocida* (Rajamanickam et al., 2019).

The use of AMD in combination with phytochemicals has been extensively studied (Monden et al., 2002; Petersen et al., 2006; Cernohorská and Votava, 2008). The antimicrobial-phytochemical cocktail strategy has exhibited the potential in eradicating complex pathogens (Lewis and Ausubel, 2006; Wise, 2006; Amyes et al., 2007; Cai et al., 2007; Gould, 2008).

AMD are powerful medicaments for maintaining human and animal health. In North America, AMD are widely used to support health of fattening cattle. Of feedlots with more than 1000 head of cattle in the USA, 87.5% administered AMD to animals by injection or in feed or water (USDA, 2019). Widespread antimicrobial use (AMU) is an important driver of selection for antimicrobial resistance (AMR), which may be threatening the ongoing effectiveness of AMD to combat disease in people and animals (Sultan et al., 2018).

Due to the need to protect the effectiveness of significant AMD for use in human medicine, the WHO has published guidelines presenting evidence-based recommendations and best practice statements on the use in food animals of “medically important antimicrobials”, defined as antimicrobial classes used in human medicine. Medically important AMD are categorized according to appropriate criteria as “important,” “highly important, or “critically important”, for human medicine. The WHO recommends that the overall use of medically important AMD in food animals be reduced. Their use for growth promotion and in animals in which clinical disease has not been diagnosed is prohibited. The WHO further suggests that critically important AMD should only be used for treatment of individual sick animals, and that highest priority critically important AMD should not be used in food animals (WHO, 2017).

Use of chemical preservatives have negative impacts (human health hazards, chemical residues in food & feed chains and acquisition of microbial

resistance to the used chemicals) in prevention of food spoilage and food poisoning pathogens. The necessity to find a potentially effective, healthy safer and natural alternative preservatives is increased. Plant extracts have been used to control food poisoning diseases and preserve foodstuff (Duffy and Power, 2001; Mostafa et al., 2018).

The use of natural AMD is preferred by consumers, due to their ability to inhibit the growth of foodborne pathogens. Among natural AMD, plant extracts are used to inactivate *L. monocytogenes*. There is a lot of these types of extracts, and their active compounds remain unexplored. The study conducted by Ceruso et al. (2020) evaluate the antibacterial activity against *L. monocytogenes* of about 800 plant extracts derived from plants native to different countries worldwide. The minimal inhibitory concentrations (MICs) were determined, and scanning electron microscopy (SEM) was used to verify how the plant extracts affected *L. monocytogenes* at the microscopic level. Results revealed that 12 of the plant extracts had inhibitory effect against *L. monocytogenes*. Future applications of this study could include the use of these plant extracts as new preservatives to reduce the risk of growth of various pathogens and contamination from *L. monocytogenes*.

Essential oils (EOs) from aromatic and medicinal plants are receiving increased scientific attention because of their long history of being sources of natural AMD for the treatment of infectious diseases (Nabavi et al., 2015).

Our experience indicates that it is necessary to improve the investigation of the use of plant essential oils and extracts as AV agents in the future, and investigations of the new approaches, such as applying a low dose of synergistic AV combination of plant extracts. It would also be necessary to examine the mechanism of action of biologically active substances that are isolated and characterized from the examined herb extracts.

Kurčić et al. (2019) investigated the *in vitro* AV activity of five selected plant extracts against Bovine Herpes Virus-1 (BHV-1) subtype 1.1 and Bovine Viral Diarrhoea Virus (BVDV) genotype 1, which cause BRDC with other viral and bacterial agents. Two prepared samples of aqueous plant extracts/macerates had AV effect against BHV-1 as a representative of herpesvirus (DNA virus), but none of the samples showed an AV effect against BVDV genotype 1, a NADL strain, that belongs to RNA viruses. In this study, Selectivity Index (SI = CTC_{50}/EC_{50}) for aqueous extracts of *Matricaria chamomilla* (SI = 32) and *Achillea millefolium* (SI = ≥ 8) indicates a significant selective inhibition of DNA virus (BHV-1, strain TN41). The obtained results require new and more extensive research, which would reveal the active ingredients of the investigated plants and mechanisms of their AV effect.

Every program designed by experts is multicomponent, including use of different vaccines and types during vaccination/revaccination, use of AMD on-arrival in feedlots, biosecurity measures, diagnostic procedures to detect the

occurrence of disease and its treatment, animal husbandry practices, quality of feeds and feeding programs, monitoring and animal health intervention programs (Ives and Richeson, 2015; Brault et al., 2019; Lhermie et al., 2019).

Rajamanickam et al. (2019) showed that tulathromycin, gallic acid and their combination were also effective against the mixed culture of *M. haemolytica* and *P. multocida*. Pre-exposure to tulathromycin generated bacterial resistance to the antimicrobial in *M. haemolytica* but not in *P. multocida*.

Vaccination

The cattle under stress have often not been previously vaccinated against BRD pathogens, and there is frequently extensive commingling of cattle from different sources leading to exposure to infectious diseases (Abell et al., 2017). The results of 15 studies of the efficacy of different vaccines against 3 bacterial pathogens, BRDC (*M. haemolytica*, *P. multocida* and *H. somni*) were combined and analyzed. The results indicated good vaccination effects against *M. haemolytica* and *P. multocida*, while the vaccination effects against *H. somni* infection were weak (Larson and Step, 2012). Vaccination against the viral pathogens, causative agents of BRDC is also effective at preventing BRDC. Standard procedures have been developed that are an integral part of the BRDC holistic prevention program. Optimal vaccination program designed for cows during pregnancy and prior to birth can enable passive transfer of antibodies (immunity) via colostrum (Duff and Galyean, 2006).

Prescribed use of vaccines and a well-designed vaccination program can be part of a biosecurity program. Minimum requirements for efficient vaccination program should include:

- Proper storage and administration of the vaccine as indicated by the manufacturer's labeled recommendations;
- Vaccination of all susceptible animals, including both resident and incoming animals;
- Application of the vaccine to systemically healthy, well-nourished, minimally stressed, and immunocompetent cattle;
- Strategic timing of vaccination (precedes contact with new animals long enough to allow an appropriate immune response) and
- Revaccination according to the manufacturer's recommendations (Callan and Garry, 2002).

Recent observations (Cavirani, 2019) suggest that it is herd immunity, obtained through a correct immunization practice, is a pillar for BRDC control and to minimize likelihood of disease onset. Immunization of calves is pivotal to get herd immunity to BRDC microbial agents. A feasible vaccination program must take into account epidemiological features of the herd or the area of relevance, vaccines available in the market and cost-effectiveness of the program. Regarding calves immunization, it's remarkable to mention sequence and timing of vaccination treatments by assessing maternal immunity and microbial risk. Likewise, in buiatrics, vaccination to BRDC microbial agents should be retained a core practice in both dairy and beef industry.

Chamorro and Palomares (2020) draw strong high-quality evidence that vaccination of beef calves at or shortly after weaning with parenteral multivalent modified live vaccines (MLV) alone or in combination with *M. haemolytica*/*P. multocida* bacterins is effective for reducing naturally occurring and experimentally induced BRDC morbidity and mortality after weaning. The presence of BVDV1 and BVDV2 antigens in parenteral multivalent MLV plays an important role in providing clinical protection against BRDC. There is limited evidence of efficacy of vaccination of young beef calves (pre-weaning) with parenteral or intranasal (IN) MLV or killed/inactivated (KV) vaccines in reducing naturally occurring or experimentally induced BRDC morbidity and mortality before weaning age. There is an evident need for additional research to determine true effects of vaccination, type of vaccines, and routes of administration in this group of cattle. There is strong evidence that vaccination of young dairy calves with parenteral or IN MLV vaccines is effective providing clinical protection against BRDC after experimental challenge with respiratory viruses.

Biosecurity and hygiene

The term biosecurity described those management and hygiene practices that reduce introduction, exposure, and transmission of infectious viral and bacterial agents (Callan and Garry, 2002). All common measures used in the prevention of infectious diseases are also useful in preventing the development of BRDC. Good hygiene practice (GHP) and protocols for the implementation of BRDC prevention at the very entrance of cattle to the facilities must be precisely designed and set in a timely manner. It is recommended that the isolation of sick and suspicious cattle be carried out rapidly, as well as disinfection of facilities, feeders, means of transport., Low stocking density, good ventilation and hygiene, as part of good management practices (GMP), reduce stress. One way to naturally provoke a defensive reaction of a young calf is to during shifting of pens, the youngest animals are

put in a pen where the older (immune) animals have been (Valarcher and Hägglund, 2006).

Adequate ventilation and prevention of the negative impact of dust particles on the respiratory tract of cattle have a significant impact on the reduction of BRDC by strong activation of defense mechanisms.

About 91% of calves suffering of BRDC were diagnosed during the first 27 days after arrival (Buhman et al., 2000). Morbidity risks of BRDC cases in feedlot cattle was highest in weeks 1 to 3, and appear in the first 45 days after arrival to the feedlot. Morbidity decreased during the 12-week period (Edwards, 1996).

Preconditioning programs

Preconditioning programs include the implementation of preparatory and preventive measures, in a period of about 30 days after weaning calves, which aim to achieve maximum average daily gain, without the appearance of health problems. A preconditioning techniques can be implemented to ensure calves have been vaccinated, which were dewormed, and accustomed to the types of food and water troughs they will encounter during shipping. Any surgical procedures to be performed on the calves, such as castration or dehorning, should be performed with plenty of time for healing prior to transport (Taylor, 2010b).

Castration of male calves after arrival at fattening facilities is a confirmed risk factor for occurrence BRDC. If implemented significantly later, it leads to a decrease in Average Daily Gain - ADG (Zweiacher et al., 1979; Berry et al., 2001; Fisher et al., 2001), a result that was not influenced by analgesia during castration (Faulkner et al., 1992). Studies have confirmed that castration is stressful. Castration of adult bulls increased plasma cortisol concentrations (Chase et al., 1995). The immunosuppressive potential of rising levels of cortisol leads to adult bull's castration casting a higher risk of BRDC than non-castrated cattle or those castrated at a younger age. It is recommended that dehorning be performed early in life (similar to castration, due to a feeling of severe pain). Both surgical procedures should be performed to meet current industry standards, but the question of the time and procedure of their implementation remains to be considered.

Calves are often sold and transported immediately following weaning. However, experts recommend waiting an additional month while preconditioning calves to aid cattle health and help avert the need for metaphylaxis when undergoing the stresses of transport (Bovine Respiratory Disease Prevention, 2016).

Dietary manipulations and feed supplements

Recipes of diets for calves immediately upon arrival at the feedlot have to provide nutrient concentrations for low-feed intake and optimal performance during accommodation and acclimatization (Urban-Chmiel and Grooms, 2012). Duff and Galyean (2006) determined correlation between nutrition of stockers, immunoglobulin production and respiratory disease frequency. Suppression of immunological response and decreases the ADG in calves is direct consequence of low energy and protein concentration in feed. Taylor et al. (2010b) reported that the concentration of energetic substances in feed during the first 4 weeks of feedlot should be 72%. Future research on evaluating BRDC will verify the extent to which changes in feedlot management practices can contribute to maintaining the efficacy of most commonly used and available AMD (Stanford et al., 2020).

Probiotics and prebiotics

The use of probiotics (ingestion of living bacterial microorganisms which provide a health benefit) and prebiotics (types of fiber that humans cannot digest, but his gut bacteria can, and prebiotics promote his growth) to improve ADG and decrease occurrence of infections among newly-received beef calves represents an interesting field of research. Commercial probiotic include bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium*. Probiotics alter intestinal flora in a positive manner and compete with unwanted disease-causing bacteria (Duff and Galyean, 2006). Data on their use in the prevention of BRDC in cattle are very scarce, and there is precise evidence in the literature that their use in poultry meals has had positive effects on their health and production results. The reason for that may be in the fact that the cattle are larger, longer-lived, and more valuable animals compared to chickens, and it is more difficult to devise trials that will provide accurate statistical significance of the differences between the observed groups of cattle.

Remote early disease identification systems

Schaefer et al. (2012) reported that advanced monitoring systems can replace the application of the metaphylactic approach, especially when a few animals are ill. Remote early disease identification system (REDI) in general works works by marking each calf with an electronic ID tag, which allows monitoring of the cattle motion, time spent in groups, and positioning. A second monitoring system works by using a thermal camera mounted near feed or water stations. Information from either system, using an algorithm, can predict whether an individual animal is likely to be suffering from BRDC.

Remote systems require less intensive staffing, and they are very precise in detecting diseases in comparison to trained observer (White et al, 2015).

Isolating and treating diseased cattle more promptly can reduce or avoid mass use of antibiotics in the herd. Herds of calves monitored with a REDI and treated only when were detected sick animals to have similar disease rates compared to herds where metaphylaxis was applied to all newly arrived cattle (White et al, 2015).

Immunomodulatory medication

Younger calves are more susceptible to certain pathogens, because their immunity is weaker and experience more stress in the transition to the feedlot and may respond less efficiently and fully to exposure to respiratory pathogens compared to older animals (Urban-Chmiel and Grooms, 2012).

Stimulation of the immune system to respond rapidly to *M. haemolytica* infection by replacing antibiotics that have been routinely administered for preventive purposes with new, commercially available immuno-modulating agents has shown good results (Zelnate™, Bayer Animal Health). No statistically significant differences were observed in the prevalence of BRDC or in the average daily gain, in cattle to which immunostimulating agents of non-antibiotic origin were applied

<http://www.zelnate.com/static/documents/ZNT152056%20Zelnate%20vs%20Micotil%20Tech%20Report.pdf>).

Conclusions

Potential antimicrobial alternative strategies include use of probiotic, prebiotic, herb extracts and essential oil, to mitigate bovine respiratory pathogens in feedlot cattle. The increase in the MDR towards these antimicrobials that are being used as metaphylaxis in feedlots necessitates the development of novel methods to mitigate bovine respiratory pathogens in feedlot cattle as alternatives to metaphylactic antimicrobial use. Herb extracts and EOs being two major natural AMD, with powerfull potential for application against bovine respiratory bacterial/viral pathogens.

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SOME ASPECTS OF INBREEDING AND ITS EFFECT ON GROWTH TRAITS OF LAMBS –A REVIEW

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Abstract: *Inbreeding seems to be a useful tool to improve the population by increasing the frequency of desirable genes, but the first result of inbreeding is inbreeding depression. Inbreeding depression implies the reduction of additive genetic variance and phenotypic values for efficiency traits in inbred animals. The kinship degree is measured by the inbreeding coefficient and expressed in percentage (%) from 0 to 100, that as the percentage higher, the higher the kinship. Nevertheless, one thing is for sure, practice in sheep breeding has shown that the use of inbreeding causes undesirable consequences like reduction of body shape or abnormal development, developmental delay, resistance to external influences and diseases, poor fertility or infertility, metabolic disorders, negative characteristics in fattening, albinism and the appearance of degenerative traits, loss of instinct, psychophysical defects and so on. Inbreeding is an inevitable companion of livestock production. No matter how hard we try to avoid it, in practice, it is almost impossible. It could not completely eliminate, but with proper breeding and selection management, it can be kept under control.*

Keywords: *sheep, inbreeding, lambs, selection management*

INTRODUCTION

There are various researches regarding linkage growth of lambs and relationship with genetic and non-genetic factors (Caro Petrovic et al., 2013a, Caro Petrovic et al., 2020). In any genetic improvement programs applied for livestock are based on two main approaches: selection and breeding systems, the intensive selection within a single population reduces the genetic variability

and increases the rate of inbreeding, as compared to crossbreeding (*Ceyhan et al., 2011*). A selection expresses its process of choosing the animals that meet the requirements of the breeding objective that pass a particular trait onto their progeny (*Caro Petrovic et al., 2015*). Improve genetic through selection in a breeding program confide in the preciseness of identifying genetically premium animals (*Caro Petrovic et al., 2013c*). However, it is essential to maintain the genetic diversity of the adapted breeds, pure breeds, and their crosses (*Caro Petrovic et al., 2013b*).

The level of inbreeding had clearly influenced by the ratio of males to females, reproduction ability, mating system, and population size (*Barczak et al., 2009*). Inbreeding level as well is relevant genetic property of any population and must be accepted as it can influence breeding decisions and the design of livestock improvement programs (*Mandal et al., 2005*). This is an aspect of tremendous influence in the genealogical analysis of a population structure because of its effect on the probability of genes lost between generations and the consequent reduction in genetic variability (*Pedrosa et al., 2010*). Inbreeding is thought, as a useful tool, to improve the sheep population by increasing the frequency of desirable genes, yet, it also leads to economic losses (*Ceyhan et al., 2011*). The more highly inbred the individuals, the more in general, they fell behind the weights of the corresponding non-inbreds as they grew older. Inbreeding may thus have permanently stunted the sheep (*Wiener et al., 1992*). Inbreeding increases homozygosity for whatever genes are present, including the less desirable ones, and have associated with a decline in performance (*Baneh et al., 2019*).

Effectual management of genetic resources in livestock needs complete information on the features of breeds, including knowledge on real and effective population size and structure, within and between breed genetic diversity, geographical dissemination and, breeds' production environment (*Groeneveld et al. 2010*).

Barros et al. (2017) noted that the negative effect of inbreeding, such as lower productive and reproductive indices within the herds, is related to the fact that inbred individuals often have less flexibility to face environmental changes, becoming more fragile and susceptible, that the external factors such as year/birth season, sex, type of birth and mother's age considered to quantify the genetic contribution to evaluated traits.

An inbreeding depression, reduction in performance of quantitative traits, including reproduction and survival, caused by inbreeding, is a well-known phenomenon observed in almost all experimental, domesticated, and natural populations (*Curik et al., 2017*). *Hussain et al. (2006)* terminate that inbreeding showed deleterious effects only at early stages of life, but as lambs grew older, the inbreeding effect on pre-weaning traits diminished.

The estimation of the level of inbreeding in any closed population is

inevitable for designing the future breeding plan in any livestock enterprises for a breed improvement program. The inbreeding depression, expressed as the change in performance per unit of inbreeding, is associate with decreased performance and depends on the rate of inbreeding. An inbreeding process increases homozygosity for whatever genes are present, including the less desirable ones of the trait (*Mandal et al., 2004*). However, the utilization of a designed mating system could be a suitable approach to maintain the level of inbreeding under control (*Hosseini-Zadeh, 2012*).

Breeding in kinship

This method, as its name suggests, involves mating animals that are more or less related. In genetics, and in sheep breeding in general, the term inbreeding is common. Almost no issue in the history of sheep breeding has provoked as much controversy and discussion as kinship breeding. On the other hand, the past of sheep breeding testifies to the great positive importance of inbreeding in establishing and retaining desirable traits, but also to a negative experience. Science has managed to explain the phenomenon of incest to a good extent, and in this way sheep breeders have been enabled to get a clearer idea of the consequences of its application or not. The kinship degree is measured by the inbreeding coefficient and expressed in percentage (%) from 0 to 100, that as the percentage higher, the higher the kinship. Nevertheless, one thing is for sure. Practice in sheep breeding has shown that the use of inbreeding causes undesirable consequences which are reflected in the following: reduction of body shape or abnormal development, developmental delay, resistance to external influences and diseases, poor fertility or infertility, metabolic disorders, negative characteristics in fattening, albinism and the appearance of degenerative traits, loss of instinct, psychophysical defects, etc. (*James, 1993*).

To illustrate the application of inbreeding in sheep breeding, have presented in Table 1.

Table 1. Effects of the application of related breeding in sheep (source: *Petrovic et al. 2021*)

Inbreeding coefficient %	Barrenness, %	Fertility, %	Mortality, %
0	7,39	131,5	22,4
6,25	7,14	126,8	25,4
12,50	10,34	119,9	31,5
25,00	10,64	118,4	34,3

The data from the table unequivocally show that with the increase of kinship, fertility decreases, and barrenness and death of lambs increase. In the end, you should know that despite the mentioned measures, inbreeding brings a lot of uncertainty, so it is not recommended for farmers for independent use with their sheep. The planned application of inbreeding in sheep breeding has found a place, above all, in line breeding.

According by Caro Petrovic et al., (2013a), as shows in table 2., on a small closed population of Mis sheep the relationship has studied the influence of inbreeding on body weight growth from birth to the age of 18 months. Differences in body weight and daily gain related to the presence of inbreeding in the pedigree were not found significant ($P>0.05$).

Table 2. Means and standard errors for growth traits of outbreds and inbreds rams. (Source: Caro Petrovic et al., 2013a)

Growth trait	Group	Mean	Standard error ($\pm$ SE)
DG 30 (g)	outbreds	233.90	17.25
	inbreds	265.10	16.94
DG 60(g)	outbreds	269.40	18.06
	inbreds	283.60	11.69
BW 90 (kg)	outbreds	29.40	1.44
	inbreds	31.85	1.56
BW 6mos (kg)	outbreds	50.10	1.38
	inbreds	52.80	1.77
BW 12mos (kg)	outbreds	82.70	1.80
	inbreds	83.70	2.43
BW 18mos (kg)	outbreds	85.45	1.74
	inbreds	86.80	2.32

Two sides of inbreeding

Inbreeding is useful in the process of fixing desirable genes in a population, especially in the creation of new breeds (Petrovic, 2000). However, according to Yeganehpur et al., (2016) inbreeding seems to be a useful tool to improve the population by increasing the frequency of desirable genes, but the first result of inbreeding is inbreeding depression. Inbreeding depression implies the reduction of additive genetic variance and phenotypic values for efficiency traits in inbred animals (table 3).

Table 3. Regression coefficients for growth traits (in grams) on Lori lamb inbreeding for 1% change in inbreeding. (Source: Yeganehpur et al., 2016)

Lambs	BW $\pm$ SE	WW $\pm$ SE	6MW $\pm$ SE	9MW $\pm$ SE
Male	-1.7 $\pm$ 4.6 ^{ns}	-12.5 $\pm$ 12.1 ^{ns}	-92.9 $\pm$ 27.3*	114.6 $\pm$
Female	7.0 $\pm$ 4.0 ^{ns}	-11.0 $\pm$ 10.5 ^{ns}	-83.9 $\pm$ 17.4*	119.9 ^{ns}
Single	0.5 $\pm$ 3.0 ^{ns}	-16.2 $\pm$ 10.1 ^{ns}	-95.7 $\pm$ 18.7**	-233.8 $\pm$
Twin	-6.3 $\pm$ 5.4 ^{ns}	-6.3 $\pm$ 5.4 ^{ns}	-8.1 $\pm$ 13.5 ^{ns}	35.2 ^{***}
All	4.5 $\pm$ 3.1 ^{ns}	-10.3 $\pm$ 8.0 ^{ns}	-76.3 $\pm$ 13.2**	-109.5 $\pm$
				25.2*
				145.4 $\pm$
				116.7 ^{ns}
				-77.4 $\pm$ 11.3**

BW: birth weight; WW: weaning weight; 6MW: 6-month weight; 9MW: 9-month weight; SD: standard deviation. ns=non-significant, * Significant at $p < 0.05$, **Significant at $p < 0.01$, ***Significant at $p < 0.001$.

By increasing the inbreeding level, 6MW and 9MW significantly decreased to -76.3 g and -77.4 g, respectively ($p < 0.05$).

According to Ebel and Phillips (2016), The regression coefficients of BW, WW, 6 MW and YW on lamb inbreeding were estimated to be -6.34 ± 0.69 , -14.68 ± 5.33 , 48.00 ± 9.43 and 98.65 ± 15.65 , respectively ($P < 0.01$). Therefore, BW and WW decreased, respectively, by 6.34 g and 14.68 g due to 1% increase in inbreeding and 6 MW and YW increased, respectively, by 48.00 g and 98.65 g due to 1% increase in inbreeding ($P < 0.01$). The regression coefficient of 9 MW on lamb inbreeding was not significant (table 4).

Table 4. Regression coefficients ($\pm$ SE) of body weight traits (in grams) on inbreeding of lambs for a change of 1% in inbreeding (Source: Ebel and Phillips 2016)

Item	BW	WW	6 MW	9 MW	YW
Single	-1.84 $\pm$ 0.67**	-11.45 $\pm$ 6.45**	54.50 $\pm$ 12.35**	-22.00 $\pm$ 19.58	232.65 $\pm$ 27.96**
Twin	-7.22 $\pm$ 1.33**	-28.63 $\pm$ 9.16**	30.26 $\pm$ 13.68*	17.81 $\pm$ 16.52	19.59 $\pm$ 15.30
Male	-5.74 $\pm$ 0.99**	-9.23 $\pm$ 7.24	32.18 $\pm$ 12.87*	-10.72 $\pm$ 16.11	68.47 $\pm$ 17.83**
Female	-7.23 $\pm$ 0.94**	-21.66 $\pm$ 7.85**	65.23 $\pm$ 13.81**	7.23 $\pm$ 22.62	171.28 $\pm$ 29.97**
All	-6.34 $\pm$ 0.69**	-14.68 $\pm$ 5.33**	48.00 $\pm$ 9.43**	-4.09 $\pm$ 13.12	98.65 $\pm$ 15.65**

BW: birth weight, WW: 3-month weight, 6 MW: 6-month weight, 9 MW: 9-month weight, YW: yearling weight. * $P < 0.05$. ** $P < 0.01$.

Mandal et al. (2004) concluded the need to control inbreeding in the flock because the level of inbreeding of lambs reduced the survivability of the lambs (tables 5 and 6). They mentioned that inbreeding had expect to increase as a result of several changes in breeding practices such as high intensity of selection, use of artificial insemination, and more accurate genetic evaluation. The slow inbreeding occurs due to mating of relatives in the flock as a consequence of selection in closed population for a long time when numbers of males have limited.

Table 5. Least squares analysis of variance for lamb traits (Source Mandall et al., 2004)

Source of variation	Cumulative survivability (%)						
	D.F.	Up to 3 2 days months	Up to 3 3 days months	Up to 7 6 days months	Up to 15 9 months	Up to 1 1 months	Up to 12 months
	MS						
Year of birth	24	0.19**	0.30**	0.36**	0.45**		
Season of birth	0.54**	0.63**	0.87**	1.14**			1.13*
Sex of animal	1	0.01	0.0004	0.02	0.0001	0.01	*
Type of birth	0.01	0.10	0.30				0.05
Regression on lamb inbreed	1	0.001	0.06	0.001	0.01		0.07
Regression on ewe inbreed	1	0.16**	0.26**	0.20*	0.34**		0.91*
Residual	0.32**	0.28*	0.78**	0.51**			*
	1	0.02	0.07	0.30**	0.40**		
	0.32**	0.31*	0.34*	0.60**			0.77*
	1	0.05	0.10	0.04	0.10	0.07	*
	0.25	0.30	0.14				0.08
	4,59	0.02	0.03	0.04	0.05	0.06	0.10
	0.07	0.09	0.10				

*, ** Significant at $p < 0.05$ or 0.01 .

Table 6. Lamb trait least-squares means and partial regression coefficient on lamb and ewe inbreeding (Source: Mandall et al., 2004)

Trait	Mean	Lamb inbreeding coefficient	Ewe inbreeding coefficient
Survivability up to 3 days (%)	96.15±0.42	-0.08±0.09	-0.18±0.13
	94.80±0.49	-0.15±0.10	-0.25±0.15
Survivability up to 7 days (%)	93.99±0.55	-0.31±0.12**	-0.16±0.17
	92.76±0.06	-0.36±0.13**	0.25±0.18
Survivability up to 15 days (%)	91.75±0.65	-0.32±0.14**	-0.21±0.20
	90.75±0.69	-0.31±0.15*	-0.41±0.21
Survivability up to 1 month (%)	87.39±0.79	-0.33±0.17*	-0.44±0.24
	86.19±0.83	-0.44±0.18**	-0.31±0.25
Survivability up to 2 month (%)	84.74±0.86	-0.49±0.18**	-0.23±0.26
Survivability up to 3 months (%)			
Survivability up to 6 months (%)			
Survivability up to 9 months (%)			
Survivability up to 12months (%)			

*, ** Significant at $p < 0.05$ or 0.01 .

CONCLUSION

Inbreeding is an inevitable companion of livestock production. No matter how hard we try to avoid it, in practice, it is almost impossible. If we were to realistically analyzed all domestic animals in the world, we would be amazed at the percentage of animals bred in kinship. Of course, that should not encourage us and lead us to the idea that inbreeding is desirable. Objectively, it could not completely eliminate, but with proper breeding and selection management, it can be kept under control. This is actually the main message of this review paper.

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NUT FRUIT GROWING IN SERBIA – STATE AND PERSPECTIVES

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Abstract: *Fruit growing is a very important branch of agriculture in the Republic of Serbia. Fruit production accounts for about 11% of agriculture production value (Development Strategy of Agriculture of the Republic of Serbia 2014-2024). Natural conditions of our country, especially some of the regions, are very favorable for the cultivation of fruit, including nut fruit. In Serbia of nut fruit the most grown are walnuts, whereas a growing interest in hazelnut production has been observed over the last years. However, the share of plantations covered by these species is not larger than 3.93% of fruit plantation structure. According to Census for Agriculture in the Republic of Serbia (2012), Serbia has over 1,3 million walnut trees and over 1,1 million hazelnut trees. However, in the period 2013-2019, a decrease in the number of productive walnut trees, and an increase of the number of hazel trees was recorded. Namely, the analysis of the available data showed that the total production of walnuts in this period varied from 8,836 to 19,058 tonnes, while production of hazelnut ranged from 3,366 to 5,428 tonnes, depending on the year (FAOSTAT, 2021). Generally, in the period from 2013 to 2019 in the Republic of Serbia there was a decrease in total production of walnuts for 10,222 t, while for the same period there was an increase in production of hazelnuts for 1,583t. Therefore, the objective of this study was to perform analysis state and perspectives of walnut and hazelnut production in different regions of the Republic of Serbia.*

Keywords: *Republic of Serbia, nut fruit, state, perspectives*

WALNUT AND HAZELNUT PRODUCTION

Republic of Serbia especially some regions, has natural climatic conditions and fertile soils favorable for the cultivation of nut fruit. The most important species of nut fruit trees, walnut and hazel, are relatively neglected fruit species which is indicated by the total production, only about 3.93% of the fruit production. Current production is insufficient to meet the country's needs,

as it supplies less than 50% of national requirements (Paunović and Miletić, 2013). Walnut growing has a long tradition in Serbia. However, the domestic population of walnut (*Juglans regia* L.) is highly heterogeneous, considering that each tree represents a different genotype as a result of many centuries of seed propagation. Therefore, different methods of walnut propagation have been investigated in Serbia. The best and most effective results under continental climate conditions have been attained by bench grafting using the whip-and-tongue grafting method. Walnut grafting is a very complex and expensive process, due to the low rate of callus formation and unsatisfactory percentage of grafting success, which varies from 60 to 80%, while the percentage of class 1 nursery plants of as low as 50-60% of the number of grafted rootstocks (Paunović, 2010). In terms of hazelnuts, there are two systems of cultivation of hazel tree. First system is grafting hazels, when one of the cultivar is grafted on the rootstock (*Corylus avellana*), while the second system is the hazelnut shrub that is often used in intensive production because of its benefits (healthier and more resistant to insects and disease compared to grafted hazelnut). Therefore, in Serbia hazelnut plantations can be bushy form or tree form grafted. Hazelnut grafting is a less frequently used form of propagation, due to technical and environmental demands during and after grafting (Miletić, 2017). A wide range of walnut and hazelnut application in nutrition, medicine and food, as well as the application of walnuts in timber and leather industry makes them one of the most appreciated fruit species, which is one of the reasons many fruit growers start investments in orchards of walnut and hazelnut. Namely, in recent years the state stimulates the establishment of new plantations through enabling subsidies through procurement of planting material, appropriate irrigation systems and agricultural machinery. Also, a growing interest in production of nut fruit can be explained by good domestic and international market conditions.

Walnut is a strategic species of fruits for human nutrition and has been included in the list of FAO as a priority crop to cultivate. According to FAOSTAT (2021), world productions of walnut is 4,498,442 tons, with the average yield of 3446,2 kg/ha over an area of 1,305,349 ha, which indicates a consolidating growing trend observed over the previous years (Figures 1 and 2). China is the world's biggest producer of walnuts of 2,521,504 tonnes (42%), while United States is second with 592,390 tonnes (29%) of production. Countries with over 100,000 tonnes of productions are Iran (321,074 t), Turkey (225,000), Mexico (171,368 t) and Ukraine (125,850 t), whereas Serbia takes the sixteenth place with production of 8,836 tonnes. In terms of Europe, Europe's leading producers of walnuts are Ukraine (125,850 t), followed by Romania (49,580 t), France (34,950 t), Greece (31,040 t) and Belarus (18,431 t), while Serbia ranks tenth by production of walnuts.

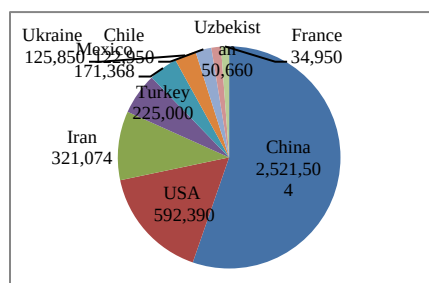


Figure 1. Production of walnut in the world

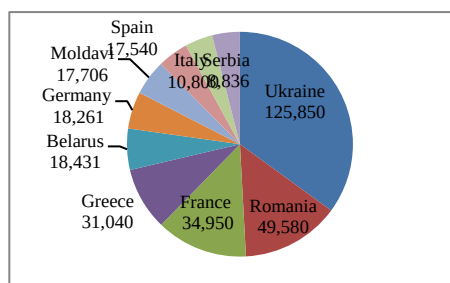


Figure 2. Production of walnut in Europe
(tonnes)

The hazelnut is a significant fruit species, which fruits are extremely appreciated on domestic and foreign market. Based on the FAOSTAT (2021), world production of hazelnut is 1,125,178 tons, with the average yield of 1124,9 kg/ha over an area of 1,000,231 ha (Figures 3-4).

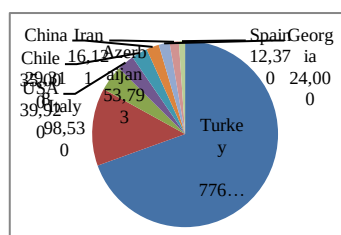


Figure 3. Production of hazelnut in the world

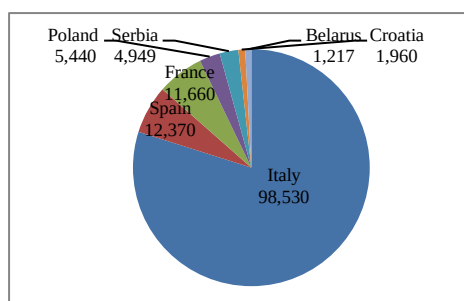


Figure 4. Production of hazelnut in Europe (tonnes)

In spite of the high number of producing countries, the highest of hazelnut production is still concentrated in two Mediterranean countries, Turkey (nearly 67% or 776,046 tonnes) and Italy (nearly 20% or 98,530 tonnes), covering more than 80% of the world production, followed by Azerbaijan (53,793 t), USA (39,920 t), Chile (35,000 t) and China (29,318 t). Serbia does not belong to the group of 25 countries in the world that are most important in the production of hazelnuts. As for the European countries, Italy is the leader in production of hazelnuts with 98,530 tonnes, followed by Spain (12,370 t), France (11,660 t), Poland (5,440 t) and Serbia (4,949 t) which ranks in the 5th place.

In Serbia, walnut is relatively neglected fruit species considering that the share of plantation is not larger than 1.53% of fruit plantation structure. It is grown in all parts of Serbia. Plantations are mostly extensive, with low level of agro- and pomo-technical measures or even without any measures in plantations. The most common reason of neglecting plantations is a long period of investments and late fruiting period, from fifth to tenth year.

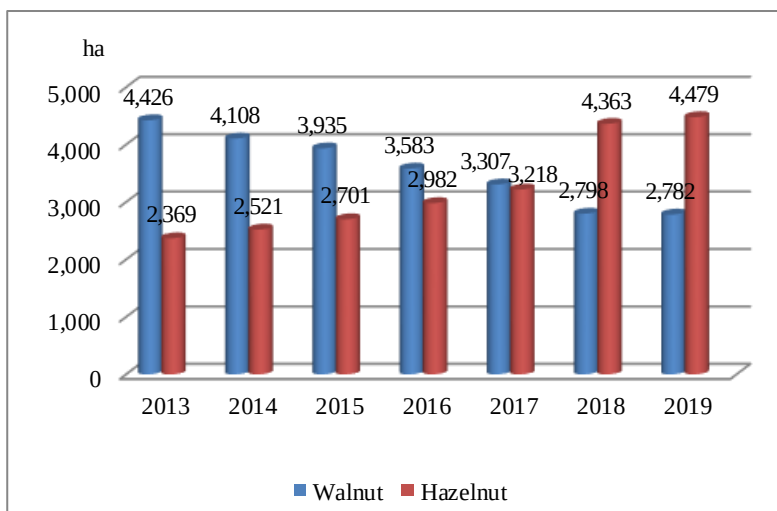


Figure 5. Growing area of walnut and hazelnut in the Republic of Serbia

In the observed period (2013-2019) the average production of walnuts in the Republic of Serbia amounted to 13,701 t on the average area of 3,562.7 ha, and with the average yield of 3.78 t/ha recorded (Figures 5-7).

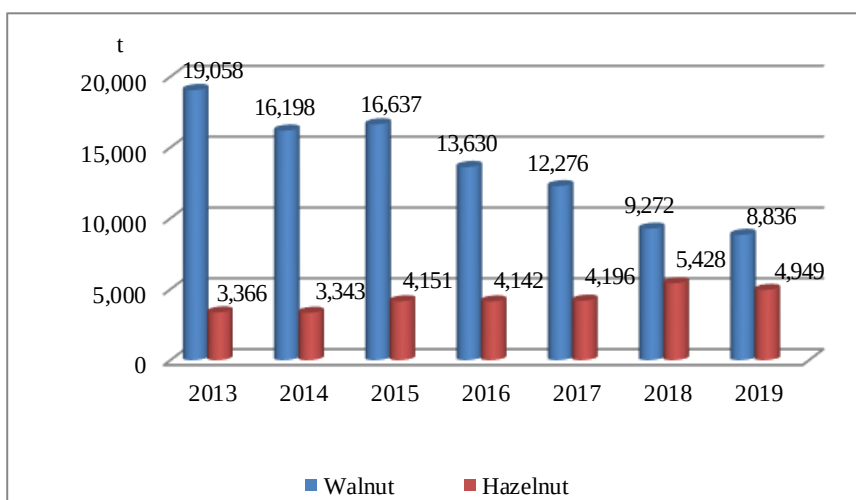


Figure 6. Production of walnut and hazelnut

According to the FAOSTAT (2021), the average areas under walnut orchards oscillated during the study period between 4,462 ha (2013) and 2,782 ha (2019). The highest productions were recorded in 2013 (19,058 t) and the lowest in 2019 (8,836 t). The analyzed data indicate a significant decrease of areas under walnut by 1,680 ha and production by 10,222 tons. In terms of yield, the highest yields per 1 ha amounted to 4.31 t/ha in 2013, while the lowest was recorded in 2019 (3.18 t/ha). The reasons for reduced areas and productions under walnuts can be consequences of walnut production on small private areas, in home gardens as individual trees, a larger number of extensive walnut orchards, clearing old orchards and establishment of small number of new ones. However, the encouraging fact is that even though a small number of new walnut orchards are established adjusted to the conditions and biological requirements of the grown cultivars, they are modern with irrigation systems and anti-hail nets.

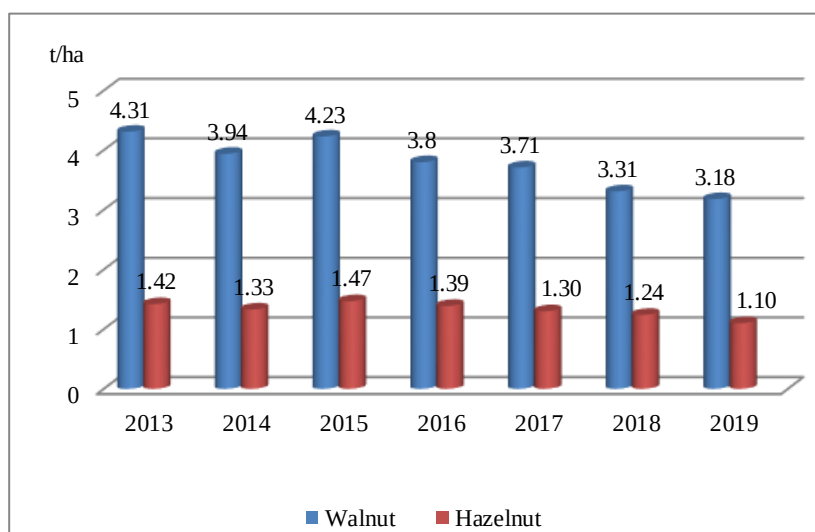


Figure 7. Yield of walnut and hazelnut

In Serbia, the share of plantations covered by hazelnut is not larger than 2.40% of fruit plantations. Production under hazelnut in Republic of Serbia is recording increase from year to year. This can be explained by favorable natural conditions for hazelnut production, from one side, and quite stable and constant market price, on the other. The average area under hazelnut orchards for the research period (2013-2019) is 3,233.3 ha, with production of 4,225 t, or yields of 1.32 t per ha (Figures 5-7). The highest productions were recorded in 2018 (5,428 t) in the areas of 4,363 ha and lowest in 2013 (3,366 t) under areas of 2,369 ha, which represents an increase in production by 2,062 t and areas by 1,994 ha (FAOSTAT, 2021). Annual increase in production indicates that extensive production is increasingly replaced with intensive ways of growing.

In terms of yield, the highest yields of 1.47 t/ha were achieved in 2015 and the lowest of 1.10 t/ha in 2019. Many factors can affect the oscillations of hazelnut production, such as occurrence of low winter temperatures and early spring frosts, damages from hail, drought, occurrence of surface water, abrupt changes of temperature in a dormant period, alternate bearing, etc. The risks for a yield can be reduced by an increase in intensity of production through installing irrigation systems, anti-hail nets, anti-frost systems, etc.

The soil and climatic conditions in most regions in Serbia is suitable for walnut and hazelnut cultivation. Data on areas, production and yield of walnut and hazelnuts by regions are shown in Figures 8-15. Production of walnut is prevalent in all parts of Serbia. The largest areas of walnut orchards are located in Šumadija and Western Serbia regions with production from 43.19%, although large areas in these regions belong to extensive plantations (Statistical Office of the Republic of Serbia, 2018).

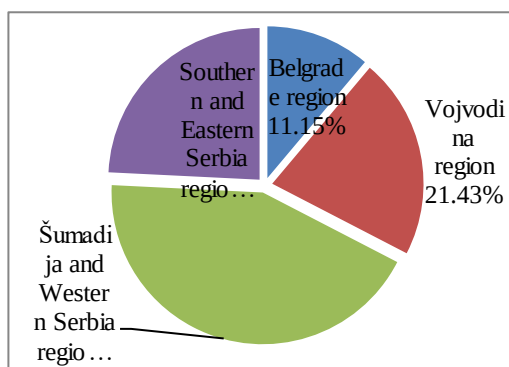


Figure 8. Share of the regions in the production of walnut

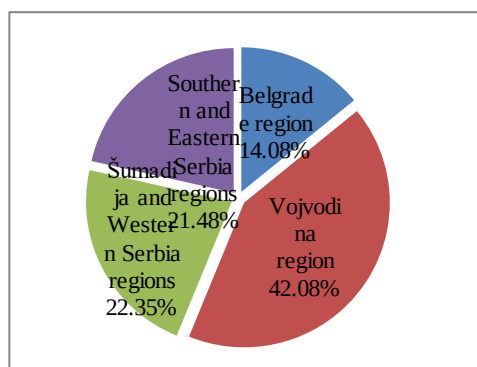


Figure 9. Share of the regions in the production of hazelnut

In Šumadija and Western Serbia regions in the observed period 2013-2018, the average production of 5,444 t over an area of 2,088 ha was achieved, including an average yield of 2.58 t per ha. The highest production was obtained in 2013 (6,969 t) over an area of 2,419 ha with the yield of 2.88 t/ha. Conversely, the minimum output was recorded in 2018 (4,005 t) on the surface of 1,713 ha, with the yield by 2.34 t/ha. The analyzed data show a decrease in walnut production considering that large areas in these regions belong to extensive plantations.

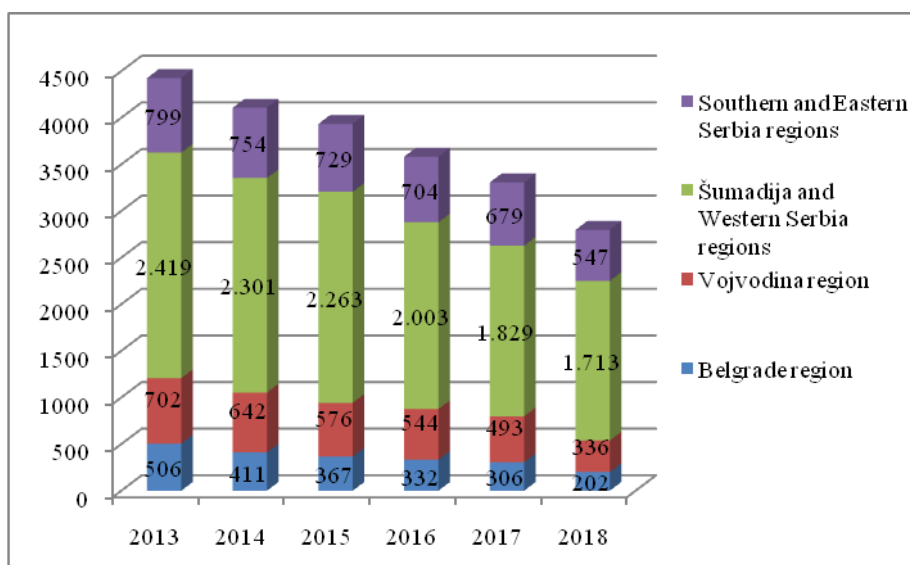


Figure 10. Areas under walnut by regions (ha)

On the other hand, Šumadija and Western Serbia region involvement in hazelnut production is 22.35%. The average hazelnuts productions for 2013/2018 have been of 1,010.3 t on areas of 816 ha was achieved, including an average yields of 1.28 t/ha. The highest area was recorded in 2018 and it was 1,142 ha, while the lowest one was in 2013 (663 ha), which represents increased by 479 ha. The total production varied from 955 tonnes in 2013 to 1,213 tonnes in 2018, whereas yield ranged from 1.57 t/ha (2015) to 1.06 t/ha (2018).

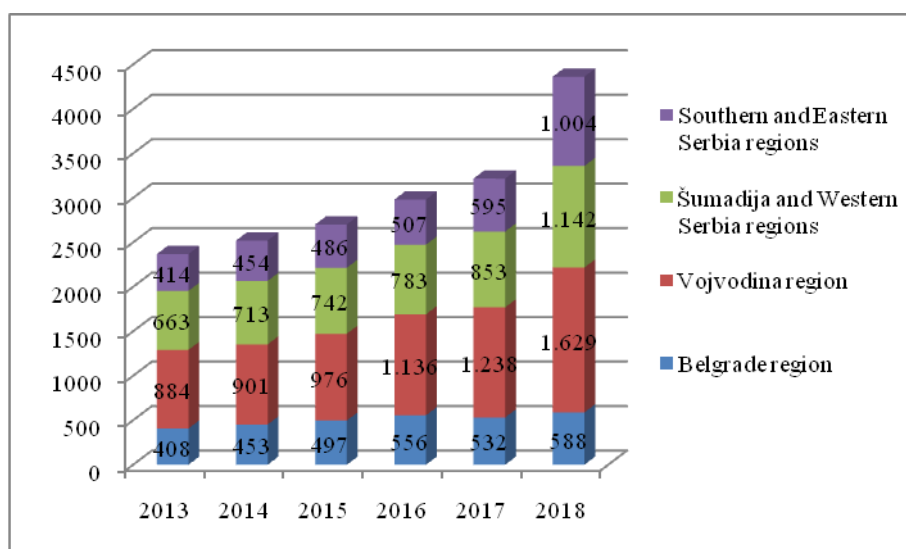


Figure 11. Areas under hazelnut by regions (ha)

In Belgrade region for the period 2013–2018, the average production of walnut of 2,173 t and hazelnuts of 720.8 t was achieved, under areas of 354 and 505.7 ha, respectively, and yields of 6.02 and 1.43 t per ha, respectively. Areas of walnut in this region were drastically decreased, from 506 ha in 2013 to 202 ha in 2018, which resulted in reduced areas over 50%. Also, significantly decreased production from 3,318 t (2013) to 1,034 t (2018) and decreased yields from 6.56 t/ha (2013) to 5.12 t/ha (2018) has been recorded. In contrast, areas under hazelnuts are increased from 408 ha (2013) to 588 ha (2018), while production slightly decreased with 595 tonnes (2013) on 588 tonnes (2018). The yield under hazelnut orchards ranged from 1.30 t/ha to 1.56 t/ha, depending on the year.

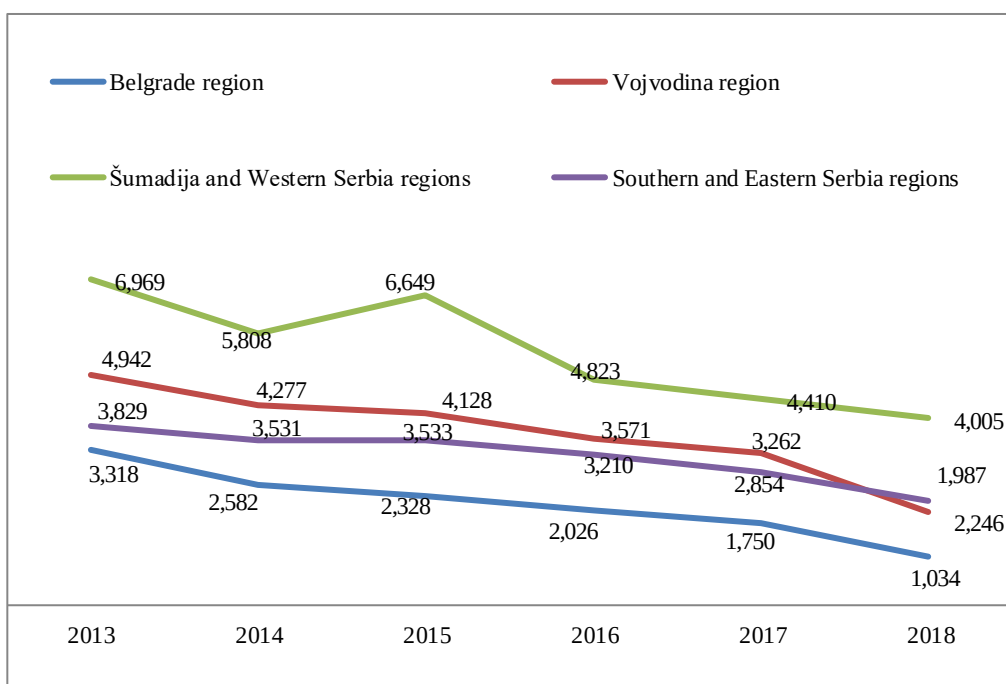


Figure 12. Production of walnut by regions (t)

Region of Vojvodina is characterized by significantly lower production of walnut (21.43%) compared to Šumadija and Western Serbia regions. The average production of walnut for the research period amounted to 3,694.5 t under areas of 548.8 hectares, although average yields (6.66 t/ha) were higher than in other regions, due to the small share of extensive plantations. The highest production was recorded in 2013 (6,969 t) in the area of 702 ha, while the lowest production achieved in 2018 (1,987 t) over an area of 336 ha; therefore, the data show that the average yield was 7.04 t/ha in 2013, and only 5.91 t/ha in 2018.

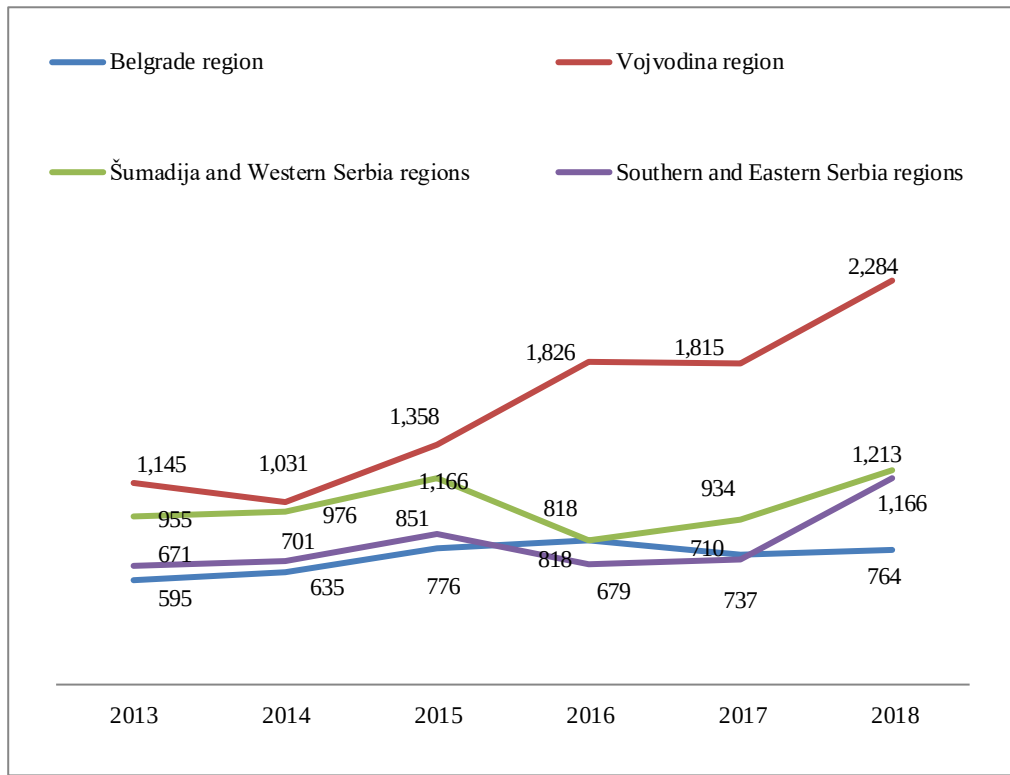


Figure 13. Production of hazelnut by regions (t)

Although natural conditions of different parts of central Serbia are favourable for hazel growing, most modern plantations of this species are in Vojvodina region. They are mostly large plantations partially of bushy form and partially of tree form grafted. The production of hazelnuts grown in Vojvodina is significantly higher than in other parts of Serbia and amount to 42.08%, i.e. 1,127.3 ha, including productions of 1,576.5 t and yields of 1.38 t/ha. The average lowest value of areas planted with hazelnut was 884 ha in 2013 and highest of 1,629 ha in 2018. Production was highest in 2018 (2,284 t) with yield of 1.40 t/ha, while the lowest was in 2014 (1,031 t) with yield of 1.14 t/ha.

In Southern and Eastern Serbia regions, fruit growing is a less prevalent branch of agriculture, except nut fruits, which are grown in a higher percentage. Out of the total production, 24.22% of walnuts are produced in this region. The areas under walnut for the observed multi-year period amounted to 702 ha, with the average production of 3,200.5 t and yield of 4.53 t/ha. The largest volume of output was in 2013 – 3829 t with yields of 4.79 under areas of 799 ha, whereas the smallest production recorded in 2018 (2,246 t and 4.11 t/ha, respectively) under areas of 5.47 ha.

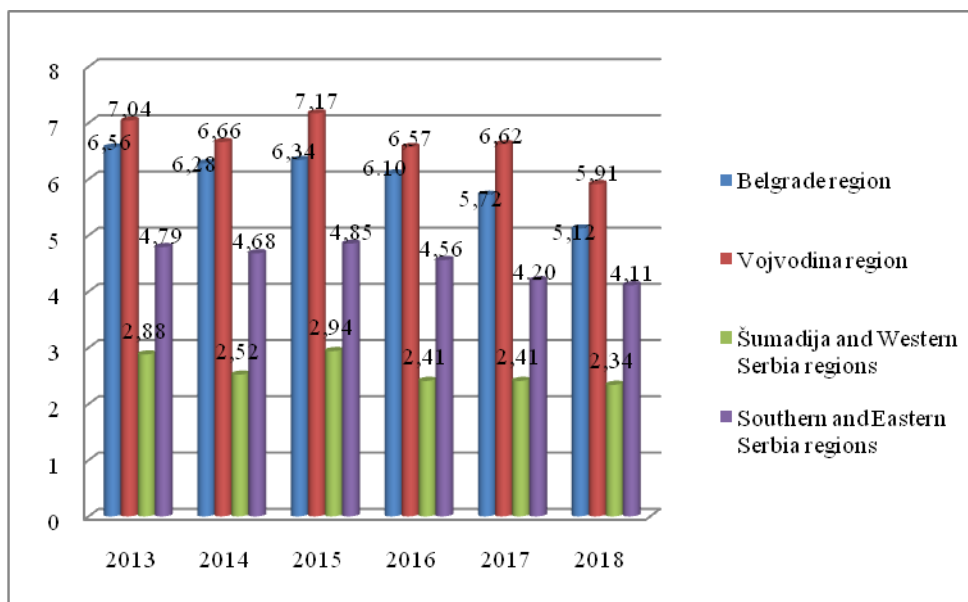


Figure 14. Yield of walnut by regions (t/ha)

In terms of hazelnut, region involvement in production was 21.48%. In the period 2013-2018, the total hazelnut production amounted to 796.3 t, including yield of 1.43 t/ha and an area of 576.7 ha. The highest production was achieved in 2018 (1.166 t under areas of 1,004 ha) and the lowest in 2013 (671 t under areas of 414 ha), while yields varied between 1.16 t/ha (2018) and 1.75 t/ha (2015).

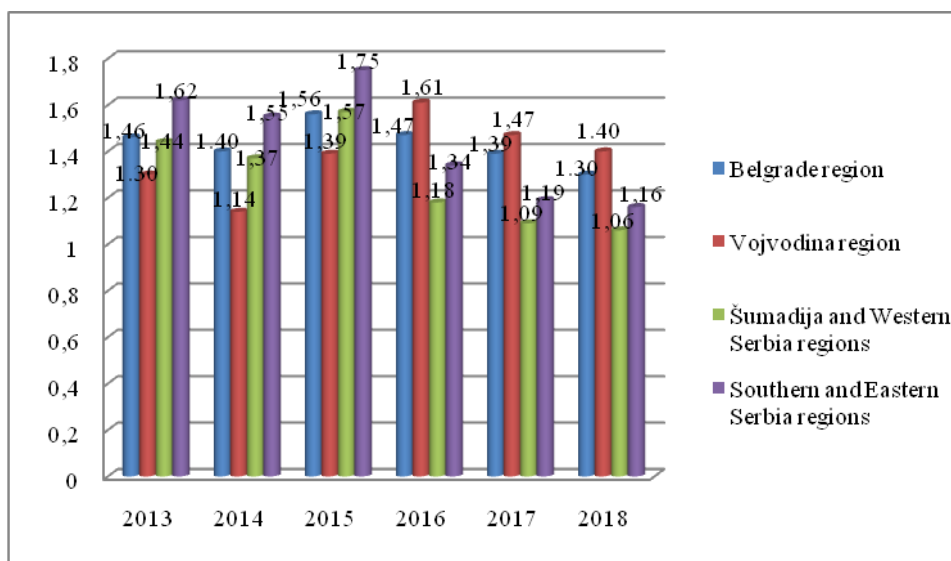


Figure 15. Yield of hazelnut by regions (t/ha)

ASSORTMENT OF WALNUT AND HAZELNUT

Walnut cultivar assortment is relatively old; it is changing slowly and it is not dynamic like the ones of other fruit species. In addition to standard cultivars with which producers have had positive experience, new cultivars and selections should be recommended as suitable for commercial growing. Leading cultivars and selections in walnut orchards are 'Šejnovo', 'Rasna', 'Šampion', 'Kasni Rodni' and 'Ovčar' and pollinators 'Geisenheim 139', 'Geisenheim 251', 'Jupiter' and 'Elit'. Modern plantations of walnut should be planted with new cultivars and selections suitable for commercial growing, such as 'Chandler', 'Fernor', 'Fernette', 'Franquette', 'Lara', 'Pedro' etc.

Expansion of hazelnut growing should be supported by the cultivars of good biological and economic value that would be adequate for growing under agroecological conditions of Serbia. Leading hazelnut cultivars in new orchards are 'Ennis', 'Tonda Gentile delle Langhe', 'Tonda di Gifoni', 'Istria Long', 'Mogul', 'Multiflorum', 'Cosford' and 'Lambert', while pollinators are 'Tonda Gentile Romana', 'Noccione' and 'Hall's Giant'. Grown cultivars respond to the requirements of the confectionery industry, i.e. expansion of cultivars with smaller and rounder fruits.

These trends in the improvement of the cultivar assortment should be accompanied by positive moves regarding growing technology, including the use of adequate agro- and pomo-technical measures.

CONCLUSION

Demand for nut fruits is unlimited on both domestic and international markets, because its wide range application makes them one of the most appreciated fruit species. The Republic of Serbia, especially some regions, has very favorable climate and soil conditions for growing of nut fruit. However, the comparison of total production of nut fruit from 2013 to 2019 in the Republic of Serbia, shows a decline in walnut production, and increase in hazelnut production. Also, the yields from year to year vary widely. Yields are affected by many factors such as occurrence of low winter temperatures and early spring frosts, damages from hail, drought, drought, the attack of pests and diseases, occurrence of surface water, abrupt changes of temperature in a dormant period, alternate bearing, etc. The risks can be reduced by an increase in intensity of production through installing irrigation systems, anti-hail nets, anti-frost systems, etc.

More recently, our fruit farmers are interested in producing nut fruit, although these species require greater investment and a longer period of exploitation until full birth. However, an important production of nut fruit advantage is the long life of the trees, including the long productive period. For

faster improvement of the production of nut fruit, as one of the most important factors is the production of quality planting material. Also, by defining regions for growing of nut fruit in Serbia, conditions would be created for increased production, intensification and introduction of new cultivars and technologies, including agro- and pomo-technical measures, increasing areas with installed irrigation systems and anti-hail nets. Actually, good prospect for exports of walnut and hazelnut should be contributed to raising new modern plantations on considerably larger areas. In addition, it is important to emphasize the support of the state in production modernization through enabling subsidies for establishment of new plantations, procurement of planting material, procurement of appropriate irrigation systems and agricultural machinery. In general, the improvement of cultivar assortment and growing technologies, accompanied by creation of encouraging environment, can lead to an increased growing of nut fruit from extensive and semi-intensive to intensive production and increased export, which provides great opportunities.

ACKNOWLEDGMENT

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MICROBIOLOGICAL AND HOMEOPATHIC PREPARATIONS IN ORGANIC FOOD PRODUCTION

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Abstract: *Agriculture, as the most important strategic industry, is tasked with providing sufficient quantities of quality and safe food. Intensive and often excessive, uncontrolled and unskilled use of the means for protection and nutrition of plants, as well as means for the prevention or treatment of animals, is carried out to increase yields in the conventional production. This approach in food production has contributed to an increasingly common endangerment of the health of plants, animals and humans, as well as significant environmental endangerment. In response to this quantitative but not qualitative approach in food production, organic agriculture has developed. Application of microbial fertilizers, i.e. clean and multiplied cells of the most physiologically active strains of microorganisms with desirable properties in relation to plant, soil, as well as the environment, it provides numerous benefits in agriculture: it reduces overall production costs, eliminates the use of expensive mineral fertilizers and pesticides, increases the use of nutrients in the soil, contributes to better growth and increased plant yield, eliminates potential soil and groundwater pollution, increases biological soil activity, improves the production of safety food.*

Homeopathy is a holistic approach in the treatment of different pathological conditions of humans, domestic animals, but also with the potential for application in wartime production. It is based on the "principle of the like", i.e. the capacity that the substance that is the cause of a particular pathological condition, at the same time, has the potential to cure the condition, if applied in minimal doses. The application of homeopathic products in the prevention and treatment of animal diseases has great advantages because these products are not toxic, reduce and eliminate the use of antibiotics, hormones and other medicines that leave residues in animal

products and animal waste. On the other hand, the application of homeopathy in wartime production is directed in two directions. The first direction refers to the application of homeopathic preparations in healthy plants to increase their vegetative properties and yields (plant growth, increase in germination, flowering, increase in yield), as well as to reduce the negative impact of abiotic stress (high temperature, sunlight, untreated pH soils, salinity). The second direction refers to their application to control certain phytopathology, without the usual application of agrochemistry. Therefore, the application of microbial and homeopathic preparations seeks to "repair" at least one part of what has been "spoiled" by the aggressive action of man in nature, because these principles combine the best models from nature, with a high respect for environmental principles, preservation of natural resources, and with an effort to put the health of plants, animals and humans first.

Key words: food safety, agrochemicals, organic production, microbial fertilizers, homeopathic preparation

1. Food safety and use of agrochemy

The issue of food safety is coming to wide attention around the world, as a high connection between food consumed and health has been established. Just as essential a man's need to eat is, so is food safety a basic human right. Since food trade and transport today have an international character, the issue of food safety is a common problem and a subject of attention, both developed and developing countries. Unsafe food contains dangerous agents or pollutants which can lead immediately to the onset of disease in humans, or to create an increased risk of developing chronic diseases (Veskovic and Djukic, 2015). Such pollutants can get into food in many ways, during any stage of its production, transport and distribution. The most common risk for unsafe food is the presence of various physical, biological (bacteria, viruses, parasites, prions), chemical agents (pesticides, heavy metals, radionuclides, mycotoxins, veterinary medicine remedies, growth promoters, hormones, tranquilizers, chemical preservatives, etc., but also personnel and environmental hygiene (Fung et al. (2018).

On the other hand, the country's population is steadily increasing, which is also caused by an increase in global demand for food, which is particularly notable among developing countries. In such circumstances, organic agricultural production has developed, as a need to improve both health and productivity of all interconnected factors - soils, plants, animals and humans. Intensifying the development of agricultural production, in a sustainable way, is based on an overall knowledge of the diversity of the agroecosystem, as well as to look at their potential ability to mitigate the adverse effects that contribute to low soil fertility, to reduce the effects of abiotic stress, as well as the harmful effects of

potentially present pathogen and pests (Tillman and sar., Timmusk and sar., 2017). The concept of organic production, in addition to the production of safe food and the preservation and improvement of the eco system, implies a reduction of all forms of pollution (Golian et al., 2017). The use of chemical supplements is significantly cloaked or completely eliminated, at the expense of the application of permitted natural procedures and substances. This approach stemmed from a response to existing high pollution of the human environment, intensive urbanization and an imperative need to protect and preserve plant and animal life. It is not rare to know that conventional production and, often, uncontrolled use of pesticides and mineral fertilizers have contributed to the disruption of biocellability relations, increasing the cost of agricultural products, as well as the appearance of unwanted consequences on human health, most often chronic character (Veskovic and Djukić, 2015)

1.1. Pesticides

An estimated 1.8 billion people worldwide are involved in agriculture, with the majority of the population using the convention principles of production. Therefore, it is not surprising that at the core of many of the world's environmental imbalances, as well as environmental disasters, there is an unethical use of agrochemicals (Alavanja, 2009). This data is not surprising given that at the beginning of this century, more than 850 chemical compounds were registered in the world market for agrochemy, which are still applied today in different areas of agriculture, either as means of protecting crops from disease and pest, or for improving plant growth and fertility (Tomlin, 2004). In 2019, the world's agrochemical market was worth about US\$243.1 billion, with predictions that by 2024 this value will increase to nearly US\$300 billion. (Garside, 2021). According to available data from the Food and Agriculture Organization of the United Nations (FAO) on the amounts of pesticides used in the agricultural sector (last update 2020) worldwide pesticide production in 2018 was 4,122,334 tonnes. For the past 30 years, the Countries of the Asian Continent have been the biggest consumers of pesticides (52.8%), America is second with 30%, while Europe consumed 13.8% of the world's output annually. However, regardless of the application of agrochemy, the world's crop productivity losses are estimated at 37%, due to the side effects of insects, 12% due to weeds and 12% from disease (Pimentel et al., 1991).

Although the criteria by which international and national regulators approve the registration and application of pesticides are very strict, both functional and toxic, ie. health aspect, however, there is ample evidence of the presence of their remains in the environment (Haynes et al., 2020; Silva et al., 2019). At the same time, primarily, in many developing countries due to the lack of a health risk control and assessment program, as many as 25 million

agricultural workers experience unintended poisoning each year due to exposure to pesticides (Jeyaratnam, 1999). Also very troubling are data from the U.S. Department of Agriculture that 50 million people in the U.S. use groundwater potentially contaminated with pesticides and other agricultural chemicals (Ward et al., 2000). It is amazing that children aged 3-6 playing with toys on carpets in their homes bring most of these harmful substances into the body (Whyatt et al., 2002; Bradman et al., 2005).

The remnants of pesticides, or their undegradable factions, can be found everywhere in nature, from air (Majewski and Capel, 1995), soil and water (Aydinalp and Porca, 2004), food (EFSA, 2017). It's absolutely unbelievable that pesticide residues can be found far from intensive agriculture, such as Antarctica (Tatton and Ruzicka, 1967). Depending on the type of pesticide, its quantity, the length of exposure, the input pathways, as well as the individual health status of the individual, the health impacts are different. In the human or animal body, pesticides metabolize, secrete or, even, bioaccumulate in body fat tissue (WHO, 1990), while the adverse effects of their effects occur dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive and endocrine disorders (WHO, 1990). Sanborn et al., 2007). What is very worrying is the fact that high exposure to pesticides can lead to hospitalization and death (WHO, 1990).

In addition to the potential proven negative effect on human health, some pesticides can negatively affect land and water biodiversity, even at sublethal doses. The negative effect of atrazine on the sexual development of amphibians (Rohr, 2017) is known, while neonicotinoids act negatively on the diet of bees (Muth and Leonard, 2019).

1.2. Inorganic mineral fertilizers

Conventional agriculture is unimaginable without the use of inorganic mineral fertilizers, which are often used to achieve higher crop yields, but only 10% – 40% of applied fertilizer plants can be directly absorbed and used. It has long been a rule that "land should be restored to what the harvest takes away." As "macro" elements nitrogen, phosphorus and potassium are extracted, followed by sulfur, calcium and magnesium, like others, in traces, the necessary elements for plants. Global consumption data on total inorganic mineral fertilizers ($N+P_2O_5+K_2O$) indicates that this is quantity of more than 313,000 tonnes (FAO, 2019). Nitrogen is the most used fertilizer nutrient in the European Union's agricultural industry in terms of volume. In 2018 and 2019, some 11.8 and 11.5 million metric tons of nitrogen fertilizer were used to encourage optimal crop production on Europe's farmlands.

Inorganic mineral fertilizers are relatively inexpensive, have high but known nutrient content, usually nitrogen, phosphorus and potassium, which plants

quickly assimilate. However, the use of their larger quantities can lead to a number of problems, such as the loss of nutrients and soil acidification, surface and groundwater pollution, the reduction of beneficial microbial communities while increasing plant sensitivity to the effects of harmful insects (Chen, 2006). Very often, the residual mineral fertilizers in the soil (most commonly in the form of insoluble anorgan salts) are rinsed into the nearest water flows, leading to a reduction in soil biodiversity (Barazani et al., 1999), which significantly harms the health and life of plants. Namely, soil microorganisms, primarily rissospheres, participate in the biogeochemical cycle of nutrients in the soil (McNear, 2013), helping plants to undertake abiotic stresses (drought, low temperatures, salinity, metal toxicity, and high temperatures) (Milošević et al., 2012), produce phytohormone that improve their growth (Costacurta and Vanderleyden, 1995), while endophiles prevent diseases caused by phytopathogens (Dutta et al., 2014). Mineral fertilizers lower the number of ammonificators (Pešaković et al., 2003), and high doses of nitrogen and potassium mineral fertilizer (especially in the case of their long-term application) lead to an increase in the number of toxicogenic microorganisms in the soil, with the following occurrence of microbial toxicity of the soil (Atlas and Bartha, 1992; Djukic et al., 2007). Any disruption of qualitative and quantitative microbial composition has a direct impact on plant yields. The fertilizer industry is considered a potential source of natural radionucleids and heavy metals (Cd, Pb, Hg) (Asghar et al., 2002), which absorb plants from the soil, thus bringing them into the food chain, which becomes unsafe and risky to the health of animals and humans.

In addition to serious environmental problems, many diseases occur in humans, such as stomach cancer, asphyxiation and several vector diseases. In newborns, the uncontrolled application of nitrogen fertilizers and their finding in the drinking water is associated with the emergence of "blue baby syndrome" (Majumdar, 2003).

It is important to note that mineral fertilizers are permitted in organic agricultural production, but only natural ones (e.g. raw phosphate, aluminum calcium phosphate, calcium sulfate), and with the approval of a certification body.

2. Organic agricultural production

Organic production as part of ecological, sustainable development includes food production (primary agriculture, processing to distribution), but also some other products of plant and animal origin (fabric, leather). The term organic agriculture (U.S.), biological agriculture (Germany, Denmark, Western European countries) or ecological or organic agriculture (R. Serbia and countries in the region) indicates activities aimed at introducing an ecological dimension into agricultural production, i.e. management of agriculture with

respect to the principles of environmental preservation (Tabaković, etc., 2017). Organic food is now increasingly attracting interest from modern consumers, and we can see it as the food that is grown with the utmost consideration for the environment and animals, which is safe and quality (Carlson and Jaenicke, 2016; EC, 2017). Organic agriculture is based on the application of the principles of agroecology, which includes a prohibition on the use of artificial mineral fertilizers and pesticides, as well as genetically modified organisms (Gomiero, 2018). Similarly, related to animal production, organic production regulations prohibit the use of hormones, growth promoters, etc., while the use of antibiotics is severely restricted (only in special cases). It also prohibits the use of many other synthetic compounds that, like food additives, are used in the conventional food chain (e.g. preservatives, coloring agents) (EC, 2016; IFOAM, 2017). In organic animal production, all actions are directed towards the health and well-being of domestic animals.

However, organic agriculture is not only producing without artificial fertilizers and other agrochemicals, as well as antibiotics and hormones. It is more of a holistic production system that functions as a sustainable unit, and unites interconnected and conditioned actors: plants, animals, microorganisms, insects, organic and mineral soil matter and humans (Mirecki et al., 2011). In other words, the role of organic agriculture is to sustain and improve the health of the ecosystem as a whole, as well as all present organisms, from the smallest ones found in the soil, through plants and animals, to man. On this basis, the International Federation of Movements for Organic Agriculture (IFOAM, 2017) has defined the basic principles of organic agriculture development, whose primary goal is to produce high quality healthy food, with preventive environmental action and well-being: the principle of health, the principle of ecology, the principle of fairness and the principle of nurturing and aging. Based on these principles, all current documents in the field of legislative are based, starting with Codex Alimentarius (FAO/WHO), through ISO documents, European laws (EEC) No. 2092/91 on organic production of agricultural products and indications referring to agricultural products and foodstuffs - revised in 1999, to our domestic regulations (Law on Organic Production) Official Gazette of RS", no. 30/10, Rulebook on Control and Certification in Organic Production and Methods of Organic Production, Official Gazette of RS", No. 95/20, Rulebook on Documentation submitted to an authorized control organization for issuing of certificate, as well as on the conditions and manner of sale of organic products, Official Gazette of RS, No. 88/16).

The growing level of educating modern consumers, accompanied by expressed awareness of the need for healthy food and healthy lifestyle, has contributed to increased interest and demand for organic products. This legality is particularly pronounced in highly developed countries where we have

significantly higher demand relative to supply, which provides great opportunities for the development of this agriculture sector.

Organic production in Serbia has a growth tendency. Data from 2019 indicate that the total organic status has 13,726 ha, whereby the arable land on which crops are grown has 9,880 ha, while under meadows and pastures there are another 3,845 ha. In the period of food conversion there are another 7,539 ha (source: MPŠV, 2021). Largest organic production surfaces are still in Vojvodina, where grains and industrial herbs are grown the most, while fruit species are grown in Sumadija and Western Serbia, as well as in Southern and Eastern Serbia.

In organic cattle, the largest number are bees (9969), chickens (17751) and sheep (6099). The total number of organic producers has risen from 434 in 2017 to 513 manufacturers in 2019. Exports of organic products in 2017 amounted to 23,113,073 euros, and growth of more than 5.5 million euros (\$29.75 million) was found in 2019. Most organic products, primarily raspberries, were exported to European Union countries (mostly Germany).

2.1. Microbiological fertilizer in organic agricultural production

By analyzing the current state of agriculture and objectively looking at the facts that indicate the existence of certain negative scenarios in modern food production, it imposes the need for certain measures that would contribute to ecological stabilization, as well as the immediate restoration of agricultural land. In this field of action, there is a particularly important aspect of the application of achievements in the field of biotechnology, especially in the field of agricultural microbial biotechnology. Activities in this area are based on the application of useful interactions of plants-microorganism-microbiome that would enable a sustainable solution for the restoration and improvement of agricultural production (Timmusk et al., 2017) in the future. Although there are now more than 100,000 species of microorganisms in nature, there are only a few hundred in the biological application, of which those microorganisms that have the potential to produce microbial fertilizers (Popov, 2007) are very important.

The production of microbial fertilizers involves the application of "clean", multiplied and physiologically active cells of selected strains of microorganisms that show desirable properties in relation to plant, soil and environment (Luci et al., 2004; Bargaz et al., 2018). This added active cells act as biocatalysts present in microorganisms in the soil, allowing nutrients from the rhizosphere to become available to plant roots while increasing the assimilation of nutrients (Spanoghe et al., 2020).

Depending on the selected microorganisms, i.e. the bioactivity of their different metabolism products, there are different mechanisms for encouraging

and improving biological processes within the plant-microorganization-soil complex (Singh et al., 2016). The biological properties selected by microorganisms are most often directed towards four fields of activity: (1) active nitrogen action processes, (2) phosphorus dissolving/mobilization procedures, (3) composting processes and (4) bioactivities that classify them as biopesticides (Pathak and Kumar, 2016). It should be noted that in addition to their main function for which microorganisms or their groups are selected, they may also show other positive properties on the plant, which contributes to increased yield.

A pioneering venture in the production of microbial fertilizers with the properties of nitrogenfixators was a commercial risobi-based preparation called *Rhizobium leguminosarum*, which Nobbe and Hiltner patented and called "nitrogin". Since then, for more than a century, many scientists have been working to explore new species of microorganisms that have the ability to stimulate the growth of different types of plants. As components of microbial fertilizers, there can be different bacteria from the genus *Azotobacter*, *Bacillus*, *Pseudomonas* and others.

Given the importance of nitrogen in plant nutrition, it is clear that a special place is attached to bacteria located near the roots and which have the ability to translate elemental, atmospheric nitrogen into those forms that are accessible to plants. In addition to nitrogen, plant growth directly depends on phosphorus, which is most commonly present in the soil, for them, in inaccessible forms. Bacteria from the genus *Bacillus* and *Azotobacter* synthesize organic acids and phosphates, which translate this inaccessible form of phosphorus into a form suitable for use. Some bacteria, thanks to the presence of *siderofo*r, contribute to the supply of plants with iron. It is also known that bacteria from the *pseudomonas* genus transform organic forms of sulfur into inorganic, rendering it accessible to plants. In the world market for microbial fertilizers, nitrogen-resistant bacteria and phosphorus solubilising/mobilizing microorganisms make up more than 75% of biostimulants on a microbial basis (Agro News, 2014).

Useful rhizospheric microorganisms, as *part* of microbiological formulations of fertilizers, they can increase plant growth through several regulatory biochemical mechanisms: a) planthormonal signals, b) by preventing the growth of present pathogenic strains of microorganisms in the earth, and c) by increasing the bioavailability of nutrients in the country (Van der Heijden et al., 2008; Jacobi et al., 2017). Of course, they must possess a good ability to survive and adapt to the specific conditions of the new environment while possessing a high ability to compete with the present indigenous populations of microorganisms. After the extinction of microorganisms entered the land, the amount of total biomass increases, which indirectly contributes to increasing land fertility and providing the necessary

mineral nutrients to plants.

Definitively, the application of microbial fertilizers achieves numerous benefits in agriculture, starting with the reduction of total production costs, eliminating the use of expensive mineral fertilizers and pesticides, nutrients from the soil are better used, growth and yield of plants is better, eliminating potential pollution of soil and groundwater, increasing biological activity of the soil, establishing ecological balance in the soil, and thus increasing the production of health safe food. This agrees with the saying "big potential in small packages" by Matt Kleinhenz (Third world congress on the use of bio stimulants in agriculture 2017, Miami)

2.2. Homeopathic preparations in organic agricultural production

Homeopathy is currently an evolving holistic approach in the treatment of various pathological conditions both in humans and domestic animals as well. Its application continues to grow throughout the world thanks to advances in the field of quantum physics. It has even been proposed to rename homeopathy as „adaptive network nanomedicine” (Bell and Schwartz, 2015). Homeopathy is a division of alternative medicine based on the „principle of the like” (latin: „*Similia Similibus Curentur*”), in other words, “like treats like”, and in minimal doses. It actually means that a given substance which in a huge dose generates pathological symptomatology, in the same time has the potential to cure it, if applied in the minimum doses. These minimal doses are gained through the process of dilution and intense agitation, also well known as homeopathic succussion (Toledo et al., 2011). Through this process, medicinal products were obtained, called potencies, dilutions or dynamisations in decimal, centesimal, thousands and others (Teixeira, 2013). However, despite its high dilution, it is still possible to detect a few or even a single molecule or nanoparticles of the „*ponderable active principle*” in dynamisations, even in high centesimal dilutions.

The principle of similarity should be understood as the parallelism between the toxic effect of a given substance and its therapeutic use for the treatment of similar, but not necessarily identical symptomatology. For the example, some drugs used for the treatment of vertigo, muscle cramps, anxiety or headache, can induce similar symptoms in a healthy person when they have been homeopathically energized by means of a dilution-succussion process (Serretti et al., 2010).

The most controversial aspect of homeopathy is that these serial dilutions have a measurable effect when they are given to an animal or even a plant in infinitesimal doses, which leave no possibility to placebo effect. The use of homeopathic remedies in the prevention and treatment of animal diseases has great advantages in organic food manufacturing because they are

non-toxic, they reduce and eliminate the use of antibiotics, corticosteroids and hormones, which leave residues in animal products and animal waste. In veterinary medicine, homeopathy is used, among others, for the treatment of fetal death in pigs (Day, 1984), control of mastitis in cattle (Ruegg, 2009; Varshney and Naresh, 2005) and sheep (Day, 1986), elimination of ticks in bovines (Figueiredo et al., 2018), salmonellosis in chickens (Berchieri et al., 2006), gastroenteritis in dogs (Epstein and Bell, 2016). Generally, clinical veterinary practice showed that homeopathic remedies could also be highly effective in the treatment of eye infections, eczema, allergies, cough, gastrointestinal, urinary, locomotor, hormonal and neurological disorders, diarrhea in calves, respiratory infections in piglets and so on (Hektoen, 2005). Homeopathic remedies can be administered in food or added directly to culture water (Mazon-Suastegui et al., 2018a) to promote resistance to pathogens of the biotic or abiotic stress conditions. Recently published results (Mazol-Suastegui et al., 2017; Ortiz-Cornejo et al., 2017) have shown that aquacultural homeopathy is able to produce measurable biological effect in marine molluscs, shrimp and fish, which trigger physiological, humoral and genomic responses (Mazol-Suastegui et al., 2018; Garcia, 2017; Garcia-Corona, 2018; Khuda-Bukhsh, 2012) to recover the lost internal homeostasis, i.e., health. Homeopathic treatment of the plants dated from the pioneering work of Lili Kolisko in 1923, to the nowadays (Dinelli et al., 2012). In late 20th century Kaviraj (2011) opened a new dimension in agricultural sciences using homeopathy. He focused on Agrohmeopathy and made it attractive to the scientific community for research purposes. According to him, treatment of plant is actually similar to the treatments of animals and humans. Generally, all experimental findings could be divided into two groups:

- effects of homeopathy on seed germination, growth of seedlings, flowering, fruiting of different crops, and soil health as well (Sen et al., 2018). It is generally conducted on healthy plants or on plants subjected to abiotic stress such as high temperature, solar irradiation, high aridity or high salinity (Feres et Soriano, 2007; Frahm et al., 2004; Majewsky et al., 2009; Mazon-Suastegui et al., 2018b; Ojeda-Silvera et al., 2015);
- applicability of homeopathic remedies to the control of phytopathology and managed on artificially infected plants (Betti et al., 2009).

The most replicated study is the protective effect of homeopathically ultradiluted arsenic versus the effect of arsenic itself in ponderable doses (pre- and post-treatment) on maize seeds (Brizzi et al., 2005). Positive results have been achieved in prevention and control of plague-organisms in the *Solanum quitoense* Lam crop (Narvaez-Martinez et al., 2014), in germination and initial

growth of *Hancornia speciosa* Gomes (Pinto et al., 2014) and in remediation of soils affected by heavy metals (Dos Santos et al., 2016). The homeopathic medicine Natrium muriaticum has been successfully used to manage high aridity (Frahm et al., 2004; Ojeda-Silvera et al., 2015) and to increase resistance to salt stress in *Solanum lycopersicum* (Giardini-Bonfim et al., 2012).

Homeopathic treatments also showed activity against *Fusarium oxysporum* who caused one of the most important diseases affecting tomato crop, i.e. vascular wilt. The control of fungal diseases in agriculture is controlled by agrochemicals including toxic pesticides whose excessive and indiscriminate use has caused the decrease or loss of fertile soil layer and death of microorganisms in soil. Additionally, the pathogens have developed resistance to the active ingredient of the agrochemical that generally has a high cost (Maneses, 2017). From the work of Betti et al. (2003) it has been proposed that decimal homeopathic potencies induce significantly increased resistance to tobacco mosaic virus. Moreover, the significant results obtained with potentized water treatments suggest that solvent potentization alone is able to induce effect similar (but weaker) than homeopathic arsenic trioxide, As_2O_3 (Brizzi et al., 2000).

3. Instead of a conclusion

Excessive and uncontrollable, and often unskilled, use of plant protection and nutrition agents, as well as agents meant for prophylactic or therapeutic purposes in animals, has led to problems with food safety, human health, and to negative environmental impacts. As a result of the non-selective use of agrochemy, today natural cycles, necessary for the survival of the plant world, are significantly impaired and endangered so different environmental imbalances and environmental disasters are not rare in various parts of the world. In such circumstances, organic agricultural production combines the best models from nature, with a high respect for environmental principles and the preservation of natural resources, as an imperative to implement natural procedures and substances, while limiting or completely eliminating synthetic chemical substances.

Today in R. Serbia organic agriculture is increasingly popular and has a tendency to grow. Thanks to the potential, which are primarily reflected in small properties, land that is not contaminated with harmful substances, this type of agriculture can contribute significantly to the development of rural areas, and thus, in general, agriculture in the country. The main focus in the coming decades, both in our country and elsewhere in the world, would be the development and production of microbial fertilizers, i.e. useful, safe and environmentally acceptable microorganisms and their application in ecological

production of crops and cultures. Their application would affect reducing production costs, reducing the use of expensive mineral fertilizers, better utilising nutrients in the soil, improving plant growth and plant development with a substantial increase in yields. In addition, the importance of the application of microbial fertilizers is reflected in the fact that they do not pollute soil and groundwater, to increase the biological activity of the soil by maintaining its ecological balance, thereby comprehensively improving the production of health-safe food.

The application of homeopathic preparations in the prevention and treatment of animal diseases is an extraordinary potential, because these products are not toxic, they reduce and eliminate the use of antibiotics and other medicines that leave residues in animal products and animal waste, are efficient, affordable, have a low price and, often, solve problems in cases where conventional therapy is not appropriate. Organic animal production is a mandatory choice for the treatment of animals. Agrohomoepathy represents a new approach in controlling certain plant diseases without the use of common agrochemistry. Also, this holistic principle may act on improving productivity, i.e., improving productivity growth and yield of healthy plants, as well as on reducing the impact of negative soil properties and external environmental factors. The results obtained so far suggest that veterinary and agricultural homeopathy are viable alternatives from the economic, ecological and social point of view to contribute to the environment and start to reduce the harmful footprint left on the planet by the indiscriminate use of various toxic mineral fertilizers and agrochemicals. Therefore, it is necessary to search for ecological and less polluting alternatives for the control of pests and diseases in agriculture, ensuring the safety and future of the agroindustry, particularly when we think about organic food manufacturing.

However, despite all the benefits and numerous scientific and field evidence of their overall agronomic and veterinary efficacy, efforts are still needed to make them full agro – veterinary substances that farmers and livestock farmers will use without thinking in their production.

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INDIGENOUS BREEDS OF ANIMALS FOR BREEDING IN ORGANIC LIVESTOCK IN SERBIA

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Abstract: *The genetic diversity of domestic animals includes different species and breeds of domestic animals, and thus represents a very important resource that provides food for humans. However, the modern market emphasizes the breeding of a very small number of breeds that have been selectively developed in the direction of achieving high one-way and specialized production. Reduced economic interest leads to the extinction of interest in breeding those breeds, and even species of domestic animals that do not meet the requirements of intensive production. In order to preserve the variability that is the basis for biological adaptation and vitality of species, as well as to preserve global habitats and human habitats, it is necessary to preserve traditional and locally adapted domestic animals and to find new economic sense for their breeding and sustainable exploitation of local resources. Due to the specific practice that encourages the conservation of variable animal resources and conservation of agrobiodiversity in general, with the application of controlled grazing which prevents the succession of natural meadows and pastures, organic livestock is recommended which focuses on preserving not only domesticated biodiversity but also living communities. biodiversity conservation. In addition to preserving agrobiodiversity, organic livestock is the basis for the renewal of the rural community and the improvement of traditional production of indigenous animal products from geographically defined areas, with the application of production and quality standards. From the autochthonous species of animals, cattle, horse, donkey, sheep, goat, pig, chicken are bred on the territory of Serbia. According to the data of the Ministry of Agriculture, Forestry and Water Management, there is a tendency for almost all indigenous species of animals for the period from 2013-2020. In the same period, there is also a*

tendency to increase the breeders of indigenous species in our country.

Keywords: *indigenous breeds, organic livestock, protected genetic resources, Serbia*

1. INTRODUCTION

The intensification of livestock production has led to the industrialization of this type of agricultural production with numerous negative consequences for the health of animal products, animal welfare and the environment, and thus the concentration of profits in large global companies. Livestock production used to be an ideal picture of the symbiosis of man and animals on small rural farms, where animals played an irreplaceable role in providing quality food for human consumption, beneficial impact on the environment, and therefore animals were recognized as socio-cultural initiators of society.

Today, animal husbandry is seen in a different context, in which keeping and exploiting animals is recognized as a potential danger to the environment (climate change), a source of various diseases - zoonoses that can be transmitted from animals to humans, a source of "unhealthy" foods for human use (animal fat) and, as paradoxical as it may sound, a generator of increasing world hunger. A special problem is the relationship between man and animals, where they (on modern production systems) are exposed to various emotional and physical treatments. The negative consequences of the industrialization of animal husbandry imposed the need for change. A significant change in society's attitude towards animal production and agriculture in general has been evident in recent decades. The interest of society and consumers has increasingly shifted from the primary "how much something is produced" to the way "something is produced". Environmental protection, animal welfare and sustainable rural development have become an indispensable part in planning and organizing livestock production (Mirecki, 2014).

Organic livestock production is based on a harmonious relationship between land, plants and animals, protection of ecosystems, as well as respect for the behavioral and physiological needs of animals, and as such represents a production system that almost completely meets the growing demands of society. Organic livestock production does not only mean the prohibition (and restriction) of the use of medicines and various chemical agents in livestock. Organic animal husbandry is characterized by a holistic approach based on understanding nature in which this production is seen as a way of "living with nature", the principle of sustainable development (social, economic and environmental aspects) and respect for the values and inviolability of animal integrity.

This type of livestock production is not a production system that will solve all livestock problems and is primarily a production method "designed" for a

specific market, one that requires high quality standards throughout the entire production system. A special value aspect of organic livestock is, in addition to preserving biodiversity (proclaiming locally raised animals and plants for their nutrition) and a clear position on banning transgenic organisms (animals and plants) in this type of production. The consequences of gene modification on human and animal health and the preservation of biodiversity cannot yet be fully understood. In addition, such biotechnological methods lead to the growth of a wide range of ethical concerns about the place and role of domestic animals in the agroecological system.

2. ANIMAL WELFARE

As a consequence of the strong intensification of animal production with all the negative implications of industrialization in animal husbandry, in the second half of the twentieth century there was a strong interest of the world public (consumers, zoologists, philosophers) in the way domestic animals are treated on farms. as animal welfare. Animal welfare is a complex bioethical issue and includes both aspects of science (biology, veterinary medicine, agronomy) and aspects of ethics (Marie et al., 2005). Welfare means quality of life and harmonious relationship between animals and the environment. It has been scientifically proven that animals are sentient beings, which can feel pain, suffering and stress (Sharapatika and Urban., 2009). Welfare is achieved when the animal is healthy, fed, safe, able to express its natural behavior and when it does not suffer from unpleasant conditions such as pain, fear and stress. In 1965, the Government of England established the Branel Commission, which established the so-called "Five freedoms" which imply ensuring the minimum needs of animals in production (standing, lying, turning, cleansing the body and stretching). However, the discussions that took place about the welfare of those years focused mainly on the listed spatial elements, and forgot about other parameters that endanger animal welfare. The five freedoms were subsequently adapted and in the early 1990s the British Farm Animal Welfare Council (FAWC) revised them (Box 1). The term "freedom" implies the moral responsibilities of humans to animals. Five freedoms are used to assess animal welfare on a farm:

1. Free access to food and water which, with their quantity and quality, ensure good health;
2. Appropriate accommodation and place for rest;
3. Appropriate prevention (prevention of injuries, pain and illness) and rapid diagnosis and treatment;
4. Enabling the expression of the natural behavior of the animal (appropriate space, equipment and the presence of other animals of the same species);

5. Protection from stress and fear by providing conditions and treatments that will not cause mental (psychological) suffering to animals.

Animal protection and welfare are an integral part of organic livestock. In organic production, animals must be raised in accordance with their basic needs. Organic production as an alternative to conventional is precisely harmonized with the natural needs of animals.

3. CHOICE OF ANIMAL BREEDS IN ORGANIC LIVESTOCK

Organic livestock production is organized according to the principles prescribed for this type of production. The principles are designed to enable the organization of animal production in the most natural way. The principles cover all segments of animal production, which includes: selection of species and breeds of animals, method of animal breeding, facilities for accommodation, nutrition and health care, transport and slaughter, and treatment of animals procured from other farms.

When choosing animal breeds for organic breeding, the basic criteria are the adaptation of the breed to local conditions, vitality and resistance to diseases. Preference is given to indigenous breeds of animals. Indigenous breeds of domestic animals represent a unique genetic heritage. Preservation of indigenous breeds of domestic animals is a complex process that integrates the general public interest with the interests of farmers, (Stojanovic, 2019). Breeds susceptible to certain health problems, such as stress syndrome in pigs, sudden death syndrome, miscarriages or complications in childbirth, are not suitable for organic farming.

Basically, the breeds of domestic animals have specially adapted to the habitat they are in originated and, in many parts of the world where bioclimatic conditions are extreme, represent the only source of income for the population, and the link between the local race and the region in which it developed often influenced the definition of cultural identity and reflects the history of symbiotic relations between humans and their domestic animals (Hoffmann and Scherf, 2005). Cultural heritage is a key component of the agroecosystem, especially in developed European countries, where rural tradition testifies to the historical heritage and enables the development of rural tourism.

Serbia, as part of the Western Balkans, can be seen as a space in which historically intertwined different historical and cultural trends, which has influenced the rural tradition and great biological diversity, which is reflected in the richness of species of domestic animal breeds that are locally adapted to different localities - regions characterized by different bioclimatic conditions, which led to very diverse rural traditions.

That is how they are on the territory of Serbia, under the influence

of bioclimatic factors, immune challenge and demographic migratory flows, have been developed by many local animal breeds for centuries. All species of indigenous animals in Serbia belong to the groups of indigenous ruminants, ungulates and pigs.

Table 1 shows the different local breeds of animals and their current endangered status (Trailovic and Savic, 2019).

Tab.1. *Current endangered status of local animal species (Trailovic and Savic, 2019)*

Species	Breed	Status
Cattle	Podolian cattle	Endangered
	Busha	Endangered
Buffalo	Domestic buffalo	Endangered
Horse	Domestic-mountain horse	Endangered Risky
	Nonius	Endangered Risky
Donkey	"Balkan" donkey	Endangered
Pig	Mangulica	Endangered
	Moravka	Endangered Risky
	Resavka	Endangered Risky
Sheep	"Lipska" sheep	Endangered
	"Krivovirska" sheep	Endangered
	Bardoka	Endangered
	"Vlach-vitoroga" sheep	Endangered
	"Pirot" sheep	Endangered
	"Karakacan" sheep	Endangered Risky
	Cigaja	Not Endangered
Goat	"Balkan" goat	Endangered

3.1. Indigenous ruminants

Busha used to be the most common breed of cattle on the Balkan Peninsula. Busha and its crossbreeds are located in undeveloped hilly, mountainous and karst areas. It was also used for work in the production of milk and meat. It is characterized by quite good fertility and resistance to diseases. He can survive without human help. It belongs to the critically endangered breeds of cattle.

In the lowlands, Podolian cattle were represented, which were raised in the most extensive conditions. The characteristics that characterize this breed are the ability to adapt to climatic conditions, good fertility, resistance to diseases, the ability to compensate after unfavorable conditions of care and nutrition (Stojanovic, 2012). The number of individuals of this breed is maintained, but it belongs to the endangered categories of domestic animals in Serbia. In 2019, only in the special nature reserve Zasavica, 40 specimens of Podolian cattle were registered cattle (of which 2 are males), which is why Podolian cattle belong to critically endangered breeds ("Official Gazette" of Serbia No. 33/17).

"Kolubara" beef is considered to have disappeared, and it was characterized by good fertility and resistance to diseases. It is believed that it was created by crossing bush and Podolac beef. However, Urosevic et al., (2019), believe that the "Kolubara" cattle is not extinct and that its status should be redefined and returned to the list of autochthonous breeds of Serbia. The "Balkan" and "Carpathian" buffalo represent relict populations of domestic animals. It is assumed that buffalo arrived in the Balkans in the middle century. They acclimatized to a colder climate and spread and settled in the area of southern and southwestern Serbia. Data on the buffalo population show that these animals belong to the critically endangered species of domestic animals. Pramenka represents the largest population of indigenous sheep breeds in Serbia, with a total of 11 strains. They are characterized by resistance to climatic conditions and infectious agents. "Sjenica" sheep is one of the most numerous and most productive strains, and the "Svrljig" strain is characterized by the best wool quality. The populations of "Piro" sheep, "Krivovirska" sheep, "Lipska" sheep, Bardoka and Vitoroga pramenka have the status of critically endangered pramenka. And these representatives are characterized by well-developed resistance to poorer living conditions. "Karakacan" sheep and "baljusa" belong to the risk endangered breeds of sheep, and there is no data on the status and current population about the "Sharplaninska" pramenka. Individual populations of "Srem" and "Sombor" cigaja are also at risk.

The "Balkan" goat is a typical example of primitive breeds with poor productive traits. It is most widespread in the hilly and mountainous areas of Serbia. It is very resistant to low temperatures. The status of the "Balkan" goat is critical.

3.2. Indigenous ungulates

In the mountainous region of Serbia, domestic mountain horses and domestic horses are primarily present "Balkan" donkey, local, natural breeds that have evolved to adapt to the harsh mountain climate of the Balkan Peninsula and have been valued since ancient times for their endurance, strength, health and temperament (Trailovic, 2009; Trailovic et al., 2010 and 2011; Djermanovic et al. ., 2011). Indigenous species and breeds of equidae are facing erosion of the population and gene pool, and are threatened with irreversible loss. Trailovic and Savic (2019) list three more breeds of horses: domestic cold-blooded, Yugoslav trotter and domestic half-blood, while Staniscic et al., (2020), list another breed of donkey in Serbia, the "Banat" donkey. In 2019, 220 individuals of the "Balkan" donkey (of which 30 males) were registered in the special nature reserve Zasavica, 58 individuals in the municipality of Dimitrovgrad, and two breeders of the "Balkan" donkey (one head each) were registered in Babusnica. Despite the slight increase in numbers, this breed is according to the Regulation on the List of genetic reserves of domestic animals, the way of preserving genetic resources of domestic animals, as well as the List of indigenous breeds of domestic animals and endangered indigenous breeds ("Official Gazette" of Serbia, No. 33/17). belongs to group III, ie is considered a potentially endangered race.

When it comes to resources in Serbian horse breeding, there is a lack of data necessary to assess the status of individual populations and the geographical distribution of individual breeds, the representation of different types, traits including morphological, physiological and genetic characteristics of certain indigenous and native equidae of Serbia. A large number of traits have not been recorded, databases on local breeds and types are incomplete, and data on the manner of exploitation of these animals are missing, especially in relation to the conditions in the characteristic environment. Selection measures in equine breeding in central Serbia are still based on a small number of heads, especially when they refer to indigenous breeds of equidae such as the domestic mountain horse and the Balkan donkey (Djermanovic et al., 2011). Thanks to the existing descriptions, the domestic mountain horse is defined as a special breed in relation to the mountain horses in the area (Bosnia and Herzegovina and Bulgaria).

3.3. Indigenous breeds of pigs

In the 19th century, pigs were one of the basic export products of Serbia, and the origin of mangulica is connected to that period. Mangulica (created by crossing a pig of the Shumadinka breed (which Prince Milosh gave to the Hungarian Count Jozef Archduk) with bacon breeds and a salantor on the Kish Jenő estate. It was very important for the total pig breeding in Serbia.

Its area is related to areas with corn production (Mitic et al., 1973), which are calm and intelligent animals, which are not demanding in terms of nutrition. There are two strains of mangulica in Serbia, lasso and white. Mangulica has advantages over other breeds in terms of free grazing in extensive housing systems when its robustness, resistance to disease and stress and endurance in terms of climatic conditions come to the fore. Maintaining the status of this race is critical. Moravka and Resavka belong to the critically endangered breeds of pigs. Shishka was grown extensively in beech and oak forests on pasture, as well as shumadinka, where it remained until winter. It is assumed that these breeds of pigs have disappeared from these areas and that they can hardly be found in remote mountainous areas.

4. INDIGENOUS BREEDS OF DOMESTIC ANIMALS IN SERBIA FOR THE PERIOD 2013-2020

It is considered that for the preservation and development of the rural community in hilly and mountainous regions that are an unfavorable area for conventional livestock, it is necessary to strategically encourage production systems that suit small farms and limited arable land with the existence of natural meadows. Therefore, it is considered that sustainable organic production is very suitable for hilly and mountainous areas and that the development of organic production enables the survival not only of mountain villages but also to improve the area and contribute to the well-being of the population. Bearing in mind that the possibilities for intensification of production decrease with altitude, as well as the areas covered by nature protection measures, it can be said that mountain villages in Serbia, but also in most of Europe, survive thanks to state subsidies that encourage agriculture in general, and additionally stimulate ecological - organic production and conservation of genetic resources and the development of rural tourism.

The main problem in the sustainability of organic production in the conditions of a mountain village is the economic viability to the extent that would enable the reconstruction and stability of human communities in this area. The economic sustainability of organic livestock relies on the proper selection of animals and due to natural habitat conditions, herbivores, in our conditions cattle, buffalo, sheep and goats, then equidae and to a lesser extent pigs and poultry, in order to obtain high quality organic milk, meat and eggs were a suitable choice for mountain units. Breeding locally adapted, indigenous breeds that make excellent use of food in their natural habitat, are resistant to climate stress and locally present pathogens and parasites in holistic farming, implies diversified organization of mountain farms with simultaneous breeding of several species of domestic animals and controlled grazing through rotation and pasture rest (Van Diepen et al., 2007). Organic farming is based on nutrition with natural resources obtained from certified plant organic

production, and that the cultivation system focuses on maintaining good health due to stress reduction and improving well-being in conditions of limited conventional treatment, which is regulated by the Law on Organic Production (2010) in accordance with the EU Directive (EC 889/2008).

Sustainability of organic sheep and goat breeding in hilly and mountainous conditions is based on an assessment of factors that positively contribute to production and risk factors. Factors that promote production are: proper selection of resistant animals - indigenous breeds and strains of cattle, sheep and goats to minimize the risk of disease outbreaks; reliance on local and available sources of organically certified plant food, whereby the available green mass in the hilly and mountainous region provides herbivorous nutrition and contributes to the preservation of ecosystems through ecological grazing; encouraging the preservation of traditional processing of cow's, sheep's and goat's milk, meat and other products on the farm and in the rural community, which provides conditions for the promotion of small traditional production, the formation of a market niche and the development of rural tourism; as well as income for the entire rural community, especially for the vulnerable population of women, which contributes to the survival of the village. Of special importance for the preservation of the mountain village are material incentives for organic production, for breeders of indigenous breeds, then for grazing on public green areas, etc., which increases the interest of the rural community for organic production and thus significantly contributes to ecotourism, agrobiodiversity, land and directly and indirectly contributes to the national economy. Factors that threaten the sustainability of organic cattle, sheep and goat breeding are: lack of labor force due to pronounced negative population trends that are especially visible in the mountain village; then abandoning the livestock tradition, especially grazing, as well as the traditional processing of animal products on the farm; small and endangered populations of indigenous races; a small amount of products per head with an underdeveloped market for organic products and for small craft products, as well as a small purchasing power of the urban population. The problems faced by organic, traditional livestock production in Serbia have also been described in developed countries (Nardone et al., 2004; Escribano et al., 2016).

Table 2 presents data for the population trend of indigenous races in Serbia, from 2013-2020. According to the data from Table 2, there is an increasing trend in almost all indigenous breeds of animals for the period 2013-2020, in Serbia such as breeds: busha, domestic buffalo, domestic mountain horse, "Balkan" donkey, mangulica, moravka, all types of indigenous sheep; "Lipska" sheep, "Krivovirska" sheep, Bardoka, "Vlach vitoroga" sheep, "Piro" sheep, "Karakacan" sheep, cigaja, goat and "Banat" golosijan. Indigenous breeds of domestic animals in the Republic of Serbia and their population trend are shown in Table 2.

Tab. 2. Population trend of indigenous animal breeds for period 2013-2020

Species	Breed	Period 2013-2020							
		2013	2014	2015	2016	2017	2018	2019	2020
Cattle	Podolian cattle	229	230	240	263	286	304	420	304
	Busha	550	550	669	815	916	1274	1748	2105
Buffalo	Domestic buffalo	850	800	423	499	729	1031	1106	1374
Horse	Domestic-mountain horse	75	85	110	182	416	1039	1621	2765
	Nonius	80	85	74	71	83	91	113	92
Donkey	"Balkan" donkey	280	300	281	332	436	541	567	628
Pig	Mangulica	2000	2000	780	1394	1964	2105	2101	2935
	Moravka	50	80	103	272	417	402	350	956
	Resavka	30	30	16	35	64	44	65	62
Sheep	"Lipska" sheep	456	600	687	822	992	1302	1705	2284
	"Krivovirska" sheep	425	550	532	538	790	1112	1488	1754
	Bardoka	55	65	81	108	143	198	155	236
	"Vlach-vitoroga" sheep	441	600	468	487	834	838	892	961
	"Pilot" sheep	30	100	101	97	177	194	218	232
	"Karakacan" sheep	125	300	165	145	193	213	211	228
	Cigaja	520	500	650	213	381	1236	1327	1625
Goat	"Balkan" goat	200	300	521	513	528	781	836	1080
		/	/	/	/	/	/	70	160
Chicken	"Svrljig" ckicken	180	200	250	90	150	82	113	100
	"Sombor" kaporka	140	250	260	440	269	273	251	182
	"Banat" golosijan	600	700	560	670	730	522	444	1474

Source: Ministry of Agriculture, Forestry and Water Management of Serbia

By constantly monitoring the trends of the population of endangered indigenous races, it is possible to identify problems in a timely manner and, if necessary, activate mechanisms for their protection. Certain diseases, natural disasters and impending climate change can be a major challenge to conserving protected genetic resources. It is necessary to continue the practice of including indigenous races in protected natural areas in which they effectively restore and maintain landscape diversity and appropriate habitats, and accordingly prevent the erosion of overall biological diversity (Stojanovic, 2019).

Table 3 presents data for the trend in the number of breeders of indigenous breeds of animals in the Serbia for the period from 2013 - 2020. According to the data from Table 3, there is an increasing trend in the number of breeders of almost all indigenous breeds of animals in the Serbia, for the period from 2013-2020, which is an extremely favorable situation regarding the preservation of indigenous animal breeds in organic livestock.

Tab. 3. *Number of breeders of indigenous breeds for period 2013-2020*

Species	Breed	Period 2013-2020							
		2013	2014	2015	2016	2017	2018	2019	2020
Cattle	Podolian cattle	6	8	12	12	15	20	25	29
	Busha	16	21	29	53	64	72	81	101
Buffalo	Domestic buffalo	10	73	143	202	282	329	392	432
Horse	Domestic-mountain horse	9	18	19	34	81	329	515	622
	Nonius	6	9	10	10	17	21	25	28
Donkey	"Balkan" donkey	7	14	23	28	42	57	77	93
Pig	Mangulica	12	28	41	66	82	97	115	131
	Moravka	3	5	14	23	24	36	53	91
	Resavka	1	1	2	5	5	6	8	10
Sheep	"Lipska" sheep	9	11	15	22	30	39	50	53
	"Krivovirska" sheep	7	7	12	16	24	38	42	49
	Bardoka	1	1	2	3	6	7	9	11
	"Vlach-vitoroga" sheep	3	4	8	8	9	13	15	15
	"Pirot" sheep	2	2	4	6	7	11	11	9

	"Karakacan" sheep	1	2	4	5	5	7	9	8
	Cigaja	3	3	3	6	4	16	20	21
Goat	"Balkan" goat	4	10	15	17	18	24	29	35
		/	/	/	/	1	1	3	3
Chicken	"Svrljig" ckicken	/	/	/	1	1	1	1	1
	"Sombor" kaporka	1	1	1	1	3	4	4	4
	"Banat" golosijan	2	2	2	2	4	5	5	8

Source: Ministry of Agriculture, Forestry and Water Management of Serbia

In order to increase in the number of breeders of indigenous breeds of animals kept in the period to come, it is necessary to create conditions for the establishment of associations of breeders, so they have a more active influence on the conservation of protected genetic resources, notably the development of a program of economic valorisation of indigenous breeds as a basis for their long-term sustainability. Establishing a model of sustainable use makes the program for the conservation of protected genetic resources less dependent on incentives.

5. CONCLUSION

Livestock is a key activity of the rural population important for the conservation of agroecosystems and the vitality of the village, especially in hilly and mountainous areas and in areas of protected nature. The benefits of controlled, ecological agriculture are huge in terms of preserving biodiversity, preserving tradition, maintaining the landscape and creating conditions for the development of eco-tourism and the promotion of the state at the international level. Due to adaptation and resistance to environmental conditions and pathogens, the production process based on traditional, indigenous races is crucial, especially if it is seriously accepted that Serbia is primarily a country affected by climate change. In order to preserve and revive villages, as well as natural wholes of global importance, budget support is needed in the form of incentives for production itself, production through conservation of indigenous breeds of domestic animals, as well as support for grazing in public areas.

In addition, assistance and support is needed during geographical certification and conversion to an organic production system. Only if the demographic balance in the countryside is achieved, it is possible to count on the recovery of the agrobiocenosis and the creation of conditions for the selection of indigenous breeds in order to obtain a larger quantity of products.

Small rural farms (households) will not be able to achieve self-sustainability in the foreseeable future, and funds for incentives for rural development should be directed for a longer period (several decades) to rural renewal, breeding of indigenous breeds and promotion of market objectives: organic products, products with protected geographical origin and products prepared according to traditional recipes.

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USE OF TREATED WASTEWATER FROM THE SEWERAGE SYSTEM FOR IRRIGATION OF GREEN AREAS AND IN INDUSTRIAL PROCESSES

Stojan Srbinoski

Summary: *The needs for clean drinking water and ventilation are increasing on the one hand while on the other hand the amount of wastewater that is already used by the population, agriculture and industry is increasing. In the modern practice of using water for various human needs, the treated waste from the sewerage systems is increasingly used, which with the high degree of purification can be used as technical water in many economic activities. The treated wastewater also allows for the use of quality quantities of drinking water that can be used without the necessary treatment for drinking water treatment. The landfill for the use of treated wastewater is large and is most often used for irrigation of green areas, in industrial processes and in maintaining communal hygiene in settlements.*

Keywords: *Wastewater treatment, Green area irrigation, Supply pipeline, Wastewater treatment.*

❖ Introduction to the problem

Wastewater generated as a product of the use of sanitary water in households and industry, increasingly occupies a large volume of liquid and solid phase at the points of its discharge, for which it is necessary to find optimal solutions for their treatment and reuse in other water economy branches.

Foul wastewater is treated or processed in wastewater treatment plants and the treated water is discharged into the recipient which is usually the nearest riverbed. The problem with the removed sediment from the treated wastewater is solved in several ways by applying various technological procedures that reduce the volume of the sediment and enable its reuse. The most common forms of use of the obtained sediment after the treatment of the wastewater is its use for obtaining methane from which electricity is obtained and the use of dehydrated sludge or dried sludge in agriculture as artificial fertilizer for feeding crops.

The treated wastewater is usually discharged in the recipients to a category of treatment that will not disturb the natural biodiversity of the riverbeds, ie will not negatively affect the river flora and fauna.

The treated wastewater that is discharged into the recipients should at least be of category II according to the Decree on water classification 18/99 which is a legal framework in the field of surface waters and the values in it, are the concentration of pollutants expressed in mg/l. Recently, attempts have been made to convert the treated wastewater for reuse in other water economy branches, because the treated wastewater has great potential for reuse and use in certain technological processes such as not requiring category I water quality and for irrigation of large areas of park greenery or agricultural areas located in the immediate vicinity.

In order to use the possibility of reuse of the treated wastewaters, it is necessary to consider all conditions such as topographic and infrastructural in order to create all the necessary preconditions for reuse of treated wastewater in other branches of water economy.

❖ **Methods and material**

The real possibility for reuse of the treated wastewater is seen in the existence of the already constructed sewerage infrastructure, the degree of wastewater treatment and of course the need for reuse of the treated wastewaters.

In this study, the possibilities for reuse of the treated wastewater from the sewerage system of the City of Skopje will be considered.

The sewerage system of the City of Skopje is a separation one and is divided into fecal and storm sewage. The trend of constructing a separation sewer system began immediately after the 1963 earthquake. In 1965, the new GUP for the City of Skopje was adopted, which it was confirmed the development of the sewerage system as a separation one.

The Skopje sewerage system for collection and discharge of fecal wastewater covers more than 80% of the urban settlements and suburban settlements with fecal sewage, while the system for discharge of storm wastewater covers about 35% of the urban areas of the City of Skopje.

In addition to the completion of the construction of the existing sewerage system, a major problem for the functioning of the system is the non-treatment of the discharged fecal wastewater, which is now discharged without any treatment in the recipient, the river Vardar.

The main recipient of the discharge of fecal wastewater is the river Vardar which receives all the toxic substances that are discharged into the river. Besides the river Vardar, the rivers Treska and Saraj also serve as recipients of the discharged wastewater, participating with less than 20% of the discharged quantities of wastewater.

The river Vardar is the largest recipient of wastewater of the City of Skopje and more than 80% of the total wastewater is discharged into the river.

According to the feasibility studies prepared so far, the level of pollution of the river Vardar at the entrance to Skopje at the Saraj bridge, measured by the parameter BOD 5 (Biochemical oxygen demand for 5 days) is 2-3 mg/l, which corresponds to class II of surface waters in accordance with the legislation of the Republic of Macedonia. Downstream at the bridge Jurumleri - Dolno Lisiche the amount of BOD 5 is 4 mg/l, which means that the waters of the river Vardar belong to class III, but it is still within the legal framework for surface water quality.

But if the wastewater from households and industry continues to be discharged uncontrollably into the river Vardar without any treatment, then by 2020 the pollution of the river Vardar can reach a value of 18 mg/l, which would mean that the water from the river Vardar belongs to class V according to the legislation of the Republic of Macedonia.

This prediction for the degree of pollution of the waters of the river Vardar is based on the simulation of the software package QUALK2, prepared by the expert team of Dzajka.

Table 1 shows the values of surface water pollution in the Republic of Macedonia according to the pollution parameters.

Table 1 shows part of the Decree on water classification 18/99 which is a legal framework in the field of surface water and the values in it are the concentration of pollutants expressed in mg/l.

Table 1

Indicator	Class I	Class II	Class III	Class IV	Class V
HPK	< 2.50	2.51-5.0	5.01-10.0	10.0-20.0	>20.0
BPK5	<2.0	2.01-4.0	4.01-7.0	7.01-15.0	>15.0
r-en O ₂	>8.0	7.99-6.0	5.99-4.0	3.99-2.0	<2.0
pH	6.5-8.5	6.5-6.3	6.3-6.0	6.0-5.3	<5.3
Suspended substances	<10	10-30	30-60	60-100	>100
Dry residue	350	500	1000	1500	>1500
Total phosphor	<0.004	0.004-0.007	0.0071-0.01	0.01-0.05	>0.05
Phosphates	<0.007	0.007-0.011	0.0111-0.02	0.02-0.075	>0.075
Total nitrogen	<0.2	0.2-0.325	0.326-0.45	>0.45	>0.45

According to the hydrogram of the flowages of the river Vardar, the following values can be ascertained for the average annual flowages, for minimum flowages $Q = 25\text{--}30$ (m^3/sec) for the months of July, August, September and October. The maximum average flowage is $Q=100$ (m^3/sec) in May and the medium average flowages are $Q=50$ (m^3/sec) in January, February and March.

The graphic representation of the hydrogram of the river Vardar through Skopje is shown in Figure 1.

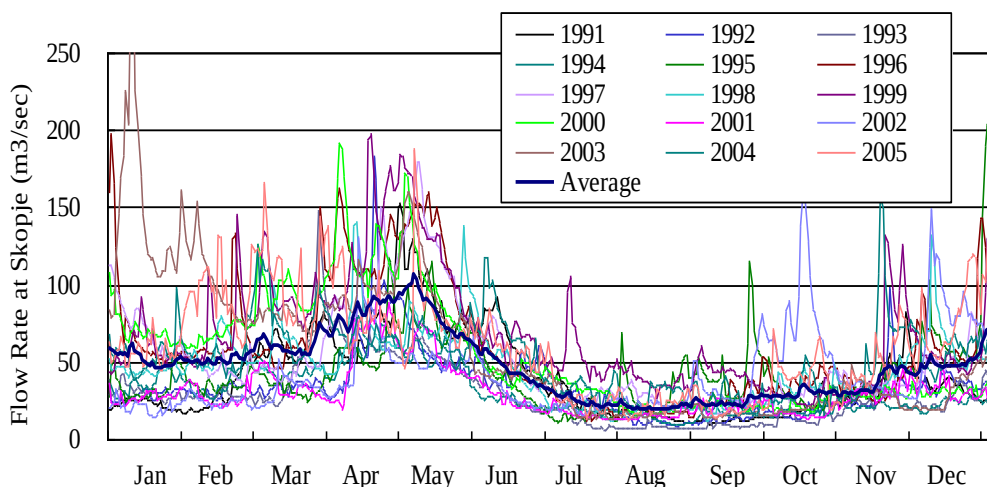


Figure 1

From the hydrological analysis it can be concluded that the small flowages through the river Vardar negatively affect the degree of pollution of river waters because the minimum flowages do not provide the required degree of self-treatment of river waters.

The average amounts of precipitation are highest in May and December and amount to 50 to 60 mm of water sediment. The minimum amounts of precipitation are the lowest in February and August with 30 mm of water sediment.

In the dimensioning of the storm sewage of the city of Skopje it is necessary to make a correction of the input parameters for the intensity of the rain from the current $q_r=110$ (l/sec/ha) to a value of $q_r=120$ (l/sec/ha) to $q_r=140$ (l/sec/ha), in order to obtain a more realistic amount of input values for dimensioning of storm collectors, which would avoid frequent floods that occur in certain parts of the city as a result of insufficient construction of the storm sewage, which in total covers only 35% of the territory of the city of Skopje.

The hydrological analysis of precipitation for the city of Skopje has been prepared over a period of 26 years, from 1980 to 2006.

Figure 3 shows the graphic representation of the intensity of precipitation for the city of Skopje.

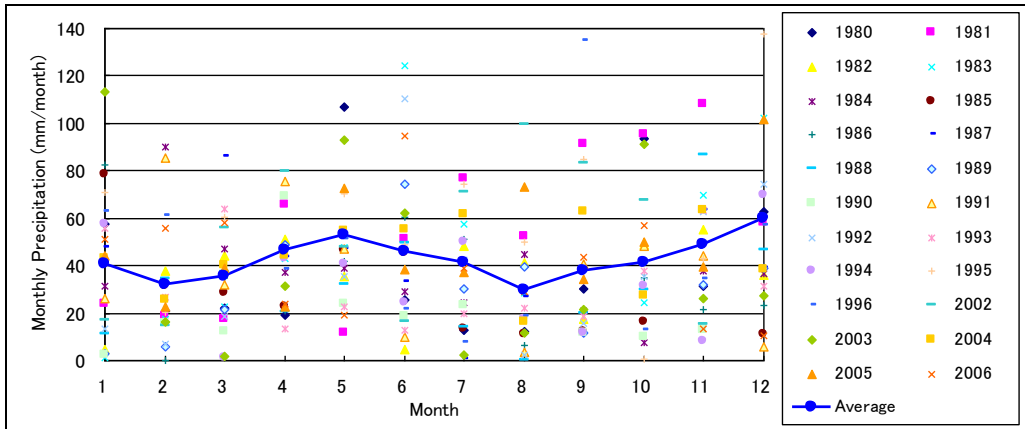


Figure 3

From the analyses conducted so far, it has been concluded that the situation with the pollution of the river Vardar has reached a critical level, having in mind that the inflow of fecal wastewater into the river Vardar is constant with a tendency of slight increase.

Figure 4 shows a schematic representation of the fecal sewage of the City of Skopje together with the fecal pumping stations.

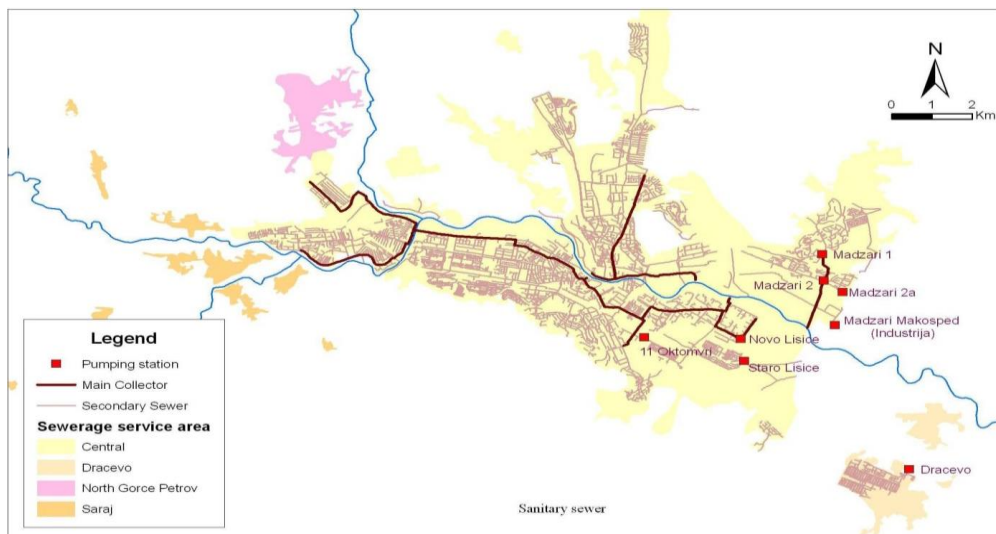


Figure 4

Figure 4 shows the layout of the fecal collectors in the City of Skopje and the geographical division of the fecal system on the right and left bank, spread around the river Vardar.

Figure 5 shows a schematic representation of the existing storm sewage in the city of Skopje.

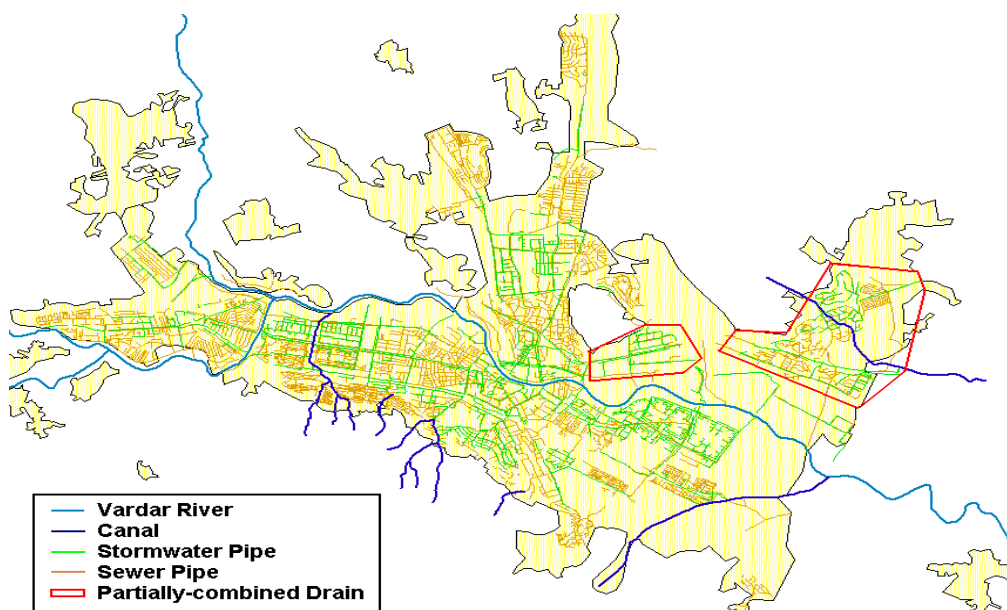


Figure 5

Figure 5 gives a clear overview of the prevalence of storm sewage of the City of Skopje and accurately locates the parts of the city where there is no storm sewage at all.

As input parameters for calculating or dimensioning the size of the fecal wastewater discharged into the river Vardar, are taken the data from the water supply system of the City of Skopje, which give a realistic picture of the size or leaks that burden the river Vardar, after discharge of the fecal wastewaters in the river Vardar.

The average annual quantities of produced, distributed and invoiced water from the Skopje water supply system for a period of 25 years are shown in Table 2.

Table 2

Years from 1993 to 2018	Water production (m ³ /year)	Invoiced- household (m ³ /year)	Invoiced- economy (m ³ /year)
Average value	95.670.343	27.854.713	12.971.227

Table 3

Year 1993/2018	Produced water (m ³)	Invoiced water (m ³)	Lost water (m ³)	Qo1 total	Qo2 required	Losses P%
Average	95.670.343	40.825.940	54.844.403	510.67	219.75	57.0

Table 3 shows the average annual values for the produced, invoiced and lost amount of water in the Skopje water supply system. In general, the quantities of water that are used usefully can realistically be expected to occur in the fecal sewage system and are divided into the following categories:

1. Water supply norm according to Q1 **total produced water.**
2. Water supply norm according to Q2 **total invoiced water.**
3. Water supply norm according to Q3 **invoiced household water.**
4. Water supply norm according to Q4 **invoiced water from economy.**

Table 4 shows the magnitudes of the water supply norm according to the four given parameters, for a time period of 25 years.

Table 4

Year 93/18	Water production	Invoiced- household	Invoiced- economy	Qo1	Qo2	Qo3	Qo4
Average	95.670.343	27.854.713	12.971.227	510.67	219.75	148.85	70.91

For the hydraulic dimensioning of the size of the fecal wastewater discharged into the recipient river Vardar, the following sizes have been adopted for the initial dimensioning. Water supply norm of $Q_o=250$ (l/day/c), is the total norm, while for households the norm is $Q_o=150$ (l/day/c) and for the economy the norm is $Q = 100$ (l/day/c).

Given the population growth in Skopje, which now is at around 540,000 inhabitants, for the next 30 years the population growth should result in a figure of 640,000 inhabitants.

The total volume of wastewater discharged untreated into the river Vardar is $W=166,000$ (m^3/day), ie expressed in (m^3/sec) the inflow of wastewaters into the river Vardar is $Q=1.922$ (m^3/sec). By adding the coefficient of unevenness K_o the maximum quantities of fecal wastewater discharged into the river Vardar are:

$$Q_{max} = qsr/day * K_o = 1.43 * 1.922 = 2.752(\text{m}^3 / \text{sec})$$

Table 5 shows the parameters used in hydraulic calculations to determine the inflow of wastewaters.

Table 5

	m^3/day	m^3/hour	m^3/min	m^3/sec
Maximum hourly flow	237 740	9 906	165.1	2.752
Average daily flow (rain)	166 000	6 917	115.3	1.922
Average daily flow - dry weather	135 000	5 625	93.8	1.563
Backup flow	302 400	12 600	210	3.500

It is clear that the most critical part of the river Vardar, which is most exposed to pollution, is the part between the Stone Bridge in the center of Skopje together with the outlets in the settlement Novo Lisiche, up to the exit from the Skopje valley near the village Taor.

On this stretch there are no natural tributaries but only outlets from the fecal and storm sewage. After this stretch, the waters move mixed in order to obtain pollution of the river waters of category III at the third measuring point.

Figure 6 shows the schematic layout of the fecal sewage outlets with the amount of wastewater discharged into the river Vardar.

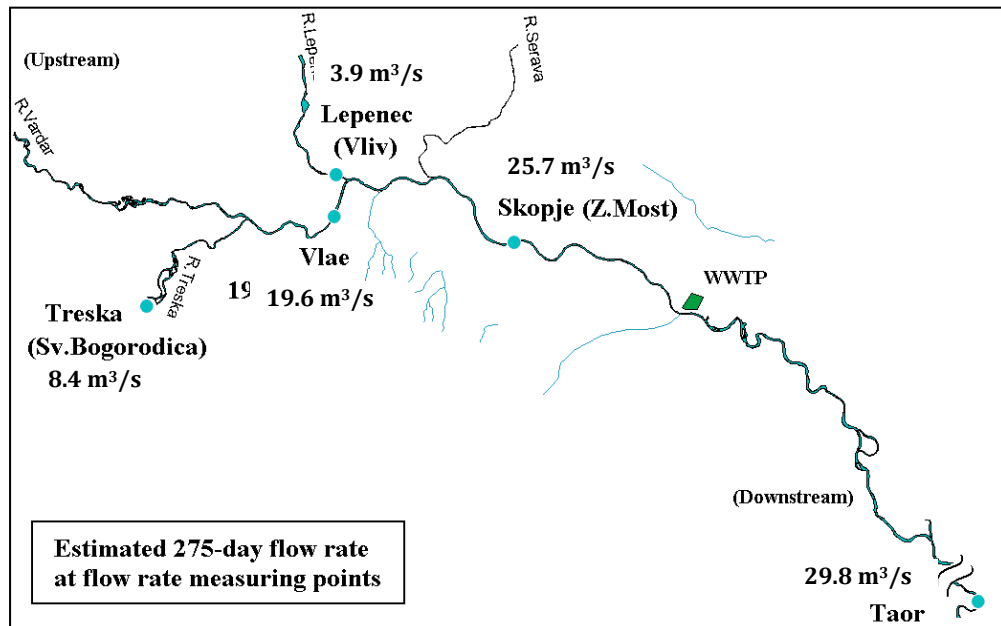


Figure 6

Figure 7 shows the schematic representation of the change in the degree of pollution, ie the change in the water class of the river Vardar depending on the location of the fecal outlets along the river.

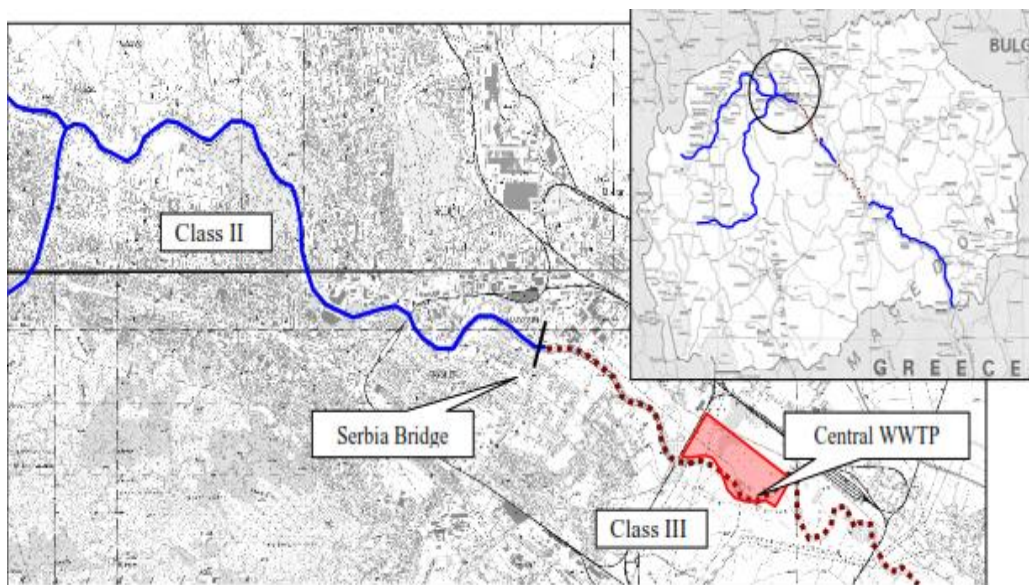


Figure 7

The quality of wastewater discharged into the recipients is subject to special physical, chemical and biochemical analyses, which can be unified in rulebooks and which can be used for mutual comparison.

Wastewater discharged from fecal sewages according to European norms has the following degree of pollution.

1. BOD 5 150-500 mg/l. (Biochemical oxygen demand for 5 days).
2. COD 300-1000 mg/l. (Chemical Oxygen Demand).
3. SS 250-850 mg / l. (Suspended solids in water).
4. TN 20-85 mg/l. (Dissolved nitrogen in water).
5. TP 4-15 mg/l. (Dissolved phosphorus in water).
6. Value for pH 6-8.

Also according to the European technical norms, the expression for Equivalent resident has been adopted as a measure of critical pollution that needs to be reduced by wastewater treatment. The values of pollutants contained in 1 IE (Inhabitant Equivalent) are:

1. BOD 5 60 g/IE/day.
2. COD 120 g/IE/day.
3. N (Nitrogen) 11 g/IE/day.
4. P (Phosphorus) 1.8 g/IE/day.

According to all previous measurements of fecal wastewater discharged from the Skopje fecal and storm sewage has the following values which are shown in Table 6.

Table 6

Contaminating Parameters	Unit	Concentration		
		Weak	Medium	Strong
Total solids	mg/l	350	720	1,200
Suspended solids	mg/l	100	220	350
BOD 5 (Biochemical oxygen demand for 5 days)	mg/l	110	220	400
COD (Chemical Oxygen Demand)	mg/l	250	500	1,000
Total nitrogen expressed as N	mg/l	20	40	85
Total phosphorus expressed as P	mg/l	4	8	15
Fats and oils	mg/l	50	100	150

The degree of treatment or reduction that needs to be done if the Central Wastewater Treatment Plant for the City of Skopje is built is given in Table 7 which is regulated by law on the quality of wastewater discharge.

Table 7

Number	Parameter	Unit	Limit value
1	BOD ₅	mg/l	25
2	COD	mg/l	125
3	Undissolved solids	mg/l	35
4	TN	mg/l	15
5	Nitrogen	mg/l	10
6	TP3	mg/l	2

By comparing the two tables, it is immediately concluded that the wastewater discharged into the river Vardar is repeatedly polluted in relation to the permitted legally prescribed concentrations.

The obtained data on the degree of pollution of the river Vardar by the discharged fecal water refers to four measuring points, ie four outlets. These outlets are:

1. Fecal outlet Usje on the right bank.
2. Fecal outlet Aerodrom on the right bank.
3. Fecal outlet Keramidnica on the left bank.
4. Fecal outlet Novo Lisiche on the right bank.

The difference in the quality of the discharged water from the fecal sewage and the required allowed concentration of waste materials is shown in Table 8 where the deviations of the wastewater in percentage of the legally prescribed norms are shown.

Table 8

No.	Unit	Parameter	Permitted concentrations	Measured values of concentrations (weak)	Percentage of pollution
1	mg/l	BOD ₅	25	110	77.27%
2	mg/l	COD	125	250	50.00%
3	mg/l	Undissolved solids	35	350	90%
4	mg/l	TN	15	20	25%
5	mg/l	Nitrogen	10	110	90.9%
6	mg/l	TP	2	4	50%

The percentage of pollution is given for the low concentration of pollutants. At higher concentrations of pollution, the percentage of pollution also increases, which of course adversely affects the water quality of the river Vardar.

If the construction of a Central Station for treatment of fecal wastewater of the city of Skopje is approached, then we can expect a reduction of pollution of the recipient, ie the river Vardar in two time phases.

Table 9 provides a tabular representation of the reduction of pollutants that will be discharged into the Vardar River after the conducted complete treatment of wastewater treatment. Table 9 accurately lists the quality expectations of the treated wastewater after the treatment. Only with the right treatment for fecal wastewater treatment can conditions be created for the reuse of the treated wastewater. The treated wastewater should first of all be safe for the users in its further use.

Table 9

Parameter	Short-term/medium-term goal (until 2030)		Long-term goal (until 2040)	
	Maximum concentration (mg/l)	Minimum percentage of reduction (%)	Maximum concentration (mg/l)	Minimum percentage of reduction (%)
BOD ₅	25	70-90	25	70-90
COD	125	75	125	75
VSM	35	90	35	90
N total	40	-	10	70-80
P total	5	-	1	80

Having in mind the level of the treated wastewater, which by its characteristics corresponds to water of category II, it can be concluded that the possibilities for reuse of the treated wastewater are real, for the other water economy branches.

❖ **Use of treated wastewater for irrigation of city greenery**

Urban greenery in the city of Skopje is represented in all municipalities and is divided into two basic categories:

- Urban greenery.
- Non urban greenery.

The representation by the municipalities of the city of Skopje will be best shown in a tabular representation which is shown in Table 10.

Table 10

Municipality	Surface area in Ha
Centar	19.50
Karposh	61.00
Gjorche Petrov	9.2
Kisela Voda	20.80
Aerodrom	80.4
Gazi Baba	18.6
Chair	29.7
Butel	7.4
Shuto Orizari	1.6
Total	248.5

The green areas that are under the jurisdiction of the City of Skopje, urban and non urban greenery, are shown tabularly in Table 11.

Table 11

Public areas in the city	Ha
City park	38.8
Park „Woman Warrior“	0.9
Boulevard greenery	90.4
Characteristic landscape Gazi Baba	105.0
French cemetery	7.0
Zajchev Rid	5.0
Sports and recreation centers	Ha
Lake Treska	18.6
Saraj	24.0
Non urban greenery	Ha
Park Vodno forest	4.537,00

According to the two tables, table 10 and table 11, it can be established exactly how much is the green area in the city of Skopje that can be irrigated with treated wastewater. The irrigation system can not include the entire available green area, but only the green area under the jurisdiction of the municipalities which is 248.50 Ha and the public areas in the city of Skopje without the non urban greenery and sports and recreation centers. The total green area that can potentially be irrigated with treated wastewater is $248.5+247.1=495.60$ Ha or approximately about 500 Ha green public area.

Figure 8 shows the distribution of the green areas on the territory of the city of Skopje.

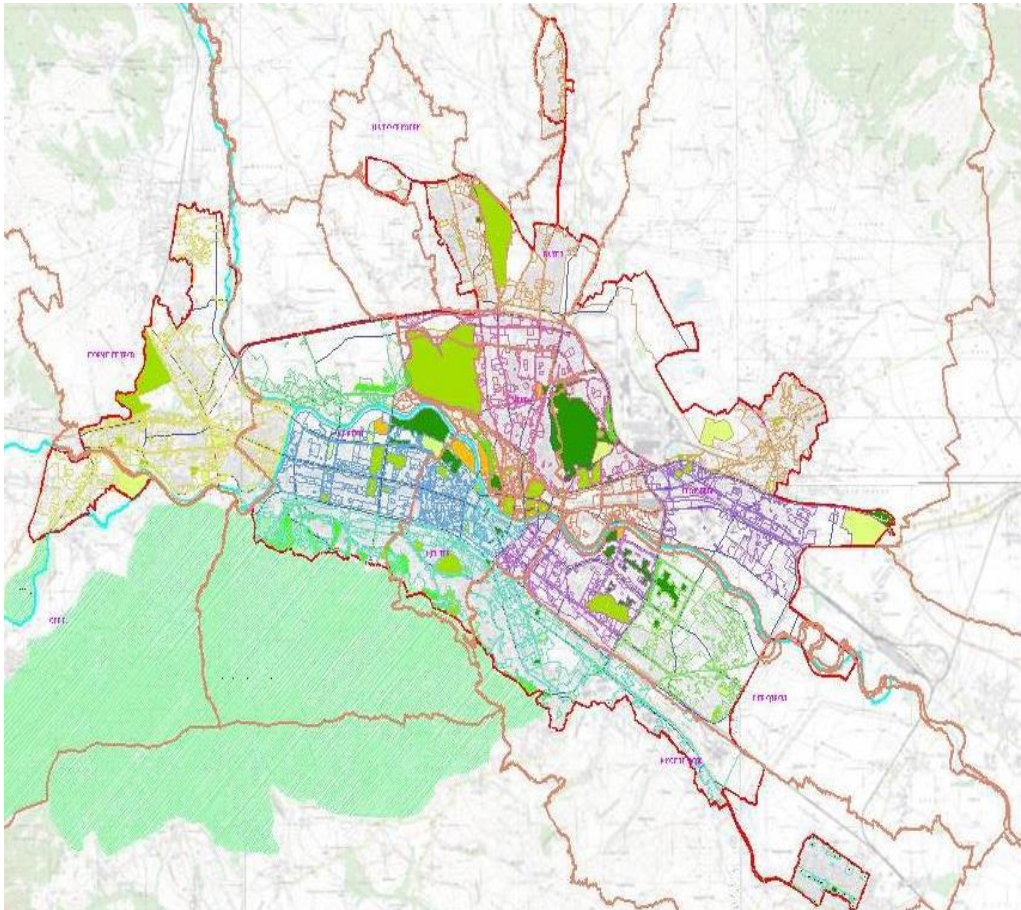


Figure 8

The following amount of water is needed to irrigate these green areas.

$q = \frac{\alpha \cdot m}{3.6 \cdot n \cdot t} \left(\frac{l}{ha} \right) = \frac{1.0 \cdot 150}{3.6 \cdot 10 \cdot 8} = 0.521 \left(\frac{l}{ha} \right)$, this is the required Hydromodule for irrigation of the green areas.

The values in the form for calculating the hydromodule for irrigation are the following:

α - Percentage of representation of the appropriate greenery $\alpha = 1$

m- Watering norm in one irrigation turn $m=150$ (l/sec/ha).

n- Number of hours of watering in one day $n=10$ hours

t- Number of days of watering during one month $t=8$ days

The total amount required for irrigation is

$$Q = F \cdot q = 500 \cdot 0.521 = 260.5 \left(\frac{l}{sek} \right).$$

Having in mind that in the recipient river Vardar it is discharged from $Q=1563$ (l/sec) to $Q=1922$ (l/sec), then it is clear that the treated wastewater can meet the needs for irrigation of the city greenery.

For the realization of this idea it is necessary to construct a pressure pipeline from the location where the wastewater treatment plant is designed in the area of the village Trubarevo, to the already existing lines of the park network for irrigation of green areas, which are an integral part of the only water supply system of the city of Skopje.

Currently, green areas are irrigated with clean and quality drinking water coming from the Rashche spring. According to the official data from PE Water Supply and Sewerage Skopje, the quantities for watering the green areas on an annual level are $W=8.905.523$ (m³/year) or expressed as a percentage, irrigation is 8.97% of the total annual production of drinking water in the Skopje water supply system.

This amount of water used annually for watering green areas, converted into daily flow is $Q=282.40$ (l/sec). Compared to the calculated quantities of water that should be used for irrigation of green areas with treated wastewater, the difference in percent is only 7.75% less compared to the official data from PE Water Supply and Sewerage Skopje.

The treated wastewater that can be used for irrigation of urban green areas is only 18.07% of the minimum average daily amount of wastewater discharged into the recipient river Vardar. The average daily quantities of wastewater discharged into the river Vardar are $Q=1563$ (l/sec).

❖ **Use of treated wastewater to wash streets and sidewalks**

According to the official data of PE Water Supply and Sewerage Skopje, 4,651,620 m³ is consumed annually for washing public traffic areas, sidewalks, paths and squares. This amount of water is 4.68% of the total amount of water produced annually in the water supply system of the city of Skopje.

These quantities of water converted into daily consumption are $Q=147.50$ (l/sec). If we take into account the average daily amount of wastewater discharged into the recipient the river Vardar, then the required water for washing public traffic areas, sidewalks, paths and squares is 9.44% of the discharged water.

At present, the vehicles of PE Communal Hygiene use the fire hydrants located on the territory of the city of Skopje to fill the vehicles with clean water, with which they wash the public traffic areas.

From these basic calculations it follows that the use of treated wastewater for regular communal services in the city of Skopje will be more than welcome and of course these amounts of water will relieve the water supply system of the city of Skopje from unnecessarily increased amounts of water consumed by the system.

❖ **Use of treated wastewater for use in the technological process in the former complex RZ Skopje**

The former metallurgical complex RZ Skopje from its beginnings in 1964, directly participated in the construction of the Dam Rashche 1 and the steel pipeline Rashche 1 with diameter $F\ 1600\text{ mm}$ and length $L=20\text{ Km}$.

In the golden times of the operation of RZ Skopje, the consumption of water from the Rashche spring for the needs of the technological process for obtaining iron and steel (cooling of iron and steel sheets during production), was $Q=2000\text{ (l/sec)}$. This trend of water consumption in the technological process in the last 20 years has drastically decreased and today is $Q=400\text{ (l/sec)}$ or only 20% of the former capacities.

The Rashche 1 steel pipeline distributes the flowing quantities of water in the urban areas of the city of Skopje.

The treated wastewater with its quality fully satisfies the needs of the technological process that takes place in the plants of the former complex RZ Skopje. Given that the average daily wastewater flow is $Q=1563\text{ (l/sec)}$, then the total amount of treated wastewater that will be used for the technological process can be calculated. $Q=1563-400=1163\text{ (l/sec)}$. Or only 25.60% of the total treated wastewater will be used.

❖ **Total quantities of water that can realistically be used as technical water after wastewater treatment**

The average daily flow of fecal wastewater discharged into the recipient Vardar River ranges from $Q=1563\text{ (l/sec)}$ to $Q = 1922\text{ (l/sec)}$.

The total water needs for irrigation of public green areas, for washing of public traffic areas, sidewalks, paths and squares and for use of water in the technological process is, according to the available data, total $Q=282.4+147.5=429.9=430\text{ (l/sec)}$.

The quantities of water required for cooling during the technological process for obtaining steel and iron at the moment are $Q=400\text{ (l/sec)}$. The total quantities of water that should be used as technical water in total are $Q=430+400=830\text{ (l/sec)}$ or only 53.10% of the average daily flow discharged into the recipient river Vardar. Table 12 shows all the calculations for the needs of the quantities of water that can be used as technical water.

Table 12

No.	User	Q (l/sec)	Q _{min} otp	Q _{max} otp	Q1 (l/sec)	Q2 (l/sec)
1	RZ Skopje	400	1563	1922	1163	1522
2	Green areas	282.40	1563	1922	1280.6	1639.6
3	Streets, sidewalks	147.50	1563	1922	1415.5	1774.5
4	Total	830.0	1563	1922	733	1092

From table 12 it can be seen that, without any problem, the necessary quantities of technical water can be provided after the treatment of fecal wastewater in different weather conditions, in dry period and in period of rain.

With the use of the treated wastewater for the other needs of the population, the Skopje water supply system will be directly relieved with the following quantities on a daily and annual basis.

$$Q_{sr}/d = 3.033,69 - 400 - 282.4 - 147.5 = 2.603,78 \text{ (l/sec)}.$$

$$W = 95.670.343 - 8.905.523 - 4.651.620 - 12.614.400 = 69.498.800 \text{ (m}^3\text{/sec)}.$$

The percentage of saving quality drinking water will be $P = 27.35\%$. This high percentage of saving quality drinking water should certainly be taken into account in future planning of the development of the water supply system of the city of Skopje.

From the study itself it can be concluded that it is necessary to start the construction of the Wastewater Treatment Plant and to use the treated wastewater as technical water for the needs of other water economy branches.

Conclusions and recommendations that need to be applied for reuse of treated wastewater from the sewerage system, for irrigation of green areas and use in industrial processes in the Skopje region

The Study for reuse of treated wastewater from the sewerage system, for irrigation of green areas and use in industrial processes in the Skopje region, provides a good opportunity to encourage the construction of the Plant for treatment of fecal wastewater and use of treated wastewater as technical water in other water economy branches.

1. The average annual production of drinking water in the Skopje water supply system for a period of 25 years is $W = 95,670,343 \text{ (m}^3\text{/year)}$. The entire water production is used for all the needs of the citizens and the economy of

Skopje, without taking into account whether certain quantities of water can be replaced by using technical water.

2. The losses in the water supply system of the city of Skopje are huge and they are $P=57\%$ of the average annual water production.

3. The pollution of the river Vardar that passes through Skopje is great and with all the measurements so far, a degree of pollution between II and III category has been established. All wastewater (fecal and storm) is discharged into the river Vardar without any treatment.

4. The need to construct a Wastewater Treatment Plant is necessary and should be given priority in the development plans of the City of Skopje.

5. Green areas are irrigated with drinking water which is used directly by the only water supply system in the city of Skopje. $W=8.905.523$ (m^3/year) are used annually, which is $P=8.97\%$ of the total annual water production.

6. For washing public traffic areas, sidewalks, paths and squares, clean drinking water from the only water supply system with an annual size of $W=4.651.620$ (m^3/year), which is $P=4.68\%$ of the total annual production of water.

7. The former industrial plant RZ Skopje still uses clean drinking water for the needs of the technological process used for cooling iron and steel sheets during production. RZ Skopje annually uses $W=12.614.400$ (m^3/year), which represents $P=13.7\%$ of the total annual water production.

8. The total volume of water used, for other needs and can be used for the same purpose as technical water is annually $W=26.171.543$ (m^3/year), which represents $P=27.35\%$ of the total annual water production. These are large quantities of water for which it is necessary to find an alternative to using technical water and that is the use of treated wastewater from the wastewater treatment plant.

9. The average daily amounts of wastewater discharged into the river Vardar without any treatment the range from $Q=1563$ (l/sec) to $Q=1922$ (l/sec). These quantities of water can easily meet the needs of technical water for the needs of other water economy branches such as irrigation of green areas, washing of traffic arteries and use of water in the technological processes for production.

All these recommendations and conclusions should be implemented immediately after the construction of the Wastewater Treatment Plant of the City of Skopje. With the realization of the conclusions, in addition to providing a clean environment for the citizens, additional reserves of clean and healthy water will be provided, which is increasingly deficient in the natural environment.

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EFFECT OF SEX ON BODY WEIGHT OF LAMBS FROM BIRTH TO WEANING IN VARIOUS STRAINS OF SHEEP PRAMENKA

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Abstract: *This research was conducted on domestic pramenkas, which are the dominant breed of sheep in Serbia. The purpose is to determine the effects of sex on lamb growth. The research was conducted on 1800 lambs of four strains (Pirót, Svrlijig, Sjenica, Sharplanin) of Pramenko sheep (450 lambs per strain). The weight of lambs was measured at birth, then 30, 60 and 90 days after birth during three years of the experiment. The obtained results refer to the variability of body weight of lambs at birth, 30, 60 and 90 days after birth, depending on the sex of lambs within each of the four strains of Pramenko sheep. Sexual dimorphism in most cases affects the body weight of lambs at birth. There was a difference in the body weight of lambs at birth in all four genotypes of pramenka. The body weight at birth in male lambs of the Pirót strain pramenka was 3.71 kg, while the body weight of female lambs was 3.69 kg. The difference was not statistically significant ($R > 0.05$). In the population of Svrlijig lambs, male heads had an average birth weight of 4.02 kg, and female 3.6 kg. The existing difference was very significant ($R < 0.01$), which means that gender as a fixed factor had a significant influence on this trait. The body weight of the male lamb of the Sjenica genotype was 3.85 kg, while the weight of the female lambs was 3.48 kg. The existing difference is under a very significant influence of the sex of lambs ($R < 0.01$). Sharplanin pramenka lambs had an average birth weight of 3.91 kg in males and 3.64 kg in females. The difference was very significant ($R < 0.01$), so that in this case, a significant influence of gender on body weight at birth can be confirmed. At 30 days of age, the body weight of male lambs of the Pirót strain was 9.34 kg, Svrlijig 9.92 kg, Sjenica 9.67 kg and finally Sharplanin strain 9.35 kg, while the body weight of female lambs was 9.31 kg, 9.80 kg, 9.30 kg and 9.18 kg. In all strains, male lambs had higher body weight, but this difference was not statistically significant ($R > 0.05$). At 60 days of age, the body weight of a male*

lamb of the Pirot strain was 15.74 kg, Svrljig 15.42 kg, Sjenica 15.52 kg, and at the end of the Sharplanin strain 14.48 kg, while the body weight of a female lamb was 14.81 kg, 15.36, 15.31 kg and 14.04 kg. At this age, in all strains, male lambs had a higher body weight, but this difference was not statistically significant ($R > 0.05$).

The influence of sex on the body weight of lambs at 90 days is evident in all genotypes examined, however, this fixed factor was not ubiquitous and significant. The body weight of the male lamb of the Pirot strain pramenka was 20.77 kg, while the body weight of the female lambs was 19.99 kg. The difference in favor of male heads was statistically very significant ($R < 0.01$). In other strains, male lambs had higher body weight, but this difference was not statistically significant ($R > 0.05$).

Keywords: *Pramenka sheep, body weight, lamb sex*

Introduction

The body weight of lambs has the most important role in achieving good results in sheep breeding. There are a large number of known factors that significantly affect fetal growth and weight of lambs, as well as the number of lambs per sheep (Petrović et al., 2011; Akhtar et al., 2012). In addition to the number of lambs obtained per female, the body weight of the cub at birth plays an important role as the initial energy factor for the later development of the organism (Petrovic et al., 2008; Riggio et al., 2008). Body weight also affects the vitality and mortality of lambs during development (Zapasnikiene, 2002; Berhane and Arendonk, 2006; Watankhah and Taleb, 2009). Mentioned, and many other authors state that knowledge of the factors that affect variations in body weight are of particular importance from the aspect of successful production and sustainability of the farm.

The development of lamb body weight, especially during the period from birth to weaning, is determined by the genotype and environment in which the organism develops (Bathaei and Leroy, 1996; Burfening and Kress, 1993; Notter et al., 1991; Caro Petrović et al., 2013).

In addition to external factors, the following have a significant influence on the weight of lambs: mother's age, type of birth and sex of lambs. (Kalantar, 2003; Rashidi et al., 2008; Atashi and Izadifar, 2012) found that the sex of lambs significantly affected the birth weight of lambs. Male lambs had a higher mass than females. Unal et al., (2006) observed the growth characteristics of lambs and concluded that the genotype did not have a significant influence on the examined traits. The authors also state that the sex of lambs and the type of birth had a very significant influence on the growth of lambs. Momani et al., (2002), state that the genotype of lambs had a significant influence on the body weight of lambs from birth to 60 days of age. The sex of the lambs also had a significant effect on the body weight of the lambs.

The fact is that the sex of lambs has a certain influence on body weight, but the difference in weight between the sexes is not always statistically significant. Given the great importance of pramenka for the development of sheep breeding in Serbia, the genetic potential of pramenka under the influence of various factors of paragenetic nature is intensively investigated (Ilic et al., 2012; 2013). The aim of this research is to study the effect of sex in lamb breeding and to estimate body weight in the period from birth to weaning in four different strains of pramenka.

Material and methods

Research was carried out in eastern, southern and south-western Serbia, more precisely in the areas where most pramenka is grown. Mating was in season from June to September and ewes lambing begin in December and further prolongs during the winter period.

In breeding of lambs, applied technology is that after ten days after birth, the offspring is separated from their mothers with the possibility of sucking twice during the day. Then, feeding with hay and concentrate mixture for lambs starts. These nutrients were available up to the age of 90 days.

The material for the research included four strains (Pirotski, Svrlijiski, Sjenicki, Sarplaninski) of Pramenka sheep. In each strain of sheep, mothers were divided into three groups according to their age (1- two years old; 2- three years old, 3-four years old). All experimental animals were properly marked and recorded. Data processing included a total of 1800 lambs (450 lambs per strain of Pramenka sheep).

Research was conducted during the period of three years, according to pre-established plan. In each year of the experiment, body weight of lambs of all genotypes was measured at the time of birth and 30, 60 and 90 days of age. The measurement was carried out by scale with accuracy of 0.10 kg and all data was used for statistical analysis by using SPSS (Statistical Package for the Social Sciences) statistical software, 20 (2012).

Results and discussion

Sexual dimorphism in most cases affects the body weight of lambs at birth. We will comment on the results of our research using Table 1.

It can be noticed that there was a difference in the body weight of lambs at birth in all four genotypes of pramenka.

The body weight at birth in male lambs of the Pirot strain pramenka was 3.71 kg, while the body weight of female lambs was 3.69 kg. The difference in favor of male heads was not statistically significant ($R > 0.05$).

In the population of Svrlijig lambs, male heads had an average birth weight of 4.02 kg, and female 3.67 kg. The existing difference between the sexes was

very significant ($R < 0.01$), which means that gender as a fixed factor had a significant influence on this trait.

Male lambs of the Sjenica genotype had a higher body weight, which averaged 3.85 kg, while the weight of female heads was 3.48 kg. The existing difference is under a very significant influence of the sex of lambs ($R < 0.01$). Sharplanin pramenka lambs had an average birth weight of 3.91 kg in males and 3.64 kg in females. The difference was very significant ($R < 0.01$), so that in this case, too, a significant influence of gender on body weight at birth can be confirmed.

Table 1. Influence of sex on body weight of lambs at birth

Genotype	Sex	M	SE	F	Sig.
PI	M	3.714	.052	.119	.730
	F	3.691	.039		
SV	M	4.024	.035	36.079	.000**
	F	3.674	.046		
SJ	M	3.858	.043	27.039	.000**
	F	3.488	.057		
SP	M	3.918	.041	16.664	.000**
	F	3.640	.054		

** Correlations significant at the level of $P \leq 0.01$

Table 2. Influence of sex on body weight of lambs at 30 days

Genotype	Sex	M	SE	F	Sig.
PI	M	9.342	.092	.042	.837
	F	9.310	.122		
SV	M	9.929	.110	3.221	.073
	F	9.803	.1145		
SJ	M	9.679	.165	.484	.487
	F	9.306	.125		
SP	M	9.358	.162	.721	.396
	F	9.185	.123		

At the end of the first month after birth, lambs double their birth

weight, and sexual dimorphism weakens its influence, and in many cases that influence is lost, because the growth of lambs mostly depends on the quantity and quality of food consumed.

Table 2 shows the values of the influence of sex on the body weight of lambs from 30 days of age. The body weight of a male lamb of the Pirot strain pramenka was 9.34 kg, while the body weight of a female lamb was 9.31 kg. Male lambs had higher body weight, but this difference was not statistically significant ($R > 0.05$).

In lambs of the Svrlijig population, male heads had an average body weight of 9.92 kg, and female 9.80 kg. The existing difference between the sexes was very close to significance, but still not statistically significant ($R > 0.05$).

At the age of 30 days, male lambs of the Sjenica genotype had a higher body weight, and it was 9.67 kg, while the weight of female heads was 9.30 kg. This difference is not under the fixed influence of half of the lambs ($R > 0.05$).

The offspring of the Sharplanin strain pramenka showed an average body weight of 9.35 kg in males and 9.18 kg in females. The difference was not significant ($R > 0.05$).

At the age of 60 days, Pirot lambs had a higher average body weight in male heads, namely 15.74 kg, while female lambs had a body weight of 14.81 kg. No significant influence of gender on the observed traits was found ($R > 0.05$).

The body weight of Svrlijig lambs was also higher in males and amounted to 15.42 kg, while the body weight of female lambs was 15.36 kg. The effect of sex on lamb body weight was not statistically significant ($R > 0.05$).

Table 3. Influence of sex on body weight of lambs at 60 days

Genotype	Sex	M	SE	F	Sig.
PI	M	15.745	.374	2.262	.133
	F	14.813	.494		
SV	M	15.426	.155	.110	.741
	F	15.362	.117		
SJ	M	15.529	.120	1.164	.281
	F	15.315	.158		
SP	M	14.482	.186	3.463	.063
	F	14.047	.141		

Sjenica male lambs had a higher average body weight than females and it was 15.52 kg, while female lambs had a body weight at this age of 15.31 kg. There was no significant effect of sex of lambs on body weight at 60 days

($R > 0.05$).

The body weight of the male lambs of the Sharplanin population was 14.48 kg, while the female lambs had a body weight of 14.04 kg. The influence of sex on the body weight of lambs in this case was not statistically significant ($R > 0.05$) and if it was very close, ie on the border of significance.

Table 4. Influence of sex on body weight of lambs at 90 days

Genotype	Sex	M	SE	F	Sig
PI	M	20.771	.181	6.740	.010 **
	F	19.994	.239		
SV	M	24.325	.230	.110	.740 ns
	F	24.199	.303		
SJ	M	23.528	.267	1.228	.268 ns
	F	23.157	.202		
SP	M	20.528	.267	.605	.437 ns
	F	20.267	.203		

* Correlations significant at the level of $P \leq 0.05$

** Correlations significant at the level of $P \leq 0.01$

The influence of sex on the body weight of lambs at 90 days is shown in Table 4, from where we can notice that the sex of lambs had an influence on all genotypes examined, however, this fixed factor was not ubiquitous and significant.

The body weight of the male lamb of the Pirot strain pramenka was 20.77 kg, while the body weight of the female lambs was 19.99 kg. The difference in favor of male heads was statistically very significant ($R < 0.01$).

Svrljig lambs, male heads had a higher average body weight of 24.32 kg, and female 24.19 kg. The existing difference between the sexes was not significant ($R > 0.05$), which means that gender as a fixed factor did not have a significant effect on this trait.

Male lambs of the Sjenica genotype also had a higher body weight, which averaged 23.52 kg, while female lambs had an average lamb body weight of 23.15 kg at 90 days. The existing difference is insignificant ($R > 0.05$) and is not influenced by the sex of lambs.

The offspring of the Sharplanin strain of pramenka had an average body weight of 20.52 kg in males and 20.26 kg in females. The difference in this case was on the side of male heads, but it was not statistically significant ($R > 0.05$), so

in this case, too, the insignificant influence of sex as a fixed factor can be confirmed.

A large number of foreign researchers state that the body weight of lambs is conditioned by sex (Combellas et al., 1980; Ghoneim et al., 1982; Wojtowski et al., 1990; Unal and Akcapinar, 2001; Babara et al., 2004; Notter et al., 1991, 2005).

In Our Country, Petrović (1992); Petrovic et al., (2011), Ruzic Muslic (2007), state that the sex of lambs in most cases in the Württemberg breed, Miss sheep and crossbreed pramenka with Württemberg and il de France breed, has a significant effect.

Petrović et al., (2009) state that there are no significant differences in terms of variability of quantitative traits between Svrljig, Sjenica and Pirot sheep, except in the final body mass of lambs, where Svrljig and Pirot populations lag behind the Sjenica sheep.

Also, there are foreign and domestic researchers who state that sex did not affect the body weight of lambs of different genotypes (Berra and Paimondi, 1986; Botswana, 1986; Lima et al., 1987; Iyiola-Tunji et al., 2010; Petrović et al., 2011; Caro Petrović et al., 2013).

The fact is that the sex of lambs has a certain influence on body weight, but the fact is that the difference in weight between the sexes is not always statistically significant. One part of the answer to this dilemma lies in the fact that there is a difference in the sex hormones of male and female throats, so that hormones correlate with the genes responsible for growth have some effect (Vaez Torshizi et al., 1992; Shahroudi et al., 2002, 2003 Rashidi et al., 2008).

Conclusion

The body weight at birth in male lambs of the Pirot strain pramenka was 3.71 kg, while the body weight of female lambs was 3.69 kg. The difference in favor of male heads was not statistically significant ($R > 0.05$). In the population of Svrljig lambs, male heads had an average birth weight of 4.02 kg, and female 3.6 kg. The existing difference between the sexes was very significant ($R < 0.01$). Male lambs of the Sjenica genotype had a higher body weight, which averaged 3.85 kg, while the weight of female heads was 3.48 kg. The existing difference is under a very significant influence of the sex of lambs ($R < 0.01$). Sharplanin pramenka lambs had an average birth weight of 3.91 kg in males and 3.64 kg in females. The difference was very significant ($R < 0.01$). Sexual dimorphism in most cases affects the body weight of lambs at birth.

At 30 days of age, the body weight of a male lamb of the Pirot strain pramenka was 9.34 kg, while the body weight of a female lamb was 9.31 kg. Male lambs had higher body weight, but this difference was not statistically significant ($R > 0.05$). In lambs of the Svrljig population, male heads had an average body

weight of 9.92 kg, and female 9.80 kg. The existing difference between the sexes was very close to significance, but still not statistically significant ($R > 0.05$). At the age of 30 days, male lambs of the Sjenica genotype had a higher body weight, and it was 9.67 kg, while the weight of female heads was 9.30 kg. This difference is not under the fixed influence of half of the lambs ($R > 0.05$). The offspring of the Sharplanin strain pramenka showed an average body weight of 9.35 kg in males and 9.18 kg in females. The difference was not significant ($R > 0.05$).

At the age of 60 days, Pirot lambs had a higher average body weight in male heads, namely 15.74 kg, while female lambs had a body weight of 14.81 kg. No significant influence of gender on the observed traits was found ($R > 0.05$). The body weight of Svrljig lambs was also higher in males and amounted to 15.42 kg, while the body weight of female lambs was 15.36 kg. The effect of sex on lamb body weight was not statistically significant ($R > 0.05$). Sjenica male lambs had a higher average body weight than females and it was 15.52 kg, while female lambs had a body weight at this age of 15.31 kg. There was no significant impact.

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***FARMING, HORTICULTURE AND
FORAGE PLANTS***

APPLICATION AND SELECTION CRITERIA FOR ENTOMOPHAGES IN DAMAGE CONTROL IN PROTECTED SPACE - short overview

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Abstract: *The biological control and use of entomophages in the fight against pests dates back to the distant past and represents alternatives to chemical control measures. In order for entomophage to have justification of use, it must meet several criteria, among which the most important properties are: specificity in relation to the pest – victim (host) and indicator of the ratio of the period of development before imago (OPR) which is efficient in specific conditions of entomophage and pest is < 1 . Today, there exist a large number of species that can meet these criteria, however, only a small number are present in the form of more widespread use in a protected area. What precedes the frequent selection of entomophages is experimental verification, which requires significant losses of time and resources. Due to these facts, the paper presents the selection of potential entomophages in the repression of some of the economically significant pests of the protected area, as well as the criteria for the selection of entomophages.*

Keywords: *entomophage, protected area, pest, control*

Introduction

In the protected areas, harmful insects are, as a rule, treated as species of foreign origin, because, similarly to them, they are separated from their natural enemies. Pests that are common in greenhouses are often not specific to any level of technology and are usually polyphagous, they have different plants for hosts that can damage, and are generally more problematic in a protected area than in an open field (Pilkington et al., 2010). Although the spatial distance in this case is small, the mutual isolation of pest and entomophagous populations is usually quite solid. The situation is complicated by the fact that in the protected area the effect of some factors that limit the reproduction of harmful insects, such as rain showers, wind, competition for food and space, is excluded (Jemcev and Djukić, 2000; Djukić et al., 2007). Biological control in

a protected area represents an economic and ecological alternative to chemical control measures (van Lenteren et al., 1992). Biological control is not a new concept, it has been used for about 1700 years (Gurr et al., 2000). According to Luck and Forster (2003), Réaumur advised in 1734 to release species of the genus *Neuroptera* into the protected area in order to control aphids (*Homoptera*). Today, biological control is increasingly used in many pest control systems, with more than 150 species of natural enemies available to growers worldwide (van Lenteren, 2007), only about 30 species make up 90% of total global sales of biological agents (Bolckmans, 1999). It is estimated that 80% of the global commercial revenue generated by biological control agents can be attributed to their use in protected areas (van Lenteren, 2007).

Entomophages the most important properties and selection

The most important properties of good biological control agents - entomophages, which are introduced against pests, are: important impact on the host in the region of origin; high specificity for host, good synchronization of development with host development; high search capacity; good adaptation to a wide range of environmental conditions and the ability to occupy empty ecological niches in the natural hostile community of pests in the region of introduction (Cock, 1986). It is known that narrowly specialized entomophages manifest the highest degree of efficiency during introduction and acclimatization. (Doutt et al., 1968). Parasitoids, especially those released in greenhouse systems, are often highly specialized in their hosts (Lynch et al., 2001) and it is possible that the unintended risk can be mitigated by the use of these organisms.

It is known that one of the most effective ways of biological control with foreign pests is the introduction and upcoming acclimatization of their natural enemies in new areas of expansion. Therefore, having in mind the considered specifics of the ecological situation in the protected area, it was concluded that the integration of natural enemies in closed spaces (greenhouses, hothouses, tunnels) must ensure efficient repression of pest populations, ultimately of those species that in natural conditions have regulatory importance (Берлјаров, 1974; 1978; Djukić et al., 2007). In addition, the populations of red spider mite (*Tetranychus urticae* Koch), greenhouse whiteflies (*Trialeurodes vaporariorum* Westwood) and tobacco thrips (*Thrips tabaci* Lindeman) are very often filled at the expense of active penetration of individuals from nearby plots that are placed outside the protected area or by inadvertent introduction from outside.

Insufficient crop rotation, conditioned by a limited selection of vegetable plant species and long-term use of the same soil, affects the accumulation of pests, for which cultivated plants are suitable food. This is due

to moderately high and relatively stable constant conditions of temperature, air humidity and hygiene conditions (Table 1) which are maintained in closed spaces during vegetable growing. However, long-term studies of colonization of *Phytoseiulus* various aphidophagous, Encarsia and others show that, despite the existence of favorable hydrothermal conditions, during the intensive industrial cultivation of vegetable species in monoculture there does not come to acclimatization of entomophages in the protected area. Therefore, in order to realize the potential opportunities, even the most effective pests and parasites, it is necessary to bring them into protected areas every year.

Table 1: The impact of optimal regime of basic abiotic factors on the growth and development of some vegetable crops in greenhouses

Plant species	Temperature (°C)		Relative air humidity (%)	Brightness 10 ³ lux
	Clear day	Cloudy day		
Cucumber				at least 9
before fruiting	21 - 24*	20 - 21	80	
	16 - 17	16 - 17		
during fruiting	23 - 27	21 - 22	85 - 90	
	17 - 19	16 - 18		
Tomato				19
before the onset of physiological maturity	21 - 23	21 - 22	60 - 65	
	15 - 16	16 - 18		
in the period of physiological maturity	25 - 26	19 - 21		
	17 - 18	16 - 17		
Sweet pepper				19 - 29
seedlings after dipping	21 - 24	19 - 20	75	
	20	18		
fruiting	23 - 27	21 - 24		
	16 - 19	15 - 18		

* day temperatures in the numerator, night temperatures in the denominator

The absence of entomophages and the possibility of their acclimatization in the soil of the protected area allow a combination of modes of interaction and seasonal colonization to be applied as a common strategy. When using species that are not able to reproduce independently in protected

areas, quite high efficiency is provided by targeted introduction, that is, the use of parasites and pests as a “living stimulus”. Starting from this strategy, we believe that the development of methodology and organization of mass reproduction is an important and indispensable condition for the successful application of even the most efficient entomophages in protected soil. Tab. 1: Influence of optimal regimes of basic abiotic factors on the growth and development of some vegetable plant species in a protected area.

The significance of the listed properties is undeniable. However still, experimental verification remains the only criterion for the correctness of selection of a predator or parasite, which requires significant losses of time and resources. Attempts by some entomologists to determine the relative value of entomophages as a biological method based on the property of belonging to a group of predators or parasites and thus argue the expediency of selecting perspective species based on this criterion have proven unfounded. Numerous examples of pest repression are already known, with the use of representatives of both groups of entomophages.

Under natural conditions, the number of pests is very rarely kept on a low level with one species of predator or parasite. Here, the usual reproduction of pests is regulated by the total activity of the complex of natural enemies. In contrast, in the soil of protected area, as already noted, there are practically no entomophages. Also, one should not count on their acclimatization and especially on the formation of complexes that function for a long time.

Numerous experiments on the colonization of different Hemerobiidae, ladybugs (Coccinellidae), hoverflies (Syrphidae) and Anystidae ticks in a protected area testify to the fact that polyphagous entomophages and wide oligophages do not reproduce here on their own at all. During seasonal colonization of this species, as well as in the soil of open spaces, they provide only partial pest repression. The full effect is achieved only when they are used as a “live insecticide”, that is, with a significant sharp increase in the norm and frequency of introduction.

It follows from all the above-mentioned that polyphagous predators and parasites are relatively less suitable as a means for fighting pests in protected areas. The efficiency of use of oligophages clearly correlates with the degree of specialization in relation to the victim (host). In any case, during seasonal colonization, narrow oligophages (aphid midge *Aphidoletes aphidimyza*, membranous parasites of peach and other *Aphidius matricariae* and *Aphelinus asyehis*, etc.) are able to suppress the reproduction of presumed species of victims, while the efficiency of wider oligophages (for example Syrphidae) is manifested only by targeted enhanced introduction. According to Hopper et al. (1996), the species of *A. asyehis*, which is widespread in the Old World, was introduced in the United States for biological control of Russian wheat aphid (*Diuraphis noxia* Kurdjumov).

For narrowly specialized predators and parasites, the importance of this factor is less important, that is, they are usually able to reproduce independently in protected areas. Thus, the most favourable practical goal of selecting entomophages for use in protected soil is to detect (find), preferably, a narrowly specialized species, which is able to suppress pest populations – victim (host), in a short period of time. However, selection based solely on the property of the specialization does not guarantee success in practical application. This is evidenced, for example, by the experiment of unsuccessful use against the red spider mite (*T. urticae*) of its specialized predators *Stethorus punctillum* and *Scolothrips acariphagus* Yakh (Зорин, 1934).

The species *S. punctillum* has the highest distribution in the Stethorini group, which stretches from North America, through Europe and Asia (Biddinger et al., 2009).

The main driving force of the current geographical distribution of the Stethorini group was their deliberate introduction by scientists for the purpose of biological control. For example, 12 species (some of which have never been formally identified) were introduced in California from geographically diverse areas such as Australia, China, Guatemala, India, Morocco, Pakistan, South Africa and Turkey (Hall and Fleschner, 1958; Gordon, 1982). Some of the earliest introductions of these species occurred in Hawaii, which apparently do not have a native species from the Stethorini group (Fournier et al., 2002).

Biological indicators of entomophages

The determination of basic biological indicators of entomophages, which are characterized by different efficiency, testifies to the fact that regardless of the degree of specialization in relation to the victim (host), data on developmental deadlines, sex ratio, fertility, and edacity are of little suitability as a property in selecting predators and parasites that are perspective for use. At the same time, there was established a correlation between the ratio of development before imago (from egg to imago) component to the predator (parasite) – victim (host) System and the efficiency of seasonal colonization of entomophages in specific conditions. It was determined that in all known cases of almost complete repression of pests with the introduction or seasonal colonization of one entomophage, there were used predators and parasites with shorter pre-imago term (period) than in the victim. In other words, the ratio of the period of development before imago (OPR) which is efficient in the specific conditions of entomophage and pest is < 1 . Thus, for example, at a favourable temperature (27°C) the pre-imago development of the genus *Phytoseiulus* ends in 5.5 days, and of the red spider mite *T. urticae* in 8.2 days (Begljarov, 1967; Берлјаров, Ушћаков, 1972), that is, the OPR indicator of these species (5.5:8.2) is 0.67.

Encarsia entomophages and [are highly efficient at a temperature of 25°C](#). The pre-imago ones complete development in 14-15 and 6 days, and greenhouse whiteflies and tobacco thrips in 20-22, that is, 13.7 days (Беглјаров, Лебедов, 1979; Беглјаров et al., 1982; Беглјаров, Сучалкин, 1983, 1985). In that case, the OPR indicator for *Encarsia* is 0.69, for *A. mckenzie* – 0.44. Arthurs et al (2009) examined *Neoseiulus cucumeris* and *Amblyseius swirskii* (Acari: Phytoseiidae) entomophages for biological control of *Scirtothrips dorsalis* on peppers, finding that these species consumed larvae in an average of 2.7 per day, that is, 1.1 – 1.7 imagoes of the undesirable insect. The same research recorded, under greenhouse conditions, a decrease in the population of *S. dorsalis* for 28 days after the release of the mentioned entomophages.

Very highly efficient entomophages, such as parasitoid wasp (*Aphelinus mali* Haldeman), mealybug ladybird (*Cryptolaemus montrouzieri* Muls.), *Pseudoophonus malinus* Gah., *Rodolia cardinalis* Muls, *Prospaltella berlesei* How. and others are also characterized by similar OPR indicators. In some cases, a fairly high effect is achieved by seasonal colonization of narrow oligophages, whose OPR is >1. Such species include, for example, the already mentioned parasites *Aphelinus asyehis* Walker, *Aphidius matricariae* and the aphid midge.

The existence of *P. berlesei* is recorded in the natural conditions of Serbia as a parasitoid of the mulberry armoured scale insect (*Pseudaulacaspis pentagona*), with this parasitoid being considered the most significant reductant of the populations of the mentioned pest (Erler and Tunc, 2001; Graora and Spasić, 2008).

Therefore, some of the most important properties of the suitability of entomophages for application in protected soil are: 1- specificity in relation to the pest-victim (host) and 2- OPR indicator. Data on the specificity and size of OPR can be used in the early stages of studying entomophages as basic properties of their relative suitability for seasonal colonization. If the entomophage is more specific and the OPR indicator is lower, the more perspective the use of predator or parasite in protected soil is. The OPR indicator characterizes the comparative (relative) suitability of entomophages for use as a way of seasonal colonization in specific conditions. When the conditions change, that indicator can change, characterizing at the same time the change in the usability of the species for application. Thus, for example, *Encarsia* is highly effective in greenhouse whiteflies at 25 and 30°C. The OPR indicator is 0.54 at these temperatures, that is 0.69. The efficiency of seasonal colonization of parasites decreases sharply when the temperature drops to 20°C, until the complete disappearance at 15°C (OPR indicator at 15°C is calculated based on literature data (Lanteren, Hulspar-Jordan, 1983), the OPR indicator rises to 0.83 and even 1.02-1.1).

Conclusion

For successful application in protected area, it is necessary to know all the characteristics of entomophages but also the conditions prevailing in the protected area, in order to expect efficient pest control. The selection should be made on the basis of OPR indicators, the abundance should be maintained by creating favourable conditions for the development and reproduction of entomophages and the cultivated plant species itself, in relation to the pest and by regular introduction of entomophages into the protected area.

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THE INFLUENCE OF LYOPHILIZATION CONDITIONS ON YEAST VITALITY AND FERMENTATION ACTIVITY

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Abstract: *The aim of this reserach was to consider the influence of the age of yeast culture on their survival after lyophilization and in the process of further storage. Two-, three-, seven- and thirteen-day-old cultures were used for lyophilization, which were grown in tubes on shira-agar. Properly performed lyophilization significantly affects the survival and fermentation activity of yeasts. Three-day-old yeast cultures are most vital during lyophilization. Yeasts survive best in protective media containing 10% sucrose, 1% gelatin, 0.1% agar-agar and 2% CaCO₃, or 10% skim milk and 1% sodium glutamate. Prolonged storage in the dried state and rehydration lead to decreasing in reproductive energy and fermentation activity of yeasts.*

Key words: yeasts, lyophilization, survival, fermentation.

INTRODUCTION

Lyophilization is a well known dehydration method widely used to preserve microorganisms. It is also commonly used in food preservation and for a variety of pharmaceutical applications, including protein-based drugs (Cleland et al., 2001). When a single microorganism or a microbial consortium is selected as a starter culture, its conservation for subsequent use in fermentations is required. Thus, storage at low temperatures (freezing or refrigeration) and lyophilization techniques are often applied to maintain the viability of starter cultures. Lyophilization is an easy way to keep a high number of viable microorganisms and the powder forms would require a low number of procedural steps (Pradelles et al., 2009). By its ability of combining freezing and drying in a unique operation, this process can create final dried products with the highest quality, but the freeze-drying step is especially

critical as it negatively affects both viability and physiological state of the yeasts. The formation of ice crystals induces mechanical damage that leads to cellular death during freezing (Tymczynszyn et al., 2005).

Although freezing itself has not a lethal effect for cells, it can induce physical stress that can injure a part of these cells, thus decreasing the proportion of viable cells (Pehkonen et al., 2008). If freezing is extremely slow, intracellular water can flow to the outer environment by osmosis and create extracellular crystals, thus causing extracellular water removing and solute concentration increase that lead to an osmotic imbalance (Miyamoto-Shinohara et al., 2008). Conversely, if freezing is too fast, cells cannot lose water fast enough to maintain the balance, so intracellular ice crystals can appear thus producing damaging or even lethal effects.

This is a very complex physical process in which cell survival is affected by many physico-chemical and biological parameters, such as cell growth conditions, type of lyoprotectant, freezing, sublimation and thawing temperatures, degree of dehydration achieved, reconstitution medium, and time and storage and rehydration conditions have been described to have great influence on the survival of microorganisms (Peiren et al., 2018). On the other hand, beside this factors, several biological factors affects microbial survival during this process including the intrinsic resistance traits of the strains, initial concentration of microorganisms, growth conditions, and the drying medium (Morgan et al. 2006). An enhanced stability of microorganisms can be achieved by adding many protective compounds such as disaccharides, polyols, monosaccharides, skim milk, and other organic molecules (Hubalek, 2003).

Lyophilization of yeast is one of the optimal ways of storing cultures and is widely used in many laboratories that have collections of microorganisms. Experience working with collections of microorganisms shows that the conditions of lyophilization, such as the age of the culture and the composition of the applied protective media, have a significant impact on the survival of microorganisms in the drying process and during their further storage (Kursop and Doybe, 2001; Abadias et al., 2001; Miyamoto-Shinohara et al., 2010).

The aim of this reserach was to consider the influence of the age of yeast culture on their survival after lyophilization and in the process of further storage.

MATERIAL AND METHODS

Two-, three-, seven- and thirteen-day-old cultures were used for lyophilization, which were grown in tubes on shira-agar. The yeast from the oblique agar was washed with 3 mL of protective medium (0.1% sucrose-gelatin agar). 0.1 mL of yeast suspension in a protective medium was added to

2 mL glass ampoules. In order to determine the number of surviving cells in the dried material, their concentration in the initial suspension was determined before lyophilization. Ampoules with lyophilized yeast were stored at +4 to +6 °C in the refrigerator. Through 24 hours, half a year and one year of storage, the yeast was rehydrated by adding sterile distilled water to open ampoules and keeping the cells for 60 minutes at room temperature before sowing on broad agar. The titer of live yeasts was expressed as a percentage of the number of grown colonies after 24 h, half a year and one year of storage in the lyophilized state according to their number before conservation, taken as 100%.

Statistical analysis

The obtained results were statistically processed using the software package SPSS 20. To determine the statistical significant differences between the obtained values, one-way analysis of variance (ANOVA test) was performed, followed by post hoc Tukey's test ($p = 0.05$).

RESULTS AND DISCUSSION

Lyophilisation is one of the most successful methods for preserving bacteria, yeasts and sporulating fungi (Spadaro et al., 2010). This method offers convenience of storage and postage, and it keeps the microorganisms viable for long periods of time (Miyamoto-Shinohara et al., 2006). However, lyophilisation is relatively expensive as it requires sophisticated equipment and adequate power supply.

In the Table 1 are shown the results for the the influence of the age of lyophilized yeasts on their survival. In accordance, the highest titer of vital yeasts, both one day after lyophilization and during storage for one year, was observed in three-day-old cultures. After one year of storage, it decreased from 95% to 60%, while in cultures of other ages, the vitality titer was only 20 to 30%. Therefore, three-day-old yeast cultures were optimal (by age) for successful lyophilization (Fateeva et al., 2016). Nevertheless, can be seen that there is statistically significant differences ($p < 0.05$) between the number of living cells at different yeast ages.

This is in line with the study from Donev et al. (1995) who pointed out that in the lyophilization of yeasts of genera *Candida* and *Saccharomyces* for collection purposes three-day-old cultures obtained through surfaced cultivation on solid medium have to be preferred. This fact has been proved many times in the routine work of the National Bank for Industrial Microorganisms and Cell Cultures.

Table 1. The influence of the age of lyophilized yeasts on their survival

Yeast age, days	Before lyophilization of yeast		A day after lyophilization		Half a year after lyophilization		One year after lyophilization	
	Number of living cells in mL	Survival (%)	Number of living cells in mL	Survival (%)	Number of living cells in mL	Survival (%)	Number of living cells in mL	Survival (%)
	$\bar{x} \pm SD$		$\bar{x} \pm SD$		$\bar{x} \pm SD$		$\bar{x} \pm SD$	
2	$3.4^a \times 10^7 \pm 0.01$	100	$2.2^b \times 10^7 \pm 0.03$	65	$1.4^c \times 10^7 \pm 0.03$	40	$1.0^d \times 10^7 \pm 0.02$	30
3	$5.0^a \times 10^7 \pm 0.02$	100	$4.8^a \times 10^7 \pm 0.01$	95	$3.6^b \times 10^7 \pm 0.03$	70	$3.0^c \times 10^7 \pm 0.01$	60
7	$2.2^a \times 10^7 \pm 0.00$	100	$1.5^b \times 10^7 \pm 0.02$	70	$9.0^c \times 10^6 \pm 0.02$	40	$6.0^d \times 10^6 \pm 0.01$	27
13	$2.0^a \times 10^7 \pm 0.01$	100	$1.0^b \times 10^7 \pm 0.02$	50	$6.0^c \times 10^6 \pm 0.03$	35	$4.0^d \times 10^6 \pm 0.02$	20

a, b, c, d - values marked with different letters, have a statistically significant difference ($p < 0.05$).

In order to determined the optimal protective media for lyophilization of three-day yeast cultures, 4 protecting media were prepared: 1. media - 10% sucrose, 1% gelatin, 0.1% agar-agar, 100 mL of distilled water; 2. media - first media with 2% CaCO_3 ; 3. media - 10% gelatin, 0.25% ascorbic acid, 2% CaCO_3 , 100 mL of distilled water; 4. media - 10% skim milk, 1% sodium glutamate, 100 mL of distilled water.

The titer of vital yeasts during storage on all tread media was reduced and was minimal with using of third medium (Table 2). Maximum survival was observed in yeasts, both one day after lyophilization and after one year of storage with using of second and fourth medium. On the other hand, can be seen that there is statistically significant differences ($p < 0.05$) between the number of living cells on different protective media.

Table 2. The influence of protective media on the survival of lyophilized yeasts during storage

Protective media	Before lyophilization of yeast		A day after lyophilization		Half a year after lyophilization		One year after lyophilization	
	Number of living cells in mL	Survival (%)	Number of living cells in mL	Survival (%)	Number of living cells in mL	Survival (%)	Number of living cells in mL	Survival (%)
	$\bar{x} \pm SD$		$\bar{x} \pm SD$		$\bar{x} \pm SD$		$\bar{x} \pm SD$	
1	$9.0^a \times 10^7 \pm 0.02$	100	$8.5^b \times 10^7 \pm 0.01$	90	$7.2^c \times 10^7 \pm 0.03$	80	$5.6^d \times 10^7 \pm 0.01$	62
2	$7.0^a \times 10^7 \pm 0.02$	100	$6.3^b \times 10^7 \pm 0.03$	90	$5.3^c \times 10^7 \pm 0.03$	75	$4.5^d \times 10^7 \pm 0.02$	65
3	$5.0^a \times 10^7 \pm 0.02$	100	$1.0^b \times 10^7 \pm 0.01$	20	$8.0^c \times 10^6 \pm 0.02$	16	$2.1^d \times 10^6 \pm 0.03$	11
4	$3.1^a \times 10^7 \pm 0.04$	100	$2.8^b \times 10^7 \pm 0.02$	90	$2.1^c \times 10^7 \pm 0.02$	85	$1.9^d \times 10^7 \pm 0.01$	70

a, b, c, d - values marked with different letters, have a statistically significant difference ($p < 0.05$).

The fermentation activity of yeasts, which are in a lyophilized state for one year, was checked on concentrated whey, from which proteins have been removed. Whey contains 10.5% lactose and 0.05% $(\text{NH}_4)_2\text{SO}_4$ was added, while the pH was adjusted in the range of 5.0 - 5.2. Revival of lyophilized yeasts was performed by their rehydration in distilled water for one hour (Blankov and Klebanov, 2011). After rehydration, the yeasts were sown in tubes on a broad-agar hair. After three days, the grown yeasts of the first passage were washed from the oblique agar in a 200 mL flask with 150 mL of whey. Fermentation of whey lactose was performed in flasks on a shaker with 120 rpm at a temperature of 25 °C. In the same conditions, whey was fermented with the yeasts of the second and third passages after their revival. The alcohol content in the fermentation broth was determined after distillation by the aerometric method. Moreover, stress effects on yeast cells, due to longer storage in the dried state and rehydration, lead to decreasing in the rate of fermentation of lactose milk by the yeasts of the first and second passages.

Thus, after 60 hours of fermentation, the yeasts of the first passage leaved 2.0 - 2.5% of lactose in the media, the second passage had a 0.5 -1.5% of lactose, while at the same time the yeasts of the third passage during that time practically completely fermented whey lactose. The reduction of reproductive energy and, therefore, fermentation activity in lyophilized alcoholic and wine yeasts has been observed by numerous researchers, including Burjan (2006). However, in some yeasts, lyophilized with protective second and fourth media, the fermentation activity was completely restored in the third passage.

Bearing in mind that the survival of yeasts, which were lyophilized with these protectors, is the highest, it can be considered that media 2 and 4 are optimal for lyophilization of yeasts in terms of maintaining their vitality and fermentation activity.

Thus, according to the study of Berny and Hennebert (1991), by using skim milk as a support material in combination with two compounds among honey, sodium glutamate, trehalose or raffinose, the viability of *Saccharomyces cerevisiae* cells increased from 30% to 96–98%. Abadias et al. (2001) reported a survival rate of 28.9% for *Candida sake* when 10% skim milk was used. Zhao and Zhang (2005) obtained the highest viability (53.6%) after freeze-drying of *Oenococcus oeni* H-2 by using 2.5% sodium glutamate. Generally, each single protectant has its own advantages and disadvantages which can be made up by other protectants. Therefore, several protectants are mixed together according to a certain formula for a better performance (Wang, 2000).

Fungal strains may be kept alive in freeze-drying ampoules for more than 40 years. The advantages of freeze-drying are the protection from contamination or infestation during storage, long viability, and facility for strain distribution. However, not all strains survive the process and, among those surviving, quantitative viability rates as low as 0.1% have been reported (Zhao and Zhang, 2005).

The freezing effect on cell morphology can be observed under a cryomicroscope (Smith et al., 1986). At the optimum cooling rate (3 °C/min to 10 °C/min) cells of *Saccharomyces cerevisiae* are extensively shrunken while at higher cooling rate, intracellular ice is associated with loss of viability. Smith et al. (1986) report that *Penicillium expansum* survives at all cooling rates between 0.5 °C/min and 200 °C/min, the optimum ranging between 1 and 10 °C/min. At optimum cooling rates, hyphae of *Penicillium expansum* shrink extensively. At higher rates (> 50 °C/min) intracellular ice nucleated without killing the cells.

The microorganisms viability during con-servation and storage is influenced by their nature, cultivation conditions, age, culture and protecting medium concentrations, regime of the process, etc. In order to preserve the

strain specific characteristics it is necessary to apply routine approaches, based on preliminary tested models. The main task is maximum number of initial cells to be kept and the first tool available is cultivation (Donev et al., 1995).

CONCLUSION

Based on the analysis of works in this area, it can be concluded that three-day-old yeast cultures were the most vital during the lyophilization process. The best survive was obtained in protective medias with 10% sucrose, 1% gelatin, 2% CaCO₃ and 0.1% agar-agar, ie with 10% skim milk and 1% sodium glutamate. According to that, both of second and fourth media are recommended for future use as protective media for yeasts during the lyophilization process. Moreover, the storage of yeast in a dried state and rehydration lead to decreasing in their reproductive energy and fermentation activity.

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INTERACTION OF BIOLOGICAL INDICATORS OF LAND WITH PLANTS YIELD

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Abstract: *The paper studies the relationship between the basic biological processes that take place in the soil and the yield of some agricultural crops.*

A pronounced correlation was found between the size of the yield and the degree of soil humification, respiration intensity, cellulolysis, nitrification, invertase and urease activity, as well as the degree of nitrogen accumulation, the presence of sporogenic bacteria and actinomycetes.

Keywords: *enzymes, microorganisms, yield, humus, soil.*

INTRODUCTION

In the last few decades have been published many papers which have pointed to a link between the many biological processes that take place in soil and yield of crops (Verstraeten, 2008; Domsch, 2007., Andreeva et al., 2001; Mandić et al., 2011; Romejko, 2008; Stefanjkina, 2008; Čunderova, Zubec, 2000). The relationship with invertase activity is particularly close (Vizemjuller et al., 2008; Galstjan, 2004). The connection between enzymes that mineralize nitrogenous organic compounds (protease, urease) and crop yields was also determined (Pešaković et al., 2008; Verstraeten, 2008; Iljina et al., 2007; Dubovenko, Ulasević, 2008). The level of connection of yield with enzyme activity in soil is higher than with agrochemical indicators (Đukić et al., 2007; Čunderova, 2002).

There is a positive correlation between the yield of winter rye and celulolizne activity area (Krause, 1999), the size of the yield, the total number of microorganisms, accumulation of free amino acids and the intensity of the nitrification (Kislih et al., 2005).

MATERIAL AND METHODS

In this paper, standard methods were used to determine the amount of humus in the soil, the number of the most important physiological groups of microorganisms (ammonifiers, nitrifiers, oligonitrophils, actinomycetes, yeasts), basic biological processes (cellulose decomposition, CO₂ release, nitrification intensity, free amino acid accumulation). activities (catalase, dehydrogenase, polyphenol oxidase, invertase, urease, asparaginase).

RESULT AND DISCUSSION

The results of the research show that there is a connection between barley yield and biological indicators of sod-podzolic sandy and clayey soil in the vegetation-field experiment (Table 1, 2).

Table. 1. Relationship between humus content, barley yield and biological properties of sod-podzolic soil

Biological indicators	Correlation relations			
	sandy		Clayey	
	humus	yield	humus	Yield
	1,68-3,25 %	33,58-134,1 %	1,90-3,81 %	66,35-132,52 %
Ammonification bacteria	Unreliable	0,45	Unreliable	0,49
Sporogenic bacteria	0,96	0,77	0,90	0,53
Yeasts	0,69	0,59	Unreliable	Unreliable
Actinomycetes	0,80	0,54	0,84	Unreliable
Oligonitrophiles	0,86	0,85	Unreliable	Unreliable
Potential nitrogen activity	0,85	0,70	0,61	Unreliable
Nitrification activity	0,93	0,85	0,92	0,59
Cellulolytic activity	0,75	0,63	0,80	0,63
Accumulation free aminoacids	0,61	Unreliable	0,47	0,66
Dehydrogenase	0,98	0,88	0,73	Unreliable
Polyphenoloxidase	0,91	0,86	0,95	0,70
Invertase	0,95	0,85	0,45	Unreliable

Judging by the correlation relations, in most biological tests, determined in sandy soil, there is a high degree of correlation between humus content and barley yield (Table 1). On clay soil, the connection between barley yield and sporogenic bacteria, nitrification and cellulolysis ability and polyphenol oxidase activity is especially pronounced. In soils of barley production crop, the connection between yield and activity of invertase, polyphenol oxidase and CO₂ release is especially pronounced (Table 2).

Based on the above, it can be concluded that microbiological and biochemical processes accurately enough manifest the state of fertility of the podzolic soil type and can serve as good indicators.

Comparative analysis of the studied biological tests to detect the leading processes in the examined soils showed that the most precise characteristic of the biological activity of soils from the group of oxido-reduction enzymes is given by polyphenol oxidase, and from the group of hydrolases - invertase. There is a clear agreement between the size of the yield, the degree of humification of the soil and the intensity of "breathing", cellulolysis and nitrification ability and the content of sporogenic bacteria.

Table. 2. Relationship between biological properties of sod-podzolic soil and barley yield

Indicators	Correlacioni relation			
	Sandy		Clayey	
	2008. year	2009. year	2008. year	2009. year
Limits of variation of barley yield, 100 kg/ha of yield	26,0-58,0	20,8-39,1	36,8-65,3	16,0-37,0
Invertase	0,24	0,40	0,43	0,43
Polyphenol oksidase	0,24	0,35	0,30	0,34
CO ₂ emission	-	0,40	-	0,59
Potential nitrogen fixation activity	-	Unreliable	-	0,32
Nitrification activity	-	0,32	-	Unreliable

Based on the analysis of the correlation of protease, urease, polyphenol oxidase, dehydrogenase and invertase with the yield of winter rye and potatoes, a mathematical model was developed that describes the relationship between yield and enzyme activity in soil (Čunderova, Zubec, 2010). This means that there is a possibility of predicting the yield of agricultural crops based on the enzymatic activity of the soil.

Very reliable correlations have been established which characterize the connection of certain biological indicators with the yield of some vegetable crops, such as cabbage and cucumber (Table 3). There is a particularly close relationship between yields with a group of sporogenic bacteria, with bacteria that use mobile nitrogen, with actinomycetes, with the intensity of nitrate accumulation in the soil, cellulose decomposition, invertase and urease activity.

Table.3. Correlation analysis of the relationship between biological indicators and yield

Biological indicators	2009. year - cabbage			2009. year - cucumber		
	r	η	$\eta^2 \cdot 100$	r	η	$\eta^2 \cdot 100$
MIKROORGANISMS						
Ammonification bacteria	0,75	0,86	74	0,13	0,33	11
Sporogenic bacteria	0,71	0,85	72	0,95	0,98	96
Nitrogen fixers	0,44	0,72	52	0,02	0,80	64
Actinomycetes	0,58	0,76	58	0,29	0,73	53
Nitrifiers	0,85	0,86	74	0,50	0,53	29
Yeasts	0,42	0,52	27	0,08	0,46	21
BIOLOGICAL PROCESSES						
Nitrate accumulation	0,58	0,76	58	0,13	0,62	38
Amino acids	0,76	0,69	48	0,37	0,46	21
Cellulose degradation	0,79	0,93	86	0,75	0,79	62
ENZYME ACTIVITY						
Catalase	0,54	0,54	29	0,49	0,68	46
Invertase	0,59	0,59	35	0,41	0,76	58
Urease	0,60	0,65	42	0,21	0,56	58
Asparaginase	0,65	0,65	42	-	-	-

CONCLUSION

The number and activity of microorganisms in the soil are good indicators of the condition of the soil, which especially refers to the activity of invertase and polyphenol oxidase.

There is a clear relationship between yield size, degree of soil humification and respiration intensity, cellulolysis and nitrification activity, and the presence of sporogenic bacteria.

Strong correlations were found between the yield of some agricultural crops and sporogenic bacteria, actinomycetes, cellulose degradation, degree of nitrogen accumulation, invertase and urease activity.

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EXAMINATION OF INFLUENCE OF DIFFERENT PYROPHYLLITE FERTILIZATION TREATMENTS ON VITAMIN C CONTENT IN CABBAGE HYBRID BRAVO F1 (*BRASSICA OLERACEAE* VAR. *CAPITATA*)

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Abstract: *In this study, the effect of different doses of pyrophyllite as nutrients and soil biostabilizers in combination with mineral fertilizers (NPK 15:15:15) on vitamin C content in Bravo F₁ hybrid cabbage (*Brassica oleraceae* var. *Capitata* L.) was examined. The research was conducted in 2018 and 2019 at the FBiH Institute of Agriculture. At the site of the Parsovići-Konjic pyrophyllite mine, a representative sampling of pyrophyllite was performed, and in laboratory conditions, certain physicochemical parameters were determined: granulometric composition, pH value in water and KCl, ie true and substitution acidity were determined.*

The aim of this study was to determine the effect of treatment of different amounts of pyrophyllite with a standard amount of mineral fertilizer on the content of vitamin C (L-ascorbic acid) which is extracted in the technological ripening of cabbage.

The vitamin C content ranged from 74 to 95 mg / 100 g-1 of fresh cabbage mass. Pyrophyllite treatments were: 2.200 kg/ha; 1.700 kg/ha; 1.200 kg/ha; 700 kg/ha in combination with constant ratio of mineral fertilizer (NPK 15:15:15 in the amount of 800 kg/ha). Highest dose of pyrophyllite per unit area (2.200 kg/ha) exhibited the greatest influence on the content of C vitamin (95,05 mg/100 g-1) and the lowest applied dose of pyrophyllite (700 kg/ha) exhibited the lowest content of vitamin C (74,30 mg/100 g-1 fresh cabbage mass).

Keywords: *pyrophyllite, cabbage, vitamin C, fertilizers, spectrophotometry.*

INTRODUCTION

Mineral raw materials have found application in certain branches of agriculture, primarily in plant and animal production, in the chemical industry, food industry, in the transport and storage of agricultural products. In recent years, in the most developed countries, the amount and value of materials obtained on the basis of natural - non-metallic mineral raw materials is three to five times higher than the value of products based on metal products (Harbi and Lab 2016). From the aspect of economic and ecological importance, the future will largely depend on the stable and adequate supply of non-metallic - natural mineral raw materials which will be the basis for industrial and agricultural production. As the properties of non-metallic mineral natural raw materials depend on the crystal chemical structure that can be modified by a certain technological processing, it follows that natural raw materials, especially non-metallic mineral raw materials must be very seriously processed technologically. Thus, it is not only their basic chemical component that they contain that is important, but also their special mineralogical and crystallographic characteristics that determine their structure and their process properties for the production of various products. The assessment of resources and exploitation availability of non-metallic minerals must be determined by the degree of possibility of their application (Churakov, S.V. 2006).

One of the most promising non-metallic minerals is pyrophyllite. According to its chemical composition, pyrophyllite is an aluminosilicate of the formula $\text{Al}_2\text{Si}_4\text{O}_{10}(\text{OH})_2$. It is the final member of an isomorphic series of aluminosilicates containing bound water. Hydroxyl groups (OH) are attached to the inner layer of the three-layer crystal lattice which makes pyrophyllite weakly reactive. It is insoluble in water and does not swell. It got its name from the Greek words *pyros-fire* and *phillon-leaf*, because it fan-shaped when annealed. According to the Moss scale, the hardness of pyrophyllite is 1-1.5. The bulk density of pyrophyllite is 2.20 g/cm^3 and the specific weight is 2.60 g/cm^3 . It is white to slightly colored, soft, oily to the touch like talc and hardens well. It usually appears in crystalline shales and hydrothermal wires. In crystalline shales it is formed by the transformation of aluminum silicates, and in hydrothermal wires it is formed by crystallization from hydrothermal solutions or by alteration of aluminum silicates. Pyrophyllite is one of the most powerful detoxifying clays, as it has a balanced mineral composition and extremely small particle size. It is rich in electrolytes and gives a lot of free ions that act as antioxidants (Halilović, N. 2018). Due to its high retention capacity, ability to retain nutrients, water, heavy metals, specific crystal structure, pyrophyllite has recently been used as a multipurpose additive to soil with transboundary concentrations of heavy metals. Prospects for greater use of pyrophyllite in agriculture are based on the characteristics of its high

absorption capacity, high selectivity for some heavy metals and radionuclides (Stoiljković, S. 2002). The quality of pyrophyllite in terms of physical and chemical parameters significantly affects soil fertility, improves the structure and ability to retain moisture in the soil, retains, regulates and distributes nutrients and water to plants when needed, at the same time binds heavy metals and polycyclic hydrocarbons to its crystal structure (PAHs) and as such has the role of a biostabilizer. According to a number of researchers, pyrophyllite has a decisive role in environmental protection and the production of healthy food (ITNMS Belgrade 2017). Pyrophyllite is an important resource in the agricultural sector and other economic activities and its use in a wider production practice can prevent serious environmental problems while obtaining quality agricultural products (Hasanbegović, E. 2018). Often, in the demanding production of cabbage with intentions for the highest possible yields, expensive and irrational application of mineral fertilizers occurs, which inevitably causes harmful consequences not only on the quality of the product, but also represents a potential danger of water and soil pollution. By applying pyrophyllite in combination with mineral fertilizer in the production of cabbage, not only as a nutrient, but also as a biostabilizer, it is possible to achieve better results in sustainable production, above all, yield and nutritional value. Pyrophyllite, in various combinations with mineral fertilizer, in the production of cabbage, helps and stimulates the growth of the plant, provides it with a good mineral composition without potentially dangerous leaching of harmful substances and nutrients into the soil and watercourses. A special effect, pyrophyllite as a biostabilizer, has in transplanting vegetable seedlings, which alleviates the current abiotic stress and temporary stunted growth of plants.

Vitamin C content in fruits and vegetables can be influenced by various factors such as genotypic differences, climatic conditions, sowing and planting methods. The higher the intensity of light during the vegetation, the higher the content of vitamin C in plant tissues. High applications of nitrogen fertilizers tend to reduce the content of vitamin C in many types of fruits and vegetables. Vitamin C from food is subject to degradation during processing and cooking (Gulija, M. 2016).

The aim of the study was to examine the effectiveness of fertilization on vitamin C content in *Bravo F₁ hybrid cabbage (Brassica oleraceae var. Capitata L.)* with the application of different amounts of pyrophyllite (2,200 kg/ha; 1,700 kg/ha; 1,200 kg/ha; 700 kg/ha) combined with a constant amount of mineral fertilizer (800 kg/ha NPK 15:15:15). In this context, it was possible to see whether pyrophyllite and to what extent its functional components and as a factor in various biochemical reactions affect the synthesis and content of vitamin C in cabbage and whether from this aspect its use as an organo-mineral fertilizer is ecologically and economically justified.

MATERIALS AND METHODS

The field experiment was set up on the experimental range of the Federal Institute of Agriculture in 2018. and 2019. by the block method with a random arrangement of plots in four replications. Prior to sowing, basic tillage was performed by plowing to a depth of 25 cm, followed by pre-sowing soil preparation by milling. Each plot was 6 m² (1.4 m wide x 5 m long). On the basic plot, 20 cabbage plants were planted per treatment in two-row strips at a distance of 70 cm between rows and 50 cm in a row. The distance between the plots was 100 cm. The area of the experimental plot was 360 m² with free space between the boards. In the experiment, mineral fertilizer of NPK formulation 15:15:15 was used in the amount of 800 kg/ha combined with different amounts of pyrophyllite: 2,200 kg / ha; 1,700 kg/ha; 1,200 kg/ha; 700 kg/ha. Pyrophyllite was used from the site in Parsovići Konjic in micronized form (granulation + 0 - 2 mm).

The basic properties of the soil on which the experiment was set up were: pH in water 6.96, pH in KCL 5.90; N content 0.17%; CaCO₃ content 0.5%; humus content 2.26%; P₂O₅ 7.65 mg/100g; K₂O 22.30 mg/100g. Soil analysis was performed in the Department of Agropedology of FBiH according to the standards: BAS ISO 10390: 2005, pH, CaCO₃, content N according to BAS ISO 11261: 2000, content P₂O₅ and K₂O Al - method.

During the development of cabbage, standard technological measures were taken. Protection of cabbage plants was carried out on two occasions, preventively. In the first treatment, an insecticide Mospilan was used. In the second treatment, Pužomor was used. Cabbage was preventively treated with the insecticide Decis. All treatments were performed foliar during the evening hours.

Determination of vitamin C in cabbage was performed by HPLC/DAD, using vitamin C-standard, dithiothreitol, potassium hydrogen phosphate. Conditions for instrumental analysis were applied: column RP: C18 150mm x 4.6mm; 5 µm, injection volume: 50 µl, column temperature: 30°C, mobile phase flow: 1 ml/min, UV/VIS detector; 254 nm, run: 12 min. Mobile phase: potassiumdihydrogen phosphate, pH 2.5 with dithiothreitol. The homogenized sample of cabbage was placed in a mixer, weighed to 10 g to which water was added which allows the vitamin C content to be found in the measuring sample. 1 mg of dithiothreitol was added to each ml of filtrate. The sample was centrifuged and allowed to stand for 2 hours and then filtered through a 45 µm membrane filter, then transferred to vials and chromatographed on an instrument, HPLC Agilent 1200 with chemstation software.

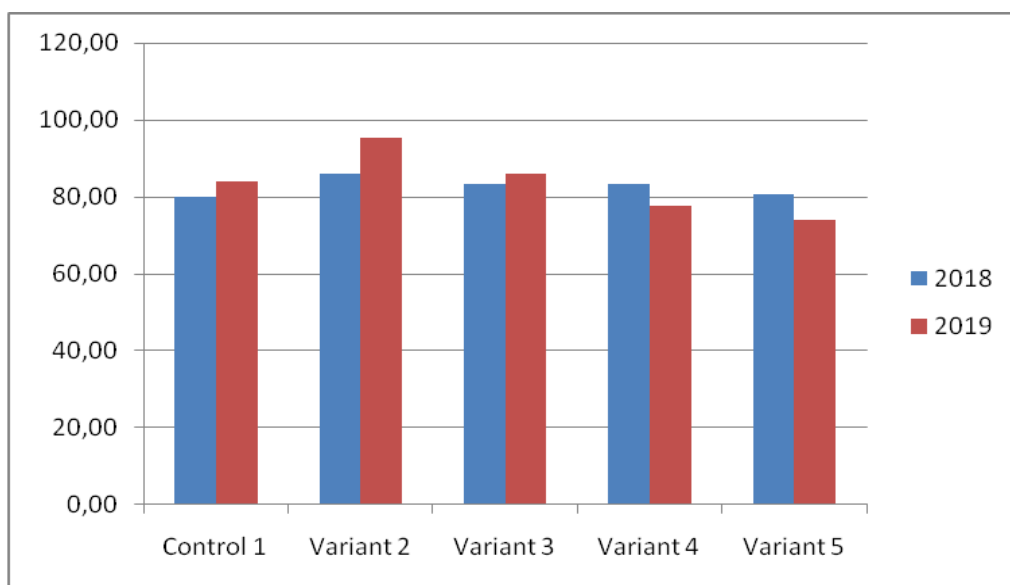
RESULTS AND DISCUSSION

After the examined samples of fresh cabbage leaves of *Bravo F₁ hybrid*, analysis and measurements, the influence of pyrophyllite as a nutrient and biostabilizer on vitamin C content, the data were statistically processed and detailed measurement results and average values of measured parameters are presented in tables and graphs. Vitamin C content in cabbage was measured at technological ripening. Values in the absolute amount ranged from 74.85 mg/100 g of cabbage in 2019. in the variant with application of 700 kg of pyrophyllite/ha up to 95.05 mg/100g of cabbage in variant 2 (with the largest applied amount of pyrophyllite of 2,200 kg/ha). Based on the average value of vitamin C content in cabbage, it can be concluded that the highest value in both years of research had plants in variant number 2 with the highest applied amount of pyrophyllite (2,200 kg/ha of pyrophyllite - 1,540 g/plot of pyrophyllite).

Table 1. Vitamin C content expressed in ml/100 g-1 fresh cabbage mass

Year	Control 1 NPK 15:15:15 - 800 kg/ha	Variant 2 Pyroph . 2,200 kg/ha	Variant 3 Pyroph. 1,700 kg/ha	Variant 4 Pyroph. 1,200 kg/ha	Variant 5 Pyroph. 700 kg/ha
2018.	80.25	86.65	83.33	82.45	80.60
2019.	79.10	95.05	86.30	77.26	74.85

The content of vitamin C of 2018. year in control group (without application of pyrophyllite) was a 80.25 mg/100g of cabbage, while in the variant 2, the highest applied amount pyrophyllite (2.200 kg/ha) was 86.65 mg/100 g cabbage. At he application of smaller amounts of phyrophilite in variants No. 3, 4 and 5, the content of vitamin C was decreasing, with the lowest amount applied pyrophyllite in variant No.5 (700kg/ha) content of C vitamin was 80.60 mg/100g cabbage and did not have statistical significance in relation to the control.



Graph 1. Vitamin C content expressed in mg/100 g-1 of fresh cabbage mass

In 2019, year according to the trends from the previous year, the highest percentage of vitamin C was in variant number 2. with the highest applied amount of pyrophyllite (2,200 kg/ha) was 95.05 mg/100 g of cabbag. Reduction of the amount of pyrophyllite in variants number 3, 4 and 5 the content of C vitamin decreased, so that in the variant number 5 with the lowest amount applied pyrophyllite (700 kg/ha) the content of vitamin C was 74.85 mg/100g of cabbage, which indicates the fact that the application of pyrophyllite in an amount of 700 kg/ha no impact to the content of vitamin C with cabbage.

Table 2. Analysis of variance for total vitamin C content

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	40.1666	5	8.033333	0.437387	0.817143	2.71089
Columns	605.4667	4	151.3667	8.241379	0.000426	2.86608
Error	367.3333	20	18.36667			
Total	1012.967	29				

Analyzing the vitamin C content in the control in 2018. and 2019. in relation to variant 5, which is also the lowest applied dose of pyrophyllite, it can be taken in relationship that, according to these results, the lower limit (dose of application) of pyrophyllite for vitamin C content was determined. with cabbage. The results of vitamin C content at the highest applied dose of pyrophyllite in the comparisons of 2018 and 2019 prove that this is still a relatively new and unexplored effect of pyrophyllite (aluminosilicate) whose application has proven to be very interesting for research in agriculture.

CONCLUSION

On the basis of the two-year research on the influence of pyrophyllite on the content of vitamin C found in cabbage hybrid Bravo F₁ (*Brassica oleraceae* var . *Capitata* L .) and the obtained results, we may conclude that:

- Content of vitamin C demonstrated a significantly different results compared to the application of pyrophyllite. Statistically, the highest significance was in variant number 2 with the highest application of pyrophyllite (2.200 kg of pyrophyllite/ha) compared to the control. Variant No. 5 with the lowest applied amount of pyrophyllite - 700 kg/ha was not statistically significant compared to the control.
- Based on the results obtained by this research, we conclude that for the successful production of cabbage hybrid Bravo F₁, we can reliably recommend the use of pyrophyllite in the amount of 2.200 kg/ha in combination with mineral fertilizer NPK 15:15:15 in the amount of 800 kg/ha.
- The application of pyrophyllite in the amount of 700 kg/ha has no statistical significance in relation to the control (without pyrophyllite) on the results of vitamin C content. The increase in the content of vitamin C in cabbage is proportional to the increased amount of pyrophyllite applied.
- Correlation analysis established a connection which is in all combinations of variants strong, positive and statistically significant, which would mean that the applied treatments of pyrophyllite significantly influenced the content of vitamin C in cabbage hybrids Bravo F₁.

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YIELD OF SOME WHEAT VARIETIES DEPENDING ON FERTILIZATION WITH A COMBINATION OF MINERAL FERTILIZERS AND ZEOLITES

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Abstract: Zeolite improves the structure of the soil and reduces acidity, which is of great significance for agricultural production that happens on soils with low pH values. It has shown exceptional results in improving soil characteristics, thus increasing the yield and quality of cultivated plants. The aim of our study was to determine the yield and some qualitative properties of numerous wheat varieties, depending on the mineral fertilizers and zeolites application. The experiments were performed in 2018/19 and 2019/20, in the area of Southern Serbia (Bojnik). The research involved 4 wheat varieties and 4 variants of fertilization, including mineral fertilizers and zeolite. The combination of mineral fertilizers and zeolites did not show noteworthy differences in the mass of 1000 grains when it comes to both varieties and fertilization variants. Hectolitre weight of grain was significantly higher on the variant with a combination of mineral fertilizers and zeolites than on the control variant. All fertilization variants had a significantly higher grain yield compared to the control variant. The variant on which the combination of mineral fertilizers and a higher dose of zeolite were applied achieved a significantly higher grain yield compared to the variant with mineral fertilizers. Varieties Pobeda and Nikol, with the application of mineral fertilizers and zeolite combination, achieved the highest yields. On acid soils, it is necessary to apply fertilizers and soil improvers so that the yields of cultivated plants will be satisfactory.

Keywords: wheat, NPK fertilizers, zeolite, yield.

INTRODUCTION

Zeolite is well known for having wide applications in agriculture, industry, and environmental protection. Due to its properties (ion-exchange, adsorption, and catalytic), it has shown outstanding results in improving soil characteristics, thus increasing the yield and quality of cultivated plants (Kulasekaran et al, 2011). Zeolite has the ability to prevent the leakage of nutrients from the soil, the capacity to adsorb soil nutrients (fertilizer ingredients), and to release them gradually during the growing season. Also, zeolite improves the structure of the soil and reduces acidity, which is of great significance for agricultural production that happens on soils with low pH values. Zeolite in the soil is a tank of water and nutrients. It improves plant nutrition, develops the root system, intensifies growth and fertility, reduces diseases, and strengthens plant immunity. Zeolite contains the essential elements for development such as magnesium, potassium, and calcium.

Ghanbari and Ariaifar (2013) indicate that natural zeolite can be used to improve soil, thus making it a vital alternative for reducing the effects of drought in arid and semi-arid regions.

According to the research of Oljača et al. (2009), the application of zeolite in combination with mineral fertilizers proved to be very efficient in barley production as it has increased yields. The research of Sushenitsa (2007) claims that zeolite combined with mineral fertilizers improves the agrochemical and agrophysical properties of the soil, shifting the pH value of the soil by 0.34-0.68 units.

Liliya et al. (2020) in the research emphasize the value of zeolite in improving the agrochemical and agrophysical properties of soil, which increases the yield of cultivated plants. Kulasekaran et al. (2011) also claim that zeolite in the soil is not only a trap for heavy metals, but is also a carrier of slow-release fertilizers, thus increasing the yields of cultivated plants. Zeolites are useful and sought after in farming thanks to their high porosity, high cation exchange capacity, and neutralization of chemicals used in plant production (Sangeetha and Baskar, 2016). Aghaalikhani et al. (2011) point out that zeolites increase the yield of many crops, particularly when combined with mineral and organic fertilizers, which may be associated with improved soil structure and nutrients. The soil is one of the most significant environmental factors whose quality has to be preserved (Ghaemi et al., 2014). Therefore, Cairo et al. (2017) emphasize the importance of repairing soil properties by applying zeolite in combination with mineral and organic fertilizers, which also increases the yields of cultivated plants. The main aim of our research was to determine the yield, weight of 1000 grains, and hectolitre weight of several wheat varieties based on the application of mineral fertilizers and zeolites.

MATERIAL AND METHODS OF WORK

The experiments were performed in 2018/19 and 2019/20, in the area of Southern Serbia (Bojnik). Four wheat varieties were involved: Nikol, Avenue and Sosthene (LG France), and Pobeda, and 4 variants of fertilization (1.Control, 2. NPK, 3. NPK + 300 kg ha⁻¹ zeolite, and 4. NPK + 500 kg ha⁻¹ zeolite). Of the zeolites, Zeo Min was used, whose chemical composition is the following: calcium (CaO) 41%; magnesium (MgO) 17%; iron (Fe₂O₃) 1.63%; zinc (Zn) 0.43%; copper (Cu) 0.25%; manganese (Mn) 0.35% and silicon (SiO) 12.8%.

The experiment was set up according to the block system in three repetitions. Corn was the preculture. The cultivation of the soil included plating in two passages where 200 kg ha⁻¹ NPK (16:16:16) fertilizer was applied. Together with NPK fertilizers, zeolite was dispersed (included) in appropriate quantities. The 2018/19 sowing was done on December 8th, and 2019/20 on October 30th. Before setting the experiment, soil samples were taken from the parcels for chemical analysis. Fertilization with KAN fertilizer in the amount of 200 kg ha⁻¹ was done in March, and treatment against weeds and diseases was performed in April with Metmark WG in the dose of 0.01% and Excor in the amount of 0.5 l/ha. The harvest was done in the phase of full maturity. The yield was calculated on each plot and reduced to 14% grain moisture. The results were statistically processed, through the analysis of variance using WASP 1.0 software and they are tabulated.

Climatic and soil characteristics

Tables 1 and 2 show the full amount of precipitation and average air temperatures during the vegetation period and the chemical analysis of the soil.

Table 1. Precipitation (mm) and mean air-temperature (°C) in Leskovac.

Mounth	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Oct/Jun
The 2018/2019 growing season										
Mm	5.0	57	19	58	55.5	8.0	38	99	68	407.5
°C	12.9	7.3	4.4	-0.3	3.7	9.3	13.8	15.5	22.2	9.9
The 2019/2020 growing season										
Mm	8	43	53	15	65	100	47	69	121	521
°C	14.5	11.9	4.6	0.7	6	8.20	11.6	16.6	19.6	10.4

During 2018/2019, the year which was unfavorable for wheat production, the total amount of precipitation during the vegetation period was small and amounted to 407.5 mm, while the average temperature was 9.9 °C. The dry period which took place during autumn throughout this vegetation year has to be emphasized, as a total of 83 mm of precipitation that fell in October, November, and December was insufficient to prepare the land for sowing. Thus, that year's sowing was not done until the beginning of December. In addition, average negative temperatures during the winter were recorded, which all resulted in a decrease in wheat grain yield that year. During 2019/2020, the total amount of precipitation during the vegetation was 521 mm, which is 113.5 mm more than in the preceding season. Throughout this vegetation year, it is essential to point out the larger total amount of precipitation, which was quite well distributed, especially in the spring part of the vegetation. Average temperatures during the vegetation were 10.4 °C. No negative average monthly temperatures were recorded in the winter months, which had a positive effect on wheat production.

Table 2. Chemical properties of the soil.

Type of soil	pH		Humus (%)	Nitrogen (%)	Available (mg/100g of soil)	
	H ₂ O	KCl			P ₂ O ₅	K ₂ O
Smonica	5.88	4.9	2.64	0.18	6.4	19.0

Soil acidity was determined using the Kappen method, humus was determined by the Kotzman method, total nitrogen by the Kjeldahl method, and available phosphorus and potassium by the Engner-Riehm Al method. According to the pH values in KCl (4.90), Vertisol belongs to the group of acid soils. When the content of humus in the arable layer is in question, they belong to the group of weakly humus soils (according to Gračanin and Škorić). According to the content of total nitrogen, Vertisol belongs to the group with a moderate amount of it. Phosphorus content (6.4) classifies this soil in the group of poorly supplied, and in terms of potassium content (19.00) in the group of medium provided. From these data, it can be concluded that Vertisol has limited production characteristics, especially in terms of acidity and low phosphorus content, which should be corrected by appropriate agro-ameliorative measures so that successful production is reached.

RESEARCH RESULTS AND DISCUSSIONS

Wheat grain yield and some of its qualitative characteristics such as weight of 1000 grains and hectolitre weight are all conditioned by a number of

environmental factors, applied agrotechnical, and variety selection. Fertilization is one of the very important agro-technical measures, which determines the wheat grain yield to a good extent. In table 3, the yield and some qualitative properties of wheat grains depending on the application of mineral fertilizers and zeolites are presented.

Table 3. Yield and some qualitative properties of wheat grains depending on fertilization and year

No.	A.Varieties	1000 grains weight (g)				Hectoliter weight (kg hl ⁻¹)				Grain yield (kg ha ⁻¹)			
		A. Fertilisers											
		The 2018/2019 growing season											
		1	2	3	4	1	2	3	4	1	2	3	4
1.	Pobeda	39.0	40.0	41.0	41.0	62.9	67.1	69.8	70.6	2.55	3.89	4.40	4.61
2.	Avenue	38.0	39.5	40.0	40.5	60.0	66.5	69.5	69.5	2.87	3.64	4.00	3.88
3.	Sosthene	38.5	39.0	40.0	40.0	61.5	67.5	68.5	68.0	2.33	3.72	3.85	3.90
4.	Nikol	38.5	39.0	40.0	40.0	59.6	66.8	69.9	68.5	2.48	3.56	4.00	4.05
Average		38.5	39.4	40.2	40.4	61.0	66.9	69.4	69.1	2.56	3.70	4.06	4.11
The 2019/2020 growing season													
1.	Pobeda	39.5	41.2	41.0	43.0	62.9	68.1	69.8	71.8	2.80	4.80	5.10	5.31
2.	Avenue	39.2	41.0	40.8	42.2	60.0	68.5	69.5	70.5	2.87	4.44	4.65	4.80
3.	Sosthene	38.8	40.5	41.6	41.0	63.5	67.7	68.8	69.0	2.77	4.42	4.50	4.55
4.	Nikol	39.7	41.0	42.5	43.0	63.9	69.8	70.1	70.5	3.08	4.76	4.90	5.08
Average		39.3	40.9	41.5	42.3	62.6	68.5	69.5	70.4	2.88	4.60	4.79	4.93

1. control; 2.NPK; 3.NPK+300 kg ha⁻¹ zeolite; 4.NPK+500 kg ha⁻¹ zeolite

The data in the table shows that the yield and the weight of 1000 grains and hectolitre weight in the 2019/2020 season were higher in all varieties than in the preceding season. These differences at 1000 grain weight and hectoliter weight are less obvious than at grain yield. Thus, the average grain yield for all varieties, in the 2019/2020 season, depending on the combination of mineral

fertilizers and zeolites, was higher by 730 to 900 kg ha⁻¹ than in the 2018/2019 season. Pobeda achieved the highest yield in both seasons (5.31 and 4.61 t ha⁻¹), while Avenue achieved the lowest (3.88 t ha⁻¹ and 4.55 t ha⁻¹). Besides Pobeda, Nikol proved to be a yielding variety (4.05 and 5.08 t ha⁻¹) in both seasons, thus making it an interesting variety for these areas. The main reason for the better results of the mentioned parameters in the 2019/2020 season is the more favorable agroclimatic conditions during this year. It should be pointed out that due to the dry period in the fall of 2018/2019, sowing was delayed and was not done until December 8th, which was reflected in the reduction of the yield of wheat varieties. Thus, the average decrease in yield was 16.6%. Nikol had the highest percentage of yield reduction of 20.2%. Many researchers emphasize the importance of the optimal sowing date, due to the fact that late sowing can significantly reduce yields, which was actually the case in the 2018/2019 season. So Ácín et al. (2016) point out that sowing in December reduces the wheat yield by 20% compared to sowing in the optimal time (first decade of October). Our results are consistent with the results of many authors (Iqbal et al., 2001; Shah et al., 2006; Qasim et al., 2008; Gupta et al., 2017; Yadav et al., 2018), who points out a significant reduction in wheat yield of 27 to 58% when sown outside the optimal period.

The following table shows the average two-year results that were processed by variance analysis.

Table 4. Yield and some qualitative properties of wheat grains depending on fertilization (2018/2020)

No.	A. Varieties	1000 grains weight (g)					Hectoliter weight (kg hl ⁻¹)					Grain yield (kg ha ⁻¹)				
		A. Fertilisers														
		1	2	3	4	Aver age	1	2	3	4	Aver age	1	2	3	4	Ave.
1.	Pobeda	39.2	40.6	41.0	42.0	40.7	62.9	67.6	69.8	71.2	67.8	2.67	4.34	4.75	4.96	4.18
2.	Avenue	38.6	39.5	40.4	41.3	39.9	60.0	67.5	69.5	70.0	66.7	2.87	4.04	4.32	4.34	3.89
3.	Sosthene	38.6	39.7	40.8	40.5	39.9	62.5	67.6	68.6	68.5	66.8	2.55	4.07	4.17	4.22	3.75
4.	Nikol	39.1	40.0	41.2	41.5	40.4	61.7	68.3	70.0	69.5	67.4	2.78	4.16	4.45	4.56	3.98
Average		38.9	40.1	40.8	41.3	40.2	61.8	67.7	69.4	69.7	67.2	2.72	4.15	4.42	4.52	3.95
LSD			A	B	AxB			A	B	AxB			A	B	AxB	
		5 %	4.7	4.7	9.4			7.4	7.4	14.8			0.33	0.33	0.67	
		1 %	6.33	6.33	12.6			9.9	9.9	19.9			0.45	0.45	0.90	

1. control; 2.NPK; 3.NPK+300 kg ha⁻¹ zeolite; 4.NPK+500 kg ha⁻¹ zeolite

1000 grains weight. Grain yield depends on this property to a large extent. The

average weight of 1000 grains for all fertilization variants was 40.2 g. The lowest was in Avenue and Sosthene varieties (39.9 g) and the highest in the variety Pobeda (40.7 g). Differences between varieties were not statistically significant. The average 1000 grains weight for all varieties ranged from 38.9 g on the control to 41.3 g on the variety where a combination of NPK fertilizer and a higher dose of zeolite were applied. No statistically significant differences were found between the fertilization variants and the control variant. Pobeda had the highest 1000 grains weight (42.0 g) which was grown in a combination of mineral fertilizers and a higher dose of zeolite, while Avenue and Sosthene varieties on the control variant had the lowest weight (38.6 g).

Hectolitre weight. This is a very important characteristic when buying wheat and it is an indicator of grain size and fullness. The average hectoliter weight for all fertilization variants was 67.2 kg. Avenue had the smallest one (66.7 kg) while Pobeda had the largest (67.8 kg), although the differences between the varieties were not statistically significant. The average hectolitre weight for all varieties ranged from 61.8 kg in the control variant to 69.7 kg in the variant where a combination of mineral fertilizers and a higher dose of zeolite were applied. The hectoliter grain weight on the variant with a combination of mineral fertilizers and a higher dose of zeolite was statistically significantly higher than on the control variant, while no statistically significant differences between the fertilization variants were observed. Pobeda which was grown in a combination of mineral fertilizers and a higher dose of zeolite had the highest hectoliter weight (71.2 kg) while the variety Avenue on the control variant had the lowest (60.0 kg).

Grain yield. That is the goal that producers strive for. It depends on a number of agroecological factors, applied agrotechnics, and variety selection. The average yield for all fertilization variants was 3.95 t ha⁻¹. Sosthene had the smallest one (3.75 t ha⁻¹) while Pobeda had the largest (4.18 t ha⁻¹) and this difference was statistically significant. The average yield for all sorts ranged from 2.72 t ha⁻¹ on the control to 4.52 t ha⁻¹ on the variant where a combination of NPK fertilizer and a higher dose of zeolite were applied. All fertilization variants had a significantly higher yield compared to the control. The variant on which the combination of mineral fertilizers and a higher dose of zeolite were applied achieved a statistically significantly higher grain yield compared to the variant with mineral fertilizers. Pobeda which was grown in a combination of mineral fertilizers and a higher dose of zeolite achieved the highest average grain yield (4.96 t ha⁻¹) while Sosthene on the control variant achieved the lowest (2.55 t ha⁻¹). The Nikol variety proved to be profitable and stable, as well as Pobeda, so we recommend them for growing on acid soils in the South of Serbia. It can be concluded that there are no good yields without

the use of fertilizers. The application of zeolite, especially the variant with increased content (NPK + 500 kg ha⁻¹ zeolite) in combination with mineral fertilizers increased the yields compared to only mineral fertilizers, by an average of 370 kg ha⁻¹. Our results are consistent with the results reported by Liliya et al. (2020) where they emphasize that the application of zeolite with mineral fertilizers increases the yield of cultivated plants by 0.3 to 0.5 t ha⁻¹. Similar results of the effect of zeolite in combination with mineral fertilizers on barley yield of 4.6 t ha⁻¹ are pointed out by Oljača et al. (2009). Being the production of wheat on heavy and acid soils is unstable, it is necessary to repair and enrich the soil using zeolite or some other improvers. Zeolites are not an expensive material, but can significantly improve the production properties of acid soils and stabilize the yields of cultivated plants on them, especially in years that are unfavorable for production.

On the other hand, in the coming period, Serbia will not be able to export any agricultural product without a certificate of land quality as a critical place in primary agricultural production. These are the conditions of the concept of “HACCP” (Hazard Analysis Critical Control Point) and “GAP” (Good Agricultural Practices) for the production of safe food (Grubišić, 2017). This is the case where zeolites can find their place as heavy metal cleaners from the soil.

CONCLUSION

Based on the research results, we can conclude the following:

- The combination of mineral fertilizers and zeolites did not show noteworthy differences in the mass of 1000 grains when it comes to both varieties and fertilization variants.
- Hectolitre weight of grain was significantly higher on the variant with a combination of mineral fertilizers and zeolites than on the control variant.
- All fertilization variants had a very significantly higher yield when compared to the control.
- The variant on which the combination of mineral fertilizers and zeolites was applied achieved a significantly higher grain yield than the variant with mineral fertilizers.
- The application of zeolite combined with mineral fertilizers increased the wheat yield compared to mineral fertilizers alone, by an average of 370 kg ha⁻¹.
- Varieties Pobeda and Nikol, with the application of mineral fertilizers and zeolite combination, achieved the highest yields.
- On acid soils, it is necessary to apply fertilizers and soil improvers so that the yields of cultivated plants will be satisfactory.
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INFLUENCE OF ACID SOIL FERTILIZATION ON THE YIELD OF SOME WHEAT VARIETIES

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Abstract: Acidic soils have poor water-air and physical-mechanical properties and represent a serious problem in plant production, so plant production is unstable on them. This study aimed to examine the effect of fertilization with mineral, lime, and organic fertilizers on the yield of several wheat varieties on acid reaction soil. The research was conducted in the vicinity of Kraljevo during 2016/17 and 2017/18. In the experiment, in addition to three wheat varieties (Renesansa, Pobeda, and NS 40 S) and 5 variants of fertilization of various combinations were included. Thus, the average wheat yields in 2018 were higher by 218.9 kg ha⁻¹ to 315.7 kg ha⁻¹ than in 2017. The average yield for all wheat varieties ranged from 1877.8 kg ha⁻¹ in the control to 3538.2 kg ha⁻¹ in the variant where a combination of mineral, lime, and organic fertilizers was applied. The application of fertilizers increased the average wheat yields from 895.1 kg ha⁻¹ to 1,660.4 kg ha⁻¹, which is a statistically very significant increase. The highest average yield (3659.9 kg ha⁻¹) was achieved by the Renesansa variety on the variant where a combination of mineral, lime, and organic fertilizers was applied. Therefore, in addition to mineral fertilizers, the use of ameliorative materials, such as lime and manure, is recommended if a satisfactory yield is to be achieved on such soils.

Keywords: mineral fertilizers, lime, manure, wheat, yield.

INTRODUCTION

Acidic soils have poor water-air and physical-mechanical properties and represent a serious problem in plant production, so plant production is unstable on them.

According to some data, over 60% of the land in Serbia is acidic. However, according to the report of the Ministry of Environment and Physical Planning of the Republic of Serbia (2009), it is stated that the percentage of acid soils in central Serbia was 52.1%, with an additional 29.2% of slightly acid soils (Deletić et al., 2010).

Liebig et al. (2018) point out that acidification must be stopped because there may be an irreversible change in soil properties, which can result in the unavailability of nutrients and the appearance of toxic metals. The appearance of toxic metals, primarily Al, and the reduced availability of some elements such as P, Mo, and B are the main reasons for the poor fertility of acid soils (Narro et al. 2001 and Sumner 2004).

Small grains are very sensitive to soil acidity, although some genotypes show different tolerances. Therefore, there is a need to grow wheat and other cereals on acid soils to isolate genotypes adapted to acid soils and different climatic conditions (Jelić et al., 2016).

Numerous studies in our country and the world indicate that the combined application of organic and mineral fertilizers is the most efficient way to eliminate unfavorable production properties of acid soils, which multiplies the yields of cultivated plants (Sharma et al., 2003; Jelić et al., 2006; Bodruzzaman et al., 2010; Biberdžić et al., 2015; Holik et al., 2018; Aula et al., 2019).

This study aimed to examine the effect of fertilization with mineral, lime, and organic fertilizers on the yield of several wheat varieties on the acid reaction of the soil.

MATERIAL AND METHODS OF WORK

Pseudogley in the Čačak-Kraljevo valley covers an area of 32.000 ha and is one of the most typical regions of its distribution in Serbia (Dugalić, 1998).

The research was conducted in the vicinity of Kraljevo during 2016/17 and 2017/18. In the experiment, in addition to three varieties of wheat (Renesansa, Pobeda, and NS 40 S), 5 variants of fertilization of various combinations were included (1. Control; 2. N 300 kg ha⁻¹; 3. NPK 300 kg ha⁻¹; 4. NPK 300 kg ha⁻¹ + 2.4 t ha⁻¹ lime and 5. NPK 300 kg ha⁻¹ + 2.4 t ha⁻¹ lime + 3 t ha⁻¹ manure). From mineral fertilizers, NPK fertilizers were represented, from lime CaCO₃ (lime) and organic manures.

The experiment was set up according to the block system in three repetitions. Before the basic tillage, mineral, lime, and organic fertilizers were applied, which were spread on the surface, and then inplowed. The other half of nitrogen fertilizers were applied in additional nutrition in the form of ammonium nitrate. The basic tillage was done classically, up to 25 cm deep. Sowing was done in October. The harvest was done in the phase of full maturity, and the yield was adjusted to 14% moisture. The results were processed statistically, by analysis of variance, using WASP 1.0 software. and presented tabularly and graphically.

Soil and climatic conditions

The following tables (1 and 2) give an overview of the chemical properties of the soil and the climatic conditions of the area.

Table 1. Chemical properties of the soil

Depth (cm)	pH		Humus	Available (mg/100g of soil)	
	H ₂ O	nKCl		P ₂ O ₅	K ₂ O
0-20	5.24	4.34	0.12	6.70	7.80
20-40	5.55	4.48	0.05	6.90	9.80

The soil at the Kraljevo location belongs to the pseudogley soil type. This soil has very bad physical properties (compacted, having a high content of silt and clay particles, with slow water percolation) and an extremely acid pH value (pH<4.5). Its total humus content is relatively fair, but microbiological activity is low, because of its poor physical properties. That causes low mineralization of organic nitrogen, and so nitrogen fertilizers show a great effect on such soils. It is characterized by low content of available phosphorus (6.70-6.90 mg/100g of soil) and potassium (7.80-9.80 mg/100g of soil).

Table. 2. Meteorological conditions during the conduct of an experiment (2016-2018)

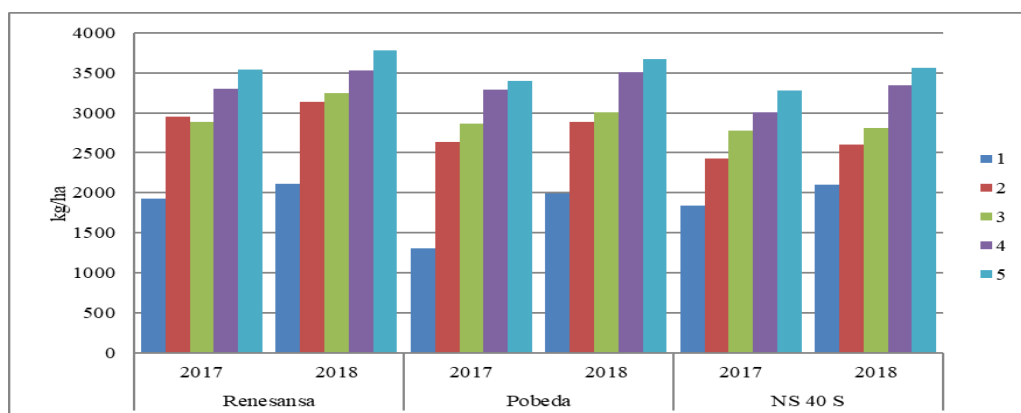
Months	The Average monthly air temperature (°C)		Monthly sum of precipitation (mm)	
	2016/17	2017/18	2016/17	2017/18
X	7.6	11.9	84.1	133.3
XI	6.0	6.8	77.6	32.4
XII	5.8	4.0	9.4	55.6
I	-5.0	2.7	22.1	51.0
II	4.5	2.0	35.3	80.9
III	10.3	6.5	57.7	111.2
IV	11.3	16.6	82.1	40.6
V	16.2	19.1	99.9	84.4
VI	22.4	20.9	56.2	169.3
Average Sum	8.8	10.0	524.4	758.7

Average monthly air temperatures in 2016/17 were slightly lower, especially in January (-5.0°C) than in 2017/18. However, in both years, temperatures were within optimal limits and had no negative effects on yield. The total amount of precipitation in the vegetation in 2016/17 was 524.4 mm and in 2017-18 758.7 mm. Higher precipitation and their better distribution in 2017/18, especially in October, December, March, and June, contributed to better fertilizer decomposition and higher wheat grain yield.

RESEARCH RESULTS

Grain yield is a complex trait that depends on many hereditary traits and environmental conditions in which the plant develops (Madić et al., 2005). The following tables show the yield of several varieties of wheat depending on the year (graph. 1) and fertilization of acid soils (tab. 3).

The average yield of wheat varieties for both years ranged from 1877.8 kg ha^{-1} in the control to 3538.2 kg ha^{-1} in variant 5. Fertilizer application increased the average wheat yields from 895.1 kg ha^{-1} to 1,660.4 kg ha^{-1} . The influence of the year on the average wheat yield was pronounced. Thus, the average wheat yields in 2018 were higher by 218.9 kg ha^{-1} to 315.7 kg ha^{-1} than in 2017, which can be attributed to more favorable climatic conditions in 2018. The smallest increase in yield was in the cultivar NS 40 S (218.9 kg ha^{-1}) and the largest in the cultivar Pobeda (315.7 kg ha^{-1}). The data of Deletić et al. (2020) also speak about the influence of favorable climatic conditions of the year on the increase of wheat yield.



1. Control; 2. N 300 kg ha^{-1} ; 3. NPK 300 kg ha^{-1} ; 4. NPK 300 kg ha^{-1} + lime 2.4 t ha^{-1} ; 5. NPK 300 kg ha^{-1} + lime 2.4 t ha^{-1} + manure 3 t ha^{-1}

Graph.1. The yield (kg ha^{-1}) of wheat depending on the year and fertilization of acid soils

Table 3. The yield (kg ha⁻¹) of wheat depending on the fertilization of acid soils (2017 -2018)

A. Fertilization	B. Wheat (variety)			Average A
	Renesansa	Pobeda	NS 40 S	
1.Control	2020.80	1644.10	1968.70	1877.90
2.N 300 kg ha ⁻¹	3043.80	2759.30	2515.70	2772.90
3.NPK 300 kg ha ⁻¹	3068.10	2935.30	2796.60	2933.30
4.NPK 300 kg ha ⁻¹ +lime 2.4 t ha ⁻¹	3412.70	3399.60	3178.40	3330.30
5.NPK 300 kg ha ⁻¹ +lime 2.4 t ha ⁻¹ +manure 3 t ha ⁻¹	3659.90	3533.50	3421.30	3538.20
Average B	3041.10	2854.40	2776.10	2890.50
LSD	A	B	A x B	
5 %	535.90	415.10	928.20	
1%	723.10	560.10	1252.30	

Fertilization variants had a greater influence on wheat yield than varieties. The average yield for all wheat varieties ranged from 1877.8 kg ha⁻¹ in control to 3538.2 kg in variant 5. Fertilizer application increased the average wheat yields from 895.1 kg ha⁻¹ to 1,660.4 kg ha⁻¹, which is a statistically very significant increase. The average yield of all wheat cultivars on variant 5 was significantly higher than on variant 2 and 3, while between variants 2 and 3, 3 and 4 and 4 and 5 there was no significant increase in yield. The highest average yield for all variants of fertilization was in the cultivar Renesansa (3041.1 kg ha⁻¹), then in the cultivar Pobeda (2854.4 kg ha⁻¹), and the lowest in the cultivar NS 40 S (2776.1 kg ha⁻¹). However, these differences were not statistically significant. All varieties significantly increased the yield compared to the control with the application of fertilizers. Fertilization variants also had a significant effect on the average wheat yield. Thus, in all cultivars, the highest average yield was achieved in variant 5 and it was significantly higher than the yield in variants 2 and 3, while between variants 4 and 5 and 2 and 3 there was no statistically significant increase in yield. The highest average yield (3659.9 kg ha⁻¹), on variant 5, was achieved by the variety Renaissance, and the lowest

(1644.1 kg ha⁻¹) on the variety Pobeda on variant 1. Since these are acid soils, the use of fields and manure in combination with mineral fertilizers led to a significant increase in the yield of wheat varieties. Therefore, in addition to mineral fertilizers, the use of ameliorative materials, such as lime and manure, is recommended if a satisfactory yield is to be achieved on such soils. Also, the Renesansa and Pobeda varieties are recommended, although a larger number of genotypes should be included in further research, to single out the most productive ones.

Agegnehu et al. (2014) point out that the application of manure with nitrogen fertilizers increases the average wheat yield by 151 and 129%, respectively, compared to the control, and by 85 and 68%, compared with the treatment of farmers, with which our results agree.

Holik et al. (2018) in their research, using a combination of NPK fertilizers and lime, obtained a wheat yield of 3100 kg ha⁻¹, while with a combination of NPK fertilizers and manure they obtained a yield of 3300 kg ha⁻¹ wheat grains. Our results are similar to the above data.

Many researchers (Jelić et al., 2004; Biberdžić et al., 2014; Agegnehu et al., 2014; Holik et al., 2018; Aula et al., 2019) emphasize the effect of applying a combination of mineral, lime, and organic fertilizers on the increased yield of cultivated plants, with which our results are in agreement.

CONCLUSION

The impact of the year on the average wheat yield was pronounced so that the average wheat yields in 2018 were higher than in 2017. Fertilization variants had a greater influence on wheat yield than varieties. The application of fertilizers significantly increased the average wheat yields. Thus, in all cultivars, the highest average yield was achieved on the variant where a combination of mineral, lime, and organic fertilizers was applied, and it was very significantly higher than the yield on the variants where only mineral fertilizers were applied. Since these are acid soils, the application of limes and manures in combination with mineral fertilizers has led to a significant increase in the yield of wheat varieties. Therefore, in addition to mineral fertilizers, the use of ameliorative materials, such as lime and manure, is recommended if a satisfactory yield is to be achieved on such soils. Also, the Renesansa and Pobeda varieties are recommended, although a larger number of genotypes should be included in further research, to single out the most productive ones.

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RESULTS OF TESTING THE QUALITY OF WORK OF POTATO HARVESTING UNITS IN THE CONDITIONS OF TOPLICA DISTRICT

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Abstract: *Potato lifting is the final stage in the production process of this crop, where the goal is to achieve the lowest possible losses and mechanical damages to the tubers that rise from the mounds, bearing in mind that the tubers also raise a large quantity of soil and stones that separate during separation on working parts. For picking potatoes in Serbia, different types of lifters are most often used, and to a fewer extent, combines. The paper analyzes the results of testing the quality of work of different aggregates for potato digging in the production conditions of the observed area. These tests aimed to determine the effects of different types of aggregates for potato harvesting, as well as which of the applied concepts achieves the most efficient and highest quality potato lifting out with the best use of technical and technological characteristics, depending on defined parameters. Based on the results of the research, it was concluded that the lowest content of lifting out the potatoes was when digging with the variant Type C and amounted to 84.97%, and the highest in the amount of 90.69% when lifting potatoes with the variant Type - A. The lowest content re-buried with soil mostly smaller fractions of potatoes were recorded during the lifting of the variant Type - C and amounted to 6.22%, and the highest during the lifting of potatoes with the variant Type - B and amounted to 8.94%. The highest total losses of potatoes were measured during digging out with aggregate Type - C and amounted to 15.03%, and the lowest while lifting with variant Type - A and amounted to 9.31% of the achieved yield. The change in the operating mode and the increase in the moving speed had a significant impact on the quality of work and the increase in the total losses of the potato lifting unit.*

Keywords: *potato harvesting, potato lifter, quality of work, losses, potatoes.*

INTRODUCTION

Potato is one of the most important vegetable species with high biological and nutritional value. In addition to the high content of starch, which gives energy and nutritional value, potatoes are also rich in vitamins A, C, B1, B2, and B5. In 2019, over 39,998 ha were planted under potatoes in the Republic of Serbia, with an average yield of 20.6 t ha⁻¹. The highest yield was achieved in Vojvodina 25.3 t ha⁻¹ and the lowest in the region of Southern and Eastern Serbia 16.7 t ha⁻¹ (Republic Statistical Office (Stat. Yearb. Serb. 2020). Potato tubers are harvested in the physiological phase maturity in the lowland areas in July, August, and September, and in the hilly areas in September and October. Potato harvesting is the final stage in the production process of this crop. For harvesting potatoes in Serbia, potato diggers are most often used, and to a lesser extent, harvesters. During the process of digging out the potatoes, the goal is to achieve the lowest possible losses and mechanical damage to the tubers that rise from the mounds, bearing in mind that the tubers also raise a large amount of earth and stones that separate during operation on the working organs. The main task of the technique and technology used in potato production is to reduce losses during harvesting and minimal damage to tubers (Budyn et al., 2002; Kielbasa, 2006). Based on the analysis of the technical and economic performance of machines used in potato harvesting, it can be concluded that they to some extent meet most requirements, with high weight and large overall dimensions (Dumych & Salo, 2013; Hevko et al., 2016). The quality of work of potato diggers during potato harvesting is very important having in mind the losses that occur during potato lift out, as well as the fact that most of the mechanical injuries occur during mechanized lift out of tubers from different parts of the digger. Losses during the lifting out of the potato tubers are reflected through the non-dug, dug, re-buried and tubers that are left on the surface. The damages that occur are grouped into incut, cut, and crushed tubers. Bearing in mind that this is a large-scale job, successful mechanized harvesting is possible only if the potato lifters are aggregated with tractors of sufficient power. When harvesting potatoes with the Effect VK 2 lifter, which was in the unit with a 42 kW tractor, the total losses varied in the range of 12.50 - 14.23% concerning the achieved yield (Mitrović et al., 2000). The moving speed during the operation of the lifter must be well harmonized with the situation on the plot and the speed of the separation elevators because at high speeds there is significant damage and crushing of tubers that reach over 23.24% (Samy et al., 2006). Lower speeds of the lifters, correctly adjusted number of revolutions of the oscillating elements and the angle of attack of the digging knives significantly affect the overall damage of potato tubers (Azizi et al., 2014). At a higher speed of operation of the separation organs of the lifter, the intensity of tuber damage decreases in the conditions of moist soil and increased flow of soil through the lifter (Bentini et al., 2006). When harvesting

potatoes with a single-row lifter, the losses on the soil surface varied in the range of 0.89 - 1.71% of the yield, while the losses of smaller fractions of tubers not removed and buried with soil were in the range of 0.78 - 2.77% of the yield. (Koprivica et al., 2007). The highest percentage of mechanical damage of 4% when removing potatoes was recorded at a speed of 2.6 km h⁻¹ and an angle of inclination of 12⁰, while the lowest percentage was recorded at a speed of 1.6 km h⁻¹ and an angle of inclination of 24⁰ (Ibrahim et al., 2008). When harvesting potato tubers with a harvester with a belt conveyor, the least damage was recorded at a speed of 3.1 km h⁻¹ and a chain's speed of 90 min⁻¹ (Khater, 2009). Potato tuber damage during the mechanized harvest depends on many factors among which the most critical are the design of the potato harvesters (Aniket et al., 2017), the material of the harvester's work tools, and their modes of operation (Aksenov et al., 2016). Damage to tubers on existing potato lifting machines occurs as a result of collisions of tubers with each other, then on the working elements of lifters and combines, as well as with lumps of earth with which they come into contact (Siberev et al., 2019). In the process of harvesting potatoes, losses must be as low as possible with minimal damage, which is achieved only by using high-performance harvesting techniques, and the result of their application is reflected in achieving a satisfactory product price (Frančak & Korenko, 2012). Increasing the speed of the potato lifting unit increases the percentage of surface damaged and broken tubers, which leads to an increase in total tuber damage, damage index, and total potato losses (Barać et al., 2014; Mordi & Dosary, 2016). When removing potatoes with a harvester in a tractor unit, 1.5% were crushed tubers, 1.5% were cut and 2-3% were damaged with an effect of 0.20 - 0.30 ha h⁻¹ (Kumari & Tripath, 2017). The potato damage is about 1-2% by use of digger cum elevator is comparatively lesser than by use of desi plow and manual lifting by spade and khurpi. It results in a 4-5% reduction in harvesting losses (Pramod et al., 2018).

These tests aimed to determine the effects of different aggregates for potato harvesting, as well as which of the applied concepts achieves the most efficient harvesting of potatoes with the best use of technical and technological characteristics, depending on the defined parameters).

MATERIAL AND METHODS

During 2019, in the production conditions of the Toplica district (43°14'36.9" N 21°38'16.9" E), tests of the quality of work of various aggregates during potato harvest were performed. Agromet Z 609/2 (type A), Bure-König J64F (type B), and Effect VK-2 (type C) lifters were tested. The previous crop, there was winter wheat, the planting of potatoes was done mechanized, and the variety Desire was used for planting. The lifters are aggregated for tractors of 41, 29.5, and 48 kW. In the first phase of testing, the

test conditions were determined, and in the second phase, the quality of work of potato lifters was determined, depending on the defined parameters. The quality of work was assessed based on analysis of samples from five passes, ie repetitions, and included the content of Lifted tubers, Un-lifted tubers, re-buried, superficially scratched tubers, and total potato losses. Losses during the operation of the lifters were determined after the passage of the unit, subsequent lifting, and collection of tubers that the lifters buried during operation. Total losses are presented as the sum of unlifted tubers, buried, and superficially scratched tubers. The achieved average yields of potatoes were 12,132 kg ha⁻¹ for the Typ A extractor, 11,950 kg ha⁻¹ for the Typ B extractor, and 11,540 kg ha⁻¹ for the Typ C lifter, used in similar production conditions. For the tests, standard methods were applied, which refer to field-laboratory and exploitation tests of potato lifters. The applied methods are adjusted to the production conditions in the areas in which the tests were performed and supplemented with the necessary assessments. The obtained results were processed and presented graphically. Technical data of the tested potato harvesters are shown in Table 1.

Table 1. Technical characteristics of tested aggregates for potato harvest

Parameters		Type of potato lifter		
		Agromet	Bure-König	Efekta
		Z 609/2 A	J64F B	VK-2 C
Number of rows	/	2	2	2
Working width	[mm]	605 - 675	600 -700	600 -700
Digger height	[mm]	1050	1000	1030
Digger mass	[kg]	730	480	900
Working depth	[mm]	100 - 200	150	100 - 220
Digger length	[mm]	4570	1500	3500
Digger width	[mm]	1650	1600	1740
Required power	[kW]	37	25	35
Rpm	[min ⁻¹]	540	540	540
Working speed (up to)	[km h ⁻¹]	6	5	5
Productivity (up to)	[ha h ⁻¹]	0,4	0,9	0,8

RESULTS AND DISCUSSION

Figures 1-5 shows the results of the evaluation of the quality of work of the tested aggregates for potato lifter, depending on the defined parameters. Based on the presented results, it can be noticed that during the lifting of potatoes the tested lifters achieved different quality of work as well as the values of realized losses, which means that the defined parameters had a significant impact on the effects of tested aggregates for potato harvesting.

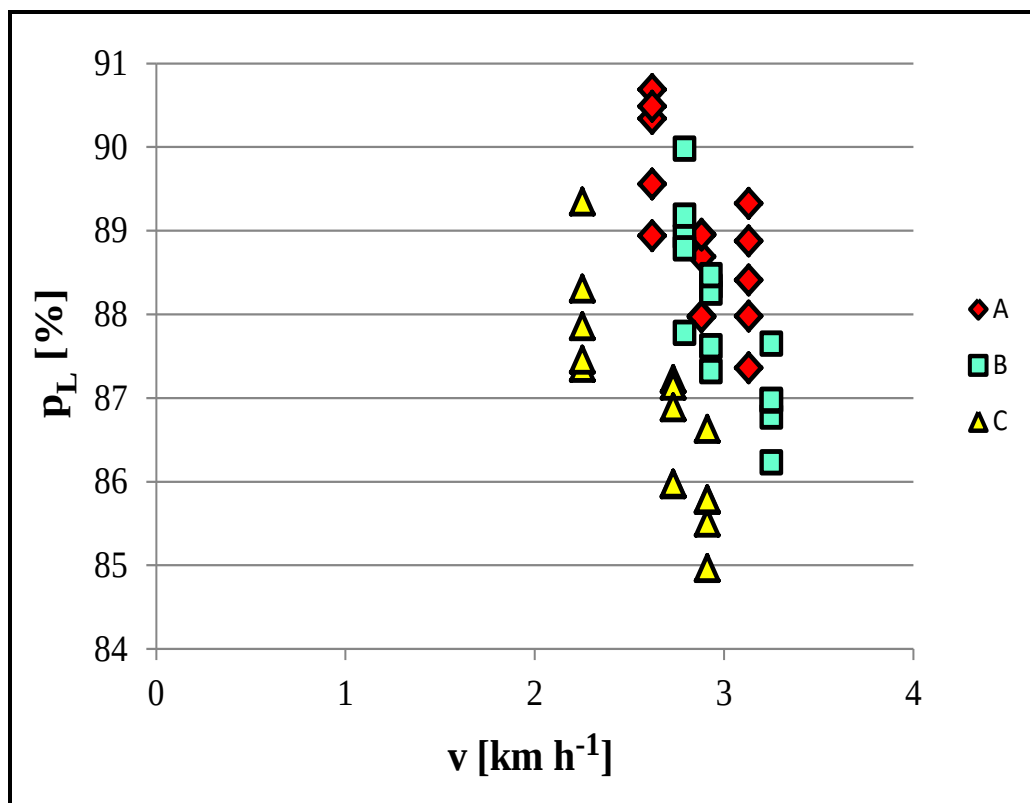


Figure 1. Content of dug tubers according to the tested variants of potato lifting aggregates

Figure 1 shows the measured values of the content of lifted potato tubers according to the tested variants, depending on the defined parameters. Based on the presented results, it can be noticed that the highest content of whole lifted potatoes was measured during lifting with variant Typ A and amounted to 90.69% (11,003 kg ha⁻¹) of the yield at a speed of 2.62 km h⁻¹ and the lowest in the amount of 84.97% (9,805 kg ha⁻¹) when lifting potatoes with variant Typ C - speed of movement of 2.91 km h⁻¹. For Typ B aggregates, the

content of whole lifted tubers varied in the range from 86.23% (10,304 kg ha⁻¹) (speed 3.25 km h⁻¹) to 89.98% (10,573 kg ha⁻¹) - speed 2.79 km h⁻¹ (Figure 1). Losses of non-lifted tubers ranged from 1.97% (239 kg ha⁻¹) of the yield of variant type A to 5.49% (634 kg ha⁻¹) of the yield of variant type C, operating speed regime of 2.62, or 2.91 km h⁻¹. In the variant of the aggregate for potato lifter type B, the losses in the unlifted tubers ranged from 2.53 - 4.19% (302 - 501 kg ha⁻¹) of the potato yield at speeds of 2.79 and 3.25 km h⁻¹ (Figure 2).

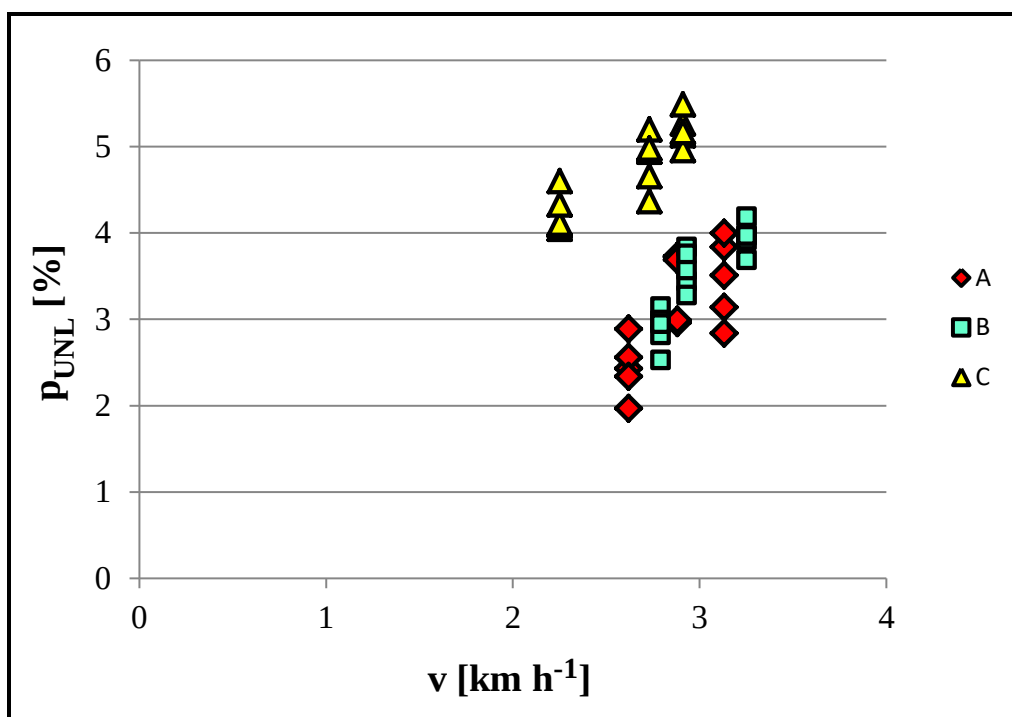


Figure 2. Content of unlifted tubers according to the tested variants of potato lifting aggregates

Based on the results shown in figures 3, 4, and 5, it can be seen that during the lifting of potatoes, different values of the realized losses were measured, which means that the examined lifters showed the different quality of work.

The highest content of mostly smaller fractions of potatoes re-buried with soil was recorded during lifting with variant Type B and amounted to 8.94% (1,068 kg ha⁻¹) of the yield - speed of movement 3.25 km h⁻¹ while the lowest content was during potatoes lifter variant type C 6.22% (718 kg ha⁻¹) speed of 2.25 km h⁻¹. Within variant type A, the content of mostly smaller fractions of potatoes re-buried with soil was in the range of 6.94 - 7.82% (842 - 949 kg ha⁻¹) of the yield at speeds of 2.62 or 3.13 km h⁻¹ (Figure 3).

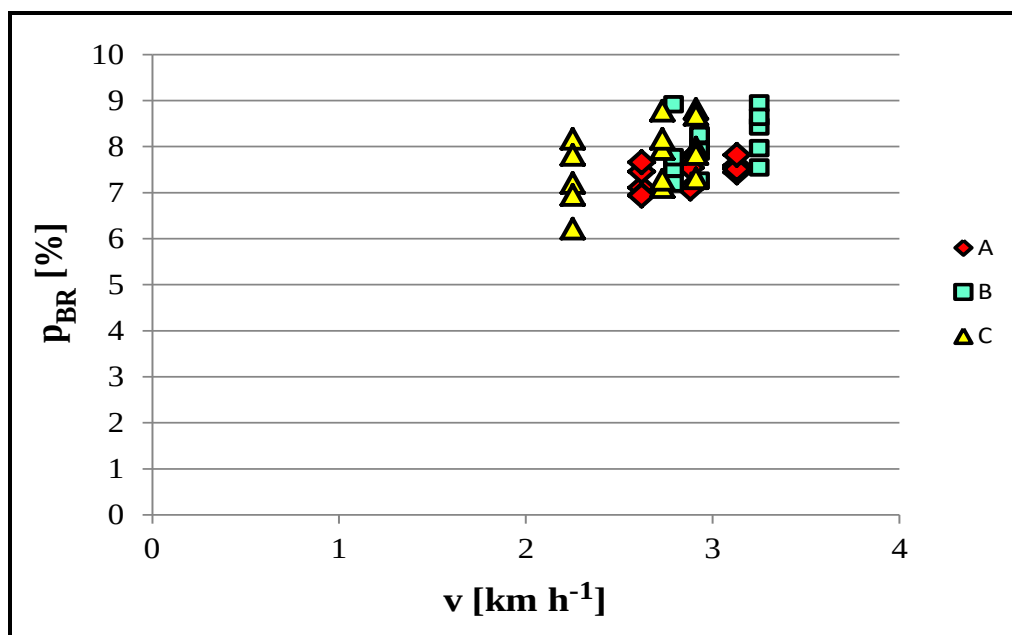


Figure 3. Content of re-buried tubers according to the tested variants of potato lifting aggregates

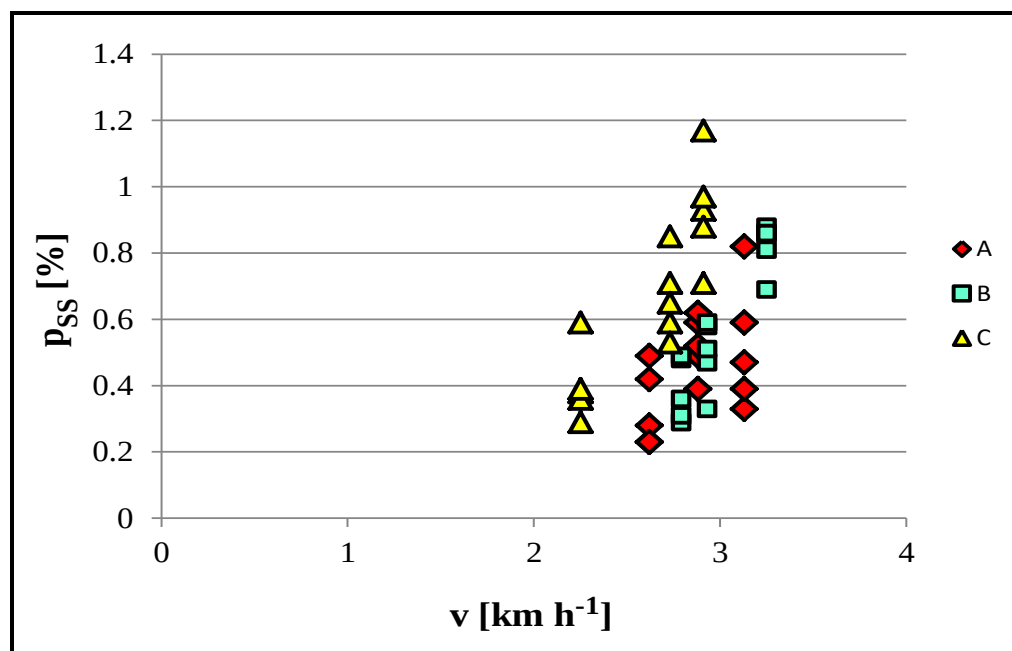


Figure 4. Content of partially scratched tubers according to the tested variants of aggregates for potato lifting

An important indicator of the quality of the potato lifting unit is the damage in the form of partially scratched tubers. Based on the results shown in Figure 4, it can be seen that the values of partially scratched tubers measured in all tested potato lifting variants are within tolerant limits, especially bearing in mind that the issue is the lifting of mercantile and not seedling potatoes. The lowest content of partially scratched potato tubers was measured when lifting potatoes with variant Type A, namely 0.23% (28 kg ha⁻¹) of the yield at a speed of 2.62 km h⁻¹ and the highest in the amount of 1.17% (135 kg ha⁻¹) of yield at variant Type C, working speed of 2.91 km h⁻¹. In the variant for harvesting Type B potatoes, the losses in partially scratched tubers were in the range of 0.29 - 0.88% (35 - 105 kg ha⁻¹) of the yield, at a speed of 2.79 and 3.25 km h⁻¹, (Figure 4).

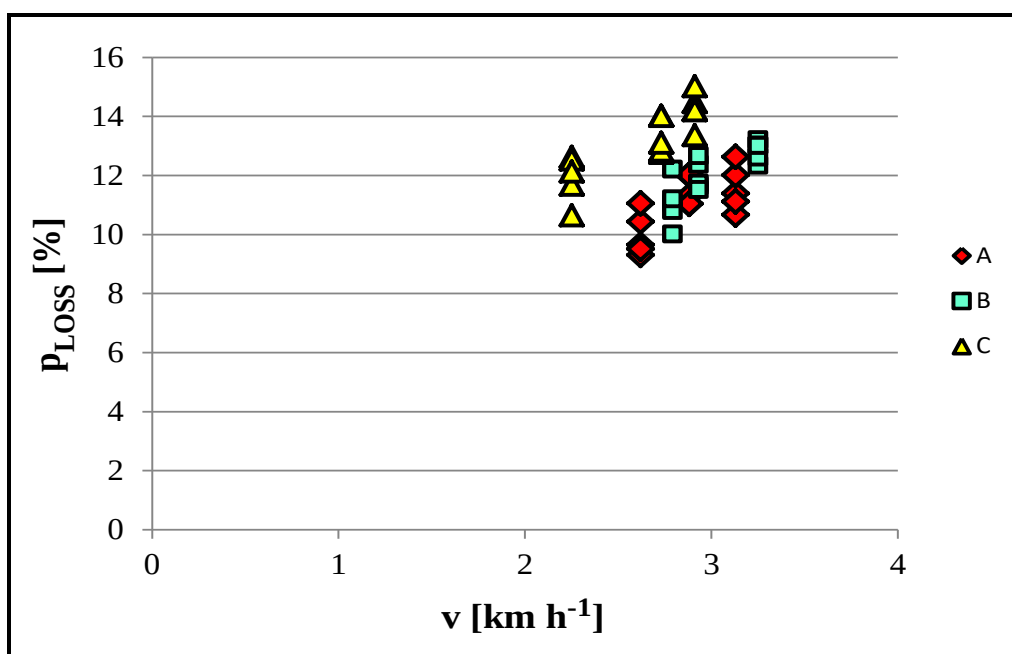


Figure 5. Total potato losses by tested variants

Based on the obtained results shown in Figure 5, it can be concluded that the values of total losses during potato lifting in all tested variants are very high, which is primarily explained by the pronounced presence of weeds and the remains on which the tests were performed, then type and humidity of the soil, planting depth, mound height, yields, and operator experience. Total potato losses are presented as the sum of un-lifted tubers, buried, and superficially scratched tubers. Based on the obtained results shown in Graph 5, it can be seen that the lowest total losses of potatoes were achieved during the liting with the variant Type A and amounted to 9.31% (1,129 kg ha⁻¹) at a working

speed of 2.62 km h⁻¹, and the highest in the lifter of the Type C variant and amounted to 15.03% (1734 kg ha⁻¹) concerning the achieved yield, at an operating speed of 2.91 km h⁻¹. In the variant Type B, the total losses of potatoes were in the range of 10.02 - 13.22% (1,197 - 1,580 kg ha⁻¹) of the yield, at the operating speed of 2.79 and 3.25 km h⁻¹ (Figure 5).

CONCLUSION

Based on the results of the research, it can be concluded that all three tested aggregates for potato lifting worked in similar production conditions, achieving different quality of work. The lowest content of lifted potatoes was when lifting the variant Type C and was 84.97% at an operating speed of 2.91 km h⁻¹, and the highest in the amount of 90.69% of the yield when lifting potatoes with the variant Typ A at the speed of movement of 2.62 km h⁻¹. Losses of unlifted tubers ranged from 1.97% of the Type A variant to 5.49% of the yield of the Type C variant, operating mode of 2.62 and 2.91 km h⁻¹. The lowest content of mostly smaller fractions of potatoes re-buried with soil was recorded during the lifting of the Type C variant and was 6.22% at a speed of 2.25 km h⁻¹, and the highest during the lifting of potatoes with the Type B variant was 8.94 %, of yield, speed 3.25 km h⁻¹. Potato losses in the form of partially scratched tubers were within tolerant limits, especially bearing in mind that it was a matter of lifting mercantile and not seed potatoes. The lowest content of partially scratched potato tubers was measured during lifting with the Type A variant, namely 0.23% of the yield at a speed of 2.62 km h⁻¹, and the highest in the amount of 1.17% of the yield with the Type C variant, working speed of 2.91 km h⁻¹. The lowest total losses of potatoes were measured during the lifting of potatoes with the Type A variant and amounted to 9.31% in the working speed mode of 2.62 km h⁻¹, and the highest during the lifting with the Type C variant, 15.03% concerning the achieved yield, at an operating speed of 2.91 km h⁻¹. Very high values of total losses during potato harvesting in all tested variants are primarily the result of the pronounced presence of weeds and thus on the plots on which the tests were performed, then the type and soil moisture, planting depth, mound height, yields, and operator experience. The obtained results show that in all examined types of aggregates for potato lifting, with the change of the mode of operation and the increase of the speed of movement, there was a disturbance of the quality of work and an increase of the total losses. The general conclusion of our research is that for the successful use and quality operation of potato extraction units, their proper inspection, proper and timely maintenance, as well as precise adjustment and handling following the manufacturer's recommendations and production conditions, especially having in mind the status of potatoes lifting aggregates used in the test area.

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THE EFFECT OF GENOTYPE AND GROWING SEASONS ON YIELD OF WINTER BARLEY

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Abstract: *The objectives of this study were to investigate: (1) the effects of cultivar, environment, and their interactions on the grain yield, test weight and 1000 kernel weight of winter barley, and (2) the correlations between these traits in different vegetation seasons. Based on the grain results during a three-year investigation with five cultivars, it can be concluded that the grain yield of barley significantly varied across years, from 3.561 t/ha in 2010/11 to 5.798 t/ha in 2012/13 in the study. The investigated period clearly indicates that a highly significant effect of year was found on grain yield and a significant effect of 1000 kernel weight. Furthermore, grain yield, test weight and 1000 kernel weight were highly significant regarding the interaction of the year and cultivar.*

Environmental conditions have had a significant effect on grain yield and quality in barley. Grain yield shows a tendency to increase in the years having a higher total amount and better distribution of rainfall during critical plant development stages.

Keywords: *barley, cultivar, productivity, quality, yield*

INTRODUCTION

Barley is one of the oldest agricultural species, which has gone through genetic alterations during the process of domestication. The basic purpose of the barley usage has been changed during thousands of years of the cultivation, i.e. from the main grain in the human diet to the very important animal nutrient

Barley cultivars that were in production until the end of the eighties were characterized by lower yields, good technological quality and higher stem sensitivity on lodging. Barley is globally cultivated on an area of about 47.5 million hectares, an average yield of 2.6 t/ha being realized making it the fifth grown crop (maize, rice, wheat and soybeans). In Serbia, barley is grown on 84.166 ha with an average yield of 2.9 t/ha and ranks third with 244.081 tons (FAO, 2010). The largest areas under barley are in Russia, Australia, Ukraine and Canada (FAO, 2010).

The grain quality of barley is affected by genetic and environmental factors as well as their interaction. Because of this, the same cultivar in different years can behave like brewing or feeding barley, according to the content of grain nutrients, which determines end-use (Đekić et al., 2017; Rajičić et al., 2019; Madić et al., 2020). It is known that individual or mutual influence of abiotic stress factors (high and low temperatures, drought, acidic and saline soil) in different barley growth stages limited expression of the maximum grain yield potential (Đekić et al., 2019). The length and intensity of the stress period vary from the environment to the environment, as well as between years in the same environment (Bratković et al., 2014).

Grain yield in barley is a complex trait that has a complex genetic basis and depends on the cultivar, applied agrotechnics and environmental conditions throughout the life cycle of a barley plant (Pržulj et al., 2010). In winter barley often variations in yield can be detected, also between the years and between locations (Đekić et al., 2015; Bratković et al., 2018). The 1000 grain weight and test weight are direct components of grain yield and change under the influence of environmental factors, but primarily varietal characteristics (Pržulj et al., 2014). They indicate the grain size and are important criteria in breeding barley.

Cultivars of the new generation exhibit the high degree of tolerance against temperature shocks during the phase of the forming and filling of grain as well as against drought. To achieve high and stable grain yields, newly cultivars are demand more precisely and more complex NPK nutrition (Popović et al., 2011; Jelić et al., 2014; Đekić et al., 2011, 2019; Madić et al., 2019, Rajičić et al., 2020). New perspective barley lines and varieties has more and better filled grain, higher yield and grain mass, while proteins were smaller compared with older varieties (Pržulj et al., 2014; Bratković et al., 2018). The interaction between the genotype and environment greatly limits the effectiveness of selection, if it is performed only on the basis of the average yield. Varieties that have a smaller contribution to interaction are less sensitive to changing environmental conditions, such varieties are stable (Đekić et al., 2012; Bratković et al., 2014; Jelić et al., 2014). New varieties are characterized by good technological quality, better resistance to lodging and diseases, shorter stem and more efficient assimilates usage (Pržulj et al., 2004).

Because of the appearance of new demanded cultivars at permanent changes in soil fertility level and environmental conditions, still exist a need to researches cultivars of barley in concrete agro-ecological conditions. The production of winter barley with a high grain yield and appropriate quality is possible only by choosing a quality assortment with appropriate growing conditions and production technology. During the three-year vegetation season on acidic soil, five varieties of winter barley were examined, in which the yield and grain yield components were analyzed, with the aim of determining the selection of the best varieties for the production conditions of Western Serbia.

MATERIALS AND METHODS

Experimental design

During the three growing seasons, five cultivars of winter barley (Nonius, NS 525, Atlas, Rekord and Grand), cultivated at the experimental field in Valjevo were investigated. The experiments were conducted in randomized block systems, with a plot size of 50 m² (5 m x 10 m) in five replications. The sowing was carried out using a machine with row spacing of 12.5 cm. The soil on which the trial was conducted was uniform and well prepared. The amount of seed per square meter amounted to 450 viable seeds, depending on the characteristics of varieties. It was sown in the third decade of October, with 300 kg/ha of fertilizer NPK 15:15:15, which was added in the fall, while during the spring fertilization soil was supplemented with CAN (27% N).

The following properties were analyzed: grain yield (t/ha), test weight (kg/hl) and 1000 kernel weight (g). Grain yields were measured for each plot and converted to yield tons per hectare on the basis of 14% grain moisture.

Soil Analysis

The trial was performed on soil that is characterized as pseudogley. According to the analysis, this is a soil of medium acidity, poor in humus, with a substitution and total hydrolytic acidity that were quite high (pH in H₂O=5.59, in KCl=4.50). The soil was moderately provided with total nitrogen, it is poor in easily accessible phosphorus (P₂O₅=<15 mg/100 g of soil), a good provided of easily accessible potassium (K₂O=23-35 mg/100 g of soil) was recorded.

Statistical Analysis

On the basis of achieved research results the usual variational statistical indicators were calculated: average values. Experimental data were analyzed by descriptive and analytical statistics using the statistics module Analyst Program SAS/STAT (SAS Institute, 2000) for Windows. All evaluations of significance were made on the basis of the ANOVA test at 5% and 1% significance levels.

Agroecological conditions

This study was conducted over a three-year period in the [Kolubara](#) district, Šumadija region, Western Serbia, on a Pseudogley soil, at Valjevo location, 176 m a. s. l. (44°19'N 19°55'E), in a temperate continental climate having an average annual temperature of 11.4°C, typical of Šumadija districts in Western Serbia and a rainfall amount of about 787.7 mm. Valjevo area is characterized by a moderate continental climate, which in general is characterized by uneven distribution of rainfall by month.

The data presented in Table 1 for the analyzed barley growing season (2011-2013) clearly suggest differences in weather conditions between the years of the study and the long-term mean for the region. The average air temperatures were by 0.35°C, 0.21°C and 1.3°C higher in 2010/11, 2011/12 and 2012/13, respectively, as compared to the long-term mean, whereas the sums of rainfall were by 253.8 mm higher in 2010/11 years and lower by 105.2 mm and 67.6 mm in 2011/12 and 2012/13 as compared to the long-term mean. Compared to the long-term mean, total rainfall values, especially in the first year, second and third year, were considerably higher in February, April and May, whereas total rainfall in April 2011/12 decreased by 26.5 mm. Given the high importance of sufficient rainfall amount during the spring months, particularly April and May, for barley production, the distribution and amount of rainfall over the growing season 2012/13 were considerably more favorable, resulting in increased yields in this year. Apart from the rainfall deficiency during the spring months and the non-uniform distribution of rainfall across months, an increase in average air temperatures was also observed. The warmest month, on average, is July with an average temperature of 21.9°C. The coolest month on average is January, with an average temperature of 0.6°C.

Table 1. Middle monthly air temperature and precipitation amount (Valjevo)

Months											
Interval	X	XI	XII	I	II	III	IV	V	VI	VII	Aver.
Mean monthly air temperature (°C)											
2010/11	9.5	10.1	2.3	0.4	1.0	6.6	12.8	16.1	21.2	22.6	10.26
2011/12	10.7	2.9	4.1	0.8	-3.8	8.7	12.3	16.6	23.2	25.7	10.12
2012/13	13.4	9.4	0.9	3.1	4.2	6.5	13.7	17.6	20.5	22.8	11.21
Average	11.7	6.1	1.9	0.6	2.0	6.6	11.6	16.8	19.9	21.9	9.91

The amount of precipitation (mm)											
2010/11	83.3	39.4	56.7	53.0	75.9	76.1	65.6	117.8	216.8	121.0	905.6
2011/12	32.7	9.3	66.6	28.2	53.7	32.4	33.4	161.4	27.5	101.4	546.6
2012/13	31.0	21.2	105.2	95.4	67.5	14.6	86.6	115.1	28.2	19.4	584.2
Average	62.9	62.7	60.6	49.9	44.6	57.9	59.9	72.1	110.2	71.0	651.8

Variations in the temperature, in the amount of precipitation during vegetation as well as in the soil moisture content are the most important factors of the yield instability (Đekić et al., 2014; Jelic et al., 2014). It is known that individual or mutual influence of abiotic stress factors (high and low temperatures, drought, acidic and saline soil) in different barley growth stages limit the expression of the maximum grain yield potential (Popović et al., 2011; Jelić et al., 2014; Đekić et al., 2011, 2019; Madić et al., 2019, Rajičić et al., 2020). In addition to the necessary reserve for the spring part of the vegetation, winter precipitation greatly influences the distribution of easily accessible nitrogen in the soil (Popović et al. 2011; Đekić et al. 2012; Jelic et al. 2014; Bratković et al. 2018; Rajičić et al. 2019).

RESULTS AND DISCUSSION

Grain yield, 1000 kernel weight and test weight

Average values of grain yield, 1000 kernel weight and test weight at investigated winter barley varieties are presented in the Table 2. The grain yield of winter barley significantly varied across years, from 3.560 t/ha in 2010/11 to 5.798 t/ha in 2012/13 (Table 2). Average grain yield of barley cultivars ranged from 4.413 t/ha in cultivar Atlas to 5.063 t/ha in cultivar Nonius. The average three-year value of grain yield was 4.818 t/ha.

During the first year of investigations, cultivar Grand achieved the highest grains yield (3.765 t/ha), followed by Nonius (3.685 t/ha), while the lowest yield was at Atlas cultivar (3.314 t/ha). During the second year of investigations, the yield of Grand cultivar was the highest with 5.340 t/ha. During the third year of testing, the Nonius variety achieved the highest grain yield (6.321 t/ha), followed by NS 525 (6.221 t/ha), while the Atlas variety had the lowest yield (5.101 t/ha). The excessive amount of moisture on the crops growing season 2010/11 (March-May-June) influenced the poorer heading and filling of grain, lodging of crops, the abundance of the weeds and the intense occurrence of the disease in the examined barley and other winter grains. Regard the high importance of sufficient rainfall amounts during the spring months, particularly May for small grains production, the distribution

and amount of rainfall over the growing season 2012/13 were considerably more favorable, which resulted in the increment of yields during that year. Considerable variation in yield depending on years of research have established Popović et al. (2011), Bratković et al. (2014), Jelić et al. (2014), Đekić et al. (2019), Madić et al. (2019) and Rajičić et al. (2019).

Table 2. Average values of investigated triticale varieties characteristics

Varieties	2010/11		2011/12		2012/13		Average	
	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd
Grain yield, (t/ha)								
Nonius	3.685	0.432	5.184	0.430	6.321	0.232	5.063	1.170
NS 525	3.481	0.065	5.047	0.597	6.221	0.365	4.916	1.221
Atlas	3.314	0.253	4.825	0.338	5.101	0.299	4.413	0.859
Rekord	3.557	0.139	5.084	0.364	5.893	0.610	4.844	1.075
Grand	3.765	0.199	5.340	0.247	5.455	0.168	4.853	0.821
Average	3.560	0.280	5.096	0.414	5.798	0.579	4.818	1.037
Test weight (kg/hl)								
Nonius	54.79	1.016	55.75	2.443	56.64	2.735	55.72	2.180
NS 525	52.74	1.494	53.72	0.796	54.68	0.658	53.71	1.271
Atlas	53.43	1.595	54.41	0.889	54.03	0.619	53.95	1.112
Rekord	55.35	1.249	56.77	1.851	59.86	2.131	57.32	2.555
Grand	55.54	1.311	57.22	2.556	53.39	1.102	55.38	2.311
Average	54.37	1.665	55.57	2.182	55.72	2.836	55.22	2.329
1000 kernel weight (g)								
Nonius	42.70	2.717	42.58	1.597	42.40	0.831	42.56	1.747
NS 525	41.53	1.771	42.20	1.698	45.80	1.179	43.18	2.426
Atlas	37.12	0.665	38.58	1.119	39.58	0.953	38.43	1.355
Rekord	38.18	1.016	40.36	0.522	40.13	1.305	39.56	1.372
Grand	38.22	0.427	40.76	0.716	39.72	0.826	39.57	1.248
Average	39.55	2.627	40.90	1.836	41.53	2.595	40.66	2.491

Table 2 presents average values for grain test weight across years and varieties. The average values for test weight significantly varied across years, from 54.37 kg/hl in 2010/11 to 55.72 kg/hl in 2012/13 (Table 2). The average three-year value of test weight was 55.22 kg/hl. During the 2010/11 and 2011/12, variety Grand achieved the highest test weight (55.54 kg/hl and 57.22 kg/hl). During the third year of investigations, variety Rekord achieved the highest test weight (59.86 kg/hl). Average test weight observed in the three-years period was the highest at Rekord variety (57.32 kg/hl), while the lowest test weight was obtained by NS 525 cultivar (53.71 kg/hl). Generally, the test weight of barley ranged from 52 to 72 kg/hl (Pržulj et al., 2004).

The thousand kernel weight of winter barley was variable, depending on environmental conditions. Thousand kernel weight in the test period was highest in 2012/13 (41.53 g) but significantly decreased in 2010/11 (39.55 g). The average thousand kernel weight observed in the three-year period of winter barley varied across cultivars, from 38.43 g in cultivar Atlas to 43.18 g in cultivar NS 525. The average three-year value of thousand kernel weight was 40.66 g. Thousand kernel weight is governed by grain plumpness i.e. grain size, grain filling degree and weight of the seed itself (Madić et al., 2011). In addition to the genetic basis, this trait is largely determined by agroecological conditions, especially during the period of grain formation and filling (Madić et al., 2018; Đekić et al., 2019; Rajičić et al., 2020).

Analysis of variance between observed traits of barley

The analysis of yield variance, test weight and 1000-kernel weight of tested winter barley varieties grown at investigated Valjevo during three growing seasons, are shown in Table 3.

Table 3. Analysis of variance of the tested parameters (ANOVA)

Effect of year on the traits analyzed				
<i>Traits</i>	Mean sqr Effect	Mean sqr Error	F (2. 72)	p-level
Grain yield (t/ha)	32.753	0.195	168.185**	0.000
Test weight (kg/hl)	13.774	5.192	2.653	0.077
1000-kernel weight (g)	25.491	5.669	4.497*	0.014
Effect of cultivar on the traits analyzed				
<i>Traits</i>	Mean sqr Effect	Mean sqr Error	F (4. 70)	p-level
Grain yield (t/ha)	0.883	1.086	0.813	0.521
Test weight (kg/hl)	32.201	3.894	8.269**	0.000
1000-kernel weight (g)	65.022	2.844	22.866**	0.000
Effect of the year x cultivar interaction				
<i>Traits</i>	Mean sqr Effect	Mean sqr Error	F (2. 24)	p-level
Grain yield (t/ha)	0.391	0.123	3.188**	0.004
Test weight (kg/hl)	10.388	2.699	3.849**	0.001
1000-kernel weight (g)	5.996	1.668	3.594**	0.002

Based on the analysis of variance, it can be concluded that there are highly significant differences in grain yield in regard to the year of investigation, while among the investigated barley varieties the differences were not significant. Analysis of variance was found significant effect of year on the 1000-kernel weight. The results of significance between years and yield of grain are in accordance with the results of other authors (Đekić et al., 2014; Madić et al., 2020; Rajičić et al., 2020). Based on the analysis of variance, it can be concluded that there are highly significant differences in test weight and 1000-kernel weight at investigated barley varieties were found relative to the cultivar of investigation (Table 3). The interaction of the investigated factors (Y x G) exhibits are highly significant effect on grain yield, 1000-kernel weight and test weight ($p < 0.01$). The present results confirm the opinion of many authors that the traits analyzed are genetically determined but are strongly modified by the environment and weather conditions (Popović et al. 2011; Madić et al., 2020; Rajičić et al., 2020).

The need for a variety of short or long growing season depends on the environmental conditions of a particular area. In winter barley often variations in yield can be detected, also between the years and between locations (Popović et al. 2011; Đekić et al. 2015; Rajičić et al. 2019). The 1000 grain weight and test weight are direct components of grain yield and change under the influence of environmental factors, but primarily varietal characteristics (Pržulj et al. 2004). They indicate the grain size and are important criterions in breeding barley.

Grain quality is a qualitative property influenced by the genetic factors and environmental factors, as well as their interaction, so the variety can behave in some years as a forage crop, which can be determined by the testing and directed for a particular purpose (Pržulj et al., 2004; Madić et al. 2011; Paunović and Madić, 2011; Bratković et al., 2014; Đekić et al., 2015; Mirosavljević et al., 2015; Rajičić et al., 2019).

Correlations between the analyzed traits

The correlations of yield, test weight and 1000-kernel weight of tested winter barley varieties during three growing seasons, are shown in Table 4. Table 4 shows the correlation coefficients between the studied growing seasons and analyzed traits. Positive correlation coefficients, in 2010/11, were found between yield and test weight (0.131) and 1000-kernel weight (0.236) and negative correlations were found between test weight and 1000-kernel weight (-0.164). During the second year of investigation (2011/12), grain yield was positive and significantly correlated with test weight (0.471*). Test weight was positively correlated with 1000-kernel weight (0.137) in 2011/12. Positive highly significant correlation coefficients, in 2012/13, were found between yield and 1000-kernel weight (0.520**) and positive correlation coefficients between test weight and yield (0.293). Positive and highly significant

correlations were found between the grain yield with test weight and 1000 kernel weight in 2011/13. Positive correlations were found between the grain yield with test weight and 1000 kernel weight in all growing seasons. Đekić et al. (2019) state that even if there is no direct dependence between the height of the plant and the yield, the decrease in height influences the yield increase indirectly, by increasing the 1000-kernel weight and increasing resistance to lodging. Most authors point to very positive effect of 1000-kernel weight on grain yield of barley (Carpici and Celim, 2012; Ebrahim et al., 2015; Mirosavljević et al., 2015). The present results confirm the statement of many authors that the traits analyzed and their correlations are genetically determined but are strongly modified by the nutrient status of the environment and weather conditions (Popović et al., 2011; Carpici and Celim, 2012; Jelić et al., 2014; Bratković et al., 2014; Ebrahim et al., 2015; Mirosavljević et al., 2015; Đekić et al., 2017; Rajičić et al., 2019; Madić et al. 2020).

Table 4. Correlations between the traits analyzed

Correlations between the traits analyzed in 2010/11			
Traits	Grain yield	Test weight	1000-kernel weight (g)
Grain yield (t/ha)	1.00	0.131	0.236
Test weight (kg/hl)		1.00	-0.164
1000-kernel weight (g)			1.00
Correlations between the traits analyzed in 2011/12			
Grain yield (t/ha)	1.00	0.471*	0.305
Test weight (kg/hl)		1.00	0.137
1000-kernel weight (g)			1.00
Correlations between the traits analyzed in 2012/13			
Grain yield (t/ha)	1.00	0.293	0.520**
Test weight (kg/hl)		1.00	-0.054
1000-kernel weight (g)			1.00
Correlations between the traits analyzed in 2011/13			
Grain yield (t/ha)	1.00	0.362**	0.451**
Test weight (kg/hl)		1.00	0.054
1000-kernel weight (g)			1.00

The importance of these components in the formation of grain yield depends on the climatic conditions in critical phases of growth and development, applied agro-technology and various combinations and relationships of NPK nutrients (Popović et al., 2011; Jelić et al., 2014; Pržulj et al., 2014; Bratković et al., 2018; Madić et al. 2018; Đekić et al. 2019; Rajičić et al., 2020). Therefore, it is important to know the effect of these properties, i.e. yield components, as well as their interdependence on grain yield.

CONCLUSIONS

Weather conditions during the experimental period had different effects on grain yield, test weight and 1,000-grain weight. Based on the gain results during the three-year investigation on winter barley cultivars, it can be concluded that the grain yield of barley ranged from 4.413 t/ha (Atlas) to 5.063 t/ha (Nonijus). More favourable conditions in 2012/13 resulted in a higher average yield value (5.798 t/ha) compared to 2010/11 (3.560 t/ha). During 2012/13, significantly higher grain yield per area unit, compared with 2010/11 year. The average kernel weight in the study was 40.66 g, with a variation of 39.55 g in the vegetation year 2010/11 to 41.53 g in the vegetation year 2012/13.

Based on these results, it can be concluded that several traits have a decisive role in the formation of grain yield. The contribution of each individual feature can be different for different genotypes and the various environmental conditions so that this results from the interaction between the features within each genotype and genotype interactions with environmental factors.

The highly significant influence of the year on grain yields, test weight and 1000-kernel weight was established at investigated winter barley varieties by variance analysis, while genotype influence on test weight and 1000-kernel weight were highly significant. The presence of interactions indicated a different reaction of the cultivars to changing agro-ecological conditions, especially to the changing climate factors. Agro-ecological conditions had a significant impact on the yield of barley so that the yield was significantly higher in the third year, that is, the year with more favorable meteorological conditions.

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SELECTION FOR LODGING RESISTANCE THROUGH STEM LENGTH REDUCTION IN BARLEY

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Abstract: Barley (*Hordeum vulgare* L.) occupies a significant place in world grain production with an area of over 50 million ha and an annual production of about 158 million tones. In addition to grain yield, which is a constant goal in the creation of new varieties, today in barley breeding special attention is paid to the components of resistance to lodging, because of all small grains, barley as a species is the least resistant. Resistance to lodging is largely determined by the genetic basis of certain characteristics of the stem (length, strength and elasticity) and development or structure of the root system. Stem height is one of the most important initial selection criteria in barley breeding, because it is a direct component of lodging resistance, and an indirect component of grain yield and quality. In barley breeding programs, *sdw1/denso* and *uzu* alleles have been widely used to shorten stem height. Semi-dwarf plants have a short, strong tree and are much more tolerant of lodging than tall plants. The barley varieties that are in production today generally have good resistance to lodging, but are still far less than the cultivated wheat varieties. One of the tasks in the following barley breeding programs is to increase the resistance to lodging, which would enable the application of more intensive agrotechnics, primarily larger amounts of mineral nutrients. In future breeding programs for winter and spring barley, the selection should be directed towards increasing the thickness of the stem as well as changes in the anatomical structure that will provide the stem with sufficient strength.

Keywords: barley, component of lodging resistance, stem height

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a very important cereal that ranks fourth in the world in terms of sown areas after wheat, maize and rice (Awika, 2016). The area under barley in the world in 2019 amounted to 51.15 million ha, with a total annual production of 158.98 million t and an average yield of 3.11 t ha⁻¹ (FAOSTAT, 2021). According to the data of the Republic Bureau of Statistics (average 2005-2015) in the Republic of Serbia, barley was grown on an area of about 96,000 ha, with a total annual production of about 362,000 t and an average yield of 3.8 t ha⁻¹. The main goal of barley breeding is mainly aimed at increasing the potential for grain yield, so that the increase in yield for the period 1977-2010 was 46 kg ha⁻¹ per year (Miroslavljević et al. 2016). In addition to increasing the potential for grain yield at the end of the last century, breeding is further extended to other properties, primarily quality and resistance to biotic and abiotic limiting factors (Knezevic et al. 2007). In the 20th century, the grain yield of winter barley was increased by simultaneous progress in the field of breeding and agrotechnical measures. Knowledge of the relationship between agronomic traits and genetic gain in yield potential is necessary to improve future breeding programs (Miroslavljević et al. 2016). Changes in yield potential were accompanied by significant changes in various physiological properties (Lillemo et al. 2010), such as an increase in the number of grains per unit area (Abeledo et al. 2008), an increase in the mass of a thousand grains and a decrease in plant height and hectoliter weight (Miroslavljević et al. 2016).

The goal of barley breeding in the coming period is to create varieties of low, strong and elastic stems, which can withstand intensive cultivation in a dense structure. The height of the stem is mostly determined by the length of the internodes, where it is important for the lower internodes to be short for greater resistance to lying down. Pržulj and Momčilović (1998) believe that, in addition to shortening the height of the stem, special attention should be paid to finding such an anatomical structure of the stem that will provide it with sufficient strength. Shortening the height of the stem would be desirable due to the pronounced sensitivity of barley to lodging. However, due to the positive correlations between stem height and grain yield, the selection for stem shortening should take into account the fact that a drastic reduction in height would lead to a significant reduction in total biomass, and thus grain yield (Pržulj et al. 2010).

Resistance to lodging is a very important property in barley, because from the group of cereals, barley as a species is the least resistant to lodging. Laying is the phenomenon that plants in a certain stage of development more or less bend or lie down. This phenomenon is usually seen as a reaction of plants to the mechanical effects of the environment (environmental factors and

diet). The damages from laying can be great and, depending on the stage of development of the plant, can cause a great decrease in yield, deterioration of quality and difficult harvesting, especially when the laying occurs at the time of flowering (earring). Resistance to lodging is largely determined by the genetic basis of certain characteristics of the tree (length, strength and elasticity) and development or structure of the root system.

STEM LENGTH AND LODGING RESISTANCE

The stem length in barley depends on the varietal characteristics, climatic conditions and applied measures of production technology (Paunović and Madić, 2011). The authors point out that stem length, in barley breeding, is one of the most important initial selection criteria, because it is a direct component of lodging resistance, and an indirect component of grain yield and quality. The height of barley has a direct positive effect on grain yield (Lalic and Kovacevic, 2010), but can also have an indirect effect, through biological yield or harvest index and number of grains per plant (Djukic et al., 2011). Madić et al. (2006), analyzing the interdependence of yield and yield components, found a strong interdependence of stem length and biological yield ($r = 0.65$).

In barley, there are four types of short stem, designated as *brachytic*, *erectoid*, *uzu*, and *sdw* (Foster and Thompson, 1987, Sears et al. 1981), which are determined by four recessive alleles of different genes (Sears et al. 1981, Thomas et al. 1984). Mickelson and Rasmusson (1994) state that the trait of a short stems in barley is determined by one, two, or three recessive genes, the most common of which is the *sdw* gene. In barley breeding programs for resistance to lodging, stem length reduction genes are essential for the creation of modern barley cultivars, in which over 30 types of dwarf and semi-dwarf mutant forms have been described so far. These genes were mapped to each of the seven barley chromosomes (Wang et al. 2010). However, only two, namely *sdw1/denso* and *uzu*, have been widely used in barley breeding programs (Hellevell et al. 2000; Zhang and Zhang 2003, Saisho et al. 2004). These two widely used stem length shortening genes are located on the long arm of the 3X chromosome, and occupy two different loci. One of these loci contains the allele *sdw1* (formerly known as *sdw*), or *denso*, and is still current in breeding programs in the United States, Canada and Europe (Rasmusson 1991; Mickelson and Rasmusson 1994).

Most European barley cultivars are of the semi-dwarf type and have the *denso* gene (also designated as *sdw1*). This gene originates from the Denso variety of a spontaneous mutant isolated in 1946 at the Abed breeding facility (Haar and Wettstein, 1976). In the initial stages of growth, the recessive alleles of the *denso* gene determine the prostratum type of growth, and the dominant

allele upright, which represents a noticeable morphological expression of this gene (Kuczyńska and Wyka, 2011). Modern high-yielding barley cultivars are mainly associated with semi-dwarf growth-type sources determined by the *sdv1/denso* locus. For example cv. Maresi, which possesses this gene, is mentioned in the literature as a variety of stable grain yield on sandy soils, which are very common in Central Europe (Górny 2001).

The barley stem height reduction genes have a different phenotypic effect compared to the *Rht* genes of wheat. They are associated with late heading, low 1000-grain weight, lower yield and increased beta-glucan content (Powell et al. 1985; Foster and Thompson 1987; Thomas et al. 1991; Mickelson and Rasmusson 1994; Hellewell et al. 2000). The phenotypic effect of the *sdv1/denso* gene depends on agroecological conditions and can reduce plant height by 10 to 20 cm (Hellewell et al. 2000).

The lodging of barley plants not only affects the reduction of grain yield and deterioration of quality, but also indirectly affects the quality of malt because the grain of fallen plants is often less mass and gives less malt extract (Day and Dickson, 1958). Hence, breeding of barley for lodging resistance is very important, and in connection with that, for such breeding programs, the identification of genes for semi-dwarf growth is necessary (Maluszynski and Szarejko 2005). Mutants of barley with reduced stem height have been frequently used in breeding programs (Khlestkina et al. 2020). For example, the Diamant and Triumph varieties, which originate from mutants selected in the M₂ generation obtained after X-rays of the cv. Valticky, are donors for over 150 varieties created in Europe in the 20th century. Today, cultivars possessing the *sdw1 / denso* gene are found in the pedigree of a large number of modern barley cultivars selected worldwide (Grausgruber et al. 2002; Dahleen et al. 2005). The influence of *sdw1/denso* locus on plant height and a large number of agronomic and physiological traits has been established in many studies (Kuczyńska et al. 2013).

Some of the for QTL for flowering time, grain yield and yield components were localized in the *sdw1/denso* region. In addition to the reduced plant height, it was observed that semi-dwarf plants are characterized by later maturation, reduced 1,000-grain weight, as well as lower grain mass per class and per plant. Grain yield per unit area is also to some extent related to the *sdw1/denso* gene, but the results are quite different; for example: Thomas et al. (1991) and Hellewell et al. (2000) noted a decrease in yield in their research, while Thomas et al. (1995), Yin et al. (1999) and Jia et al. (2011) state that genotypes with the *denso* gene are characterized by increased yield.

The gene for the semi-dwarf type of *uzu* has been widely used in breeding programs in East Asia and more recently in breeding programs in Australia (Chen et al. 2016). The results of the mentioned authors showed that this gene has a pronounced influence not only on the height of the plant but

also on a number of other traits. It is important to note that the effect of this gene depends on temperature and that its influence on certain traits is stronger at higher temperatures. For that reason, deeper sowing, which is necessary for better contact of seeds with moisture, is not recommended for barley genotypes with this gene, because the *uzu* gene has a drastic reduction in the length of coleoptiles at higher temperatures. In addition, a significant reduction in grain mass in barley genotypes with this gene in areas with high temperatures leads to a deterioration in grain quality as a raw material for malt production (Chen et al. 2016). In addition, Chen et al. (2016) also observed a significant influence of *uzu* genes on many other characteristics including leaf size and flowering time.

Ceccarelli and Falcinieli (1978), analyzing the genetic basis of plant height, found that higher height is determined by dominant genes, as well as that selection in the direction of decreasing or increasing plant height changes the length of classes in the same direction. The authors believe that this is due to genetic linkage and/ or pleiotropic effects of genes. Ceccarelli and Falcinieli (1978) state that selection in the direction of higher plant height also increases the number of tree internodes, while selection in the direction of decreasing stem height does not change the number of internodes significantly.

Malešević (1985) states that the stem length is a very important characteristic of barley, because plants with a shorter stem are usually more resistant to lodging. Therefore, by breeding, they want to create varieties of low, strong and elastic stem, which can withstand intensive cultivation in a dense structure. Pržulj et al. (2000) state that in the Novi Sad assortment of spring barley, the Pek variety has the lowest stem length, which also represents the lower limit of the stem length without a negative impact on grain yield. According to Paunović et al. (2007) and Paunović et al. (2009) the average stem length of new barley cultivars is 70 to 90 cm. Based on experiments with a large number of barley varieties created at the Center for Small Grains in Kragujevac at five locations in the Republic of Serbia (2003–2005), Madić et al. (2006) found that plant height of winter varieties of barley is from 80 to 100 cm, and of spring from 70 to 80 cm. Maksimović et al. (2000), based on the results of a three-year study, point out that the height of the new varieties of spring beer barley Kraguj is 79.40 cm, Dinarac 60.80 cm, Dunavac 73.20 cm and Dukat 72.50 cm.

Internode length is of great importance to plant height, and it is important that the lower internodes are short, because this increases the strength of the stem, and thus the resistance to lodging. Plant height can be reduced at the expense of shortening all internodes, or at the expense of shortening the first and third internodes. Of great importance for resistance to lodging is the length of the second basal internode, for the inheritance of which the additive and dominant effects of genes in the F1 and F2 generations are

significant, with the nonadaptive effect being more pronounced (Vazquez and Sanchez 1989; Knezevic et al. 1996; Madić et al. 2010 and 2014). Increased resistance to lodging can also be achieved by selecting genotypes of vertical arrangement of internodes, which form a single axis, in contrast to non-resistant ones, where angles appear at the points of connection of internodes (Krivogornicyn et al. 1984).

The barley varieties that are in production today generally have good resistance to lodging, although far less than the cultivated wheat varieties. One of the tasks in future barley breeding programs is to increase the resistance to lodging, which would enable the application of more intensive agricultural techniques, primarily larger amounts of mineral nutrients.

Varieties of winter and spring beer barley are now present in the production, which in production are generally characterized by very good resistance to lodging and an average tree height of 90 to 100 cm. In the following winter barley breeding programs, the selection should be focused on reducing the height of the tree by 10 to 20 cm. In the breeding of spring malting barley, the optimal height has been achieved, so that in future breeding programs, attention should be focused on increasing the thickness of the tree as well as changes in the anatomical structure that will provide the stem with sufficient strength.

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THE CONTENT OF AVAILABLE POTASSIUM IN THE SOIL OF THE ALEKSINAC MUNICIPALITY

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Abstract: *The territory of the Municipality of Aleksinac covers 707 square kilometers, of which agricultural land covers 45,463 ha, and forests 20,398 ha. It is a very well developed agricultural region. The aim of this research was to determine content of available potassium in the soil of the Aleksinac municipality. Potassium is vital to many plant processes, it is important for the movement of nutrients and water in plant tissue, activates enzymes in the plant and thus helps the synthesis of starch, protein and adenosine triphosphate, improves drought resistance and stimulates root growth; reduces water loss, maintains turgor; stimulates photosynthesis; reduces respiration, thus preventing energy loss; stimulates the movement of sugar and starch in the plant; grain is richer in starch; increases the protein content in the plant; builds cellulose that prevents lodging and slows the onset of plant diseases. 1732 samples were taken from 66 cadastral municipalities of Aleksinac municipality. The collected samples were examined by the AL-method and data was processed on IBM SPSS Statistic – 20 (trial version). Over 80% of samples were categorised as well supplied with readily available potassium, around 18.6% as moderately or medium supplied and 0.7% of samples as poorly supplied.*

Keywords: *potassium, municipality, content, soil, available*

Introduction

Biogenic elements play the crucial role in plants advancement. Around seventy elements have been discovered to be present in plants, some of which are essential while the others are regarded as useful elements. For an element to be considered essential to the plant, certain conditions are to be fulfilled: its deficiency prevents a further growth of the plant; it causes the plant chronic growth and development; its deficiency symptoms are more or less typical

(Kastori i Maksimović, 2008). One of the most significant biogenic elements which is essential for the plant growth and which has positive effects on plant's disease resistance is considered to be potassium. It is present in plants and soil as the monovalent cation (K^+) with reduction characteristics. It is not an integral part of the organic matter but is predominantly linked to the proteins. Potassium is vital to many plant processes, which makes it a crucial biogenic element (Jelić, 2012). Long-term negative potassium balance is today considered to be another factor in the degradation of agricultural soil productivity, after soil acidity. On average, 18.6% of the world's soil is extremely poor in potassium (Grzebisz et al., 2012). Nowadays, worldwide potassium (K) fertilizer use has grown, while the expected fertilizer use efficiency has decreased (Dhillon et al., 2019).

The following can be singled out as some of the most significant benefits of potassium: it is important for the movement of nutrients and water in plant tissue, activates enzymes in the plant and thus helps the synthesis of starch, protein and adenosine triphosphate (ATP). Lack of potassium in the soil slows down the growth of plants and reduces the yield. The roles of potassium in plants are: improves drought resistance and stimulates root growth; reduces water loss, maintains turgor; stimulates photosynthesis; reduces respiration, thus preventing energy loss; stimulates the movement of sugar and starch in the plant; grain is richer in starch; increases the protein content in the plant; builds cellulose that prevents lodging and slows the onset of plant diseases (Kaiser et al., 2016; Radulov et al., 2012). The amount of potassium that the plant removes from the soil during the growing season depends on the plant species. So for plum which is the most widespread cultivated fruit species in Serbia (Grčak et al., 2017)

There are different systems for assessing potassium bioavailability in soil, for example based on one system there is a division into three categories: high available K, medium available K and low available K (Li et al., 2016). In the surface layer of the soil where the root system of plants is located, there is a significant amount of potassium, but most of the K_2O is in an unavailable form for plants (Varga, 2015). Potassium that is available to plants originates from various primary minerals which decompose during the process of pedogenesis and are transformed into secondary minerals or clay minerals. Therefore, the clay quantity is regarded as an indicator of the potassium supply in soil (Gudžić, 2015). Other research indicated that potassium fixation showed positive significant correlation with amorphous ferri-alumino silicate content (Chatterjee et al., 2014). Soluble potassium, i.e. soil solution potassium is the form most available to plants. It is present in the soluble salts of the soil solution as well as in the ionian solution which is balanced with the salts absorbed by the colloidal complex (Radulov et al, 2014).

Aim of this paper is to determine content of available potassium in the

soil of the Aleksinac municipality. This is necessary in order to provide the soil with the necessary elements if necessary and thus avoid reduction in yield and prevent the possible occurrence of diseases.

Materials and methods

The territory of the Municipality of Aleksinac covers 707 square kilometers, of which agricultural land covers 45,463 ha, and forests 20,398 ha. Fertile alluvial deposits and groves predominate in the plains, and smonica in the hilly and mountainous parts (Agromedia, accessed 03.9.2020). In the entire Aleksinac region, out of the total number of households which is 18242, about 60%, more precisely 12,250 households are engaged in agricultural production. With that percentage, the Aleksinac region is one of the most developed agricultural areas (Zvanična internet prezentacija opštine Aleksinac, accessed 20.4.2021). In order to monitor supply of agricultural soil, Fertility control is a legal obligation of the owner, ie user of agricultural land according to the Law on Agricultural Land (Official Gazette of the Republic of Serbia 62/06, 65/08, 41/09). Samples for this research were taken in 2016 from a depth of 0-30 cm in field crops and vegetable cultures, or 0-60cm in orchards. They were taken from agricultural soils, and were collected from the territory of the 66 cadastral municipalities shown in Table 1. The total number of the collected samples was 1732. The largest amount of them were taken from cadastral municipality of Mozgovo (134 samples). The collected samples were examined by the AL-method in the laboratory of the Agricultural and Advisory Services in Niš and obtained results were processed by the computer program IBM SPSS Statistic – 20 (trial version).

Table 1. The number of samples and their percentage as regards the cadastral municipalities in which they were collected.

Cadastral municipalities	Frequency	Percent	Valid Percent	Cumulative Percent
Aleksinac Van Varoš	17	1,0	1,0	1,0
Aleksinački Bujmir	10	,6	,6	1,6
Baličevac	8	,5	,5	2,0
Bobovište	68	3,9	3,9	5,9
Bovan	97	5,6	5,6	11,5
Bradarac	20	1,2	1,2	12,7
Česta	19	1,1	1,1	13,8
Ćićina	31	1,8	1,8	15,6
Crna Bara	5	,3	,3	15,9

Crni Kao	2	,1	,1	16,0
Čukurovac	8	,5	,5	16,5
Deligrad	40	2,3	2,3	18,8
Dobrujevac	22	1,3	1,3	20,0
Donja Peščanica	6	,3	,3	20,4
Donje Suhotno	8	,5	,5	20,8
Donji Adrovac	5	,3	,3	21,1
Donji Ljubeš	16	,9	,9	22,1
Draževac	43	2,5	2,5	24,5
Glogovica	53	3,1	3,1	27,6
Gornja Peščanica	4	,2	,2	27,8
Gornje Suhotno	21	1,2	1,2	29,0
Gornji Adrovac	9	,5	,5	29,6
Gornji Krupac	7	,4	,4	30,0
Gornji Ljubeš	1	,1	,1	30,0
Gredetin	77	4,4	4,4	34,5
Jakovlje	15	,9	,9	35,3
Jasenje	33	1,9	1,9	37,2
Katun	81	4,7	4,7	41,9
Koprivnica	1	,1	,1	42,0
Korman	10	,6	,6	42,6
Kraljevo	6	,3	,3	42,9
Krušje	4	,2	,2	43,1
Lipovac	47	2,7	2,7	45,8
Ljupten	41	2,4	2,4	48,2
Loćika	62	3,6	3,6	51,8
Loznac	3	,2	,2	52,0
Luzane	18	1,0	1,0	53,0
Moravac	30	1,7	1,7	54,7
Mozgovo	134	7,7	7,7	62,5
Mramor	1	,1	,1	62,5
Nozrina	10	,6	,6	63,1
Prćilovica	8	,5	,5	63,6
Prekonozi	28	1,6	1,6	65,2
Prugovac	27	1,6	1,6	66,7
Radevce	13	,8	,8	67,5
Rsovac	24	1,4	1,4	68,9
Rujiste	3	,2	,2	69,1
Rutevac	73	4,2	4,2	73,3

Srezovac	9	,5	,5	73,8
Stanci	16	,9	,9	74,7
Subotinac	34	2,0	2,0	76,7
Šuric	57	3,3	3,3	80,0
Tešica	49	2,8	2,8	82,8
Trnjane	47	2,7	2,7	85,5
Vakup	3	,2	,2	85,7
Vrćenovica	40	2,3	2,3	88,0
Vukašinovac	37	2,1	2,1	90,1
Žitkovac	3	,2	,2	90,3
Bankovac	16	,9	,9	91,2
Mali Drenovac	44	2,5	2,5	93,8
Veliki Drenovac	7	,4	,4	94,2
Donji Krupac	9	,5	,5	94,7
Kulina	12	,7	,7	95,4
Dašnica	3	,2	,2	95,6
Vukanja	69	4,0	4,0	99,5
Porodin	8	,5	,5	100,0
Total	1732	100,0	100,0	

Prior to the chemical analysis, the samples had to be well prepared for it. The soil samples were thus first placed in separate vessels and left to dry. The elimination of impurities, soil grinding and griddle followed. Having confirmed that the soil was free from any impurities and homogeneous, it was returned to the vessels and prepared for the chemical analysis.

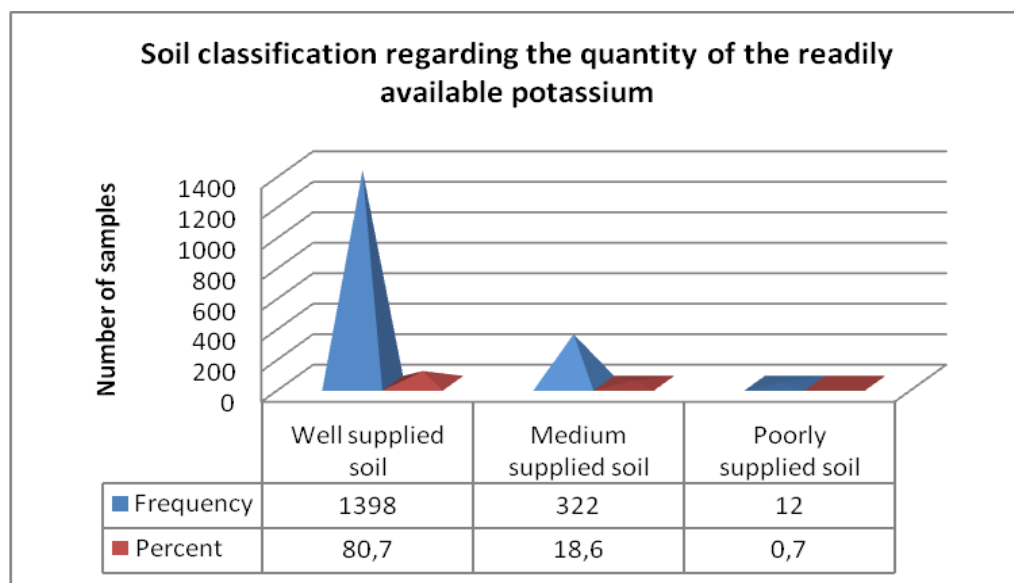
There are a lot of methods used for determining the potassium supply in soil. The AL Egner-Riehm method is considered more convenient in comparison to the other ones (Manojlović et al, 1969), since the readily available phosphorus and potassium are determined from the same extract. This method has been officially recognized by a lot of countries. Therefore, this method was used in this research in order to determine the readily available potassium supply in soil, i.e. the quantity of the soil solution potassium and of the adsorptive complex potassium. The method is based upon the element extraction from the soil samples using the extraction means. The AL solution is actually the solution of 0,1 N ammonium lactate and 0,4 N glacial acetic acid (Predić, 2011). Extraction was performed by AL solution and flame photometric method was used to determine concentration of potassium from this extract.

Results and discussion

After chemical analysis of all the 1732 samples, results of available potassium were compared to the values presented in Table 2 to determine the soil supply with the readily available potassium forms and to classify the soil in Aleksinac municipality. After that, the data was presented in the form of a graph 1.

Table 2. Soil classification as regards the readily available potassium supply showcased by Jelić (2012)

Soil classification	Values mg K ₂ O 100g ⁻¹ of soil
Well supplied soil	> 20
Medium supplied soil	10 – 20
Poorly supplied soil	< 10



Graph 1. Soil classification as regards the quantity of the readily available potassium obtained in Aleksinac municipalities

Based on presented results it can be confirmed that the results of the analyzes were satisfactory. Over 80% of samples were catagorised as well supplied with readily available potassium, that is exactly 1398 samples. Out of 1732 samples, 322 were classified as medium supplied wich is 18,6%, and onli

12 samples or 0,7% of all the samples were classified as poorly supplied soil.

These results are encouraging, given that the Aleksinac region is one of the most developed agricultural areas in eastern part of the republic of Serbia. The results recorded here are better compared to the results obtained in the municipality of Niš, where 62,9% of samples were categorised as well supplied and 35,5% as medium supplied (Grčak et al., 2020).

Conclusion

Chemical analysis of agricultural soil has become a necessary tool for monitoring the supply of essential nutrients. Such analyzes can help farmers a lot by instructing them on whether fertilization is necessary or not. This research has shown us that the supply of soil with potassium in the municipality of Aleksinac is at a very good level. Samples were taken in 2016 from a depth of 0-30 cm in field crops and vegetable cultures, or 0-60cm in orchards. They were taken from agricultural soils, and were collected from the territory of the 66 cadastral municipalities. The largest amount of them were taken from cadastral municipality of Mozgovo (134 samples). It is shown that 80,7% of samples were well supplied in available potassium while only 0,7% were poorly supplied, but research on other necessary macroelements, and also necessary microelements is certainly desirable. Because only with a complete picture of the situation of soil supply can one embark on more serious agricultural endeavors.

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THE CONTENT OF HUMUS IN THE SOIL OF NIS MUNICIPALITY IN 2016 (SERBIA)

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Abstract: *Analysis of 196 soil samples from arable land in the city of Nis, found that 37.2% belongs to the class of low humus soil with organic matter content of 1% to 3%, In the class of good supply of organic matter with a percentage of 3% to 5% is 62.8% of the analyzed samples. There was no sample with soil that had a very low percentage of humus (0-1%), but there are also no plots that had a high humus content ($\geq 5\%$). The content of organic matter in the soil is strongly influenced by man, so it is recommended to make a mandatory analysis of the soil before taking it out and burning the crop residues. Plowing them, as well as organic fertilizer, improves the content of humus in the soil, and with it the microbiological activity of the soil.*

Keywords: *humus, organic matter, soil.*

Introduction

According to the "Official Gazette of the City of Nis" (2009), there are 37,205 hectares of agricultural land on the territory of the city of Nis, of which 32,105 hectares, or 86%, have been cultivated. Humus is a source of nutrients and a factor in soil fertility preservation. Humus is a very important soil matter, due to its ability to bind large amounts of water and nutrients, which are easily available for plants (Weil & Magdoff, 2004). The humus content decreases in deeper soil.

Production of food, especially healthy food, is a global problem, because conventional agriculture creates a great number of negative effects (Pejanović, 2015). In order to preserve the natural fertility of soil, it is necessary to create a minimum of 3-3.5 t/ha of humic substances out of manure or other organic matter microbiologically. Today, the average amount of organic matter in the territory of the Republic of Serbia is around 3% (Cupać et

al., 2006), while in the north of the country it is somewhere between 3 and 5% (Sekulić et al., 2010). In earlier works (Korschens et al., 1998), larger amounts of organic matter were recommended to make the soil better structured, 5-10% organic matter. Also, a much higher amount of humus has been recorded on farms engaged in organic production (Shepherd et al., 2002).

One of the ways of losing humus is removal of or burning biomass of field plants. In this way, not only is organic matter lost, but also biogenic elements, which are fully or partially lost during the burning process (Kastori et al., 2012). Another way of losing organic matter from the soil is through climate change. Specifically, the loss has been proven of soil organic C contents may decrease greatly with global warming (Kirschbaum, 1995).

In order to stop further land degradation, organic agriculture is discussed more often. Fertilizers are not used in organic production, so it is important to maintain long-term soil fertility. The application of organic fertilizers introduces microorganisms into the soil, and the introduced organic matter is partially mineralized, then high-molecular organic compounds, humic substances are formed from the decomposition products (FAO, 2011). Soils with a higher content of total nitrogen contain more humus, so they are more fertile (Ubavić et al., 1990). Organic substances and mineral fertilizers in the soil contribute to the increase of root mass (Jaćimović et al., 2014).

In addition to regular fertilization with organic fertilizers, the best way to improve the total organic matter in the soil is to regularly plow the crop residues. Increases in organic matter may reduce compactibility by increasing resistance to deformation and/or by increasing elasticity (rebound effects) (Baldock & Nelson, 2000).

Material and Methods

In order to control the fertility of agricultural land, agricultural producers sampled their plots according to the principles of land sampling for fertility control based on the received instructions. Fertility control is also a legal obligation of the owner, ie user of agricultural land according to the Law on Agricultural Land (Official Gazette of the Republic of Serbia 62/06, 65/08, 41/09).

Sample collection and chemical analysis were performed in the Agricultural Advisory and Professional Service - Nis, during 2016. Soil samples were taken from a depth of 0-30 cm in field crops and vegetables, and 0-60 cm in orchards. An individual sample consisted of 20 to 25 individual samples (in lots up to 5 ha) from a depth of 0-30 cm in plots under field or vegetable crops or in plots where field or vegetable crops were planned to be grown, and from a depth of 0-60 cm, in plots under orchards or vineyards or in plots where orchards and vineyards were planned to be located. The content of

organic matter was determined by the modified method ISO 14235: 1998 according to Tjurin, on the principle of oxidation of soil organic carbon with $K_2Cr_2O_7$.

Results and discussion

Plots from all five municipalities of the city of Nis were analyzed. The layout of the samples taken from the plots is shown in Table 1. The total number of plots from which samples were taken is 196, while the number of farms from which the land was sampled is 49. Regarding the area of plots, the smallest area surveyed was 0.1 ha, while the largest was 2.78 ha. The average value of plots was 0.4 ha, while the sum of all plots from which samples were taken was 76.01 ha.

Table 1: Distribution of land samples by municipalities

Municipalities	Frequency	Percent	Cumulative percent
Crveni Krt	44	22,4	22,4
Medijana	35	17,9	40,3
Niška Banja	2	1,0	41,3
Palilula	40	20,4	61,7
Pantelej	75	38,3	100,0
Total	196	100,0	

In Serbia, for many years there was a practice of burning crop residues (due to increased fuel and mechanization costs when plowing crop residues and insufficient education of individual producers), which brings huge damage, both in agriculture, loss of organic matter and destruction of soil microorganism life, and significantly contributes to environmental pollution by emitting carbon dioxide and harmful combustion products into the atmosphere. The burning of crop residues is legally prohibited according to the Law on Agricultural Land (Official Gazette of the Republic of Serbia 62/06, 65/08, 41/09).

In the course of soil fertility control, the content of organic substance, i.e. humus, was analyzed as one of the parameters. Based on the processing of 196 data on the farms of Nis municipality (Table 2), it was found that a large percentage of 62.8% of the samples analyzed belonged to the class of humified soil, with a percentage of organic matter from 3 to 5. The rest, respectively 37.2% of the agricultural lands analyzed belonged to the class poorly humus content, from 1 to 3%. It is interesting that among the analyzed data there are no plots with very poorly humified (0-1%), but there are also no well-humified soils with the humus content higher than 5%.

The variability indicator was calculated through standard deviation

using the computer, and the standard deviation was ± 0.67 , which indicated that there were no major differences among the values of humus.

Table 2: The proportion of samples that belong to certain classes of humus content, based on the analysis of 196 soil samples taken in the municipality of Niš in 2016

Soil	Number of plots	Percentage (%)	Cumulative percentage
very poorly humified (0-0.99%)	0	0	0
poorly humified (1-2.99 %)	73	37,2	37,2
humified (3-4,99 %)	123	62,8	100,0
well-humified (≥ 5 %)	0	0	100,0
Total	196	100,0	

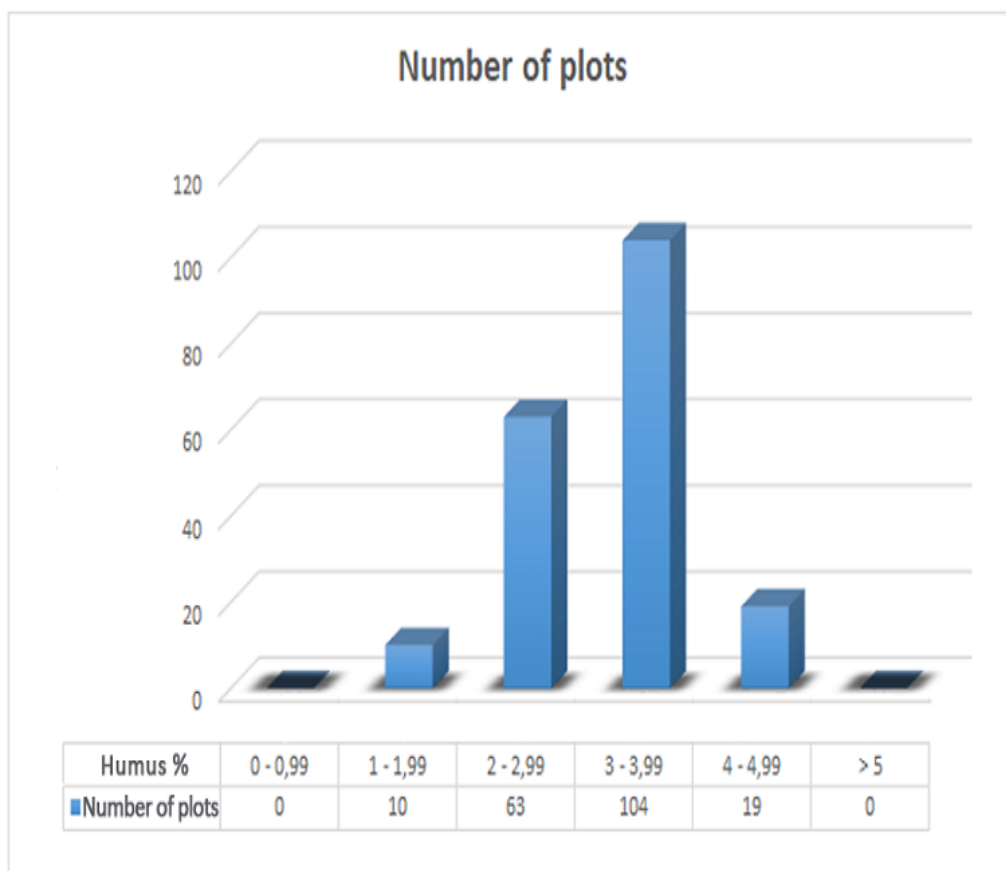
Since the existing classification of humus content is imprecise in terms of a wide interval for poorly and humified soil, and in order to better understand the dynamics of organic matter content in the soil, it is necessary to redefine the levels of soil security and introduce subclasses. Based on a more detailed classification (Table 3), the class of low humus soil is dominated by samples with an organic matter content of 2% to 3%, while the class of humus soil is dominated by soil with an organic matter content of 3% to 4%. That is, in the lands in the municipalities of Nis, the samples are distributed in such a way that they only fall into these two categories.

Table 3: Proportion of samples with different levels of humus in more detailed classification with subclasses

Soil class regarding levels	Humus %		% of soil samples
Very low in humus	0 – 1		0
Low in humus	1 – 3		37,2
	1 – 3	1 - 2	5,1
		2 - 3	32,1
Well provided by humus	3 – 5		62,8
	3 - 5	3 - 4	53,1
		4 - 5	9,7
Very high in humus	> 5		0

Also, the classification of land, where the intervals of certain groups of soil security with organic matter are more detailed, can be seen graphically in Graph 1. We notice that the limit values are completely absent, and that the densest distribution of samples is in the central part. Specifically, the mean value of all samples for organic matter content is 3.16%, which coincides with the graph. The lowest value recorded on the examined plots was 1.53% of organic matter, while the highest value was 4.92%.

Graph 1. The proportion of samples that belong to certain classes of humus content (%) in the municipality of Nis (number of samples 196).



Examination of humus content within the fertility control in 2015, 70,723 samples were analyzed in Central Serbia. The mean value of the analyzed samples is 3.73% and belongs to the class of humus soils (3-5% humus). The analyzed samples indicate that the lands under orchards, meadows and pastures belong to the class of humus soils (3-5% humus). While the lands under arable land and vineyards are in the class of low humus soils (1-3%

humus) (Ministry of Agriculture and Environmental Protection of RS, 2017). Also, the results of the humus content in the paper Milivojević *et al.*, 2012, on field crops on the arable land in the town of Kragujevac, show that 59.54% of the samples (average humus content 3.29%) belong to the class of soils with good humus content, while 35.77% of the samples (humus content ranging from 1.01 to 3.00%) belong to the class of soils with poor humus content.

Based on our results and the results of other authors, we can say that the presence of humus on the territory of the Republic of Serbia is not at an enviable level. It is encouraging that these values are still around 3%, and that an increasing number of agricultural producers are trying to improve the percentage of organic matter in the soil. In our country and in the world, on performed experiments, plowing of crop residues increases the yield and their quality, and the content of total nitrogen (N) and carbon (C) increases (Powelson *et al.* 1987). Soil microorganisms play a key role in creation and maintenance soil fertility. By taking out, or even worse by burning the harvest residues, we greatly reduce the microbiological activity (Šarčević-Todosijević & Živanović, 2017). So in addition to fertilizing with organic fertilizers, one of the key things in order to improve the organic matter in the soil is plowing the crop residues.

Conclusions

Based on the results of the analysis of 196 soil samples from 76,01 ha, or 49 farms, in the municipality of Nis (Serbia), it can be concluded that the fertility of the soil with humus is quite uniform. It was found that:

- 37.2% of the sample belongs to the class of low humus soil, with the content of organic matter from 1% to 3%.
- 62.8% of the analyzed soil samples were classes of good humus security, with a content of 3% to 5%.
- There was no sample with soil that had a very low percentage of humus (0-1%), but there are also no plots that had a high humus content ($\geq 5\%$).
- The humus content dominates above 3%, but it should still be maintained and increased by plowing crop residues and organic fertilization.
- Based on the test results, it can be concluded that more organic fertilizers should be used in the future.

Analysis of humus content shows that most of the examined soil belongs to the class of humus soil (62.8%) and weakly humus soil (37.2%). The obtained results are a consequence of inadequate agricultural techniques, ie insufficient application of organic fertilizers (insufficiently developed animal husbandry), as well as incineration and uncontrolled removal of crop residues.

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INFLUENCE OF HEAVY METALS ON MICROORGANISMS

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Abstract: *The technosphere that man created and the biosphere that he inherited are constantly in conflict. The ecological crisis that arose as a consequence of environmental pollution, concerns, above all, all of us. If we do not change our behavior, it is certain that we will not survive. Therefore, in this paper, the accumulation and other abilities of microorganisms, which should contribute to solving this great problem.*

Keywords: *biosphere, microorganisms, technosphere, pollution.*

INTRODUCTION

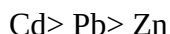
Of the large number of different chemical substances that reach the environment from anthropogenic sources, a special place is occupied by heavy metals. All heavy metals, and there are over 40 of them, are not equally dangerous for living organisms. According to the toxicity and ability to accumulate in various links of the food chain, only some of them are priority pollutants of the biosphere - mercury, lead, cadmium, copper, vanadium, tin, zinc, molybdenum, cobalt and nickel (Kastorii et al., 1997). mercury, lead and cadmium are the most dangerous.

During evolution, microorganisms, plants, animals and humans have adapted to the basic (natural) content of heavy metals in the soil. However, the intensive development of industry, transport, urbanism and the application of various chemical plant protection products have led to the accumulation of significant areas, even entire areas, of our planet, which negatively affects the soil, water, atmosphere and all living organisms that inhabit them (Đukić et al., 2013, 2018, 2020).

One of the most important tasks, which is related to the prevention of negative consequences of pollution of certain components of the environment, is to clarify the conditions and nature of the action of heavy metals on microorganisms (on their quantitative and qualitative composition) and enzyme activity.

Interaction between living and non-living matter in the soil

The interaction between living and non-living matter takes place most intensively in the soil, in which microorganisms play a significant role. Upon reaching the soil, heavy metals have an impact on the structure (number, composition of species) and functional state of microbial coenosis (Jemcev, Đukić, 2000; Mikanova, 2006; Đukić et al., 2007) and the activity of the enzyme catalase, urease, dehydrogenase, amylase, invertase (Balyaeva et al., 2005; Wang et al., 2007). The toxicity of heavy metals to different enzymes is not the same. In sod-underground soil, the most sensitive enzyme is catalase (Levin, 1989), while urease activity does not change under the same conditions. According to the influence on the activity of enzymes, heavy metals are classified into the following series:



Heavy metals change the total number of microorganisms. Under the influence of heavy metals, the number of oligonitrophils, ammonification and beneficial bacteria decreases. Cellulose bacteria are relatively resistant to heavy metals. It should be noted, however, that a change in the number of certain groups of microorganisms occurs only if the presence of heavy metals is twice as high as the basic (natural) level (Govedarica et.al., 1997). Heavy metals cause certain changes in the composition of microbial coenosis species (Jemcev, Đukić, 2000; Đukić et al. 2007).

Pollution of soil with heavy metals reduces the diversity of the microbial community. At the same time, species that are resistant to heavy metals appear in the micromycete community (Gavrilesca, 2004; Zafar et al., 2007). Species of the genus *Bacillus* are the most sensitive to heavy metal pollution, while *Pseudomonas*, *Streptomyces* and many species of cellulosic microorganisms are somewhat more resistant. Therefore, it is recommended that the change in the composition of the microbial coenosis be used as a criterion for assessing the polluting effect of heavy metals. The number of species of microorganisms decreases in soil contaminated with heavy metals, while the absolute dominance of *Penicillium purpurogenum*, which exhibits a strong phytotoxic property, is emerging among micromycetes. When soil is polluted with heavy metals, the share of typical soil yeasts decreases sharply. Sechromogenic (pigmented) microorganisms are characterized by increased tolerance in relation to heavy metals.

At low concentrations of heavy metals, there is a certain stimulation of the development of the initiated community of microorganisms, followed by their partial inhibition and finally, complete suppression. Soil microorganisms are differently resistant to heavy metals and are in constant interaction with each other and in interaction with the soil. Therefore, the reaction of microbial coenosis to heavy metals depends on their interaction with the soil, the action on microorganisms and the competitive interaction between microorganisms. In order to assess the degree of influence of heavy metals on the microbial coenosis of the soil, four zones of resistance are singled out (Guzev, Levin, 1991).

The first zone of stability (adaptive zone), which is characterized by the lowest level of load, is the zone of homeostasis. This zone includes the range of cadmium concentration of 0-7 mg /Kg of soil, in which only the total biomass of microorganisms changes while the composition and organization of the community do not change. According to the size of the homeostasis zone, soils can be classified into a certain series. Chernozems are characterized by maximum stability, and podzolic soils with minimal stability.

The second zone of stability, which is characterized by a medium level of load, is the stress zone of microbial soil coenosis. The concentration of agents in that zone causes a redistribution of populations of microorganisms according to the degree of dominance. With significant changes in the organization of microbial coenosis, its composition does not change. Toxinogenic microorganisms mostly develop in that zone. They inhibit the germination of plant seeds, seedling development, etc. (Kastori, Petrović, 1993). At the same time, the biomass of these microorganisms is not used by terrestrial invertebrates. There is a close relationship between the degree of microbial toxicity of cultivated crops. The occurrence of toxicosis is considered to be negative and is assessed as a polluting effect of heavy metals on the soil (Verešbaranji, et al., 1993).

The third zone of stability of microbial coenosis is characterized by a high level of load and is manifested at high doses of heavy metals - zones of resistance. In this zone, the diversity of microorganism species decreases sharply and resistant populations of microorganisms develop predominantly. In this zone, heavy metals have a direct toxic effect on soil microorganisms, causing the death of most of them.

The fourth zone of stability, which is characterized by the highest level of load, which leads to the complete elimination of the growth and development of microorganisms in the soil, is called the zone of repression. Complete suppression of the activity of soil microorganisms is a diagnostic property of its strong (catastrophic) heavy metal pollution.

Therefore, the size of the homeostasis zone should be accepted as an objective criterion on the basis of which the effect of heavy metals on the

microbial coenosis of different soil types should be assessed and the potential stability (resistance) of microorganisms to pollution should be determined.

The accumulation of heavy metals in the cytoplasm of microorganisms depends on the seasonal variations of their biomass and on the content of the respective element in the soil. In biogeochemical areas with a high content of heavy metals, the microbial community of soil accumulates 3-150 times more metals than the microbial community of soil from areas with their basic (natural) content. Heavy metal pollution causes a decrease in the nitrification ability of the soil. The increase in copper content in acidic podzolic soils is accompanied by a decrease in the amount of mineral nitrogen. However, in soils with a high humus content, cadmium and lead have contributed to the increase in the amount of mineral nitrogen. High doses of cadmium and zinc suppress the urease and dehydrogenase activity of the soil.

As the content of heavy metals increases, the spatial variability of nitrogen-fixing activity decreases. In soils where the concentrations of heavy metals are more than ten times the base (natural), the variability of nitrogen-fixing activity is significantly lower.

When soil is contaminated with cadmium, the release of CO₂ from the soil is reduced by 2-3.5 times, and when chromium is contaminated, it is reduced by 2-6 times. According to the strength of the effect on the number of nitrifying bacteria in the soil, heavy metals can be distributed in the following order:



The increased content of heavy metals in the soil inhibits the processes of nitrogen transformation and leads to a decrease in the activity of nitrifiers, which slows down the processes of transformation of nitrogenous substances and reduces the utilization of mineral forms of nitrogen (Hu et al., 2002; You et al., 2008).

The microbial coenosis of the soil adapts to the pollution of the soil with heavy metals depending on their concentration. At certain concentrations, the mechanism of adaptation is not functional and the microbial community undergoes significant changes. Under the influence of increasing concentrations of cadmium and chromium, the number of nitrifiers in chernozem decreases and the activity of cellulose bacteria decreases, while under the influence of nickel, the activity of cellulose bacteria does not change (Đukić et al., 2007, 2020).

Therefore, a characteristic feature of soils that are not contaminated with heavy metals is the wide spatial and temporal variability of the application of all parameters of microbial coenosis functioning. During soil pollution, which is several times higher than the basic (natural), certain indicators of enzyme activity change. The biomass of the amylolysis microbial community

grows. When the soil is polluted with heavy metals, which are an order of magnitude above the basic one, the biochemical activity of soil microorganisms decreases and the degree of dominance of the amylolysis microbial community that actively functions in the soil is redistributed. When the soil is polluted with heavy metals, which exceed the basic by one or two orders of magnitude, there is an obvious decrease in the whole group of indicators. There is an absolute dominance of one type of micromycetes and the participation of epiphagous yeasts is growing rapidly. When soil is contaminated with heavy metals, which is three orders of magnitude higher than the basic one, there is a sudden change in all microbiological processes, as well as the death of microorganisms that are normal for unpolluted soils. At the same time, the dominance of micromycetes increases (Đukić, Mandić, 2000). If the content of heavy metals in the soil increases four or more times above the basic one, there is a sharp decrease in the microbiological activity of the soil, which borders on the complete death of microorganisms.

The mutagenic activity of a large number of heavy metals was also determined: cadmium, copper, mercury, lead, chromium, nickel and manganese. Mutations in microorganisms, caused by heavy metals, are manifested by the appearance of resistance to these elements. For example, the formation of resistance in rhizobium to cadmium is due to the mutant action of that metal. However, at a concentration of less than 10 mg /L, cadmium stimulates the growth of rhizobium. Soils that contain the highest amounts of cadmium, despite their high adsorption capacity, exhibit the greatest mutagenic effect compared to less polluted soils.

Thus, the most toxic elements for soil microorganisms are mercury and cadmium, while nickel lead and copper are less toxic. Because heavy metals exhibit high toxicity in plant tissues (Kastori, 1997). for food products, the minimum values of MDK of heavy metals are provided.

CONCLUSION

Based on the results of research related to the role of intensive industrialization, urbanization, transport and chemicalization of agriculture in environmental pollution by heavy metals and their impact on soil microorganisms, the following conclusions can be drawn:

- numerous technogenic and anthropogenic agents have led to, often, a significant increase in heavy metals in the environment;
- in low concentrations heavy metals can stimulate the development of microorganisms, and in higher concentrations they lead to a decrease in their number and a change in the qualitative composition of their species;
- some microorganisms, especially some micromycetes have the role of

hypercumulators of heavy metals, thus leading to a decrease in their concentration in the environment;

- chromogenic microorganisms are especially characterized by the hyperaccumulation ability of heavy metals;
- many microorganisms appear as indicators of the type and degree of environmental pollution by heavy metals.

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BEHAVIOR AND BIOLOGICAL TRANSFORMATION OF ENVIRONMENTAL TOXICANTS IN THE ENVIRONMENT

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Abstract: *This paper examines the behavior of environmental toxicants in the environment and their biological transformation. Organic and inorganic environmental toxicants undergo hydrolysis, reduction, oxidation, photoisomerization, photoionization, photomineralization, etc. reactions, either spontaneously or under the influence, primarily of microorganisms, but also of plants and animals.*

Key words: *ecology, environment, microorganisms, toxicants.*

INTRODUCTION

The basis of the processes that condition the current state of the biosphere are the processes of chemical and micro (biological) transformation of toxic substances, by which man constantly pollutes the environment (Ložničenko et al., 2008). To solve various environmental problems imposed by the modern state of the environment, it is very important to apply precise methods of chemical analysis as well as other methods of bioindication and biotesting of various objects from the environment - air, soil, water and living organisms (Pöllumaa et al., 2004; Kuczyńska et al., 2005; Đukić et al., 2013). Thanks to that, it is possible to monitor and direct the mechanisms of chemical and biological transformation of certain compounds or groups of compounds in the conditions of action of natural and anthropogenic factors.

The aim of this informative review paper is to point out the behavior of environmental toxicants in the environment and possible pathways of its chemical and especially biological transformation.

Behavior of environmental toxicants in the environment

Upon arrival in the environment, heavy metals undergo various transformations with a change in valence and solubility. Metallurgical companies release heavy metals, mostly in insoluble form. However, during atmospheric transfer, they are extracted in the form of aerosol particles and converted into ionic, water-soluble form. This is conditioned by the presence of ionic acids (HNO_3 , H_2SO_4) in the atmosphere. The intensity of extraction is evidenced by the fact that the Antarctic aerosol contains metals exclusively in water-soluble form.

Ecological toxicants of organic nature undergo the following basic transformations: hydrolysis, reduction, oxidation, photoisomerization, photoionization, photomineralization, etc.

Hydrolysis involves the saponification of complex ethers and the replacement of halogen atoms with a hydroxyl group. A typical example of the hydrolytic replacement of chlorine atoms with the $-\text{OH}$ group is with pesticides with chlorine. The rate of their hydrolysis depends significantly on soil pH and temperature. In acidic soils with low temperatures, organochlorine pesticides are retained for several years.

Reduction processes in the environment usually take place in an anaerobic environment, for example in sediments. As a result of the life activity of some, where oxygen is released, the reduction of Fe (III) to Fe(II) and some other processes.

Oxidation of chemical substances in the gaseous and aqueous phases, and also in the adsorbed state, occurs primarily at the expense of various activated forms of oxygen. Chemical reactions with the participation of (singlet) excited oxygen are conditioned by the electronic structure of its molecule: the existence of an unfilled π -orbital determines its electrophilic character, due to which singlet oxygen enters reactions with unsaturated compounds, e.g. $-\text{C}=\text{C}-$ position. Concentrations of singlet oxygen in the atmosphere are at the level of 10^8 cm^{-3} , and in aquatic environments $10^{11}-10^{12} \text{ cm}^{-3}$, and such small concentrations still do not allow for a detailed analysis of their role in the ecosystem.

In the upper layers of the atmosphere during the photodissociation of oxygen molecules, and in the troposphere into which light with wavelengths below 290 nm does not penetrate, atomic oxygen is created during the photodissociation of nitrogen dioxide. It can be combined with double alkene bonds. In reactions with alkenes, another active form of oxygen competes - ozone, whose concentration in ground air is at the level of $10^{12}-10^{13} \text{ cm}^{-3}$. The reaction of alkenes, for example, chlordiene, with ozone has several phases. As a result of the first of them, ozonide is formed, further decomposition with the termination of the $\text{O}-\text{O}$ bond leads to the formation of reactive radicals.

The return from the excited to the ground state, when light is absorbed by molecules, can take place in several ways: at the expense of photoisomerization, photoionization, energy transfer to another molecule, which is then chemically transformed, and photomineralization, which is the complete decomposition of chemicals into simple inorganic molecules (CO₂, H₂O, HCl). In the aqueous and gaseous phase, organochlorine pesticides are relatively resistant, adsorbing on the surface of silicate materials, they are quickly mineralized.

Biological transformation of environmental toxicants in the environment

Inorganic environmental toxicants. Many organisms possess natural mechanisms for metabolizing and removing heavy metals, most commonly in the form of organic heavy metal compounds (Bentley, Chasteen, 2002). One of the mechanisms of biomethylation is the transfer of the methyl group from the modified form B12. As donors, the methyl group also serves as products of N-methyl-tetra-hydrofolate and S-adenosylmethionine.

The process of biological methylation is most intensively performed by microorganisms (bacteria similar to pseudomonas), which live in sedimentary rocks. Therefore, organometallic compounds, in the largest quantities, enter aquatic ecosystems.

Due to the rather high chemical stability and lipophilicity, organic forms of mercury and some other heavy metals accumulate in adipose tissue and are transmitted through food chains.

Mechanism of organic environmental toxicants. Enzymatic reactions can lead to the detoxification of environmental toxicants, as well as to the formation of metabolites with higher toxicity, so in the latter case we are talking about activation. Three types of enzyme-induced transformation are possible: complete mineralization and degradation of xenobiotics to low molecular weight compounds, which are then released into the environment and incorporated into the natural environments of matter; chemical transformation with accumulation of metabolites in the cells of organisms.

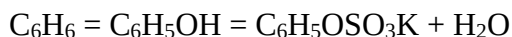
Nonspecific enzymatic transformations. Living organisms, with the exception of some other bacteria, do not possess specific enzymes for the transformation of xenobiotics. Nonspecific enzymatic transformations usually involve oxidation, reduction, and hydrolysis reactions (Parkinson, 2001). Oxidation processes are the most common. A typical example of oxidative metabolism is the oxidation of alcohol ethanol followed by aldehyde dehydrogenases ($\text{CH}_3\text{CH}_2\text{OH} \rightarrow \text{CH}_3\text{CHO} \rightarrow \text{CH}_3\text{COOH}$). Reduction processes in organisms have been less studied than oxidative ones. An example of a reduction transformation is the reduction of disulfide. In plant tissues, nitroso compounds are probably reduced by nitro- and azoreductases to amines.

Hydrolysis reactions include, above all, the breakdown of starch, proteins and neutral fats. These reactions are catalyzed by hydrolase enzymes (enzymes that break down fat-type complex ethers called esterases). Among the means for chemical protection of plants from insects, there are many substances with complex ether groups, especially interorganophosphorous compounds. This is explained by the fact that in insect organisms, unlike mammalian organisms, the set of esterases is poor, which is why insects do not detoxify rapidly, but accumulate to a lethal concentration.

Conjugation. In plants that do not have excretory systems, analogous to the excretory organs in animals, there is usually conjugation of xenobiotics (or their metabolites) with carbohydrates and deposition in fats that are not related to the basic metabolism (Parkinson, 2001).

An important stage of biological transformation in vertebrates is the conversion of hydrophobic compounds into water-soluble ones, which cannot be excreted in the urine. The previous stage of transformation of a hydrophobic xenobiotic must be the introduction into the molecules of a polar group, for example its C-hydroxylation. Further, conjugation-binding of xenobiotics or products of their transformation with hydrophilic molecules, such as sulfate ion and glucuronic acid, takes place. These molecules are present in biological environments in small concentrations.

The summary process of C-hydroxylation and conjugation with sulfate ion is given for the benzene molecule:



Finally, toxic benzene is removed from organisms with urine in the form of calicum salt of phenol sulfoether.

Unfortunately, no attention is paid to biological bodies and processes in the curricula. Not only specialists or agronomists must have knowledge about them, but literally every person, because the ecological security in any region (area) is closely connected with humic substances, and the stability of land and water areas, soil and biocenoses depends on them. Knowledge of such processes and bodies must be significantly reconciled.

CONCLUSION

The human impact on the environment has reached worrying proportions. In order to radically improve the state of the environment, it is necessary to introduce very well-thought-out and focused activities. Responsible and effective policy in relation to the environment will be possible only if we collect comprehensive data on the current (current) state of the environment, apply knowledge about the interaction of important

environmental factors and develop new methods that will reduce and even prevent human damage. inflicts on nature, counting on the possibility of living systems, especially microorganisms, to play the most important role in it.

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IMPORTANCE OF BIOLOGICAL SUPPLEMENTS BASED ON *BACILLUS SUBTILIS*

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Abstract: *In order to fully upgrade the ecological state of the environment, we must resort to alternative agricultural production systems that protect, maintain and improve the processes of pedogenesis and soil bioproductivity, implementing organic fertilizers and microbiological supplements. Factors unducing plant diseases may incur significant damage to cultivated species, by diminishing the quality and destroying the yield. Due to negative impact of chemical pesticides on the environment and human health, the possibility of implementing biopesticides in intensive plant protection has lately become the object of intensive studies. This study points out the anti-microbe effect of biological supplementproduced on the basis of Bacillus subtilis.*

Key words: *Bacillus subtilis, microbiological supplements, plant pathogenes*

INTRODUCTION

Numerous technogenous and anthropogenous factors exert highly negative impact on the environment (Đukić and Mandić, 2000; Đukić et al., 2007a), accordingly the issue of diminishing the impact is being raised. According to Solanka et al. (2015), microorganisms that exert antagonist properties to pathogenes parasitizing the cultivated plant species, have the ability to substitute chemical pesticides with negative environmental impact. Ecological state improvement may be contributed to by transition of certain types of agricultural production to harmless methods, including partial or full substitution of chemical plant protection agents for biological ones, such as microbiologically originated supplements (Đukić et al., 2007b). Here not only

the live cultures of microorganisms-introducents, but the products of their metabolism may also act as basis for the supplement.

Microorganism-based biopesticides may represent an alternative course of crop protection, due to its safety for people and untargeted organisms, both in individual applications and within an integrated pest management (IPM) system. Gašić and Tanović, 2013; Seiber et al., 2014; Mnif and Ghribi, 2015). Marketing share of biopesticides in the global market of pests and pathogenes eradication products is still low, but there is a constant increase in their imoplementation and production (Damalas and Koutroubas, 2018). For a biopesticide to be accepted in broad application, it has to fulfill several conditions: to be economical to produce, be consistently stable in warehousing, have high residual activity, be easily managed, mixed and applied, and provide efficient control of targeted pests (Gašić and Tanović, 2013).

The aim of this study is to point to great potential of alternative systems of managing agricultural production that lead to increase in biological productivity of plants and to protection of the environment.

Advantages of biological supplements

Advantages of microorganism-based biological supplements are numerous: they are ecologically harmless, do not pollute the environment, are neither harmful to humans and animals nor phytotoxic, and do not act as a mutagen. Live microorganism cultures, constituting ingredients of biological supplements, are able to fixate the atmospheric nitrogen, synthesize plant growth stimulators and vitamins, convert certain chemical compounds (e.g. phosphates) to forms that are better absorbed by plants, as well as colonize the root system or plant phyllosphere.

The foundation of anti-microbe activity of biological supplements based on microorganisms lies in their ability to synthisize biologically active compounds, such as antibiotics and similar compounds, pigments, siderophores (catecholates and hydroxamate type), etc.

Practically speaking, the most important group of biological supplements is the one intended for protection of plants against bacterial and fungal infections. Diseases of thess types are the most common in agricultural plants and do not affect only vegetable crops of open and closed spaces, but also the cereal grains, leguminosae and a large number of other plants, causing significant reduction in quality and yield loss (Đukić et al., 2007c).

A spectrum of biological supplements for struggle against fungal diseases is still narrow. The most well known among them are: Trihodermin, Trihocetin, Fitolavin-100, Mikolitin, Baktofit, Rizoplan, Bacifit. As for the group of supplements active against diseases of plant bacterial nature, there are only a few of them, most prominent of which are: Agrofil – biological

supplement based on bacteria *Agrobacterium tumefaciens* and Mikolin – based on *Bacillus mycoides*. Reviewing the Register of pesticides in agriculture and forestry of the Republic of Serbia for 2020, biofungicides that have been registered and licensed for the market are the following: Ekstrasol F (*B. subtilis* Č 13), F stop (*B. subtilis* ST 1/III), Bacillomix aurum B (*B. subtilis* BS10), Erwix (*B. subtilis* BS10) and Polyversum (*Pythium oligandrum*) (Tim priređivača, 2020):

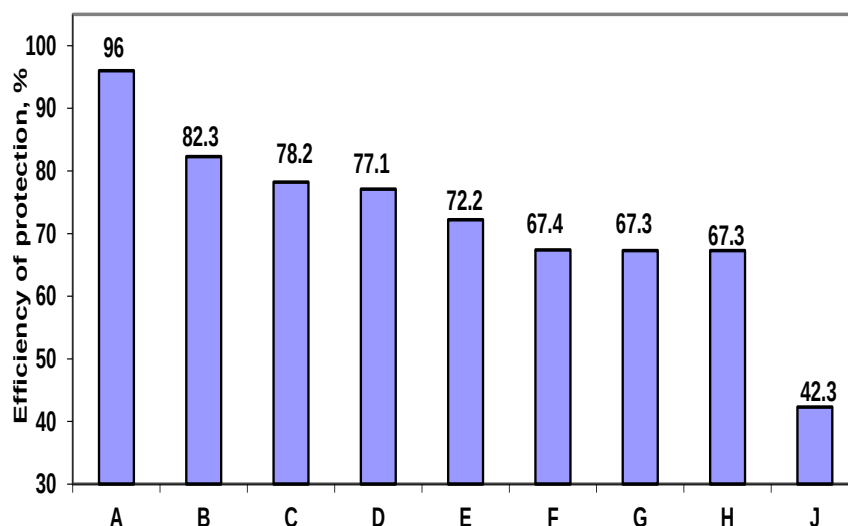
Suppressing of pathogenic bacteria and fungi by implementing biological agent *Bacillus subtilis*

There's been an ever increasing use of biopesticides lately in control of phytopathogenic bacteria (Agrios, 2005; Alabouvette et al., 2006). Assis et al. (1996) have studied the antagonism between 32 epiphyte isolates of *Bacillus* spp. from cabbage, kale and radish, 13 of which have reduced by 100 % the occurrence of black rot in kale in greenhouse conditions. In field experiments the occurrence of black rot in cabbage has been reduced by 48 - 78 % by applying the same 13 isolates of *Bacillus* spp. (Assis et al., 1997). Representatives of the *Bacillus* spp. genus have been involved in control of plant diseases, through various mechanisms of operation, such as competition over food and space, induction of systemic resistability, production of antibiotics (Monteiro et al., 2005). This genus consists of diverse group of gram-positive, aerobic or facultative anaerobic bacteria, with extreme ability to quickly adapt to various environmental and nutritive conditions, and to produce a wide spectrum of bioactive metabolites (Perez-Garcia et al., 2011). Among the well-known representatives of the mentioned genus, *Bacillus subtilis* stands out, being able to produce numerous antibiotics (bacilisin i fengimicin, difucidin i oxidificidin, bacitracin, bacilin i balilomicin b and iturin) affecting numerous types of aerobic and anaerobic bacteria (Grahovac et al., 2009). Certain strains of *B. subtilis* genus, the cultures of which synthesize antibiotics, exert 100% anti-microbe activity in relation to a high number of phytopathogenic bacteria, such as *Erwinia*, *Pseudomonas*, *Xanthomonas* (Vlajić et al., 2016), *Clavibacter* (Milijašević-Marèić et al., 2018), but also of the phytopathogenic fungi of genera: *Sclerotinia* (Radujkov et al., 2015), *Verticillium* (Milijašević-Marèić et al., 2018), *Fusarium* (Feldeždi et al., 2016), *Trichoderma pleuroti* and *Trichoderma pleuroticola* (Potoènik et al., 2019), *Rhizoctonia solani* (Solanaki et al., 2015), *Alternaria*, *Ascochita*, *Botrytis*, *Phytophthora*, etc. Here it was determined that both the vegetative and generative functions of mold are suppressed.

Selected strains of this bacteria genus synthesize an antibiotic of aromatic nature, with aminoglycoside component. The antibiotic is resistant to proteolysis, temperature and pH of the environment. The metabolism products,

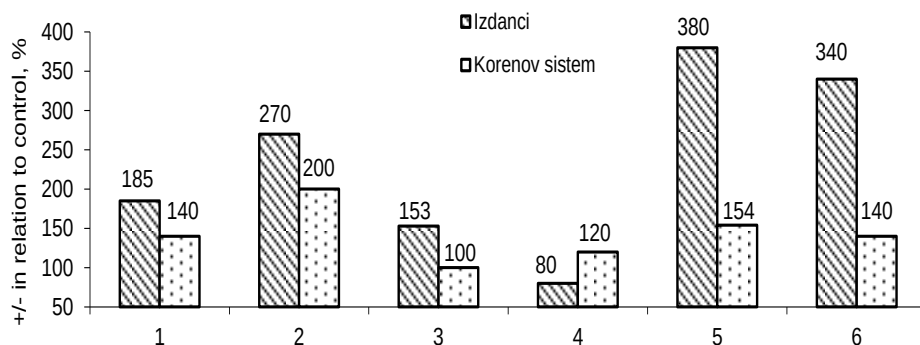
lipopeptides, affect various components of the cell wall, preventing adhesion to plant organs, while the enzyme *subtilin* obstructs the development of pathogens (Klokočar - Šmit et al., 2003). However, toxin production slowing down the growth of pathogens - the antibiosis mechanism, is one of the most important mechanisms (Thomashow and Weller, 1996).

Biological supplements, produced on the basis of this bacteria, that is, some of its strains, such as Bactogen, for example, exert high anti-microbe activity in closed space conditions, and can therefore be considered as highly efficient in protection of plants against bacteriosis, fungal infections and nematodes (Graph. 1).



Graph. 1. Efficiency of bactogen application in protection of vegetables against bacterial and fungal infections: A-Bacteriosis; B- Root rot; C- Powdery mildew; D- Ascochitosis; E-Peronosporosis; F- Cladosporosis; G- White rot; H- Black rot; J- Nematodes

Apart from the anti-microbe activity, this supplement is capable of stimulating plant growth and development (Jemcevic and Đukić, 2000). Its effect is particularly evident in early stages of plant development (Graph. 2). The supplement helps increase the vegetable crop yield (1,1 - 1,2 kg/m² in cucumber; 2 kg/m² – in tomato, for example) – Maksimova et al. (2000).



Graph. 2. Phytostimulating effect of bacterial supplement produced on the basis of *Bacillus subtilis* (bactogen): 1- cucumber; 2- wheat; 3- corn; 4- barley; 5- flax; 6-rye

CONCLUSION

Biological supplements, produced on the basis of *Bacillus subtilis*, exert manifest a multifold positive effect :

- protect plants against bacterial and fungal infections;
- stimulate plant growth and development;
- to a certain extent represent an alternative to chemical means of plant protection, contributing to protection of the environment.

Due to all of the above, if used correctly and timely, the supplements based on the mentioned biological agent can be an adequate substitute for chemical pesticides. This will respectively lead to production of health-safe food and preservation of the environment.

ACKNOWLEDGEMENT

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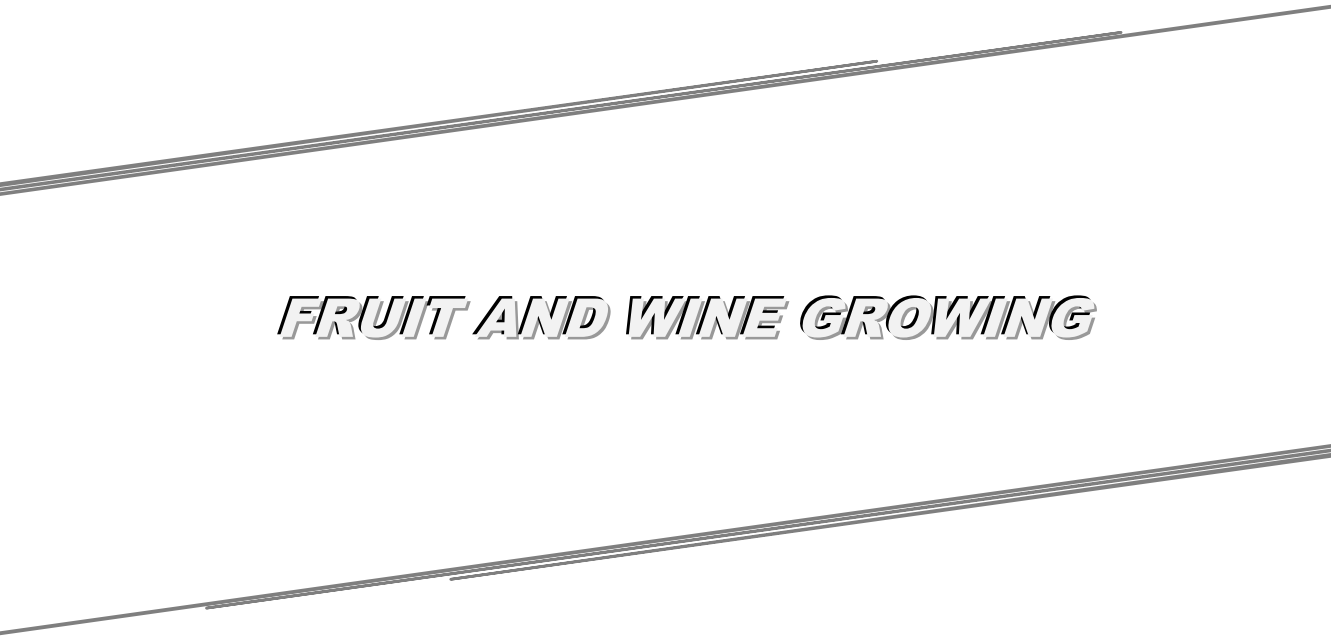
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FRUIT AND WINE GROWING

INFLUENCE OF DIFFERENT FERTILIZATION TREATMENTS ON PHYSICAL CHARACTERISTICS OF GALA SHNIGA APPLE FRUIT (*MALUS DOMESTICA L.*)

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Abstract: *Apple is the second most widespread fruit crop in terms of representation immediately after plums and has an important role in the total fruit production in Bosnia and Herzegovina, with a total area of 19,040 ha, yields of 3.43 t/ha, total production of 65,307 t with a tendency of a slight decline at a rate of 0.96% per annum. The production and supply of apples on the BiH market is less than the potential and does not meet domestic needs, and the lack of apples is imported. In the world, the apple is one of the most popular fruit species, and in Europe it is produced with about 60% of total world production, and it is mostly grown in Poland, Italy and France. Apple production, compared to other fruit species, ranks third in the world.*

The Gala Shing apple (Malus Domestica L.) originates from New Zealand and was obtained by crossing Golden Delicious and Kidd's Orange Red varieties.

The aim of the research was to determine the effect of the treatment and the influence of different fertilization methods on the physical characteristics of the fruit of the Schniga variety. The research was conducted in 2019 and 2020 at the Institute of Agriculture of the FBiH in Sarajevo at the Butmir site on 18-year-old apple trees (Malus domestica L.- cv. "Gala Shniga"), on a substrate M9 according to the block system (10 trees per treatment), at a density of 1300 trees/ha. The results of research show that they and spitivani treatments fertilization and years of study had statistically significant differences in the physical characterization of tics fruit apples.

Keywords: *Apple, Gala Shinga, treatments, fertilization, fruit characteristics.*

Introduction

Apple is one of the most important fruit species in the world in terms of production and processing. The use of apples throughout the year in the human diet as a table variety or apple product is classified as a primary fruit species. In some countries it is the basic measure of the development of fruit growing in

general. Apple is one of the most useful and widespread fruit species. It is native to Asia and neighboring European countries. It thrives in different areas, because it adapts well to climatic conditions (Milić, D. 2003). World apple production is about 70 million tons. The largest producers are China with 44, followed by EU countries with about 10, USA with 4.7, Turkey with 2.8 and India with 2.3 million tons. In the EU, Poland leads with 2.9, Italy with 2.1, France with 1.4 million tons, and Germany with 990,000 and Spain with 550 thousand tons.

Soils that are most often unfavorable in terms of their physical, chemical, microbiological and other characteristics, and on which, for example, field crops would not achieve satisfactory production and economic results, can also be used for apple growing. Fruit growing is one of the most productive agricultural branches, which far exceeds the productivity of other crops (Keserović, 2004).

The Gala Shniga apple variety originates from New Zealand and is one of the leading varieties in production in the world. Depending on the agro-ecological conditions and localities, it is harvested in BiH during August. It is moderate to strong lushness and is very fertile. The fruits are regular, sweet in taste, with a complementary red color in the form of stripes or spots. It is less susceptible to powdery mildew, susceptible to soot scab and bacterial blight, and is very susceptible to bark cancer (*Nectria galligena*). Complementary color in the basic variety is not sufficiently developed and better colored clones are constantly distinguished. Leading clones are Brookfield, Schniga, Bakej, Fendeka (Cvetković, M. et al. 2016)

The application of agrotechnical measures, above all, fertilization is crucial for the yield and quality of apple fruit. It is necessary to plan the nutrition of the apple tree from the time of planting in order to stimulate proper growth and development until the time of fruiting (Lončarić, Z. 2016). For this reason, it is necessary to balance the diet of apple growing, which depends on several factors, primarily on planting material (category), assortment of age, etc. In this context, it is necessary to know what are the requirements and norms of fertilizers it should also be applied in which way a certain type and amount of nutrients (Milatović, D. 2009).

Therefore, it is necessary to know the phenophases of apple development in order to nourish, protect and other measures of apple fruit tree care. For adequate growth and development of apples, basic nutrients are necessary, primarily nitrogen (especially in the development phase), phosphorus, potassium, calcium, magnesium and sulfur. Adequate application of nutrients is based on the assessment of expected and desired yields, and certainly the pedological analysis of the soil that is the basis of any production, and therefore apple production. It is important to determine the method of nutrient application, especially the application of phosphorus and potassium

which are poorly mobile in the soil. Fertilization of all fruit species, therefore apples, primarily affects the balance of nutrients in the soil and their availability, the effect of which largely depends on the physical and chemical properties of the soil, especially the pH value. The strategic commitment of apple producers is to obtain quality fruit that would meet market demands (Latal, M. 2004) .

The aim of researching the influence of different fertilization treatments on the physical characteristics of apple fruit is to determine which quantities, types of fertilization and methods of treatment (applications) affect the physical characteristics of Gala Shniga apples.

Material and method of work

The research was conducted during 2019 and 2020 in the orchard of the Federal Institute of Agriculture (Butmir - Sarajevo), on 18-year-old apple trees (*Malus domestica* L.- cv. "Gala Shniga"), according to the block system (10 trees per treatment), at a density of 1,300 trees/ha. The inter-row planting distance is 3.0 m and the distance within the row is 0.8 m. The fertilization experiment was set up and carried out in the orchard on 4 rows on the Gala Shniga apple variety.

Physical characteristics of soil were determined by standard methods that are used in the laboratories of the Federal Bureau of Agropedology. Location on whom he set up an experiment (Butmir) by the type of soil is brown soil on alluvial application, and the texture composition of the clay.

Fertilization treatments were determined based on the results of soil analysis. Quantities of it fertilization treatments were performed by blocks (variants and control):

- Variant-control: (standard fertilization NPK 15: 15: 15-300 kg/ha);
- Variant-A: foliar fertilizer - YaraVita Universal Bio (P_2O_5 - 35 g/l, K_2O - 60 g/l, B 0.2 g/l, Cu 1 - g/l, Mn - 1.1 g/l, Mo - 0.3 g/l, Zn - 0.6 g/l);
- Variant-B: KAN (27%) 400 kg/ha + NPK (15:15:15) 200 kg/ha.

All treatments were dissolved in 8 l water/block. Measured values of apple fruit were determined on the analytical balance. The circumference, height and width of the apple fruit were measured with a meter.

For each variant and control, an average sample of 20 apple fruits was made for analysis. The obtained values of the measurement parameters were statistically processed by two - factor analysis of variance using the software package SPSS 23.0 according to the ANOVA procedure, and the differences in the means were tested by the LSD test.

Results

The aim of research on influence of different fertilization treatments on the physical characteristics of apple fruit is to determine which quantities, types of fertilization and methods of treatment (applications) affect the physical characteristics of Gala Shniga apples.

The area where research was conducted is the experimental polygon Institute for Agriculture FBH – Butmir. The chemical characteristics of the soil where measured by standard methods that are used in the laboratories of the Federal Bureau of Agropedology. The land was sampled according to the Instruction on the procedure, actions and conditions for performing soil fertility control. The limit values (Wiegner) of the active reaction (pH in H₂O) range from 6.96 to 7.44. The values of the substitution reaction (pH in 1M KCl) range from 5.90 to 6.22, whereby the soil shows a neutral to weakly acidic reaction. Sadržaj importance of humus in % had a value of 2.26 % to 2.28 %, indicating that this is not enough soil humus. The CaCO₃ content in % is in the values of 0.3 to 0.5 and it is a moderately carbonate soil. Content of physiological active (approachable) phosphorus is in the range of 27.50 to 33.39, potassium is between the values of 20.50 to 27.32 which indicates on a good provision of soil with this element.

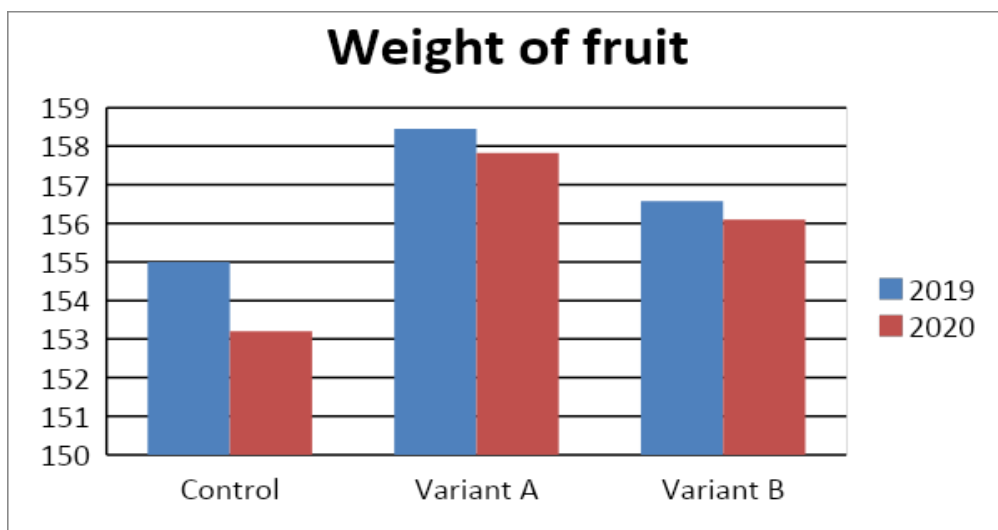
After the analysis of the examined samples of Gala Shniga apple fruit, the data was statistically processed, and the results with average values were presented in tables and graphs. In the context of the study, the mass of 20 average apple fruits taken for each variant and repetition was analyzed.

Table 1. Characteristics of apple fruit

Variants	Fruit weight in g.		Fruit circumference in mm.		Fruit height in mm		Fruit width in mm.	
	2019	2020	2019	2020	2019	2020	2019	2020
Control	155.00	153.20	67.60	66.65	62.40	60.84	68.28	65.21
Variant A	158.45	157.83	69.50	67.85	65.95	62.41	69.31	67.73
Variant B	156.58	156.10	69.81	68.15	66.36	61.40	68.76	66.90

It was found that in both years of testing variant A (foliar fertilizer - YaraVita Universal Bio (P₂O₅ - 35 g/l, K₂O - 60 g/l, B 0.2 g/l, Cu 1 - g/l, Mn -

1.1 g/l, Mo - 0.3 g/l, Zn - 0.6 g/l) showed a higher mass (weight) of apples compared to variant B (KAN (27%) 400 kg / ha + NPK) (15:15: 15) 200kg/ha) and control (standard fertilization NPK 15:15:15 - 300 kg/ha). The values of apple mass between the applied fertilization norms in control and variant B are not statistically significant.



Graph 1. Weight of the apple fruit

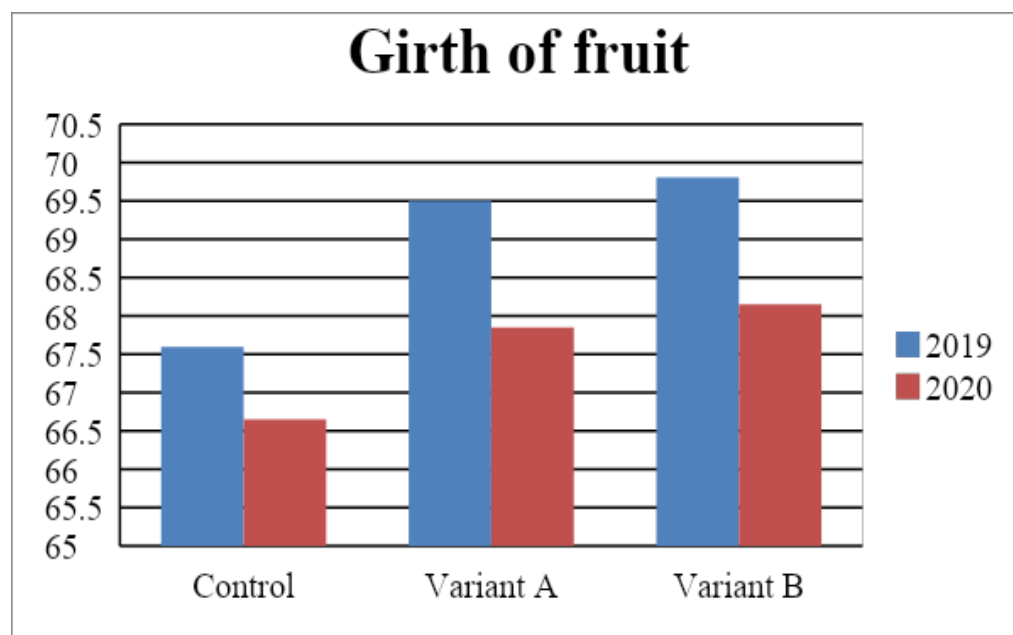
In terms of the examined years and with variant $p > 0.05$ (controls and B variant) there were no statistically significant differences in weight in relation to the applied variant and year. Therefore, the Post-hoc test was not taken.

Table 2. Analysis of variations for the fruit weight - Tests of Between-Subjects Effects

ANOVA					
Source of Variation	SS	df	MS	F	Sig.
Year	, 092	1	, 092	, 019	, 891
Variant	18,450 th most common	2	9,225 th most common	1,943 th most common	, 186
Error	56,987 th most common	12	4,749 th most common		
Total	442353,152	18			

a. R Squared = , 287 (Adjusted R Squared = -, 011)

The years of testing ($p < 0.05$) had a statistically significant effect on girth. On the other hand, when it comes to the differences between the fertilization variants themselves, there are no statistically significant differences. Due to the absence of statistically significant differences between the variants themselves ($p = 0.114 > 0.05$), no Post-hoc tests were developed.



Graph 2. Apple fruit girth

Table 3 . Analysis of variations for the fruit girth - Tests of Between-Subjects Effects

ANOVA					
Source of Variation	SS	df	MS	F	Sig.
Year	9,003 th most common	1	9,003 th most common	3,983 th most common	, 039
Variant	11,805 th most common	2	5,903 th most common	2,612 th most common	, 114
Error	27,122 th most common	12	2,260 th most common		
Total	83930,435	18			

a. R Squared = , 287 (Adjusted R Squared = -, 011)

Fruit height shows statistically significant differences in relation to the year of the study ($p = 0.046 < 0.05$). Therefore, we can conclude that the fruits are harvested and tested in 2019 were higher than those harvested and tested in the 2020 year. When it comes to fruit width, there are no statistically significant effects of the test year and variants on fruit width ($p > 0.05$).

Conclusion

On the basis of the two-year research on influence of different treatments of fertilization on the physical characteristics of the fruit of the apple varieties Gala Shniga (*Malus domestica* L.) it can be concluded:

- It was found that in both years of testing variant A (foliar fertilizer - YaraVita Universal Bio (P_2O_5 - 35 g/l, K_2O - 60 g/l, B 0.2 g/l, Cu 1 - g/l, Mn - 1.1 g/l, Mo - 0.3 g/l, Zn - 0.6 g/l) showed a higher mass (weight) of apples compared to variant B (KAN (27%) 400 kg / ha + NPK) (15:15: 15) 200 kg/ha) and control (standard fertilization NPK 15: 15: 15-300 kg/ha).
- The values of apple mass between the applied fertilization norms in the control and variant B are not statistically significant.
- Years of testing had a statistically significant effect on fetal volume, while different treatments did not have statistically significant differences.
- The height of the fruit shows statistically significant differences in relation to the year of examination, which indicates the fact that the fruits harvested in 2019 were higher than those harvested and examined in 2020.
- When it comes to fruit width, there are no statistically significant effects of year and questioning and variants on fruit weight ($p > 0.05$).

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DEFINING THE MOST SUITABLE MODEL OF GLASS FOR CONSUMING PLUM BRANDY

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Abstract: *This research examined and defined the most suitable form of glass for consuming plum brandy. Three types of spirit glasses were used from Riedel company, which are different in shape and intended for consumption of stone fruit brandies, cognac and whiskey. These types of glasses were chosen as typical representatives of different forms of glasses from which spirits are consumed. Sensory evaluation of 51 samples from five most commonly used plum varieties in Serbia, Čačanska Rodna (12 samples), Čačanska Lepotica (5 samples), Crvena Ranka (11 samples), Požegača (9 samples) and Stanley (14 samples) was performed. Special attention is dedicated to odor as an important parameter of quality, so the odor of each analyzed plum brandy was evaluated using all three glasses. After analyzing all the results, it was determined that the most suitable glass for consuming plum brandy is a tulip-shaped glass for Cognac VSOP.*

Key words: *plum brandy, glasses for spirit drinks, plum variety, sensory characteristics*

INTRODUCTION

Plum brandy (Slivovitz) is a traditional Serbian product obtained by double distillation of fermented plum mash. The produced plum distillates are usually aged in oak barrels for a certain period of time, which can be from one year, and sometimes up to 15 or more years. Aging is intended to improve the product in synergy with the components of the oak. (Nikićević and Paunović, 2013) In some parts of Serbia, it is common to consume colorless brandies, which primarily comes into play a variety of plum brandy which is produced. Since there are a large number of plum varieties, there is a wide range of aromatic compounds that plum brandy can have (Popović et al., 2009).

Traditionally, brandy in Serbia is consumed from glasses of different shapes and volumes. Most often, they are narrow, cylindrical glasses, then vessels of a specific shape (so-called čokanj and fraklić) in which typical sensory characteristics often cannot be fully expressed. Due to the increase in supply and demand for quality plum brandy and raising the culture of

consumption to a higher level, there is a need to define the adequate shape and dimensions of the glass from which these products are consumed. Following the example of world-famous spirit drinks such as cognac, grappa and whiskey, registered brandy producers gathered in the Association of Brandy Producers of Serbia have expressed the need for a closer definition of the shape of the glass for the consumption of plum brandy. Due to the large number of plum varieties used for the production of plum brandy and the way it is aged, there are different types of final products.

This indicates that the task of determining an adequate glass for consumption is very complex and requires a scientific and professional approach. The starting point for that would be the analysis of the sensory characteristics of plum brandies, mono distillates aged for one year, produced from the most commonly used plum varieties. Considering that Požegača and Crvena Ranka, as domestic varieties, and Čačanska Rodna, Čačanska Lepotica and Stanley, as varieties with combined properties, are most often used for the production of plum brandy (Popović et al., 2012), it is necessary to analyze as many as possible samples from different manufacturers. The aromatic complex of plum brandy consists of a large number of volatile compounds (Vyviurska et al., 2017) which differ in composition and intensity depending on the plum variety (Satora et al., 2013) so different types of glass can be expected to be differently suitable for different plum varieties.

The aim of this paper is to examine and define the typical and most adequate shape of a glass for the consumption of plum brandy. In that way, the possibility of a new, hedonistic approach to plum brandy and its classification in the category of drinks with an identity is realized.

MATERIAL AND METHODS

For the analysis of sensory characteristics, plum brandies from the varieties Crvena Ranka (CR), Požegača (PO), Stanley (ST), Čačanska Rodna (ČR) and Čačanska Lepotica (ČL) were used, with ethanol contents of 45 vol%. Samples of these brandies were obtained from registered commercial brandy producers from central and western Serbia. All examined plum brandies were produced from plum fruits in full technological maturity and by double distillation of fermented mash (Nikićević and Tešević, 2010). Copper pot stills were used for distillation with separation of the head, heart and tail fractions during second distillation. After distillation, all distillate samples had an ethanol content between 60 and 65 vol%

For sensory analysis, the ethanol content in the produced distillates was reduced with demineralized water to 45 vol%. The total number of evaluated samples was 51, of which the variety Stanley (14 samples), Čačanska Rodna (12), Crvena Ranka (11), Požegača (9) and Čačanska Lepotica (5).

Sensory evaluation was performed by a five-member expert commission, using a modified Buxbaum method. The following parameters of sensory quality of plum brandy were evaluated: color (0-1 points), clearness (0-1 points), typicality (0-2 points), odor (0-6 points) and taste (0-10 points). Based on the total points, plum brandies are classified into quality groups. They receive different medals: bronze medal (14.01-16.00), silver medal (16.01-18.00) and gold medal (18.01-20.00). (Nikićević, N. 2005)

In order to obtain the most accurate results in terms of the influence of the shape of the glass on the odor, three different types of glasses were used, which are characteristic of different spirits according to their dimensions and shapes. The glasses were used from the production program Sommelier of the Austrian glass company Riedel, namely 1) “Stone fruit”, 2) “Cognac VSOP”, 3) “Single malt whiskey”. All brandy samples were tasted from all three glasses.

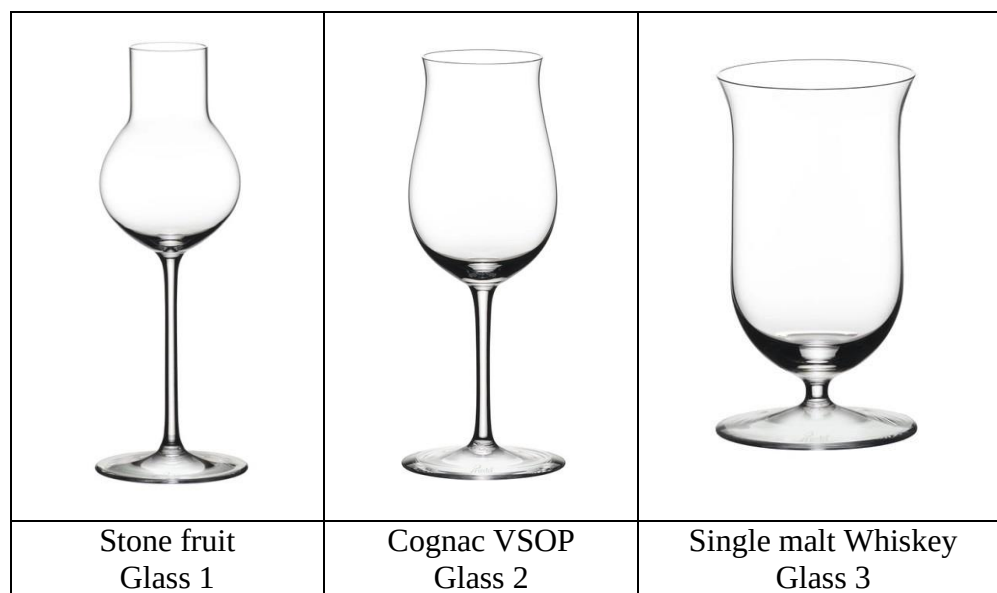
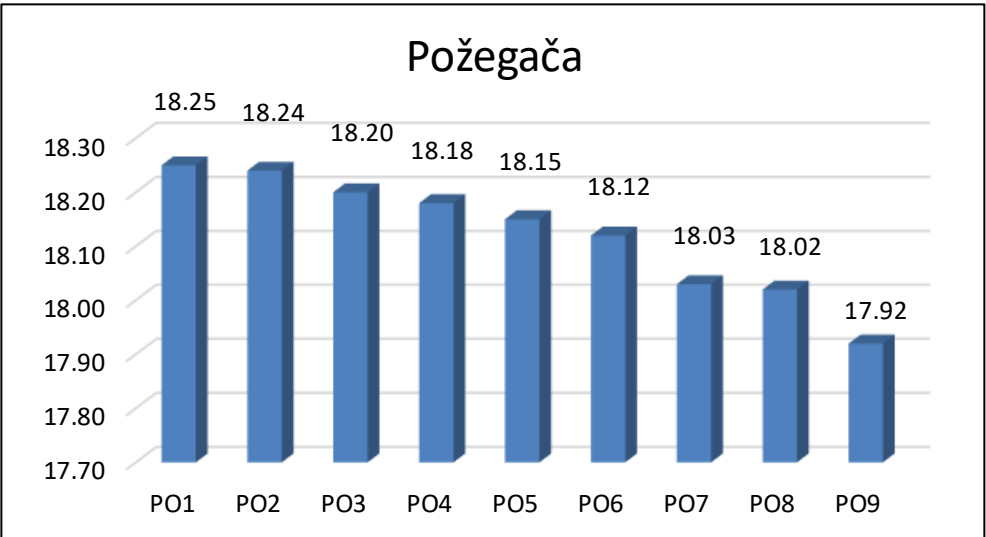


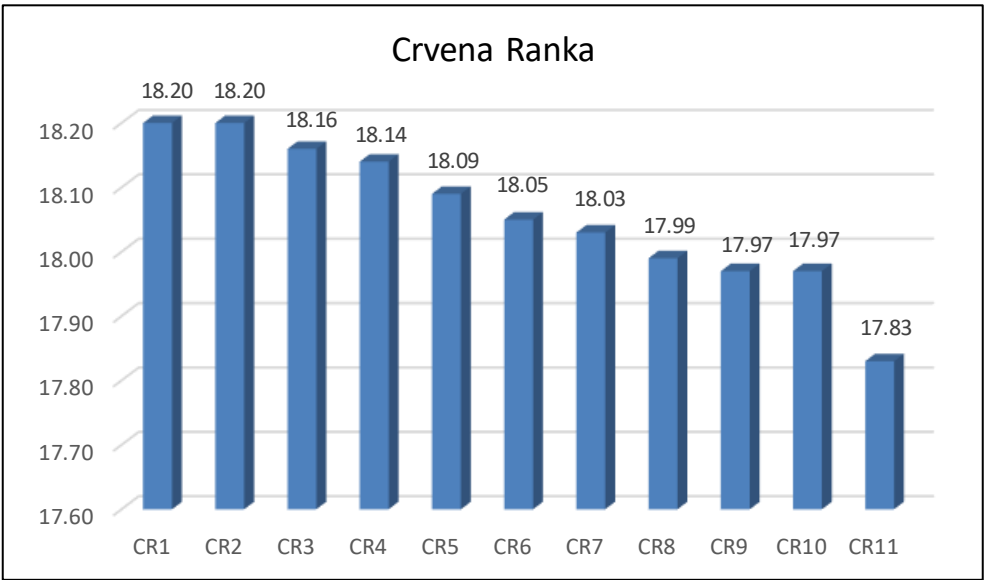
Figure 1. Glasses for plum brandy tasting

RESULTS AND DISCUSSION

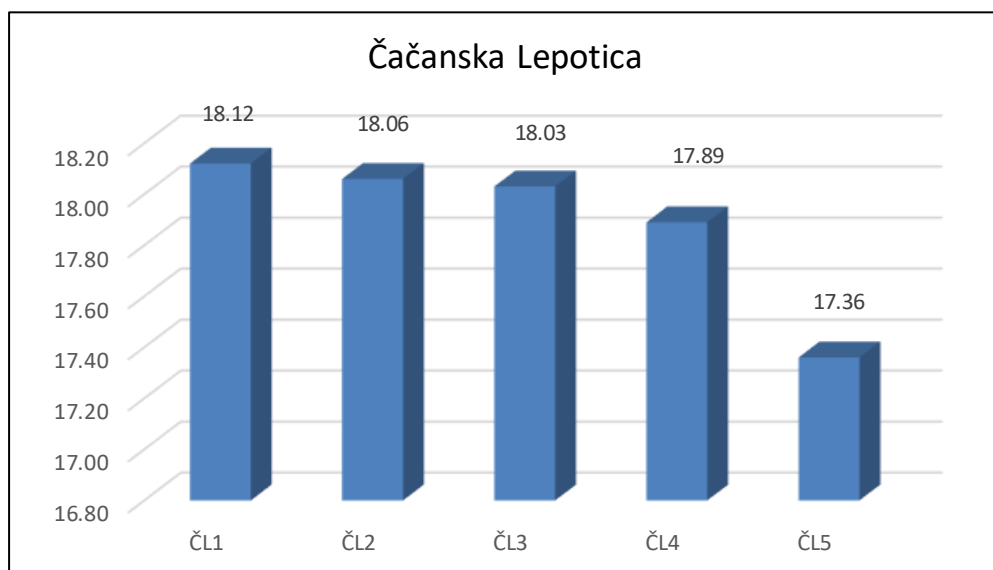
After sensory analysis of plum brandy samples of different varieties, the obtained results shown in graphical diagrams, from Graphic 1 to Graphic 5. The largest number of samples was evaluated with a score above 18.01 point, which are in the gold medal interval and indicates that the evaluated brandies were of very good quality. The sample of Požegača was rated the best with 18.25 points, while the sample of Čačanska Lepotica received the lowest rating with 17.36, points which belongs to the silver medal interval.



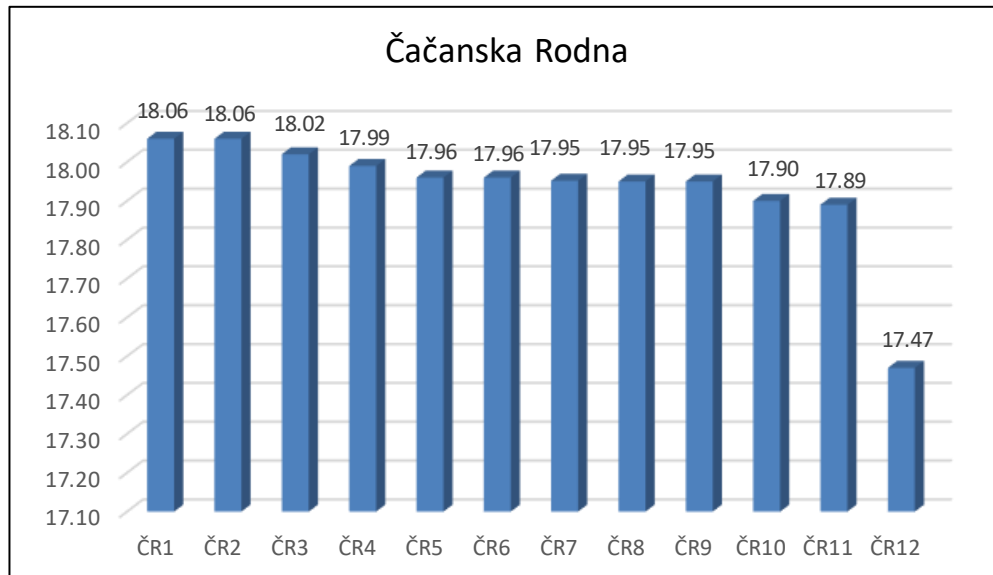
Graphic 1. Sensory evaluations (points) of plum brandy samples of the Požegača variety



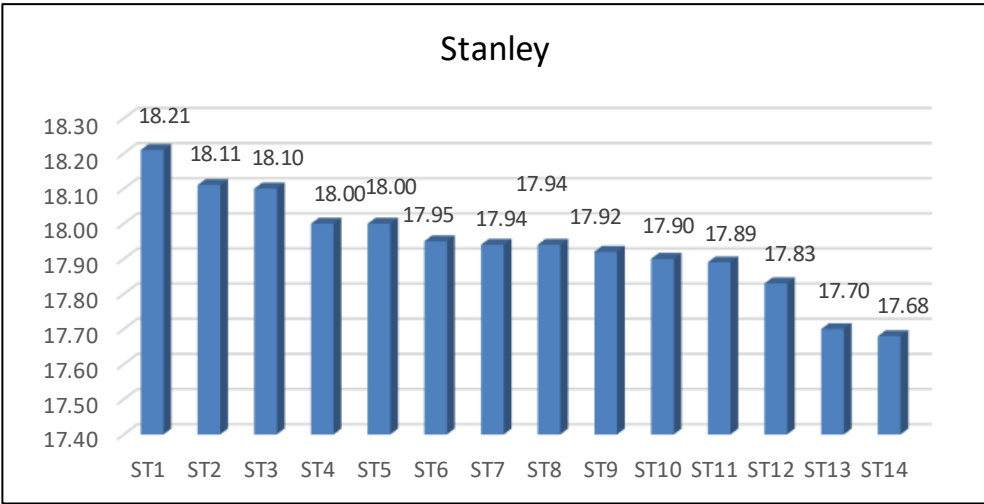
Graphic 2. Sensory evaluations (points) of plum brandy samples of the Crvena Ranka variety



Graphic 3. Sensory evaluations (points) of plum brandy samples of the Čačanska Lepotica variety



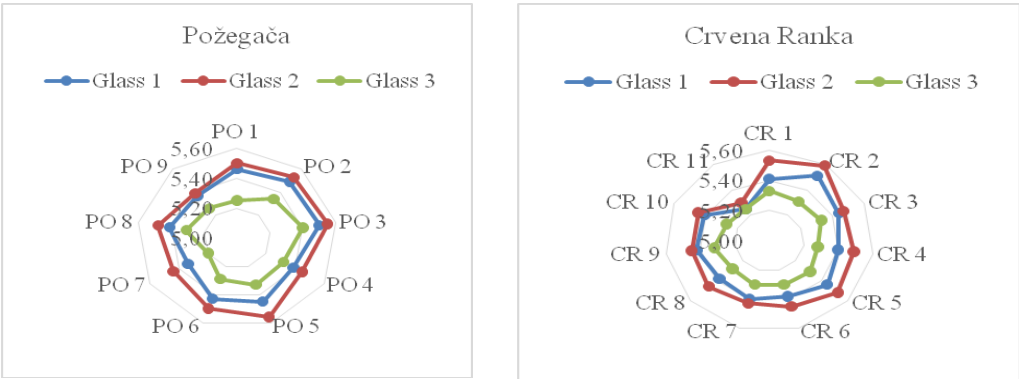
Graphic 4. Sensory evaluations (points) of plum brandy samples of the Čačanska Rodna variety



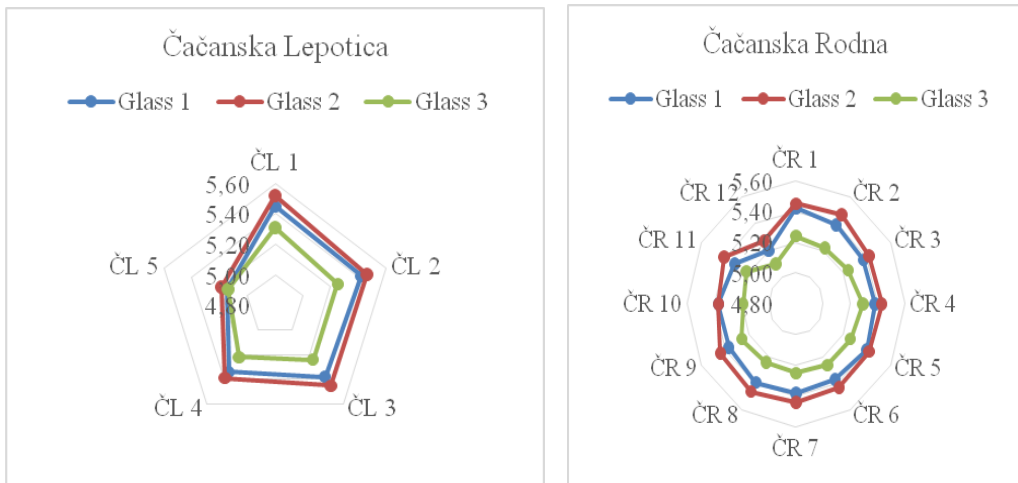
Graphic 5. Sensory evaluations (points) of plum brandy samples of the Stanley variety

All samples evaluated the maximum number of points for the parameter of colour, clarity and typicality. Differences in overall scores among the 51 samples analyzed were based on differences in odor and taste.

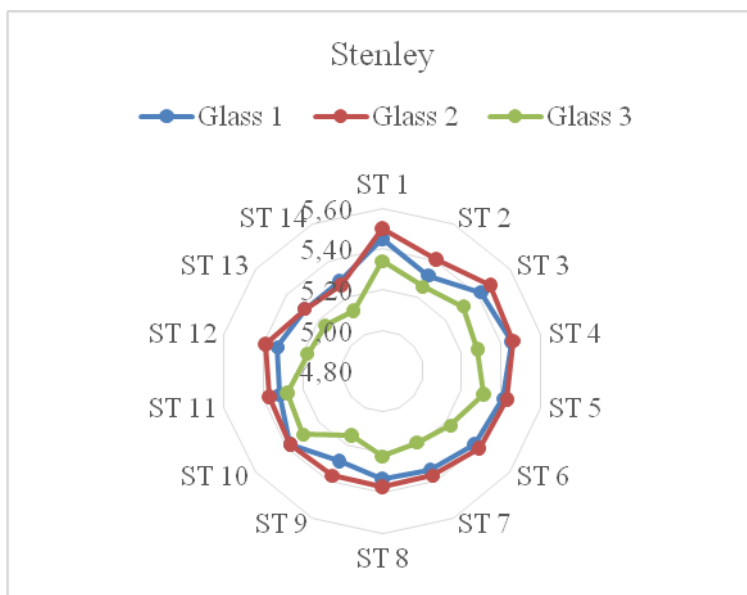
As the shape and dimensions of the glass are crucial for the sensory impression of the odor, the biggest differences between the samples were noticed with the quality parameter - odor. The results of odor evaluations by varieties are shown in graphs from 6 to 8.



Graphic 6. Sensory analysis of odor for plum brandy samples from the Požegača and Crvena Ranka varieties



Graphic 7. Sensory analysis of odor for plum brandy samples from the Čačanska Lepotica and Čačanska Rodna varieties



Graphic 8. Sensory analysis of odor for plum brandy samples from the Stanley variety

As can be seen from the graphs for the Požegača plum variety of a maximum of 6 points for the odor parameter, the best grade was sample PO5 with an average score of 5.56 in a glass of “Cognac VSOP”, while the lowest score was sample PO7 with a score of 5.20 in a glass of “Single malt Whiskey”. Crvena ranka samples were evaluated in the range of 5.59 in

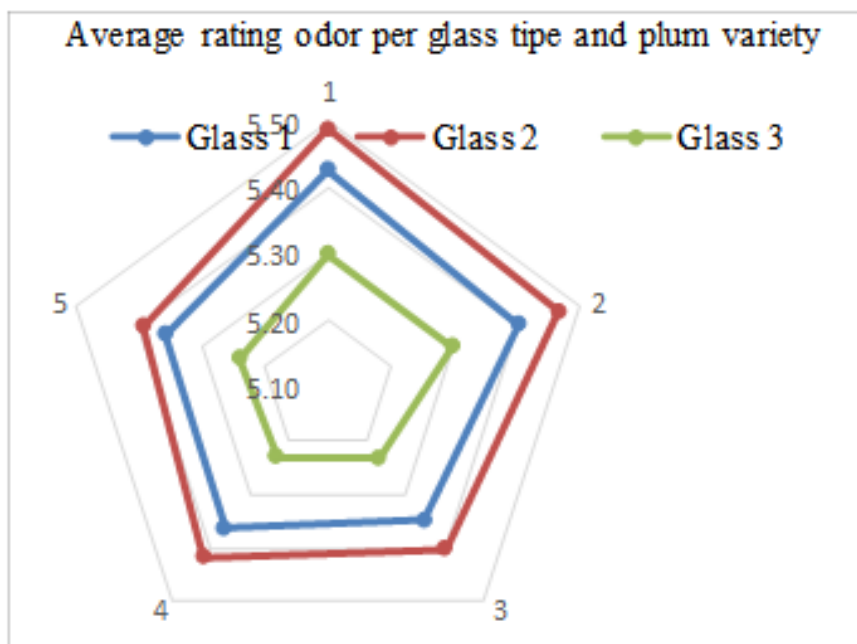
“Cognac VSOP” glass for sample CR2 to 5.25 for sample CR11 in “Stone fruit” and “Single malt Whiskey” glasses. The best rated sample for Čačanska rodna was the CR2 sample in the “Cognac VSOP” glass and was rated at 5.47, while in the “Single malt Whiskey” glass, the lowest rated odor was 5.10 in sample CR12. From five samples of Čačanska lepotica, the highest grade was sample ČL1 with 5.52 point in the sample in “Cognac VSOP” glass, and the lowest rated was sample ČL5 with 5.14 point in “Single malt Whiskey” glasses. Samples of plum variety Stanley, which is also the least aromatic variety of all other evaluated varieties, was also highest rated in “Cognac VSOP” glass with a grade of 5.50 point in ST1, and lowest in “Single malt Whiskey” glasses with a score of 5.13 point in sample ST14. It is noticed that all samples were evaluated with very high odor ratings (> 5.0), which indicates the general high quality of the evaluated samples. The obtained results of the analysis indicate that the samples in glass “Cognac VSOP”, were the highest odor points in all varieties, while the samples in “Single malt Whiskey” glass, were evaluated with the lowest points for odor.

When analyzing the average odor scores obtained for each of the varieties (Table 1), it is clear that in the samples of all varieties in glass “Cognac VSOP” (glass 2) had the highest score while the samples in “Single malt Whiskey” glass (glass 3) were rated with the lowest scores. It is also recognized that the average ratings for odor in varieties Požegača and Crvena Ranka are higher than in varieties with combined properties. This indicates that in plum brandies from extremely aromatic plum varieties, the influence of the glass is more pronounced than in plum brandies from varieties with combined properties.

Table 1. Average odor rating depending on the variety of plum and type of glass

Variety	Glass		
	1	2	3
Požegača	5.43	5.49	5.30
Crvena Ranka	5.40	5.47	5.30
Čačanska Lepotica	5.35	5.40	5.23
Čačanska Rodna	5.36	5.42	5.23
Stanley	5.36	5.39	5.24

In addition to the tabular presentation, odour ratings depending on the type of glass are illustrated graphically (Graph 9).



Graphic 9. The average point of the odor of all plum brandy samples depending on the type of glass

(1 – Požegača; 2 – Crvena Ranka; 3 – Čačanska Lepotica; 4 – Čačanska Rodna; 5 – Stanley)

“Cognac VSOP” glass No. 2, intended for Cognac VSOP, provided the most pronounced odor impression in all analyzed samples, which resulted in the highest odor ratings. The shape of this cup is such that all the fragrant components are concentrated in the narrowest part of the glass, after which it opens slightly. This reduces the intensity of alcohol, apropos its sharp smell. The impression of the odor in the “Stone fruit” glass (glass 1) in some samples was similar to that in the “Cognac VSOP” glass (glass 2). However, the size of the odor rating for the same samples was slightly lower in the “Stone fruit” glass than in the “Cognac VSOP” glass.

In the “Single malt Whiskey” glass, the evaluated samples had the lowest odor ratings. One of the reasons for this is that the glass construction at the top opens and there is no concentration of odor as in the case of “Stone fruit” and “Cognac VSOP” glasses.

The impression of tasting commission was that the samples in the “Single malt Whiskey” glass had lower odor intensity as well as less complex odor properties. In the case of the “Stone fruit” glass, conclusions of tasting ' commission were that a somewhat stronger intensity of alcohol could be felt in certain samples, but that the fragrant tones were always pure. The “Cognac VSOP” glass in terms of shape satisfies the largest number of criteria necessary for quality assessment, intensity and purity of the aroma in all varieties of plum brandy. This shape of the glass allows the concentration of aromatic compounds in the narrowest part of the glass, after which the feeling of alcohol intensity decreases with a slight expansion at the top. In this way, it can be achieved a balance in the odor which emphasizes the varietal characteristics.

CONCLUSION

Based on the presented analysis of the research results, the following conclusions are drawn. The shape of the glass that would be universally acceptable for plum brandy produced from different varieties of plums is the shape of a tulip, as with the “Cognac VSOP” glass. It is very acceptable to consume varietal plum brandy from a glass like “Stone fruit”, while a glass of “Single malt whiskey” contributes the least to the quality of the aroma impression.

ACKNOWLEDGEMENTS

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ZOOTECHNICS

THERAPEUTICAL STUDY REGARDING THE ROLE OF SOME ACARICIDAL SUBSTANCES AGAINST *VARROA DESTRUCTOR* UNDER LABORATORY CONDITONS

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Abstract: *The honeybee, Apis mellifera, is the world's most important managed pollinator of agricultural crops, however, Varroa mite, Varroa destructor infestation has threatened honey bee survivorship. Low efficacy and development of Varroa mite resistance to currently used Varroacides has increased the demand for innovative, effective treatment tool options that exhibit high efficacy, while minimizing adverse effects on honeybee fitness. The aim of the paper is to classify the effectiveness of acaricides in conditions where the result is not influenced by the microclimate factors inside the hive, by external factors and by the way of application. Under laboratory conditions, the results of this researche revealed that 15% lactic acid and thymol are the most effective, acaricide substances.*

Key words : honeybees, Varroa, acaricides, in vitro sudy

INTRODUCTION

Varroa destructor is a formidable enemy for which there is no miraculous treatment, although research aimed at stopping its spreading has been going on for more than a quarter of a century. Recent researches are looking forward to discovering an effective way to control this parasite [6, 9]. Nowadays, we are able to improve control strategies to limit the development of parasites and their damage and at the same time take care to minimize drug residues in bee products, which are dangerous to human health.

The first official identification of *Varroa* mites in a country is followed by intensive actions of specialists, aimed at controlling the spreading of mites and preventing the collapse of bee colonies. During the first phase, the losses of bee colonies are maximum due to the lack of knowledge about control

methods. In most cases, the selection of beekeepers based on their ability to include antivarroa treatments in practice is made during the first 10 years. Beekeepers who do not adapt to these measures will lose their bee colonies. Normally, the situation of beekeeping stabilizes in a few years, but periodical losses of over 30% of bee colonies, mainly in winter, are common and seem inevitable. In this context, *V. destructor* mites play an essential role in these losses [2].

V. destructor is involved in the massive losses of bee colonies in some European countries and the USA. Recent studies of bee CCD found that acaricides are either banned or ineffective in controlling *V. destructor* and concluded that the usual methods of treatment used by beekeepers are often inadequate for the control of varroosis [3, 10].

Undoubtedly, most colonies of *Apis mellifera* in temperate regions will be severely affected or even die within a few years if effective control methods are not applied. Fortunately, beekeepers today have a wide range of substances, application techniques and methods to keep mite populations under control [4].

The massive invasion of the colonies, the enormous losses due to him *V. destructor* and the need to maintain colonies at an economically viable level has led to the need to use various synthetic acaricides that are more effective and easier to use than the products used in alternative control. All substances used for the control of parasitic diseases of bees have advantages and disadvantages and their study is necessary to contribute to the design of a control protocol as effective as possible. Treatments can be administered in various forms: in medicated feed, sprays, evaporation, fumigation or aerosols [8].

Due to their lipophilic and decomposition resistance characteristics, synthetic acaricides accumulate massively in wax and less in honey. Also, mite resistance to strong synthetic acaricides, which include pyrethroids, organochlorines, formamidines, has long been identified in many countries [2, 6, 8, 9]. These are the main reasons for using of alternative natural substances to organic acids and volatile oils. The effectiveness of these substances is lower but without the risk of accumulation and installation of resistance.

Numerous studies have been performed on the acaricidal efficacy of substances used in the alternative treatment of varroosis. Testing these substances directly in the bee colony is influenced by many factors and therefore the results of the treatments are very different [1, 2, 5, 7].

The aim of the paper is to classify the effectiveness of acaricides in conditions where the result is not influenced by the microclimate factors inside the hive, by external factors and by the way of application.

The study of in vitro acaricidal efficacy leads to results that can be oriented towards the most effective substance with its advantages and disadvantages.

Materials and methods

The study was performed in the Parasitology and parasitic diseases Laboratory of the Faculty of Veterinary Medicine Timisoara. The *Varroa destructor* mites were collected from the captive drone (*Apis mellifera*), from untreated bee colonies.

The substances tested were menthol, thymol, oxalic acid 3.5%, formic acid 60%, lactic acid 15%, oxalic acid 3.0% mixed with alcoholic propolis extract 15%, and distilled water was used as a control.

Seven Petri dishes were prepared by applying filter paper so as to cover the entire surface of the inside of the plate, and the lid was labeled (Fig. 1). One ml of the substance used was applied to each plate, respectively 0.5 g of menthol and crystallized thymol. The control group was prepared with 1 ml of distilled water.



Fig. 1. Petri dish prepared for study

In each plate, 10 adult females of *Varroa destructor* were introduced (Fig. 2) and the time in which the mites died in laboratory conditions was recorded. The mites were examined under a magnifying glass; their activity and the time elapsed until death were monitored.

The values obtained were expressed as a percentage per unit time, obtaining mortality in minutes. The results were compared to highlight the efficacy of each test substance.

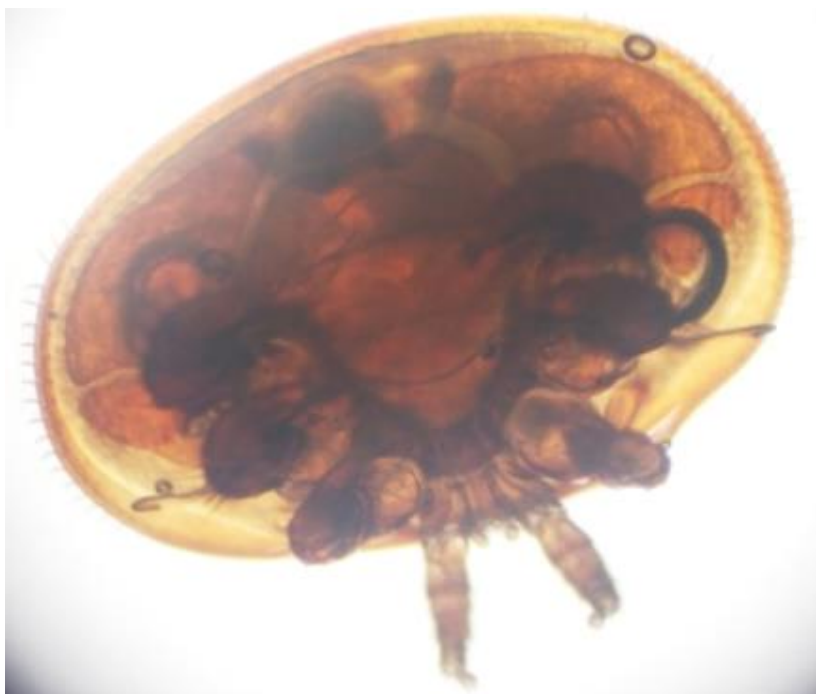


Fig. 2. *Varroa destructor* - female

Results and discussions

Under laboratory conditions, 15% lactic acid acted the fastest, killing 10% of mites in 3 minutes, and menthol was the least effective, taking 59 minutes to kill the same percentage of mites. For the other substances, the acaricidal activity on the percentage of 10% of the mites was between 5 and 59 minutes, 3 minutes for lactic acid, 5 minutes for thymol and Bee Vital, 6 minutes for formic acid and 7 minutes for oxalic acid.

The lethal effect of the test substances followed the same graph when mortality reached 50%, in 10 minutes for thymol, 11 minutes for lactic acid, 14 minutes for Bee Vital, 15 minutes for formic acid, 19 minutes for oxalic acid and 157 minutes for menthol.

All substances except menthol killed all the mites in an acceptable time: 18 minutes thymol, equal to lactic acid, 22 minutes formic acid, 28 minutes oxalic acid + propolis, 29 minutes oxalic acid with propolis and 170 minutes menthol (Fig. 3).

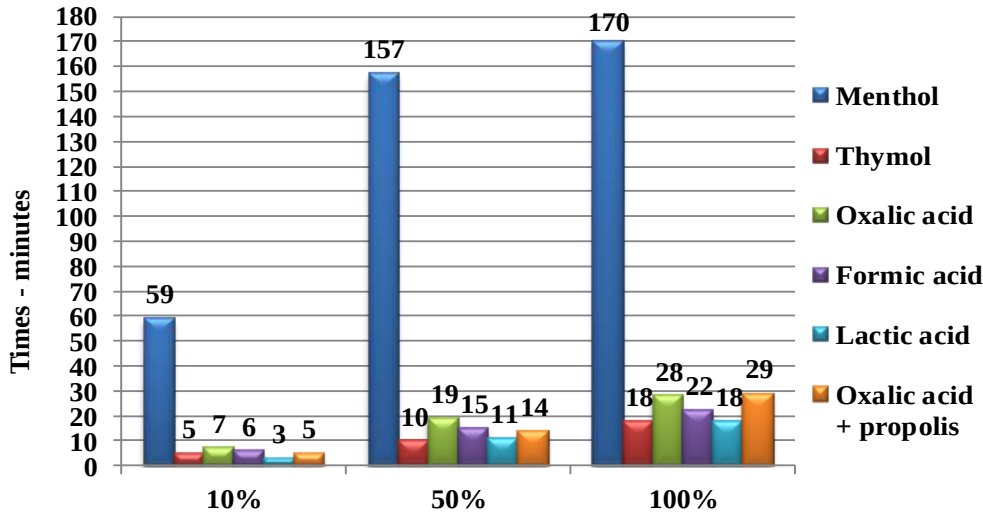


Fig. 3. *Varroa destructor* mites mortality

In this study, it was found that the death of *V. destructor* mites was preceded by a state of hypermotility followed by a state of latency in which the agony lasts, on average, five minutes.

In contact with the distilled water from the control sample, the mites did not undergo any behavioral change and survived during and after monitoring.

In comparison, a study performed by *Bahreini R. et al, 2020*, has been evaluated the toxicity of 16 active ingredients and 9 formulated products of registered miticides for use on crops from 12 chemical families were evaluated in comparison to amitraz on *Varroa* mites and honey bees using contact surface and topical exposures. It was found that fenpyroximate (93% mortality), spirotetramat (84% mortality) and spirodiclofen (70% mortality) had greater toxicity to *Varroa* mites, but high dose rates caused high bee mortality (> 60%). Further research is needed to investigate other options to minimize the adverse effect of these compounds on bees. The results also found high toxicity of fenazaquin and etoxazole against *Varroa* mites causing 92% and 69% mortality, respectively; and were found to be safe on honey bees. The authors emphasized that fenazaquin and etoxazole are candidates for a potential Varroacide and recommended for further testing against *Varroa* mites at the colony level [1].

It is well known that the using of yearly treatments for the control of varroosis is the most common answer to prevent collapses of honey bee colonies due to the mite. However, the number of effective acaricides is small

and the mite tends to become resistant to these few active molecules. In this context, *Riva C. et al*, 2019, have been looking for a new original varroacide treatment inhibiting selectively *Varroa destructor* acetylcholinesterase (vdAChE) with respect to *Apis mellifera*. To do this an original drug design methodology was used applying virtual screening of the CERMN chemolibrary, starting from a vdAChE homology sequence model. By combining the in silico screening with in vitro experiments, two promising compounds were found. In vitro tests of AChE inhibition for both species have confirmed good selectivity toward the mite vdAChE. Moreover, an in vivo protocol was performed and highlighted a varroacide activity without acute consequences on honey bee survival. The two compounds discovered have the potential to become new drug leads for the development of new treatments against the mite varroa. The method described clearly shows the potential of a drug-design approach to develop new solutions to safeguard honey bee health [7].

The results of this study have been joined by those performed by *Balint A.*, 2011 [2] and *Dulin F. et al*, 2014 [5].

Conclusions

Under laboratory conditions, 15% lactic acid and thymol are the most effective, all mites died in 18 minutes.

Oxalic acid kills 100% of mites in 28 minutes, ranking it at a middle level of effectiveness.

Mites exposed to 60% formic acid die 100% in 22 minutes.

The acaricidal effect of 3% oxalic acid with propolis is good, killing 29% of mites in 29 minutes.

The acaricidal activity of menthol is slow and ranks last, acting lethally on all mites in 170 minutes.

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THE CURRENT STATE AND PROSPECTS OF LIVESTOCK SECTOR IN CAMBODIA

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Abstract: Cambodia remains predominantly agrarian and livestock is the main asset for approximately 70% of rural households. However, in recent years, growth in the agriculture sector has stagnated with farmers facing several challenges including declining prices for traditional crops and irregular rainfall patterns. Livestock contributions are not restricted to meat and milk; they are also a source of wealth, employment and transport to smallholder farmers in the rural communities. The livestock sub-sector in Cambodia has great potential for growth, due to the country's increasing meat consumption brought about by urbanization and its fast-growing population but growth depends on how effectively Cambodia is able to improve the efficiency and competitiveness of its livestock industry. Livestock numbers between 2015 and 2019 shows that cattle production as well as buffalo and small ruminant (predominantly goat) and poultry production (68.7%) rely on smallholder livestock keepers. With regard to pigs, in the last years a shift from family farming to commercial farming can be observed (families 47.1% and commercial farms 52.8%) as free range pig production systems are increasingly replaced by intensive rearing systems with the use of artificial insemination leading to successful and sustainable value chains. Cambodia is, despite of some exports of cattle, a net importer of live cattle, pigs and poultry. Imported beef and chicken are needed to bridge the gap between the national meat production and the consumption of its population. MAFF estimates the consumption needs to be at least 290,924 during 2019, representing the need of 18% of the consumer needs be covered by imported meat. The government of Cambodia has enacted a new Law on Animal Health and Production in 2016 and is about to update existing by-laws and decrees of implementation.

Introduction

The Kingdom of Cambodia is situated in the southern portion of the Indochina peninsula in Southeast Asia with 181,035 square kilometres of area. Cambodia is divided into 25 provinces, including the capital. The provinces are

subdivided into 162 districts and 27 municipalities. Provisional results from Cambodia's 2019 census²⁵, released in August, 2019, show a total population of 15.29 million people. The provisional figures indicate 7.42 million men and 7.87 million women. 78 percent of the population live in rural areas.

The country remains predominantly agrarian and livestock is the main asset for approximately 70% of rural households. However, in recent years, growth in the agriculture sector has stagnated with farmers facing several challenges including declining prices for traditional crops and irregular rainfall patterns. This has led to a need for farmers to diversify income sources with livestock promoted as a more viable livelihood activity, particularly the raising of cattle and poultry. In fact, In Cambodia, livestock contributions are not restricted to meat and milk; they are also a source of wealth, employment and transport to smallholder farmers in the rural communities.

The agricultural subsector which comprise crops, livestock, fisheries and forestry/wildlife contributed to approximately 21% (current price) of the Gross Domestic Product (GDP) in 2019, and it employs a large part of the workforce. However, this is now decreasing as consequence of mechanization and also of the growing industrial sector such as the garment industry which attract an increasing number of rural people, in particular women. Rice is also Cambodia's major crop, its principal food, and also its most important export commodity. Livestock including poultry ranks third with 11.11% contribution to the GDP behind fisheries (24.3%).

According to Chuon (2014) and Chuon et al.(2014), fermented fish products, commonly consumed in south east Asia, are used as condiments that contribute to people's nutritional sources and as seasonings to improve food taste and flavour. Among these, the Cambodian products *prahok* (fish paste), *kapi* (shrimp paste) and *toeuk Trey* (fish sauce).

Current situation of the livestock sector

The development of global animal husbandry is an imperative of every country and government, which, through science that deals with the problems of food production, finds ways and methods of progress. The livestock sector globally is highly dynamic. In developing countries, it is evolving in response to rapidly increasing demand for livestock products. Therefore, there are great challenges, with an emphasis on genetic improvement of animals, in order to obtain as many quantities of quality products as possible (Petrovic et al., 2017). For that purpose, modern technique and laboratory analyzes are applied (Caro Petrovic et al., 2013, 2017). In countries of the European Union attention is directed towards production systems and marketing (Gabina, 2006).

²⁵ <https://opendevelopmentcambodia.net/topics/population-and-censuses/>

The livestock sub-sector in Cambodia has great potential for growth, due to the country's increasing meat consumption brought about by urbanization and its fast-growing population but growth depends on how effectively Cambodia is able to improve the efficiency and competitiveness of its livestock industry.

The major constraints of the livestock industry in Cambodia include the high costs of local animal production, including lack of access to quality breeding stock and endemic diseases, but are also related to poor infrastructures' presence and high market volatility.

The animals are typically free ranging without any biosecurity. While such a situation is suitable for rural and geographically limited economies, this poses problems for harnessing livestock-rearing as a driver of economic growth: small-scale, family-based livestock husbandry does not easily lend itself to expansion or to the development of regional trade and export industries.

Table1. Livestock numbers in smallholder farm and commercial farming between 2015 and 2019

Species	Year					Comparison 2018/2019
	2015	2016	2017	2018	2019	
TLU						
Cattle	2903420	2897126	2951359	2917302	2769885	-5.0%
Pigs	2357839	2371283	2331512	1934917	1030494	-46.7%
Poultry	26688675	28402486	28652409	28956342	27763479	-4.1%
Tropical Livestock Unit (TLU) Total						-14.0%
Commercial farming						
Cattle	13289	23188	20363	11232	9877	-12.1%
Pigs	416525	599341	742771	812938	1155431	42.1%
Poultry	7830398	7331275	7592530	9210409	12631974	37.1%
TLU total						-2.0%
Total						
Cattle	2916709	2920314	2971722	2928534	2779762	-5.0%
Pigs	2774364	2970624	3074283	2747855	2185925	-20.5%
Poultry	34519073	35733761	36244939	38166751	40395453	5.8%
TLU Total						-8.0%

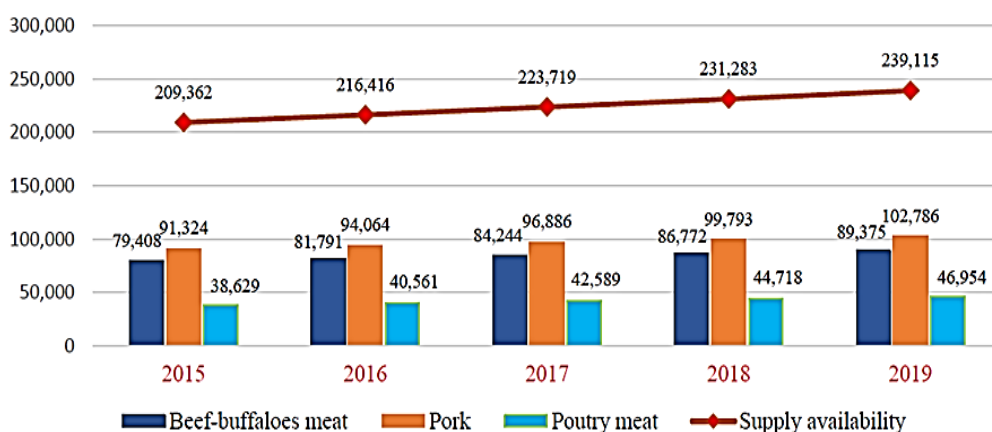
From table 1 we see how livestock numbers between 2015 and 2019 shows that cattle production as well as buffalo and small ruminant (predominantly goat) and poultry production (68.7%) rely on smallholder livestock keepers. With regard to pigs, in the last years a shift from family farming to commercial

farming can be observed (families 47.1% and commercial farms 52.8%) as free range pig production systems are increasingly replaced by intensive rearing systems with the use of artificial insemination leading to successful and sustainable value chains.

The provinces with the highest number of large ruminants (cattle and buffalo) are

- Prey Veng ((351000 heads)
- Takeo (325000 heads)
- Kampong Spue (257000 heads)
- Siem Reap (242000 heads)

In spite of a reduction in the livestock number the total meat supply from cattle, pigs and poultry has increase (see graph 1 hereafter).



Graph 1: Total meat supply by species

According to Darith et al. (2016), it is important to optimize the livestock development model for rural areas. Pen et al. (2010) researching small-holder farmers, they draw the following conclusion: The cattle production of small-holder farmers was assessed as very low in terms of management and feeding. Farmers raised their cattle in the traditional way with low health care intervention. Moreover, there was no breeding program to manage the genetic improvement in small-holder cattle production systems. Cattle feeds were mainly based on natural grasses and rice straw. Providing locally available feeds was a major challenge for farmers which required high labor input, especially during the dry and flooding seasons. Lack of feed was a critical factor limiting the ability of farmers to expand cattle numbers. Better housing of cattle with proper health care and improved feeding system is

recommended to improve cattle production. Planting forages including grasses and legumes is a simple solution for farmers to respond to the lack of feed. Keeping cattle in pens for fattening before sale and improving cow-calf production can help improve management of cattle in order to increase income and expand the number of cattle to respond more to market demand.

Small scale farming is the most common way of producing pigs in Cambodia. This type of production often means one or two pigs at the most. By-products from rice production, such as rice bran and broken rice, are used as feed. Therefore the rice production affects the small scale pig production substantially. Cambodian pigs are also fed with kitchen waste and if enough money, piglets are offered concentrate. Often, pigs in small scale productions grow slow as a consequence of deficient feeding (Emilia, W., 2011). In Cambodia, indigenous chickens are reared under backyard systems and are kept mainly for home consumption and also as a source of income. During the survey, a wide variation was observed in the age of farmers, which ranged from 15 to 76 years, but only a very small proportion of people under 30 engage in this activity, which supports the general trend for fewer and fewer young people to adopt agriculture as a mode of livelihood. The high usage of mobile phones shows that people are willing to adopt new user-friendly technologies, and illiteracy is not a barrier for this. From the survey, it is clear that the adoption of management techniques such as housing, feeding and health care is closely linked to the literacy level of farmers. The literacy level is highest in Kampot, where farmers are well ahead in adopting improved management techniques such as providing good housing and extra feed, utilizing veterinary facilities, and seeking to improve the productivity of their birds. Another parameter that substantiates this is flock size; the average flock size is highest in Kampot, with 40.16 birds per farm. The impact of literacy can also be seen on family size; more than 66 percent of the households in Kampot have fewer than five members. Rice is the main agricultural crop and most farmers keep cattle. Only five households rear goats and no sheep were found in the entire survey area. Very few farmers use improved varieties to improve their flocks; the majority rely on local birds, which they buy from their neighbours. Body weight and number of eggs laid are the two main criteria for selecting breeding birds. House construction is done mainly with on-farm materials, and about 80 percent of farmers provide housing. In most cases, traditional chicken farmers do not keep production records, but were able to report that an average hen starts laying eggs when it is between 5.9 and 6.3 months of age, and has 3.6 to 4 production cycles per year, with 11.6 to 12.3 eggs per cycle. Regarding the survival of chicks, on average each hen hatches 9.86 chicks, of which 7.44 survive the first month and 6.1 survive for six months. In Cambodia, indigenous chickens are reared under backyard systems and are kept mainly for home consumption and also as a source of income. During the survey, a wide

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Research of Denish et al. (2009) show that backyard chicken production is important for many rural households. Although it is a small and sometimes unorganized sector, it makes a substantial contribution to meeting the protein requirements of the majority of Cambodia's population. The mentioned authors further state that local breeds are considered a possible reservoir of important genes/traits that may not be found in many commercial breeds and that could be potentially utilized in current commercial breeding programmes.

Livestock regulatory framework and relevant standards

The government of Cambodia has enacted a new Law on Animal Health and Production in 2016 and is about to update existing by-laws and decrees of implementation.

- Sub decree 14 on the inspection of animal and animal products (1988)
- Sub decree No.16 (2003) on Sanitary Inspection of Animal and Animal Products
- No.108 (2007) on the Management of Slaughterhouse and Sanitary Inspection of Animal, Meat and Animal Products
- No. 227 (2008) on Domestic Protocol of Sanitary Inspection of Animal and Animal Products, dated on 23 June 2008
- No.178 (2009) on Procedure on Sanitary Inspection of Animal and Animals;
- No.224 (2008) on Internal Regulation of Slaughterhouses
- No. 225 (2008) on Location and Technical Standard for Building Slaughterhouse;

- No. 463 (2008) on Protocol and Veterinary Measures for Transportation of Meat and Animal Products from Slaughterhouse, dated on 22 October 2008;
- No.178 (2009) on Procedure on Sanitary Inspection of Animal and Animals;
- Sub-decree No 26 on the ‘Creation and Regulation of VAHWs’
- Inter-ministerial Prakas No.868 (2010) on Food Safety Management
- Directive number 115, titled ‘Taking action to strengthen livestock and fishery production management’ signed by MAFF which allows the operation of multiple slaughterhouses in the communes and districts of each province.

Policies, strategies and laws impacting the livestock sector:

- Rectangular Strategy for Growth, Employment, Equity and Efficiency, Phase IV, 2018
- Policy on Environmental and Social Safeguards for Sub-National Democratic Development
- National Environmental Strategy and Action Plan (NESAP) 2016-2023
- MAFF Gender Mainstreaming Policy and Strategic Framework in Agriculture 2016 to 2020
- Law on Environmental Protection and Natural Resources Management of 1996
- Sub-decree on EIA Process of 1999
- Climate change action plan
- Prakas N. 021 on Environmental Impact Assessment Classification for Development Project

A joint multisectoral action plan to fight AMR had been put in place by the Ministries of Health (MoH), MAFF and Ministry of Environment (MoE). Several joint technical working groups, namely (i) AMR Technical Working Group (AMR Technical Working Group (TWG)), (ii) Zoonosis TWG, (iii) Food Safety TWG, and (iv) an Inter-ministerial Foodborne disease outbreak Investigation and Response Team, ‘FORT’ were established to respond to “One Health” or :”Health in All” policy promoted and supported by FAO, OIE and WHO.

Table 2. Standards for the livestock and products of animal origin sector

CS 083:2010	ASAEN Common Principles for Food Control System
CS 084:2010	ASAEN Common Principles and Requirements for Food Hygiene
CS 085:2010	ASAEN Common Principles and Requirements for the Labelling of Prepackaged Food

CS 111:2014 CAC/GL 83-2013	Principles for the use of sampling and testing in international food trade
CS 417:2014 CAC/GL 30-1999	Principles and Guidelines for the Conduct of Microbiological Risk Assessment
CS 420:2014 CAC/GL 33-1999	Recommended Methods of Sampling for Pesticide Residues for the Determination of Compliance with MRLs
CS 425:2014 CAC/GL 40-1993	Guidelines on Good Laboratory Practice in Pesticide Residue Analysis
CS 426:2014 CAC/GL 41-1993	Analysis of Pesticide Residues: Portion of Commodities to which Codex MRLS Apply and which is Analyzed
CS 432:2014 CAC/GL 49-2003	Harmonized IUPAC Guidelines for Single-Laboratory Validation of Methods of Analysis
CS 434:2014 CAC/GL 53-2003	Guidelines on the Judgment of Equivalence of Sanitary Measures associated with Food Inspection and Certification Systems
CS 441:2014 CAC/GL 60-2006	Principles for Traceability / Product Tracing as a Tool Within a Food Inspection and Certification System
CS 444:2014 CAC/GL 63-2007	Principles and Guidelines for the Conduct of Microbiological Risk Management
CS 446:2014 CAC/GL 65-1997	Harmonized Guidelines for Internal Quality Control in Analytical Chemistry Laboratories
CS 450:2014 CAC/GL 69-2008	Guideline for the Validation of Food Safety Control Measures
CS 452:2014 CAC/GL 71-2009	Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programs Associated with the Use of Veterinary Drugs in Food Producing Animals
CS 458:2014 CAC/GL 77-2011	Guidelines for Risk Analysis of Foodborne Antimicrobial Resistance
CS 460:2014 CAC/GL 79-2012	Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food
CS 461:2014 CAC/GL 80-2013	Guidelines on the Application of Risk Assessment for Feed
CS 462:2014 CAC/GL 81-2013	Guidance for governments on prioritizing hazards in feed

CS 463:2014 CAC/GL 82-2013	Principles and Guidelines for National Food Control Systems
CS 464:2014 CAC/GL 84-2012	Principles and guidance on the selection of representative commodities for the extrapolation of maximum residue limits for pesticides to commodity groups.
CS 465:2014 CAC/MRL 1	Maximum Residue Limits (MRLs) for Pesticides
CS 466:2014 CAC/MRL 2	Maximum Residue Limits for Veterinary Drugs in Food
CS 468:2014 CAC/RCP 1-1969	General Principles of Food Hygiene
CS 488:2014 CAC/RCP 45-1997	Code of Practice for the Reduction of Aflatoxin B1 in Raw Materials and Supplemental Feeding stuffs for Milk-Producing Animals
CS 497:2014 CAC/RCP 54-2004	Code of Practice on Good Animal Feeding
CS 501:2014 CAC/RCP 58-2005	Code of Hygienic Practice for Meat
CS 519:2014 CAC/MISC 5-1993	Glossary of Terms and Definitions (Veterinary Drugs Residues in Foods)
CS ISO 22000:2010	Food safety management systems — Requirements for any organization in the food chain
CS ISO 22000:2019 (IDT/ED 2018)	Food safety management systems-requirements for any organization in the food chain
CS ISO 22002-1:2019 (IDT/ED 2009)	Prerequisite programs on food safety -Part 1: Food manufacturing

Imports, exports disease control and transit of livestock

Cambodia is, despite of some exports of cattle, a net importer of live cattle, pigs and poultry. Imported beef and chicken are needed to bridge the gap between the national meat production and the consumption of its population. MAFF estimates the consumption needs to be at least 290,924 during 2019, representing the need of 18% of the consumer needs be covered by imported meat. Table 3 show the import, export and transit of live animals (by species) and of animal products. Currently, no Disease Control Zones (DCZ) exist in Cambodia. For animals on transit additional veterinary checkpoints should be established for interprovincial livestock movement control. Special attention is paid to the prevention of swine diseases as highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) in medium-scale swine farms (Tornimbene et al.,2015).

The establishment of DCZ is a first step towards achieving Disease Free Zones (DFZ). Preconditions are a clear delimitation of the zones preferably through geographical barriers, vaccination of all susceptible animals for a given diseases.

Table 3 : Livestock Trade (imports, exports, transit)

Live Animal s	Unit	2018			2019			2020 (11 months)		
		Import	Export	Transit	Import	Export	Transit	Import	Export	Transit
Cattle	Head	0	6786	31,130	200	56,218	84,050	22,376	8,887	47,630
Pig	Head	630,776	0	87,710	573,798	0	109,740	1,654,899	0	785,520
Sow-pi	Head	2,170	505	0	1,960	200	0	16,040	200	0
Piglet	Head	37,155	5,000	0	21,165	0	0	275,039	0	0
Goat	Head	300	0	0	5,839	0	3,300	3,660	0	6,520
Chicken	Head	3,981,303	0	0	6,914,620	0	0	4,716,230	0	0
Chick	Head	2,637,604	0	0	4,711,315	0	0	7,003,465	0	0
Animal Product s	Unit	2018			2019			2020 (11 months)		
		Import	Export	Transit	Import	Export	Transit	Import	Export	Transit
Beef-buffalo	Kg	122,776	12,076	0	603,116	0	0	1,438,013	0	0
Beef-cattle	Kg	459,562	0	0	469,342	0	0	244,006	0	0
Pork	Kg	18,26	0	0	168,	0	0	58,2	0	0

		2			281			47		
Chicken meat	Kg	1,591,427	0	0	711,312	0	0	1,843,735	0	0
Quail meat	Kg	120,000	0	0	60,000	0	0	45,000	0	0
Hide-salted	Kg	0	1,274,960	0	0	1,439,824	0	0	1,471,443	0
Hide-dried	Kg	1,625,531	0	0	1,234,860	0	0	888,191	0	0
Pig skin-dried	Kg	83,975	0	0	0	0	0	0	0	0
Cattle feed	Kg	0	0	50,000	0	0	45,000	0	0	0
Fresh milk	Kg	0	7,558,072	0	0	3,984,250	0	0	0	0

Information of required procedures for the import, export and transit of livestock and products of animal origin can be found on

- Cambodia National Trade Repository²⁶
- SPS/TBT requirements from Cambodia SPS-TBT Enquiry points at General Department of Camcontrol²⁷, e.g. as to for existing trade agreements and animal health requirements for the livestock to be imported.

The registration and licensing as trader authorised to import and export livestock and products of animal origin involves several services, namely the department of business registration, the department of taxation, the department of livestock, and the department of customs and excise.

Meat markets

Buying, selling, and slaughtering of animals for food in unhygienic conditions can cause transmission of pathogenic animal-borne diseases to humans, known as zoonosis. Animals sold in wet markets are often unhealthy and kept in dirty, cramped conditions such as in stacked cages. Where animals

²⁶ <https://www.cambodiantr.gov.kh/index.php?r=site/display&id=3>

²⁷ <http://www.camcontrol.gov.kh>

are under stress, viral pathogens can intermingle and mutate making them more transmissible between species. Many food vendors in wet markets are not aware of public health and food safety standards necessary to maintain good hygiene while distributing food. Insanitary conditions for processing and storage of food products, a lack of vendor access to clean water and inappropriate disposal of animal fluids including blood and faeces encourage the proliferation of pathogens, leading to heightened risks of foodborne diseases. In the case of respiratory diseases, such as COVID-19, the virus can jump to food handlers or customers through exposure to an animal's bodily fluids. Close interactions with wild animals have caused numerous disease outbreaks in humans, such as Ebola, Avian Influenza, SARS, and HIV.

The level of hygiene and food safety control in the food industry has been greatly improved worldwide by the implementation of the global hazards analysis and critical control points programme (HACCP). To help prevent the spread of food-related pathogens and diseases in wet markets statutory Food Health and Safety regulations using the HACCP model need to be implemented and enforced by local governments to regulate food safety standards. Both vendors and consumers need to be educated to recognise the importance of food hygiene and safety in disease prevention and control. Initiatives to improve food safety in developing countries by trade in a clean and safe environment can generate opportunities to support locally produced food for producers and consumers. Assistance through the project to implement training in international hygiene and food safety standards for vendors in wet markets with course completion certificates followed up by regular inspections from local authorities could help to enhance awareness of the need for global food safety and hygiene.

Livestock Development Strategy

The MAFF has developed the key medium-term policy and strategic plan ASDP 2019-2023. It road maps identifying agricultural development directions with clear goals outputs, indicators, and clustered activities. The ASDP also reflects the Royal Government's policy, especially Rectangular Strategy Phase IV, and is in line with National Strategic Development Plan (NSDP) 2019-2023. This strategic plan was approved and declared officially effective on December 25, 2019. The vision is for agricultural development to be competitive, inclusive, resilient, and sustainable, leading to increased farmers' income and the prosperity and well-being of the Cambodian people. In 2015, the Department of Animal Health and Production (DAHP) was upgraded to General Directorate of Animal Health and Production (GDAHP). One of its first major activity was the development of the "Strategic Planning Framework for Livestock Development: 2016-2025" which identified strategic

issues and planning to address Cambodian livestock sub-sector during the next ten years, such as: animal production, animal health with improvement of access to animal health and extension services, markets growth and access to markets for smallholders, food safety and increasing resilience to climate change and other shocks in poor rural households and communities, public health amelioration, poverty alleviation and food security, strengthen of the private sector, support to farmer groups/associations, strengthen of the Village Animal Health Workers (VAHWs), regional economic integration and important cross cutting issues such as gender and climate change.

In order to attain this vision, two priority policy objectives are identified as follows:

- (i) Enhance productivity, diversification, competition, and commercialization;
- (ii) Increase the effectiveness of sustainable land, forestry, and fisheries management and development.

To realize the policy objectives, seven strategies will be developed and implemented focusing on:

- Strengthen animal production, including the promotion of animal management and breeding;
- Strengthen animal health and public veterinary health
- Promote the dissemination of laws, strengthen the implementation of laws and policies
- Promote research and development of animal health and animal production
- Develop institutional and human resource capacity
- Promote public-private partnerships in animal production
- Strengthen national and international economic and technical cooperation on animal health and production subsector

Three main strategic goals shall be implemented:

- boost animal production at an annual growth rate of 3% (240,000 tons in 2019 to 270,000 tons in 2023) through management and development of animal husbandry,
- Increase the rate of commercial livestock production at 23% in 2019 to 30% in 2023,
- Reducing of infectious diseases to under 5% through strengthening animal hygiene and animal product monitoring.

Conclusions

Cambodia has good potential for the development of livestock production. The current level is not completely satisfactory, so a great effort is being made by the state in this area of agriculture. The government has a clear plan to improve the production of meat and milk by farmers, considering all aspects of food quality and safety. International scientific cooperation and quality control laboratories play a very important role now and in the future.

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PHENOTYPIC AND MOLECULAR PREDICTION AND SELECTION OF DOMESTIC ANIMALS

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Abstract: *Eenvironmental factors are always present, it is difficult to reliably determine the value of an animal's genotype. The fact that genotypes are not passed from generation to generation, but numerous genes that exhibit average effects in relation to the mean value of the population also complicated genotype assessment. In order to estimate the genotype of an individual as accurately as possible, we must apply a series of procedures that collectively at the end of the journey can give us a clearer picture of what we are looking for. The basis of selection work is the assessment of the breeding value of domestic animals on the basis of which the selection of individuals or groups for further reproduction and use in production. With the development of computer technology, statistical software was obtained, which enabled faster and more reliable data processing and assessment of breeding value, using linear models. From the first ideas of DNA molecule analysis for that purpose until today, many procedures have been applied and improved and many genetic markers and methods have been discovered, so that in practical terms it is necessary to accept a common platform called genomic selection. Genomic selection - is a powerful tool for future use. Currently, the efficiency of genomic selection is limited by various interactions between the locus of quantitative traits, the variability of quantitative traits, and the influence of environmental factors. However, research results in many countries*

have confirmed that the use of standard statistical methods together with genomic selection increases reliability in predicting the breeding values of animals.

Key words: *Animal selection, breeding value, genomic selection*

INTRODUCTION

The most important goals of animal breeders is rapid genetic progress, ie the improvement of production traits that are of economic importance (Petrović et al. 2017).

In that sense, it is necessary to rank animals according to productivity, in order for some animals to be kept for further reproduction, and individual heads must be scrapped (Petrović, 2000).

Modern selection is possible only if we have reliable indicators of the breeding value of the individuals we breed, ie which are the subject of selection measures. In the language of genetics, data on quantitative traits whose basic category is phenotypic value, ie phenotype, must be possessed.

The value of the phenotype of the animal is the result of the action of hereditary factors and the environment. Hereditary factors include summary or additive gene action, dominance and epistatic effects.

Knowing that environmental factors are always present, it is difficult to reliably determine the value of an animal's genotype. This is also complicated by the fact that genotypes are not passed from generation to generation, but numerous genes that exhibit average effects in relation to the mean value of the population. In this way, the individual and the population stand in a certain relationship, so the breeding value is determined, both by assessing their own phenotype, and on the basis of relatives or data from the population (Falconer, 1981).

The accuracy of the assessment of the breeding value is higher if the indicators of the individual itself are combined with relatives. At the same time, a more reliable assessment is obtained if the degree of kinship of the observed animal and relatives is higher. Bearing in mind that each individual inherits the komple gene complex of both parents, the breeding value is, in fact, the average of the breeding values of the parents.

Without much wisdom, it can be said that all the paths that man wants to go with his animal population, lead to the same goal. It is an increase in production from generation to generation. So, the whole process is in motion and all the actions in it are interconnected. Therefore, in order to estimate the genotype of an individual as accurately as possible, we must apply a series of procedures that collectively at the end of the journey can give us a clearer picture of what we are looking for. About the quality or breeding value of the animal. Great hope is placed on genomic selection.

Basic principles of selection

The estimated breeding value is obtained from the following expression:

$POV = b \cdot (P - M)$, where are

POV - Estimated breeding value

b - Regression coefficient of additive genotype to phenotype

P - Average value of the observed individual

M - Average phenotypic value of the observed trait in a particular population

From a brief insight into the basic principles that characterize the additive genotype of an individual, we can conclude that the question of the value of individual individuals or populations, in terms of their ranking and selection, is very complex.

The basis of selection work is the assessment of the breeding value of domestic animals on the basis of which the selection of individuals or groups for further reproduction and use in production.

Starting from the fact that we cannot determine the actual genetic value of the throat, we perform an assessment based on productive indicators obtained by measurement (Pantelić et al., 2011, Petrović et al., 2009., 2011., 2015).

In order to increase the productivity of animals, and thus get better offspring from generation to generation, the accuracy of the assessment of the breeding value and the choice of superior parents is extremely important.

The reliability of the assessment of the breeding value can, to some extent, be influenced by good organization by providing the animals we compare with approximately equal breeding conditions. However, since the measured productivity of sheep is influenced by a wide range of factors, it is quite clear that in production conditions we cannot ensure complete equality even with the best organization.

Since the phenotypic manifestation of animal traits, as we have already explained, is characterized by continuous variability due to the interaction of all hereditary and environmental factors ($P = G + E$), one genotype can give more phenotypes (Petrović, 2000).

Starting from that, it raises a logical question: how to determine the breeding value of an individual, when the influences of the external environment (E) make it impossible to directly measure the genetic value (G)? Only records on the production of the animal and its relatives are available to us.

An attempt to answer that question is contained in the mathematical procedure of eliminating environmental factors, where we usually include the

farm, year and season. In addition to these, many other factors affect the reliability of the assessment of the breeding value, such as: sex, age, type of birth, etc.

With the development of computer technology, statistical software was obtained, which enabled faster and more reliable data processing and assessment of breeding value, using linear models.

Phenotypic prediction and selection of domestic animals

In the selection of domestic animals, before the use of artificial insemination, there was no need to use a better method of analysis of variance than the conventional ANOVA method. Favorable results could be achieved by these methods as well. With the introduction of artificial insemination, there has been a need for some more complex methods that could evaluate the components of variance without error, and can also include both fixed and random effects, as well as their interaction. If these treatments were evaluated by the conventional ANOVA method, parameter estimates would be obtained with errors that would be significant, therefore these effects are estimated by the method of least squares.

According to this method, each property can be represented as a linear function of the general average of the effects of individual factors that affect that property and the error that occurred during its measurement. In considering the least squares method, we will start from the general model of analysis of variance, which has the following form:

$$Y_{ij} = \mu + a_i + e_{ij}, \text{ where:}$$

Y_{ij} - The value of the property of the y -th observation, the i -th influence class A

μ - General average of the population with the same number of repetitions by classes of influence A

a_i - Deviation of the i -th mean value of impact class A from the general average

e_{ij} - Other undetermined effects or error

In domestic animal selection, the least squares method has found great application, especially thanks to Harvey (1987, 1991) who adapted the least squares method for software application on computers.

Depending on the type of data and the desired goal, models are also defined. Thus, for example, if we want to estimate crossbreeds with different proportions of imported race genes, the general statistical model will have the following expression (Petrović, 2000):

$$Y_{ijklmnp} = \mu + K_i + G_j + L_k + O_l + F_m + T_n + B_p + b_1(x_-) + E_{ijklmnp}$$

With the development of ideas and results in terms of assessing the breeding value of animals, and especially those related to the assessment of the data dispersion components of various dependencies and imbalances, the BLUP method was created.

Its very name indicates that it is the method that is the best of all the previous ones, especially when the error that is the smallest is taken as a criterion. BLUP combines the good properties of least squares methods and selection indices. It includes the phenotypic characteristics of relatives and explains the differences between animals due to the influence of mixed external factors, such as flock or year of birth, which allows comparisons between the estimated breeding value of sheep born or raised in different groups.

The BLUP method is objective in terms of the significance of the estimated breeding value in relation to the actual one. BLUP implies that the breeding value is a linear combination of measured productivity indicators (Kenedy 1981, 1989, Van Vleck 1989, Petrović 2000).

In sheep and goat breeding, the BLUP method is used in many countries around the world, especially for assessing the breeding value of rams and goats. Depending on the volume of data and the number of factors included in the procedure, a model is defined, such as:

$$Y_{ijk} = \mu + O_i + G_j + e_{ijk}, \text{ where:}$$

Y_{ijk} - Data obtained on the k -th descendant, j -th years, i -th fathers

O_i - The effect of the i -th father

G_j - The effect of the j -th year

e_{ijk} - Residue or error

In the further procedure, many equations are solved, which depends on the number of animals, the number of fixed effects and other relevant factors.

The most accurate estimate of the breeding value of sheep was obtained by applying animal models (Henderson and Quass, 1976). It involves assessing the individual breeding values of all individuals within a population. The animal model uses all available data, both ancestors, relatives, descendants, and the results of their own productivity. It is suitable for rams and goats with a smaller number of daughters, but it is also used to assess females. It is also suitable for assessing the breeding value of parents who do not yet have the results of testing their offspring, but also individuals who do not have offspring.

The main reason for the introduction of animal models in practice is the shortcoming that the usual procedure of ranking animals has. Thus, for example, the evaluation of offspring based on the performance of the offspring is carried out according to the model:

$$Y_{ij} = \mu + G_i + S_j + e_{ij},$$

where farm or year effects are involved, then random father effects, etc.

Despite the fact that the mentioned model is important for practical application in sheep and goat breeding, it contains some limitations that have become unrealistic over time (changes in population, kinship, etc.).

One of the properties of the animal model is the ability to adapt to the required or given situation, whether it is to assess the breeding value of sheep and goats on the basis of reproductive performance or to assess the additive genetic value of dairy sheep (Barillet, 1986).

Quass and Polack (1980) reduced the complex computational procedure of the animal model, with the emergence of the so-called RAM or reduced animal model, which provides the same solutions.

The selection index is proportional to the value of an individual and is therefore usable as a criterion for animal selection. It represents a combination of advantages and disadvantages in the overall contribution of a particular animal in a population. The index (I) is compiled so that the correlation with the net genetic contribution (G) of the individual is, as far as possible, maximal. Any index is a function of the traits included ($X_1, X_2, \dots, X_n$) in the aggregate genotype. Statistically, it represents multiple regression equations that serve to predict, ie estimate the value of G based on the included features. Particular difficulties arise only in defining the properties of G and determining the correlations between G and the included properties X.

The basic characteristics of selection indices are reflected in the simultaneous selection of several traits, with more attention being paid to the property that is, from an economic point of view, the most interesting. The economic value of a property can be expressed in several ways, which to some extent creates difficulties. It is important to determine the effect of increasing the yield of milk, meat, wool, eggs, and the economic contribution of increasing the value of a trait.

Due to the unequal value of the heritability of individual traits, the selection effect will not be the same for all observed traits. Success is also influenced by genetic and phenotypic connections between traits.

However, selection indices have an advantage over the previous two procedures due to a more accurate assessment of the relative economic value of each trait covered. Also, here it is possible to make compensation between the characteristics of the observed animals because we know that no head is

superior in all properties of interest to the breeder. Finally, selection indices allow the use of data from their ancestors and descendants, thus increasing the accuracy of the assessment of breeding value.

The genetic contribution of an individual in a population depends on a number of factors. If the selection is applied separately for each trait, then it may happen that the selection pressure on certain traits is greater than we would like. It must also be taken into account that it is not economically justified, and even impossible to apply selection to only one trait, since the economic value of an animal depends on several traits.

The process of calculating selection indices is complex and mathematically represents a function of the properties included in the index.

The general form of selection indices has the following appearance:

$$SI = b_1x_1 + b_2x_2 + b_3x_3 + ... + b_nx_n,$$

where the symbols have the following meanings:

$b_1, b_2, b_3 \dots b_n$ - multiple regression coefficients

$x_1, x_2, x_3 \dots x_n$ - phenotypic values of traits

Molecular prediction and selection of domestic animals

Phenotypic prediction and selection of domestic animals are the most widespread method of genetic improvement. However, the same approach may be suboptimal for selection, because the best predictor of phenotype is not always the best predictor of genetic merit of the same trait.

The genome of domestic animals contains 20,000-35,000 useful-purpose genes.

In general, genomic selection should mean the process of analyzing DNA molecules in order to look for markers or individual nucleotide polymorphisms (SNPs) to obtain information about the genetic potential of the production traits of an individual, which will serve as a selection criterion.

From the first ideas of DNA molecule analysis for that purpose until today, many procedures have been applied and improved and many genetic markers and methods have been discovered, so that in practical terms it is necessary to accept a common platform called genomic selection.

The term "genomic selection" was first introduced by Haley and Visscher (1998). Meuwissen (2001) developed and presented a methodology for the analytical assessment of breeding values using marker maps that cover the entire genome.

Genomic selection (GS) is at today's level of science, a method by which high-density markers cover the entire genome. Thus, an individual

genetic evaluation is performed - genomically assessed breeding values (GPOV) (GEBV-English). Genomic selection can increase selection accuracy, shorten the generation interval by selecting individuals at an early stage of life, and accelerate genetic progress (Christensen et al., 2014).

Practical work in genomic selection involves taking a sample of biological material (blood, seeds, hair) from which DNA is isolated in the laboratory. The process of isolating pure DNA is very complex and includes cutting with restriction endonucleases, radioactive or non-radioactive labeling, ligation, use of special detergents, bases, organic solvents and enzymes. After DNA isolation, a genotyping procedure was performed using the Illumina SNP50K chip. Genotyping is a method by which genetic variations are determined without sequencing the entire genome. Genotyping results in signals for each of the 50,000 SNPs that are computationally converted to the SNP genotype (AA, AB, or BB). Thus, for an individual that has been subjected to genotyping, a result is obtained (more precisely, a genotype) for each of the 50,000 SNPs. The fact that we came to know about the genotype for a large number of SNPs, however, does not give a complete answer about the breeding value of the animal. To solve this, it is necessary to estimate the impact of an individual SNP using the SNP equation. Thus, the genomic breeding value (GOV) of a genotyped animal is the sum of the influences of all SNPs, obtained by applying the SNP equation (Spehar, 2013).

In many countries, Genomic Selection is becoming the leading method of genetic evaluation (Hayes et al., 2009, 2012). Especially in dairy cows, GS may soon completely replace the traditional system of genetic evaluation of animals.

Genomic technologies make it possible to decipher the genotype of an animal at birth. This allows the selection of individuals for further cultivation even then. SNP as the latest technology is designed to further improve the accuracy and reliability of selection and assessment of animal breeding value. The precursors of SNP genomic selection are selection markers, which enable the determination of the presence or absence of certain genes (alleles) of carriers of quantitative traits in the animal genome.

The idea and theory of the use of markers in breeding were first given by the Russian scientist AS Serebrovski during the second decade of the 20th century. The term "marker" (then called "signal", the English term "guard") began to be used later. According to Serebrovski, a marker is a allele of a gene with a pronounced phenotype, localized next to another allele that is responsible for an economically important trait, but does not have a clear phenotypic expression. Thus, the selection for the phenotypic manifestation of this allele-signal is possible by the selection of related alleles that determine the manifestation of the observed trait. However, the direct marker is far more reliable than the associated marker for predicting phenotypic variations of a

particular trait of domestic animals (Dekkers, 2004).

Genetic markers were initially based on morphological characteristics (phenotypic markers). However, quantitative traits are the result of a complex inheritance process. Their manifestation is determined by environmental conditions and the number of markers used. This limits their use.

Polymerase chain reaction (PCR) and DNA sequencing (nucleotide sequencing) have played a significant role in the genomic selection of animals (Awise, 2004). In recent years, several new molecular techniques have been developed, where, in addition to genetic markers such as microsatellites, the single Nucleotide Polymorphism (SNP) has taken the lead, thus significantly expanding and deepening selection possibilities.

The application of DNA technology and genetic markers in animal husbandry began in the 1980s. Molecular genetic markers have been used for programs to preserve endangered genes breed of domestic animals. Also, markers have found application to solve the problem of the origin and parentage of the prevalence of species, genetic causes of hereditary diseases. The application of markers promised to accelerate the selection of individual characteristics - resistance to certain factors from the impact on productive performance.

During the ninth decade of the XX century, marker assisted selection was developed, ie MAS. Because the number of markers that were available at that time was insignificant. The use of the MAS method was limited, but still gave a positive effect in selection. Using this method, the superior bull “Ha-Ho Cubby Manfred” was selected which had a great influence on later generations in the Holstein cattle population. The disadvantages of the MAS method are the fact that it uses a small number of markers for certain properties, and in addition, it is no less important that it prolongs the duration of the method itself. When using this method, it is important to focus on a number of specific markers that have a direct effect on the required properties.

In the early 1990s, genetic markers were also used in pigs to identify the halothane gene that causes stress syndrome.

Microsatellites are very popular because they are widely distributed in the genome, and have a high level of polymorphism. Microsatellites, such as SSR (Simple Sequence Repeats) or STR (Simple Tandem Repeats) are repeated in tandem many times per genome at more than one hundred thousand loci (Weber and May, 1989). They are highly polymorphic and suitable for individual DNA typing, ie making the genetic profile of an individual. Genetic markers are thought to be the most informative.

New method of genome analysis is the SNP polymorphism (Single Nucleotide Polymorphism) of a single nucleotide, which shows the change of only one nucleotide base into a DNA molecule. In the genome of animals thousands of SNPs- point mutations have been discovered. SNPs have a low

mutation rate per generation ($\sim 10^{-8}$) in contrast to microsatellites, which makes them suitable markers for population genetic analyses (Petrovic et al., 2018).

Significant progress for livestock has been made by the SNP using automatic detection methods for testing of large numbers of animals to find links between known point mutations and productivity. It turned out that a large number of polymorphic genes have influence on the animals production.

The application of genomic selection, however, is limited by many interactions between quantitative traits and the influence of environmental factors. Recent findings suggest that use of standard statistical methods together with genomic selection increases reliability in predicting the breeding values of animals (Bai Sartor and Cavalcoli, 2012, Petrovic et al., 2018).

The meaning of the application of genomic selection is reflected in the desire of researchers and breeders to increase the genetic progress of the population. Therefore, they try to connect all known sources of information (phenotype, origin and genetic markers), in order to obtain the highest possible accuracy of the estimated breeding value (POV).

Recent research shows that genome sequencing of several species of domestic animals is applied. This gives farmers hope to achieve their goals on animal genetic improvement (Burt, 2005; Archibald et al., 2010; Zimin et al., 2009; Wade et al., 2009; Bai et al 2012).

CONCLUSION

The selection of domestic animals is an imperative for the preservation and improvement of populations. At the end of the last century, there was a rapid development of modern laboratory methods that detect the DNA molecule itself. Genetic markers and genomic selection procedures have been developed. However, despite the impressive breakthrough, classical methods of population genetics are necessary for a complete and accurate assessment of the animal's breeding value. Therefore, quantitative phenotypic parameters should be combined with molecular ones.

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RELATIONSHIPS BETWEEN CONCENTRATIONS OF LIPIDS, PROTEINS AND UREA IN BLOOD AND MILK IN DAIRY COWS DURING LACTATION

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Abstract: *The objective of this study was to determine blood and milk metabolites and their interrelationships to evaluate the metabolic status in dairy cows for early diagnosis of subclinical metabolic disease at different stages of lactation. For this study, blood and milk samples were taken from 24 Holstein dairy cows during morning milking. The cows were divided into 2 groups according to the production period. Group 1 comprised early lactation cows (n=12); group 2 included mid-lactation cows (n=12). The concentrations of glucose, triglycerides (TG), total cholesterol (TChol), total protein (TP), albumin and urea in the blood and the contents of fat, protein and urea in milk were determined. Statistically significant differences ($P < 0.05$) were observed in blood TG, TChol and urea levels and milk fat and urea concentrations among experimental groups of cows. Significantly negative correlations ($P < 0.05$) were found between TG in blood and fat ($r=-0.525$) and urea ($r=-0.711$) in milk; and between TP in blood and urea in milk ($r=-0.366$). Significantly positive correlation ($P < 0.05$) was observed among TG in blood and protein ($r=0.381$) in milk and between TChol in blood and fat ($r=0.362$) and urea ($r=0.406$) in milk. Based on changes in blood and milk metabolites at different stages of lactation, the present results suggested that early lactation cows showed a mild degree of hepatic lesions, probably due to fat infiltration. The results showed that changes in blood and milk metabolite levels and their interrelationships can be helpful in the herd monitoring of metabolic status in dairy cows and for the early diagnosis of subclinical metabolic disease.*

Key words- dairy cows, blood and milk metabolites, different stages of lactation.

INTRODUCTION

Constant monitoring of high-yielding cows kept in large herds is useful for assessing animal welfare, and health as well as for early diagnosis of many health problems (Cook *et al.*, 2006). A metabolic profile, a series of specific blood analytical tests, is routinely used to reveal metabolic problems in dairy cattle (Oetzel, 2004; Overton, and Waldron, 2004).

Evaluation of blood and milk biochemical parameters to assess animal health and milk yield has always attracted attention, and various discrepancies have been observed in both blood and milk yield results (Eicher, 2004; Nozad *et al.* 2006, 2007; Liu *et al.* 2012; Jozwik *et al.* 2012). Milk parameters originate from blood and feed components, and clarifying appropriate relationships among these parameters individually in feed, blood, and milk is useful in understanding the health and production status of animals (Hamann and Kromker, 1997; Nozad *et al.* 2007).

The metabolic profile test is known as an applicable approach in assessing blood biochemical parameters, such as glucose, protein, urea, TG, cholesterol, BHB, and macro-minerals (Gross *et al.* 2001). The same test is used for milk parameters including protein, fat, urea, lactose, and macro-minerals as major animal products (Hamann and Kromker, 1997; Nozad *et al.* 2006, 2007; Samanc *et al.* 2006; Jozwik *et al.* 2012).

Major health disorders in high-yielding cows occur around parturition and during lactation (ketosis, fatty liver, etc). However, under lipidosis conditions, endogenous liver synthesis decreased, resulting in reduced levels of blood glucose, TP, albumin, globulin, tChol., TG and urea (Stang *et al.* 1995; Sevinc *et al.*, 2003; Bobe, 2004; Djoković *et al.*, 2011).

Milk, the outcome of various biochemical activities in mammary secretory cell is composed of fat, protein, carbohydrates, enzymes, vitamins and various minerals. The composition of milk is influenced by various factors, i.e., stage of lactation, lactation number, breed, feeding pattern, environmental and diseased condition of the udder (Brinez *et al.*, 2003; Eicher, 2004).

The fat, protein and urea contents of milk vary according to stage of lactation. Fat content is high immediately after calving but soon begins to fall, and continues to do so for 10 to 12 weeks, after which it tends to rise again until the end of the lactation (Samanc *et al.* 2006). Therefore, the amounts of urea nitrogen in the blood or milk could have an effect on the quality and quantity of milk and their determination would be useful in understanding the health status of the animals (Stang *et al.* 1995; Jilek *et al.* 2006). Variations in urea are based dietary energy and protein, animal breeding methods, lactation period, pregnancy and parturition (Brinez *et al.* 2003; Nozad *et al.* 2011).

The objective of this study was to determine blood and milk metabolites and their interrelationships to evaluate the metabolic status of dairy

cows for early diagnosis of subclinical metabolic disease at different stages of lactation.

MATERIAL AND METHODS

A total of 24 dairy cows were randomly selected from the same Holstein herd containing 445 cows. Two groups of clinically healthy cows were chosen from the herd. Group 1 comprised early lactation cows ($n=12$) in the first month of lactation (22 ± 12 days) and group 2 included mid-lactation cows ($n=15$) between 90 to 150 days of lactation (133 ± 75 days). The experimental cows were housed in open-stall barns. Diet and housing facilities were adapted to research purposes, with diet suited to the energy requirement of early lactation, and mid-lactation cows.

Blood and milk samples were taken simultaneously from each lactating cow during morning milking. Blood samples (10 ml) were taken by jugular vein puncture into sterile tubes from each animal, and blood serum was separated by centrifugation at room temperature ($1,800\times g$, 15 min). Blood samples collected into fluoride-containing tubes were immediately centrifuged in the same manner, and plasmas were assessed for glucose concentrations. Milk samples were centrifuged at $12,000\times g$ for 30 min at 4°C , and the supernatant was transferred into new sterile tubes. Blood and milk sera were stored at -20°C until biochemical analysis.

The levels of glucose, triglycerides (TG), total cholesterol (TCh), total proteins (TP), albumin and urea were measured by spectrophotometric techniques using a BT 1000 spectrophotometer (Biotechnica Italia) and the corresponding commercial kits (DIALAB, YUNICOM). The content of fat in milk was determined by the acidobutrics method (Gerber), total protein by volumetric method according to Kjeldahl (QP-11.52), and urea concentration by spectrophotometry (QP-11.50).

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc. Warrenton, VA, USA). The analysis of t-test was used to evaluate the probability of the significance of statistical differences between mean blood and milk metabolites in each group and the Pearson test was performed for evidencing significant correlations. Differences were considered as significant when P values were below 0.05 or 0.01.

RESULTS

The present study compared organic parameters in blood and milk sera and their interrelationships in dairy cows. Results of blood biochemical metabolites for all groups of cows are shown in Table 1.

Table 1. Blood metabolites in early lactation (Group 1) and mid-lactation (Group 2) dairy cows (n=12 per group). Results are expressed as mean $\pm$ standard deviation (SD).

Variables	Group 1	Group 2
Glucose(mmol/l)	2.83 $\pm$ 0.53 ^a	3.00 $\pm$ 0.28 ^a
TG(mmol/l)	0.04 $\pm$ 0.04 ^A	0.13 $\pm$ 0.05 ^B
TChol. (mmol/l)	2.93 $\pm$ 0.59 ^A	5.93 $\pm$ 1.17 ^B
TP(g/l)	68.36 $\pm$ 7.62 ^a	72.36 $\pm$ 5.5 ^a
Albumin(g/l)	32.64 $\pm$ 4.46 ^a	34.63 $\pm$ 2.61 ^a
Urea (mmol/l)	3.67 $\pm$ 0.71 ^a	4.70 $\pm$ 1.09 ^b

Legend: Mean values within a row with no common superscript differ significantly; values followed by small letters differ significantly ($P < 0.05$); values marked with capital letters differ highly significantly ($P < 0.01$).

Biochemical testing for metabolites in the blood serum showed significantly lower values ($P < 0.05$) of TG and urea in early lactation cows than in mid-lactation cows. In postpartal cows, serum concentrations of TChol. were statistically lower ($P < 0.01$) than in mid-lactation cows. No significant difference ($P > 0.05$) was observed in the blood values of glucose, TP and albumin, among experimental cows. The results of milk biochemical analysis for groups of cows are given in Table 2.

Table 2. Milk metabolites in early lactation (Group 1) and mid-lactation (Group 2) dairy cows (n=12 per group). Results are expressed as mean $\pm$ standard deviation (SD).

Variabile	Group 1	Group 2
Fat (%)	3.52 $\pm$ 0.28 ^a	3.80 $\pm$ 0.04 ^b
Protein (%)	3.39 $\pm$ 0.99 ^a	3.52 $\pm$ 0.39 ^a
Urea (mmol/l)	4.55 $\pm$ 0.67 ^a	5.68 $\pm$ 0.36 ^b

Legend: Mean values within a row with no common superscript differ significantly ($P < 0.05$).

Fat contents and urea concentrations in milk were statistically lower ($P < 0.05$) in cows in early lactation compared to mid-lactating cows. No significant difference ($P > 0.05$) was observed in milk level of protein, among the experimental groups of cows.

Correlations between blood and milk biochemical metabolites for all cows in this experiment are given in Table 3, which shows coefficients of correlation among biochemical parameters calculated for all cows in this experiment.

Table 3. Correlation coefficients for biochemical metabolites in milk and blood calculated for all cows in the present study.

	Milk fat	Milk urea	Milk protein
Blood glucose	-0.224	-0.318	0.155
Blood TG	-0.525	-0.711	0.381
Blood TCho.	0.362	0.406	-0.113
Blood TP	-0.186	-0.366	0.113
Blood albumin	-0.189	-0.164	0.107
Blood urea	0.236	0.342	-0.041

Legend: Significant correlations ($P < 0.05$) are presented in bold.

Significantly negative correlations ($P < 0.05$) were observed between blood TG and milk fat ($r=-0.525$) and urea ($r=-0.711$) levels, and between blood TP and milk urea ($r=-0.366$) concentrations. Significantly positive correlations ($P < 0.05$) were determined between blood TG and milk protein ($r=0.381$), and between blood TChol. and milk fat and urea ($r=0.362$; $r=0.406$).

DISCUSSION

Modern milk production often puts the production capabilities of cows at risk, which can result in metabolic disorders. In order to predict such disorders and eventual subclinical diseases, it is necessary to determine physiological ranges of biochemical parameters in a clinically healthy herd (Oetzel, 2004; Overton, and Waldron, 2004; Eicher, 2004).

The significant decreases ($P < 0.01$) in serum TG, TChol. and urea concentrations were observed in early lactating cows compared to mid-lactation females in this study. Other biochemical parameters, at least partially synthesized in the liver, such as glucose, albumin, and total proteins, were also decreased during the puerperal period ($P > 0.05$). These results suggested an decreased liver gluconeogenesis and ureagenesis as consequence of accumulation of lipids in hepatocytes in puerperal cows (fatty liver), which was probably associated with depleted liver synthesis of VLDLs. (Herd *et al.* 1983; Stang *et al.* 1998; Reist *et al.* 2002; Sevinc *et al.* 2003).

Stage of lactation significantly influences the composition of raw milk of dairy cows (Brinez et al.2003; Nozet *et al.*2011). Factors predisposing to general or mammary gland diseases should be evaluated in combination with major milk components (fat and protein) by measuring urea in milk as indicators of a balanced diet (Samanc *et al.*2006; Nozad *et al.*2011).

In this study, fat contents and urea contents in milk were statistically lower ($P<0.05$) in early lactation cows compared to mid lactating cows. No significant difference ($P> 0.05$) was observed in milk protein levels among the experimental groups of cows. Milk fat can increase or decrease depending on ration composition. Early lactation cows have a tendency to mobilize body reserves while ingesting rations that are low in effective fiber will tend to decrease milk fat levels (Hamann and Kromker, 1997; Reist et al.2002; Samanc et al.2007).

Lower milk fat content is frequently used on farms as an indicator of sub-acute ruminal acidosis and to predict the effectiveness of diet structure for chewing(Hamann and Kromker 1997; Valk et al.2002; Nozet et al.2011). Low milk fat content is caused by a lack of major precursor, acetic acid in rumen, which is produced in insufficient quantity (Hamann and Kromker 1997; Brinez et al.2003; Samanc *et al.* 2006). Generally, the increase in milk protein content and decrease in milk fat content lead to sub-clinical acidosis (Eicher, 2004; Babaei *et al.*2007; Katsoulos *et al.*2010).

The level of urea in milk is an indicator of metabolic nitrogen balance of cows (Westwood et al.1998; Jilek et al.2006) , which characterizes their health and reproductive ability. The obtained results on milk urea were within physiological limits (2-6 mmol/l) in all groups of cows (Samanc et al.2006). Among the nutritional factors, the energy to protein ratio is the most important in cow rations. Urea concentration increases with increasing intake of rumen degradable protein, but also when energy in rations is lacking, since no optimal amount of protein can be utilized from the ration due to decreased activity of rumen bacteria. As the feed energy supply increases, the concentration of urea in milk decreases (Westwood et al.1998; Jilek et al.2006; Samanc et al. 2006). At the beginning of lactation, milk urea and fat levels were significantly lower ($P<0.05$) than in the mid lactation periods due to decreased energy supply through diet in the peripartal period, as well as due to increased lipomobilization from body reserves.

The significant correlation between milk urea and total protein in the blood ($r=-0.366$) and between blood TG and milk protein ($r=0.381$), fat ($r=-0.525$), and urea ($r=-0.711$) can indicate a correlation between protein and lipids in the blood and urea in milk, i.e., indicate the nitrogen metabolism of the mammary gland. Similar results were reported elsewhere (Westwood et al.1998; Jilek et al.2006; Samanc et al. 2006; Nozad et al.2007).

CONCLUSIONS

Based on changes in blood and milk metabolites at different stages of lactation, the present results suggest that early lactation cows showed a mild degree of hepatic lipidosis, probably due to fat infiltration. The results showed that changes in blood and milk metabolites and their relationships can be helpful in the herd monitoring of metabolic status in dairy cows, and for the early diagnosis of subclinical metabolic disease.

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COCCIDIOSIS IN LAMBS IN NORTHERN SERBIA (VOJVODINA)

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Abstract: The paper examines the results of the research on the coccidiosis in lambs in Vojvodina. The present study was conducted in 90 sheep flocks from the all territory of Vojvodina in the period from March 2014 to January 2015. Sheep of both sexes (320 males and 580 females, for a total of 900) were randomly chosen. There were 610 adults (one-year-old and above) and 290 lambs. Collected faeces samples were examined using routine coprological methods. During our examination coccidiosis were found at 32.22% (29/90) flocks. We usually occurred mixed infection with 2-3 coccidia species. At sheep most abundant species were *E.ovinoidalis* found at 87.1%, followed by *E faurei* (42%), *Eimeria ahsata* (37%), *E. granulosa* (31%), *E. intricata* (19%), and *E. pallida* (8%). Oocyst were found at adult and young animals, but clinical sign of disease were present only at young animals. The number of excreted oocysts was higher in lambs than in adult sheep

Key words: coccidiosis, lambs, sheep, Vojvodina

Introduction

Coccidiosis in sheep is parasitic infection caused by protozoa in the genus *Eimeria*. Historically, some *Eimeria* spp were thought to be infectious and transmissible between sheep and goats, but the parasites are now considered host-specific (McDougald,1979). At sheep were established next coccidial species: *Eimeria ahsata*, *E.ammonis*, *E.arkhari*, *E.crandallis*, *E.dalli*, *E.danielle*, *E.faurei*, *E.gilruthi*, *E.gonzalezi*, *E.granulosa*, *E.intricata*, *E.marsica*, *E.ovina*, *E.ovinoidalis*, *E.pallida*, *E.parva* Kotlán, *E.punctata* and *E. rachmatullinae* (Foreyt, 1987, 1990). Coccidia of sheep are present worldwide and it seems difficult to say that there is any particular geographical distribution for one or the other species of coccidia (Levine,1985, Wright and Coop,2007).

All species of coccidia are not disease causing. There are only some species that are responsible for the outbreak of the disease. *E. ovinoidalis* can be very pathogen and other species such as *E. bakuensis* (*E. ovina*) and *E.*

crandallis may exacerbate the symptoms of the former two species. Outbreaks of coccidiosis are usually acute and characterized by moderate morbidity and low mortality (O'Callaghan et al., 1987, Gregory,1990). Usually it is presented at animals at 4-10 weeks. Lamb and kids infection had moderate morbidity and low mortality rate. Environmental contamination and resulting clinical disease is generally influenced by local weather conditions and the grazing management practices of the flock (de la Fuente et al.,1993, Pavlović et al.2013). The economic impact of coccidiosis in small ruminants is not well documented and there is no published data about estimate for economic losses due to subclinical or clinical disease. The economic cost is considerable, in terms such as low growth performance, decrease in productivity, mortality, morbidity, and the cost of prevention and treatment Pavlović et al.,2009,2010). These losses can be linked to reduced production, in the case of moderate infection without clinical signs or direct consequences of diarrhea on the growth of the animals and on mortality, in the case of clinical coccidiosis (Foreyt,1990).

Coccidiosis in small ruminants (sheep and goats) in Serbia has been tested in the last fifteen years and in our paper we presented results of the our examination at norther part of Serbia – in Vojvodina.

Material And Methods

Vojvodina is situated in the northern quarter of Serbia, The region is divided into: Bačka in the northwest, Banat in the east and Srem in the southwest. Agriculture is a priority sector in Vojvodina. Traditionally, it has always been a significant part of the local economy and a generator of positive results, due to the abundance of fertile agricultural land which makes up 84% of its territory (Ognjenović, 2008). Vojvodina is rich in grasslands suitable for sheep grazing.

The present study was conducted in 90 sheep flocks from the all territory of Vojvodina in the period from March 2014 to January 2015. Sheep of both sexes (320 males and 580 females, for a total of 900) were randomly chosen. There were 610 adults (one-year-old and above) and 290 lambs. Collected faeces samples were examined using routine coprological methods (Soulsby,1977, Pavlović and Anđelić-Buzadžić,2010). Despite the general relationship between clinical coccidiosis and oocyst excretion, no threshold for treatment has been established, though a fecal oocyst count of >20,000 OPG of a pathogenic species is considered characteristic of coccidiosis in lambs. A necropsy is the most reliable method of diagnosis. The coccidia organisms themselves cannot be seen at necropsy, as they are microscopic from gut swabs and at histopathological examination. In total we examined 39 lambs slaughtered in a slaughterhouse. Determination of occidia we performed by morphological characteristic (Levine 1985, Foreyt, 1987,1990).

Results And Discussion

During our examination coccidiosis were found at 32.22% (29/90) flocks. We usually occurred mixed infection with 2-3 coccidia species. At sheep most abundant species were *E.ovinoidalis* found at 87.1%, followed by *E faurei* (42%), *Eimeria ahsata* (37%), *E. granulosa* (31%), *E. intricata* (19%), and *E. pallida* (8%). Oocyst were found at adult and young animals, but clinical signs of disease were present only at young animals. The number of excreted oocysts was higher in lambs (18325+/-23383 OPG) than in adult sheep (2597.3+/-12373 OPG).

At necropsy, macroscopic changes included mucosal hemorrhages and whitish nodular polyps in the jejunum were found. Histopathological changes were characterized by local hypertrophy and hyperplasia of intestinal villi, villus blunting and inflammatory infiltration in the lamina propria. Numerous developmental stages of the parasites were observed in enterocytes. These findings were identical to the pathological changes in lamb coccidiosis described by Khodakaram-Tafti and Mansourian (2008).

Coccidiosis, like other internal parasite problems, is directly linked to contamination of the lambing area or pastures with coccidia “eggs” (oocysts) passed in the manure of infected ewes and lambs (Pavlović,2009 Altaf and Hidayatu,2014). The symptoms observed are dependent on the species of *Eimeria*, how many are present, the animal’s age, production status, overcrowding, stress level and environmental factors. Immunity develops over time, so young animals are more susceptible than older animals (Gregory and Catchpole,1989). Adult animals can have coccidia, but not show symptoms. Lambs 1–6 month old in lambing pens, intensive grazing areas, and feedlots are at greatest risk as a result of shipping, ration change, crowding stress, severe weather, and contamination of the environment with oocysts from ewes or other lambs. When lambs are exposed to infection early in life as a result of infection from the ewe and a contaminated lambing ground, a solid immunity usually develops and problems are seen only when the stocking density is extremely high (Chartier and Paraud,2012).

In winter lambing production systems, coccidiosis outbreaks are common in 3 to 6-week-old lambs that are infected with coccidia oocysts shortly after birth (first few days of life). In our area, outbreaks of clinical disease in winter lambing flocks commonly occur when lambs are about 20 to 30 days old. Coccidiosis is also more common during the second half of winter lambing, when the wet and relatively warmer transitional weather of late February and March contributes to coccidiosis survival and spread in the environment.

Signs of clinical disease (scours) generally occur about 18 to 20 days after ingestion of sufficient amounts of coccidia oocysts from the contaminated

environment. Clinical coccidiosis occurs when damage to the gut is sufficiently severe to cause dysfunction. This normally occurs at the beginning of the parasite's sexual multiplication stage, when parasite numbers reach their peak. (Taylor et al., 2007). Due to the damage of the cells lining the intestines, the primary symptoms of coccidiosis in sheep is sheep diarrhea, which may be foul smelling and contain mucus and blood. Sheep diarrhea may have a dark tarry appearance and, in severe cases, large blood clots can be seen (Gregory,1990). The hindquarters and tail may be covered with manure. Additionally, the performance of sheep will suffer due to loss of appetite, weakness, abdominal pain and fatigue. Some animals may die before showing signs of coccidiosis if they are exposed to a large amount of coccidia in the sheep environment. Even though sheep may recover, there could be long-lasting effects due to intestinal damage that cause general unthriftiness, decreased growth, inferior sheep milk production and a greater predisposition to other diseases (Altaf and Hidayatu, 2014)

There are two aspects to preventing clinical coccidiosis. The first is eliminating or lowering the numbers of pathogenic oocysts ingested through good biosecurity, hygiene and management. Prior to lambing and between groups all housing and pen materials should be thoroughly cleaned to remove all organic material and then disinfected with a product effective against coccidia oocysts. At pasture, contamination levels are reduced by regularly moving to clean grazing and avoiding fields that carried young lambs in the previous season when possible. Lambs with ewes at lower stocking densities have a much lower risk of disease (Kusiluka and, Kambarage, 1996).

The second is timed and targeted preventative treatment. Because occurrence of coccidiosis under these management systems often becomes so predictable, coccidiostats should be administered prophylactically for 28 consecutive days beginning a few days after lambs are introduced into the environment. Treatment of affected sheep once coccidiosis has been diagnosed is not effective, but severity can be reduced if treatment is begun early. In groups of lambs at pasture, frequent rotation of pastures for parasite control also helps control coccidial infection (Vujić et al.1991).

Conclusions

Sheep coccidiosis was of great importance to health status of lambs and its performances. Lambs infection had moderate morbidity and low mortality rate. Consequence is significant increase of lamb accrescence, its weakens and less growth. The best preventive measure a lamb producer can take is to use a feed with a coccidiostat added. With careful management and sound preventive measures, the losses associated with this disease can be reduced to minimal levels.

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VALIDATION AND DETERMINATION OF PESTICIDES RESIDUES IN ORGANICALLY PRODUCED RASPBERRIES

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Abstract: *With the organic food market on the rise, organic food fraud has become an issue to consumers, producers and the market. Modern food production almost entirely depends on the use of pesticides. There are about a thousand different pesticides on the market that have different applications. Organic food however has strict guidelines on what can be used during the farming process and no synthetic pesticides are allowed to be applied in organic production. Organic food is increasingly attracting the interest of consumers, as it is perceived to be healthier than food produced by conventional agriculture, and to be more sustainable for the environment. This paper provides a review on the quality of organically produced raspberries in terms of the presence of pesticide residues. A method for the determination of trace amounts of the pesticides was developed using solid-phase extraction and gas chromatography-mass spectrometry. The linearity of the analytical response across the studied range of concentrations (0.005 - 0.05 mg kg⁻¹) was excellent, obtaining correlation coefficients higher than 0.99. The average recoveries of the pesticide ranged from 71.69 to 109.53%, for fortification levels of 0.005 and 0.05 mg kg⁻¹. The precision values associated with the analytical method, expressed as RSD values (n=5), were less than 20% for the pesticide in the raspberry matrixes. Limit of quantification were 0.005 mg kg⁻¹ for the majority of the pesticides. The results of this paper demonstrate the need to establish a pesticide residue monitoring programs for raspberry analysis to improve food safety and decrease exposure risks to consumers.*

Key words: *extraction, organic production, pesticide residues, raspberries*

INTRODUCTION

In the era of intensive chemicals of agriculture, pesticides are widely used to increase the yield of fruits and vegetables, maintain their quality and destroy pests, and prevent diseases during cropping. However, the use of pesticides often leads to the presence of pesticide residues below or above permitted limits (maximum residue levels, MRLs). From the European Union (EU), there are many efforts to achieve a sustainable use of these compounds to

avoid the increase of pesticide levels in the environment and food. Fruit and vegetables are recognized as the group of food containing higher pesticide residue levels compared with food of animal origin. During the last years the main focus in pesticide residue control laboratories has been on the improvement of mass spectrometric based instrumentation (Lawal et al., 2018). Multiresidue gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) methods are widely recognized as ideal, highly specific and sensitive for testing food products (Biziuk and Stocka, 2015). The high selectivity provided by GC and LC-MS/MS allows the determination multi-class pesticides in one run. As a matter of fact, many health implications of pesticides usage have been documented which include: disorderliness of reproductive system, birth defects, diabetes, risk of cancer in both children and adults, diseases of central nervous system and cardiovascular diseases (Brantseter et al, 2017).

Therefore, the problems caused by the continuous use of pesticides leads to developing the methods and extraction agents for determining the pesticides residues as well as efficient management practice to enforce regulations towards controlling safety and distribution of vegetables and fruits. Unfortunately, one of the most significant challenges in developing efficient methods for pesticide residue extraction is their wide range of chemical properties and different kinds of matrices such as polar, non-polar, waxy, color and fatty.

Revolutionary QuEChERS method was introduced in 2003 for the pesticide residue analysis of fruits and vegetables by Michelangelo Anastassiades (Anastassiades and Lehotay, 2003). This basic procedure is based on a liquid partitioning with acetonitrile followed by a dispersive solid phase extraction (d-SPE) cleanup with primary secondary amine (PSA). The primary challenge for the modern QuEChERS multi-residue analysis is the possibility of modifying the method and adjusting the method of preparation depending on the reliability of testing specific pesticides in the tested matrix. Hence, the modern sample preparation method, after two decades, has achieved to be a basic and inviolable method of preparation.

Since QuEChERS were released, it has been widely used in the detection of pesticides residues in vegetable, fruit, cereals and other related plant substances. The use of QuEChERS preparations for pesticide analysis has expanded over the years to fatty foods, i.e., products of animal origin (meat, eggs, milk, etc.), honey, but also samples from the environment, such as soil, sediment and water (Hildmann et al., 2015; Zanella et al., 2012; Vazquez et al., 2016; Lozowicka et al, 2017; Tankiewicz and Biziuk, 2018).

The presence of pesticides residues in organic fruits and vegetables is insufficiently reported in the scientific literature (EFSA, 2020) where an

overview of 2018 analyzes is given. Overall, 4863 organic samples did not contain quantifiable residues (84.8% of the analyzed samples), 794 samples contained quantified residues below or at the MRL level (13.8%) and 78 samples were reported with residue levels above their corresponding MRLs (1.4%), of which 0.5% (29 samples) were non-compliant in 2018. Compared to conventionally produced food (non-organic), the MRL exceedance and quantification rates were lower in organic food. In 2018, the MRL exceedance rate was 1.4% in organic food, while 4.8% for conventional food. Taking all these points into account and considering the importance of fruit in nutrition, the goal of this study was to develop and validate an analytical method for pesticides residue determination in raspberries by GC/MS.

This work is focused on new chromatography strategies that improves or facilitates the application of mass spectrometry in pesticides residue area, and the products are obtained by organic production. The aim of this research was to adapt the QuEChERS method for pesticide multiresidue analysis in organic raspberries samples using gas chromatography coupled to mass spectrometry (GC/MS). Pesticide residues were extracted using the modified QuEChERS technique with acetonitrile and then purified using the dispersive solid phase extraction (d-SPE) clean-up. For this purpose, it was conducted a monitoring study of 20 commercial organic samples originated from Serbia.

MATERIALS AND METHODS

Reagents and materials

Stock standard solution (10 mg l⁻¹) was supplied by DR Ehrenstorfer (LGC, Germany). A stock standard solution was prepared in acetonitrile and stored at -18 °C. The individual stock standard solutions were prepared in ethyl acetate. This solution was used as spike solution for recovery experiments and also to prepare the analytical curves solution for linearity studies. All high purity solvents were obtained from PanReac AppliChem (ITW Reagents, USA). Extraction kits, or QuEChERS extraction agents, were manufactured by Phenomenex (USA). Matrix-matched solutions were also prepared by serially diluting the intermediate solution with blank raspberries sample extracts containing none of the tested analytes to perform matrix-matched calibration with the same concentrations as in the solvent. The use of matrix matched calibration solution is necessary to minimize errors associated with matrix induced enhancement or suppression effects during GC-determination.

Equipment - Gas chromatographic analysis

A method using gas chromatography with mass spectrometric detection (GC-MS) was developed and validated for simultaneously determining pesticides residues in raspberries. A GC-MS analysis was carried out using a

GC Clarus 680 PerkinElmer system comprising an autosampler and a gas chromatograph interfaced with an MS Clarus SQ8T instrument under the following conditions: capillary column Elite-5MS (30 x 0.25 mm ID x 0.25 μ m df, composed of 95% dimethyl poly siloxane and 5% Phenyl), operating in the electron impact mode at 70 eV. Helium (99.999%) was used as the carrier gas at the constant pressure of 21.5 psi and an injection volume of 2 μ L was employed (a split ratio of 50:1) at the injector temperature of 250 °C; the temperature of the ion-source was 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.2 seconds and fragments from 50 to 400 Da. The oven temperature program was initially set at 70 °C for 3 minutes and then ramped up to 150 °C at 25 °C/min, then to 200 °C at 3 °C/min, and finally to 280 °C at 8 °C/min for 10 min. Total GC running time was 41.87 min. The software was adapted to handle mass spectra and the chromatograms were Turbo Mass Ver 6.1.0. The mass conditions were set as follows: ion source temperature at 250 °C and transfer line temperature at 280 °C. The mass spectrometer with EI was used in selected ion monitoring (SIM mode, and three ions were selected and monitored for each pesticide. Retention times, qualitative ion, and quantitative ion for SIM od studied pesticides are listed in Table 1.

Matrix effect

In order to investigate the matrix effect and to reduce matrix induced effects during GC, validation was performed by calibration in the matrix, so that the sample and calibration solution had the same concentration of co-extracted matrix components. To prepare matrix calibration solutions a blank matrix of raspberries was used containing no detectable residue of the analytes of interest. The blank is treated as any other sample. For blank extracts, raspberry samples were purchased at the local Serbian supermarket. To each aliquot of the blank extract were added correspondingly known amounts of pesticide standards mixture (five levels) and known and the same concentration of internal standard (ISTD). Thiphenyl phosphate (TPP) was used as an internal standard.

Sampling

Raspberry samples were produced by organic production. According to Council Regulation (EC) No 834/2007 organic production is defined as an overall system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes. The samples were collected in polyethylene bags, frozen, labeled and brought to the laboratory for analysis. European Union organic farming rules cover agricultural products, including

aquaculture and yeast. They encompass every stage of the production process, from seeds to the final processed food. This means that there are specific provisions covering a large variety of products, such as: seeds and propagating material such as cuttings, rhizome etc. from which plants or crops are grown; living products or products which do not need further processing; feed; products with multiple ingredients or processed agricultural products for use as food. Twenty raspberries' samples were in triplicate were scanned for the examination. Pesticides residues were extracted using the modified QuEChERS technique with acetonitrile and then purified using the dispersive solid phase extraction (d-SPE) cleanup. The procedural internal standard (triphenyl phosphate, 100 μL of a 10 $\mu\text{g mL}^{-1}$ standard solution) was spiked on the weighed raspberries sample, resulting in a concentration of 100 $\mu\text{g kg}^{-1}$ in the final extract.

Extraction and clean-up

Each sample was comminuted using the laboratory blender and the representative of homogenized (10.0 g) of each was then placed into a 50 mL polyethylene tube. Samples were extracted and cleaned up immediately after sampling using the QuEChERS method. Ten milliliters of acetonitrile were added into each tube. The samples were well shaken using a vortex mixer at maximum speed. Afterward, 4.0 g of anhydrous magnesium sulfate and 1.0 g of sodium chloride were added, then extracted by shaking vigorously on a vortex for 2 min and centrifuged for 5 min at 4000 rpm. An aliquot of 6 mL was transferred from the supernatant to a new clean 15 mL centrifuge tube containing with primary secondary amine (PSA) sorbent, C18 and anhydrous MgSO_4 in a simple approach termed dispersive solid-phase extraction (dispersive-SPE) cleanup. The samples were again vortexed for 3 min and then centrifuged for 5 min at 4000 rpm. Final extracts of acetonitrile were concentrated using a gentle stream of nitrogen and reconstituted into ethyl acetate. The sample was then ready for the final analysis in GC system. The prepared samples were analyzed with gas chromatography – mass spectrometry (GC/MS) technique in the selected ion monitoring mode (SIM) using one target and three qualifier ions for each analyte.

Analytical method validation

The initial full validation was based on the SANTE/12682/2019 document. The validation parameter included the linearity, matrix effect, LOQ, specificity, recovery and precision for GC amenable analytes from the standard mixture. In order to study the linearity, analyte mixtures were prepared with ethyl acetate and also in parallel in blank matrix at different levels (5, 10, 20, 30, 50 $\mu\text{g kg}^{-1}$). The reagent blank and a blank control sample were prepared as described for the spiked samples. The accuracy and reproducibility of the

analytical method were evaluated through recovery and RSD, respectively. The recoveries and precision were determined as an average of five replicates at two different spiked levels. The spiked concentrations were 5 and 50 $\mu\text{g kg}^{-1}$. The precision of the analytical methods was evaluated by RSD.

Recovery assays

Final extracts were evaluated by fast GC/MS analysis using the SIR (selected ion recording) mode with ion ratios for confirmation. Under the optimized conditions, the method was validated according to recovery. The samples employed in validation studies did not contain any of pesticides analyzed. For spiking, 10 g representative portions of previously homogenized sample of raspberries were weight in 50 ml tubes, where they were fortified homogenously with the appropriate amount of the working standard solution in acetonitrile. The validation method was performed at two fortification levels (0.005 and 0.05 mg kg^{-1}). Five replicates were analyzed at each level. We have to take in account that co-planar compound (e.g., pigment) strongly interact with the stationary phase. These need to be removed from the column after several injection, for example, at the end of a sequence. For the purpose, cleaning of the column with hexane is highly recommended. Retention time and peak shape will recover after column conditioning. In all those cases, the results of the recovery tests are in accordance with the validation and quality control SANTE criteria for pesticide residue analysis (mean recovery in the range 70–120% with an RSD < 20%), showed in Table 2.

RESULTS AND DISCATION

Before the calibration and quantification of pesticides it is necessary to set the acquisition parameters of mass spectrometer to determine the reactions for ion monitoring, to find the energy of collision cell and the fragmentation energy where the response of the studied pesticides will be highest for the given conditions (Table 1). The mass spectrometer detector was tuned by maximum sensitivity autotune. The aim of an extraction is the complete transfer of all analytes from the sample matrix to the extraction solvent. Also, solvent evaporation is essential to sample preparation for the concentration of the extracts. However, analyte losses due the high volatility are well know to residue analysis. Various procedures are applied in pesticides residue analysis to estimate the matrix content in an extract and, consequently, to evaluate the clean-up effect of the sample preparation step (Dias et al., 2016). The following extraction agents are also used in many tests: magnesium sulfate and Z-sep sorbent (d-SPE); Z-sep (columnSPE) and Agilent Bond Elut QuEChERS Enhanced Matrix Removal-Lipid (EMR-Lipid).

In the last decade, the consumption trend of organic food has increased dramatically worldwide. Since only a few pesticides are authorized in organic crops, concentrations are expressed to the range at zero or ultra-trace levels. Two authorized pesticides by organic production are Spinosad and Lambda-Cyhalothrin. The sensitivity of the method was calculated in terms of limit of quantitation's (LOQs), which was estimated as the lowest concentration of the calibration curve. The obtained LOQs ranged from 3 to 5 µg/kg and in all cases, the LOQs values achieved were far below the maximum residue levels established for conventional products.

Table 1 GC/MS acquisition parametrs

Pesticide	Quantify trace, (m/z)	Qualifier ion 1, (m/z)	Qualifier ion 2, (m/z)	Qualifier ion 3, (m/z)	Retention time, min
Dichlorvos	109	185	79	187	5.58
Acephate	136	94	42	95	7.47
Carbofuran	164	149	131	123	12.59
Diazinon	179	137	152	199	13.77
Carbaryl	144	115	116	145	16.66
Chlorpyrifos	197	199	314	97	18.46
Chlorfenapyr	59	247	249	408	24.68
Bifenthrin	181	165	166	182	28.43
Cyflutrin I (Baythroid)	163	206	165	226	31.84
Cyflutrin II	163	206	165	226	31.99
Cyflutrin III	163	206	165	226	32.06
Cyflutrin IV	163	206	165	226	32.13
Cypermethrin I	181	163	165	209	32.31
Cypermethrin II	181	163	165	209	32.45
Cypermethrin III	181	163	165	209	32.54
Cypermethrin IV	181	163	165	209	32.63
Boscalid	140	342	344	142	32.40
Azoxystrobin	344	388	345	372	35.68
TPP	326	325	77	125	27.42

Table 2 The linearities of the pesticide in the concentration levels (0.005-0.05 mg kg⁻¹)

Pesticides	Regression equation	R ²
Dichlorvos	y=3.15821x + 35.1683	0.998399
Acephate	y=0.621452x – 20.6132	0.992012
Carbofuran	y=1.00701x – 64.1507	0.993090
Diazinon	y=0.635145x – 9.79079	0.999644
Carbaryl	y=1.05677x – 57.9871	0.998398
Chlorpyrifos	y=0.687529x – 12.1225	0.999025
Chlorfenapyr	y=0.0756964x – 0.616831	0.997360
Bifenthrin	y=3.86296x – 71.4540	0.998935
Cyflutrin I (Baythroid)	y=0.179614x – 0.257887	0.998981
Cyflutrin II	y=0.248725x + 0.0581308	0.992049
Cyflutrin III	y=0.120601x + 6.69806	0.994277
Cyflutrin IV	y=0.135464x + 4.91023	0.998848
Cypermethrin I	y=0.203783x + 2.88366	0.994350
Cypermethrin II	y=0.243042x – 2.36615	0.998926
Cypermethrin III	y=0.240994x – 10.6618	0.996404
Cypermethrin IV	y=0.147977x – 1.83916	0.990083
Boscalid	y=1.96602x – 19.7533	0.998747
Azoxystrobin	y=0.877805x – 19.1200	0.999413

The results corresponding to the mean recovery (n=5) and within-laboratory reproducibility in terms of relative standard deviation (RSD) at two fortification levels (0.005 and 0.05 mg kg⁻¹) are summarized in Table 3. The average recoveries of pesticides were from 71.69% to 109.53%. Linearity of the MS system was evaluated by assessing the signal responses of the target analytes from matrix-matched calibration solutions prepared by spiking blank extracts of raspberries at five concentration levels, from 0.005 to 0.05 mg kg⁻¹. In all cases, coefficient of determination (R²) was higher than 0.99. In the fatty matrices it is more common not to achieve satisfactory recovery such as eggs where of the 174 pesticides tested in 14 pesticides recovery was less than 70%, and the reason is also strongly polarity and high molecular weight pesticides (Hildmann et al., 2015). The pesticides included in this study were selected based on their relevance in terms of high probability of being found in raspberry samples. Most of studied compounds are low and medium polarity

pesticides ($pK_{ow} > 3$).

Table 3 Recoveries (% , $n=5$), RSDs and LOQ of the pesticides in raspberry matrixes

Pesticides	Spiked (mg kg ⁻¹)	Meas (%)	RSD (%)	LOQ (mg kg ⁻¹)
Dichlorvos	0.005 0.05	74.13 72.31	6.15 5.06	0.003
Acephate	0.005 0.05	82.95 83.86	6.57 2.40	0.005
Carbofuran	0.005 0.05	71.69 80.52	5.05 2.95	0.005
Diazinon	0.005 0.05	102.23 109.52	3.27 6.39	0.005
Carbaryl	0.005 0.05	94.18 101.57	2.67 2.56	0.005
Chlorpyrifos	0.005 0.05	93.99 98.45	2.16 4.63	0.004
Chlorfenapyr	0.005 0.05	89.76 94.07	2.09 2.96	0.005
Bifenthrin	0.005 0.05	94.07 91.23	4.22 3.45	0.003
Cyfluthrin (sum of four isomers)	0.005 0.05	93.41 97.23	6.12 2.00	0.005
Cypermethrin (sum of four isomers)	0.005 0.05	93.16 100.84	5.74 6.37	0.005
Boscalid	0.005 0.05	77.96 89.51	1.46 2.56	0.004
Azoxystrobin	0.005 0.05	105.14 109.53	6.05 2.63	0.005

Table 4 shows the presents of organic samples with positive pesticide detections above the MDK. As can be seen, only two samples (10 % of samples) of all organic samples analyzed exceed the suggested reference level (10 µg/kg). The application of the developed procedure to real samples of raspberries revealed the presence of Cypermethrin, Cyfluthrin, Boscalid and Azoxystrobin at µg kg⁻¹ level (Table 4). All four mentioned pesticides were found in both raspberry samples. Contamination with these pesticides prohibits the sale of these raspberry samples as an organic product, but they can already be sold as a conventional product because the values of the obtained pesticides are below the MDK values.

Overall, 18 samples of frozen raspberries did not contain quantifiable tested pesticides residues (80.0%).

Of the tested samples results are showed in the Table 4 with the standard deviation, because the samples were determined in triplicate.

Table 4 Mean value and standard deviation of the obtained pesticides

Pesticides	Mean value $\pm$ STDEV, $\mu\text{g kg}^{-1}$
Cyflutrin (sum of four isomers)	16 ± 10
Cypermethrin (sum of four isomers)	24 ± 10
Boscalid	20 ± 15
Azoxystrobin	25 ± 8

In previous studies of organic food, chlorpyrifos was the most frequently detected insecticide at a concentration between 9.8 and 101.3 $\mu\text{g/kg}$ (Ramos et al., 2020).

The following analysis results were obtained in raspberry samples in Poland: boscalid and pyraclostrobin (2.395 mg kg^{-1} and 0.732 mg kg^{-1} , respectively), and for cypermethrin 0.235 mg/kg (Sadlo et al, 2018).

Hence, food control is necessary to monitor pesticide residues in food commodities prior to their introduction into the market and it is regulated by official directions, established by authorities such as the European Food Safety Authority (EFSA). Notwithstanding, the high usage of fungicides and insecticides during cultivation or storage of fresh fruits and vegetables has become a major concern that requires analytical attention. From the report EFSA (EFSA, 2020) the most frequently quantified residue in organic food was copper, found in 225 samples (in 28 different food items, mostly wheat, linseeds, bananas, oranges, peas with pods and maize), followed by dithiocarbamates in 90 samples (22 commodities, mostly in cauliflower and broccoli), bromide ion in 82 samples (23 commodities, mostly wheat and sweet peppers), chlorates in 62 samples (28 food items, mostly in wheat), Spinosad in 60 samples (17 commodities, mainly in tomatoes), fosetyl-Al in 51 samples (23 commodities, mostly wheat) and chlorpyrifos in 35 samples (29 commodities, mainly in bananas). Other pesticides found in organic foods are: Imazalil, Pendimethalin, Thiocloprid, Cypermethrin, Tebuconazole, Acetamiprid and Imidacloprid. Cypermethrin was reported in 6 baby food samples (0.36%), of which the MRL was not exceeded (EFSA, 2020). Cypermethrin is a synthetic pyrethroid insecticide that aims at resembling naturally occurring pyrethroids (chemicals with insecticidal properties). Consequently, due to the different input sources, pesticides residue can be transferred to fruits and vegetables,

which can be determined in them and their products.

Many of the pesticides currently being used have a tendency to survive in plants for a long time and they also do in to the food chain (Kumar, 2015). Pesticides are used almost everywhere not only in agricultural fields, but also in homes, parks, school, forests and roads. In addition, pesticides can be found in the air we breathe, the food we eat, and the water we drink. Preparation of samples for analysis is one of the most important steps in the total analytical procedures (Biziuk and Stocka, 2015). Selection of the optimal parameters for carrying out analyte extraction and extract purification is necessary in order to obtain reliable results. It is noteworthy that no specific MRLs are established for organic food produced in accordance with Regulation (EC) No 2018/848 (Council of the European Union & European Parliament, 2018) and hence the MRLs set in Regulation (EC) No 396/2005 (European Commission, 2005) apply to both organic and conventional foods.

CONCLUSION

In this paper, a new analytical procedure for routine analysis and simultaneous determination of selected electronegative pesticides was developed. For extraction, a modified QuEChERS sample preparation method was used. The obtained validation data has confirmed that QuEChERS extraction ensures satisfactory results for all investigated pesticides. These results show that although the use of synthetic pesticides is not allowed in organic production, they can be found in the tested samples. Although low concentrations, of the order of 20 $\mu\text{g kg}^{-1}$, these values confirm their presence. In general, low levels of one or two pesticides residues may be a matter of environmental contamination or cross-contamination processes. Such methods showed the advantages of simplicity, rapidness, and sensitivity, and could meet the requirements for the determination of pesticides residues in various derived foods.

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THE ANNEX TO THE KNOWLEDGE OF MICROCLIMATE CONDITIONS OF SPORTS HORSES ACCOMMODATION

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Abstract: An adequate accommodation of sport horses implies the appliance of all construction, zootechnical and hygienic norms. Stables for horses should provide optimal microclimatic conditions for horses, social contact with other horses, unrestricted access to food and water, as well as to reduce the risk to injury to a minimum. Stables are essential for wellbeing of horses, because they spend a significant part of the day in them. Lights, temperature, humidity, air flow, concentration of gases and dust, hygiene and noise intensity in the areas where the animals are, must be within limits that are not harmful for horses. Satisfying optimal microclimatic conditions in the horse stables represent one of the most important items when projecting a facility. The aim of this paper is to highlight the significance of adequate accommodation for horses in accordance with the natural ethological characteristics of horses as well as to describe the most important ambient parameters in the closed type facilities.

Keywords: *sport horses, stables, microclimatic parameters*

INTRODUCTION

Equestrian sport and horse breeding represent one of the indicators of economic development of the country and the life quality of the population in the 21st century, and that is the reason why a horsemanship is the most represented in the most economically developed countries of the world. Horses are mostly used in equestrian sports (gallop and trotting races), hurdles (show jumping) and dressage sport, and significantly less for recreational riding, as a help for people with development disabilities, tourism and as a working animals. In the past, horses were mostly used for warfare and transportation, and only after that for competition and hunting (Lang, 2005).

Horses, as the first domesticated animals are typical representatives of the herd ruled by hierarchy, so in the wild the herd consists of several mares and foals, and one stallion that is an alpha of the heard or the horse leader. Among the mares, the first place is occupied by the alpha mare which leads the herd to the water, food and when running, and the stallion is at the end of herd and it drives the remaining members forward. After domestication, the man changed the life of horses in herds and started to breed them in stable controlled conditions trying to make the microclimatic conditions of accommodation as close to physiological needs of horses as possible, as well as to provide appropriate accommodation, nutrition and care for horses. From the former use of horses for work in agriculture, transportation and warfare, the present day horses are mostly used for sports.

Short extracts from the historical development of equestrian sport in Serbia

The first equestrian association, whose main activity was proper riding competition and dressage training, was founded in Principality of Serbia in Belgrade in 1885, and sometime later in Šabac in 1888 and then in Niš, Kragujevac and Zaječar in 1898. Tournament competitions in speed were held several years later, so the first trotting races were held in Belgrade in 1909, and dressage riding and hurdles (show jumping) in 1911. Equestrian Association of Serbia was founded in 1947. and after breakup of Yugoslavia, The Association for Equestrian Sport of Serbia for Olympic and FEI disciplines (Federation Equestre International) was founded in 2002. The main activity of the association is to hold gallop and trotting races, and Serbia participated in the olimpic dressage riding discipline for the first time in 2012 (Šiljak V., 2007). There are no precise data on exact number of specific categories of horses in the Republic of Serbia, but according to official data of Statistical Office of the Republic of Serbia from 2019, about 14.000 horses of different breeds as well as about 6.000 mares and fillies (females below the age of 3) are bred in our country. Compared to 1955 when Yugoslavia had 519.000 horses, the number of horses decreased by 97%, and compared to 290.000 horses in 1975 it is a 95% decrease in number of horses. According to official data , only about 19.000 head of horses were tagged , of which there are 1.293 mares and 67 stallions (Statistical Office of the Republic of Serbia, 2019).

Horse accomodation

An adequate accommodation, besides training and nutrition of horses, is one of the most significant factors for achieving sports results. Stables for keeping sport horses should be with stalls of suitable dimensions which are comfortable for lying down, moving, feeding and should have enough light

and fresh air. Proper accommodation implies the realization of all construction, zootechnical, hygienic norms and wellbeing of horses. That is the only way to create preconditions for implementation of other zootechnological measures, and all that in order to achieve good sports results in competitions.

Facilities for horses accommodation on horse farms consists of a main stable where there are boxes for different age categories and outbuildings that are used as a placement for food, manure, mechanization, ring (exercise space) etc. The main feature of the horse farm is that the separation of the head is performed according to gender, into foals and yearlings, and that is why all categories have got separate facilities. When keeping horses in stables, where there is usually smaller number of heads, it is common for all heads to stay in the same facility and there is no strict division into breeding stock and offsprings (Brinzej, 1980; Pejić, 1996). In our country, the field of construction of facilities for horse breeding and keeping is defined by legal regulations (Official Gazette of RS, No. 81/2006).

There are several divisions of horse stables in the literature. In horse farms horses can be kept in individual stalls, in groups in stables and freely on the pasture. There is a similar division of stables according to microclimate, and based on it the stables are divided into: warm (closed), cold (open) and semi-open (one lateral side of the stable is open). The third division of the stables are based on the ability of the horse moving, and according to it the stables are divided into free way of keeping (individually or in groups) and individual keeping in the stalls on the leash. The free way of keeping horses has a number of advantages compared to other ways of keeping horses, because horses as social animals achieve better results in competitions. When you keep horses in groups, the optimal number of horses in the group is 10 heads. According to Ogrizek (1952), breeding horses on the pastures is the healthiest way to breed horses, because there is enough light, fresh air and movement for horses. Each horse needs at about 0.5 to 1.0 hectares of pastures with additional feeding or 1.25 to 2.5 hectares of pastures of appropriate quality if there is no additional feeding. We need to build awnings for horses on the pastures in order to protect them from sun, wind and natural disasters. The height of a fence on pastures are from 1.4 m to 1.8 m, and are made of wood, hedges and metal constructions (Ogrizek and Hrasnica, 1952; Pejić, 1996; Margić and Matković, 2011).

Today, horses are mostly bred in individual stalls, which facilitates work of breeders for feeding, easier monitoring and care for horses. The stalls are facing towards the hallway and that way the horses have an opportunity for communicating and socializing with other heads in the stable. All the doors of the stalls open towards the stable hallway. Doors and windows are made of wood, and more recently of PVC material. For floors, which must not be slippery and must prevent injuries, we use asphalt, rubber backing and

combination of concrete and rubber backing (Lang, 2009). Each head in the stall should be provided with a minimum of 10 to 12 m² of lying space depending on the size of the horse, where the minimum floor area of a stall is calculated according to the formula:

3 x height of the crest in meters - for wider side of a stall

1.5 x height of the crest in meters - for narrower side of a stall

According to Pejić (1996), the size of the stall should be 3.5 x 3.5 up to 4 x 4 meters. The dimension of the stall for smaller horses are 3 x 3 up to 3 x 3.5 meters, while for larger horses they are 4.5 x 4 up to 4.5 x 4.5 meters and for mares with the foals 5 x 4 meters. The data from Table 1, which is based on the measured height of the crest with the Litin's stick, can be used for more precise determination of the area of the individual stalls.

Table 1. Dimensions of the individual stalls for horse accommodation (Sakač et al., 2010)

Height of the crest	Area of the stall per head	Shorter side of the stall	Width of the stall	Height of the ceiling
up to 150 cm	8.5 m ²	220 cm	1.5 x height of the crest	260 cm
up to 165 cm	10 m ²	250 cm	1.5 x height of the crest	260 cm
up to 175 cm	11 m ²	260 cm	1.5 x height of the crest	270 cm
up to 185 cm	12 m ²	270 cm	1.5 x height of the crest	300 cm
over 185 cm	14 m ²	290 cm	1.5 x height of the crest	310 cm

Feeders and automatic drinkers in individual stalls are placed at a height of 1/3 of the height of horse's crest, while in group keeping of horses we use group feeders where each adult horse should be provided with 60 to 70 cm of space. Cribs and hammocks are used for hay, and solid containers made of different materials (wood, plastic) are used for concentrated nutrients. The practical organization of work day in stables is based on the morning feeding

of horses with forage, then with concentrated food, and after the heads are submitted to care (cleaning with the brush), mucking out of the stall is performed. After exercise and work with horses, the heads are fed with hay or they are let to a pasture in the afternoon, and after that they are allowed a few hours of free time and rest. The third feeding of horses with concentrated nutrients or forage is done in the evening.

In practice, keeping horses on leash is combined with free way of keeping horses during which the horses are trained or are on the pasture. In the most countries of EU it is forbidden to keep animals tied in boxes, but temporary tying is allowed only during cleaning, breeding and during sports competition. Modern stables have an outlet for the movement of the horses, where the area of the outlet is twice as large as the floor area of the stall. Modern training stables, alongside outlet, are also equipped with automatic treadmills so that the heads in training can walk several times a day (Ivanković, 2004).

In group keeping, horses are allowed to have a social contact with other heads and a hierarchy is created among the heads in the group. This manner of breeding horses disables the breeder to feed and approach each horse individually, but the consumption of working hours is significantly lower compared to individual horse keeping. The floor area of a group stall depends on dimensions, i.e. on the height of the horse crest and it is in average from 10 m² to 12 m² per head, and an area of the outlet should be from 10 m² to 13 m² per head (Pollman, 2000).

The stable must be lit well, because the light is incredibly important factor for the health preservation as well as functioning of horses' reproductive cycle. The length of a daylight in the horse stable must not be less than eight hours with a light intensity of at least 40 lux. The recommendation is that the area of the window on the facility is about 5-10% of the floor area of the stable, and each stall has to have a window of dimensions 1.2 x 1 x 1 m. According to Ivanković (2004), in the practice it is typical for the area of windows on the facility to be from 1/15 to 1/20 of the total floor area.

The hygiene of the stable must be in the function of maintaining optimal microclimatic conditions, in order to preserve the health and wellbeing of horses. For that reason, horse stalls must be regularly cleaned and hygienically maintained (hot water, disinfectant). Straw and sawdust bedding as well as rubber coverings are used in the stalls. It is recommended to use sawdust for a bedding because it does not contain bacteria or mold which could cause respiratory diseases. Inversely, straw contains organic dust which irritates the respiratory organs. The thicker the bedding, the better it absorbs the urine and it is less likely for a horse to injure itself when lying down and getting up. On modern horse farms, rubber floor coverings are most often used combined with sawdust.

Microclimatic conditions in the stable

In the closed type stables, optimal climate conditions are of great importance for the normal functioning of the organism, the health preservation and achieving good sports results of horses. Unlike open type stables, where complete control of climate parameters is not possible, in the closed type stables achieving optimal values of climate factors depends entirely on a man.

Temperature

As a herd animal that lives all around the world, a horse is very adaptable to climate differences and as a homoeothermal animal it is adaptable to changes in outdoor temperature in order to maintain its body temperature. Moreover, horses tolerate lower temperatures better than the higher ones, because in colder climate areas and during the winter, horsehair grows longer which protects them from cold (McCutcheon et al., 1999). Body temperature is a by-product of metabolism in all body tissues, whereby 60-70% of heat production at rest takes place in the heart, liver, kidneys and brain, although these organs have less than 10% of body mass. Metabolism and body heat production in horses are lowest between -10 and +10°C, while at a temperature of -40°C basal metabolism increases to over 140% (Sjaastad et al., 2016).

The optimal temperature in a horse facility depends on the breed, feeding intensity, humidity, number of animals and the health of horses. Horses are able to withstand temperatures ranging from -40°C to + 40°C, and critical temperatures are between -15°C and 10°C, whereby foals are more sensitive to low temperatures than adult heads (Hintz and Cimbaluk, 1994; Charles, 1994). Horses protect themselves from high outdoor temperature by sweating, which is a very efficient way of cooling, because basal energy of organism is spent on water evaporation from the surface of the skin. According to Guthrie and Lund research (1998), 2.4 MJ of heat is used for evaporation of 1 liter of water from the surface of a horse skin, which is equivalent to heat production during 2 minutes of intensive work. The optimal temperature interval, for most horse breeds, range between 10°C and 15°C (Guthrie and Lund, 1998).

Relative humidity

Relative humidity represents the ratio of the water content in the air and its water capacity, i.e. to what extent the air is saturated with water vapor. Humidity in the stable comes from horse breathing, urine and feces, bathing horses and cleaning the stable. Warmer air contains more water vapor than the cold one, and increased humidity over 80% causes respiratory disorders in horses, increases the content of harmful microorganisms in the air, and it also

leads to moisture condensation on the walls of the stable which causes corrosion and rotting of wooden elements. Optimal relative humidity in horses' facilities is 65-70% (Wathes, 1994; Pejić, 1996).

The content of the pollutants in the air of the horse stable

The content of the pollutants in the air of the stable is a very important factor in preservation of the health of horses, because horses live longer than most domestic animals, so the health of the respiratory organs are more critical for horses than other species of domestic animals. Also, horses are nowadays used for intensive work that requires maximum involvement of respiratory organs, and therefore they are more susceptible to respiratory diseases. The air in the poorly ventilated stables for horses contains various pollutants that originate from food, bedding and feces, and its concentration primarily depends on the ventilation of the stable. The most common air pollutants in horse stables are: ammonia, carbon dioxide, hydrogen sulfide, dust, microorganisms, endotoxins and other matters of organic and inorganic origin (Curtis et al., 1996).

Ammonia (NH₃) is the most common toxic gas released from the urine and manure and which in high concentration can cause pneumonia in horses, and in foals it can even cause death. The concentration of ammonia in the air increases with increasing of the temperature and humidity (Curtis et al., 1996).

Carbon dioxide (CO₂) concentration is caused by horse breathing and since it is specifically heavier than air, the highest concentration of this gas is found near the stable floor. The efficiency of the ventilation system in the stable is primarily measured by the content of carbon dioxide in the air (Curtis et al., 1996).

Hydrogen sulfide (H₂S) is a highly poisonous gas which is formed by decomposition of feces and whose increased concentration in the air can cause respiratory diseases.

Dust in the stable air consists of inorganic part (bedding, food) and organic part (bacteria, bacterial endotoxins, viruses, fungi and allergens). The most dangerous for horses is respiratory dust which can be inhaled and whose particle size is between 0,5-5 µm. Dust in the air works as a carrier for damaging microorganisms and it irritates the mucosa of the respiratory organs and eyes. Dust concentrations are twice as high if the straw is used as bedding compared to wooden sawdust or rubber coverings (Vandenput et al., 1997).

The concentration of ammonia must not exceed the value of 20 ppm or 13.93 mg/m³, and the optimal value of ammonia in the stable air is up to 10 ppm or 6.96 mg/m³ of the air. The maximum level of carbon dioxide that is allowed in the stables for horses is up to 3000 ppm or 5400 mg /m³ of the air, while the optimal values are from 500 to 2000 ppm, considering that the level of CO₂ in

the outdoor air is 350-400 ppm. The maximum level of H₂S that is allowed is up to 0.5ppm or 0.68 mg/m³ of the air, and dust in the stable air is 4 mg/m³. The optimal level of H₂S in the stable air is only in traces, and of dust is from 0.2 mg/m³ - 0.6 mg/m³.

The most important factor in providing fresh air in the stables for horses is ventilation. The ventilation system affects the concentration of pollutants in the air, water vapor content and temperature values, so without appropriate ventilation the concentration of harmful matters increases in the stable air (Elfman et al., 2011). It is necessary to provide from 40 m³ to 60 m³ of air for horses a day, where the allowed air flow speed in the zone of the animals stay is from 0.4 to 0.8 m/s in summer, and about 0.2 m/s in winter. Approximately during summer months the air in the stable should be changed at least four times a day, and in winter at least twice a day. Modern horse stables use mechanical ventilation whereby fans create noise that must not be higher than 65 dBA (Ivanković, 2004; Elfman et al., 2011).

CONCLUSIONS

Accommodation of sports horses, besides nutrition, exercise and care is one of the most important factors for achieving good sports results. Horse facilities must be airy, with enough natural light, without harmful gases and dust, and projected in a way to allow breeder unrestricted access during feeding and care of the horses as well as maintenance and cleaning of the facility. Concentrations of gases, temperature values, dust and relative humidity must be within optimal limits, all in order to achieve good sports results.

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METABOLIC CHANGES IN DAIRY COWS DURING THE TRANSITION PERIOD

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Abstract: *The peripartum period in dairy cows includes 3 weeks ante partum and 3 weeks post partum. Partus and lactogenesis are accompanied by numerous physiological changes in the activity of almost all cells in the body that facilitate the maintenance of homeostasis, and in order to meet the needs of the mammary gland through the redistribution of nutrients. Numerous metabolic adaptations take place in the organism of dairy cows, which occur as a consequence of pregnancy, parturition and the beginning of lactation (homeoretical processes), as well as the organism's tendency to preserve homeostasis. Homeoresis is the functioning of the endocrine system and metabolism in conditions when the body must provide primary physiological processes, such as fetal growth or lactation. During the transition period, peripartum stress occurs, when inflammation, insulin resistance and metabolic adaptation begin to develop. Adjustment of the endocrine system during the peripartum period is a key factor in establishing metabolic adaptation. In the blood of dairy cows, the most important indicators of metabolic stress are the concentration of glucose, non-esterified fatty acids - NEFA, the concentration of beta hydroxybutyrate - BHB. Homeoretic hormones (growth hormone, prolactin, glucocorticosteroids, thyroid hormones, insulin, glucagon and leptin) play a key role in maintaining high lactation and maintaining the health of cows.*

Key words: *metabolic changes, transition period, dairy cows.*

Introduction

Peripartal - transition period includes 3 weeks ante partum and 3 weeks post partum. It represents the period around calving, which is characterized by numerous metabolic adaptations. The metabolism of dairy cows faces a series of changes in homeostasis with peripartum stress, endocrine changes, metabolic stress and numerous pathophysiological mechanisms that develop in the peripartum period such as inflammation, insulin resistance and metabolic adaptation (Petrović et al., 2018).

Calving and the beginning of lactation in dairy cows is the most important stressor. The stress reaction develops in two directions. One direction is an increase in adrenal gland activity with an increase in cortisol concentration as a consequence of the classical stress response, and the other direction is an increase in lipid mobilization with an increase in non-esterified fatty acid (NEFA) as a consequence of metabolic stress and increased energy needs (Cincovic et al., 2017).

During the transition period, numerous defense mechanisms in the body of dairy cows are activated in order for partus and lactation to occur. However, all these processes lead to stress in cows. Stress can have a positive effect on the body of cows in terms of activation of adaptation mechanisms in the peripartum period. On the other hand, the negative side of stress is reflected in the fact that if stressors act for so long, they can lead to disruption of homeostasis and to a reduction in production characteristics and poor health (Petrović et al., 2020).

The state of stress that occurs as a consequence of imbalance of energy metabolism with numerous endocrine, biochemical, hematological, immunological and other changes in the body is called metabolic stress. The occurrence of stress in dairy cows occurs as a consequence of strengthening homeorectic processes, catabolic processes and negative energy balance of cows at the beginning of lactation (Cincović 2013). Metabolic stress in early lactation underlies many diseases in dairy cows (Cincović et al., 2016, 2017, 2018).

The endocrine system has a key function in regulating the adaptation of metabolism during the peripartum period, although the roles of both the nervous and immune systems are important in this process. Metabolic adaptation of the organism in dairy cows, which takes place during the transition from late pregnancy to early lactation, is a very clear example of homeoresis. The basic characteristics of homeostasis, as a principle of regulating the physiological functions of the organism, are the constant maintenance of certain parameters of the internal environment of the organism within physiological limits. Then the function of all tissues is adjusted to the new situation, whereby their activity is subordinated to the preservation of a

certain condition and the maximum function of the organs that play a key role in this process (Đoković et al., 2019a; Đoković et al., 2021).

The metabolic profile, as well as through specific analytical tests from the blood, are routinely used to assess metabolic disorders in dairy cows. They include the collection of blood samples from dairy cows in 4 time periods in relation to calving (late pregnancy, early lactation, full lactation and middle lactation) and the assessment of selected blood indicators (Oetzel, 2004; Gross et al., 2011).

Transition period - characteristics and metabolic status of dairy cows

Peripartum period in dairy cows is a period of three weeks before to three weeks after calving when metabolic processes in the body are adapted to provide energy, organic and inorganic precursors necessary for fetal development and for the synthesis of milk ingredients (Đoković et al., 2021).

The transition from pregnancy to early lactation (peripartum period) is a very strenuous process accompanied by numerous physiological and pathophysiological changes that burden cows to the extreme, so many adaptations can be such that the number of cows with some of the diseases (fatty liver, ketosis, metritis), mastitis, abomasum dislocation, hoof disease) increases during this period (Cincovic 2013a; Petrović et al., 2019a).

Namely, the selection in cattle breeding is very successful in the direction of increasing milk production. But the phenotype of milk yield is only 25%. The influence of paragenetic factors on milk properties, regardless of whether their nature is fixed (the breeding area of the years and the season of birth, season of calving, lactation in order) or continuous (age at first fertilization and calving) is very pronounced and significant, and it is necessary to include them in models for assessing the breeding value of dairy cows (Petrović et al., 2005, 2006, 2009, 2010, 2012; Bogdanović et al., 2012)

The main goals of modern cattle breeding are the increase in the fertility of breeding throats, the prolongation of their exploitation time, the increase in profit and the cultivation of as many genetically high-quality offspring as possible. The achievement of these goals depends on the factors determining the genetic potential of the reproductive properties of the reproductive throat and environmental factors (paragenetic factors), which enable the phenotypic expression of reproductive characteristics (Košarcic et al., 2003; Petrović et al., 2013a, b, c).

However, the genetic potential for high milk production, fattening of animals during calving and weight loss after calving are the main factors that cause dairy cows in the period before and after calving to a greater extent to disorders of organic matter metabolism.

Significant loss of body condition of cows after calving - BSC below 3.5 (3.25 to 3.75) leads to a permanent decline in cow productivity through reduced milk production and poor maintenance of lactation, which results in the appearance of reproductive disorders such as placental abruption and cysts ovaries, causing great economic damage to farmers (Petrović et al., 2019b). Today, more and more authors point out that insufficient adjustment of the endocrine system in the peripartum period is one of the key factors responsible for the development of subclinical and clinical metabolic disorders.

However, the most important health disorders of dairy cows that occur in the period around calving are sudden changes in energy metabolism. These changes can cause severe and uncontrolled disorders in the metabolism of organic matter (Grummer, 1995; Overton and Waldron, 2004; Drackley et al., 2005).

A drastic change in the metabolism of organic matter occurs in the peripartum period and follows the adjustment of the metabolism of animals to the coming calving. These changes do not affect only the mammary gland, but the entire metabolism of the cow adapts to the upcoming lactation (Đoković et al., 2003, 2007, 2010; Šamanc et al., 2005).

Also, it is characteristic that already during pregnancy there is a gradual decline in food intake, so that for each week of pregnancy food intake decreases by 1.53% (0.17 kg) to three weeks before calving, while newer models have shown that the negative trend in food intake continues after calving (Ingvarlsen et al., 1992).

Metabolic changes in the peripartum period adjust the metabolism of dairy cows to start lactation.

At the beginning of lactation, the needs for food and energy are significantly increased. However, the biggest problem with postpartum cows is the imbalance between body reserves and milk production (Petrovic et al., 2019a).

And it is during this period that cows cannot get enough of the food they need, resulting in a negative energy balance - NEBAL and metabolic stress. Metabolic states of negative energy balance (starvation, partus and the beginning of lactation) lead to accelerated and uncontrolled mobilization of fat from body depots and their increased accumulation in liver cells, disrupting its physiological and morphological integrity (Reist et al., 2002; Dann et al., 2005; Djoković et al., 2011; 2015a). Fats are deposited in hepatocytes in the form of triglycerides (TGC), forming fatty vacuoles, which leads to fatty liver hepatocytes (Mihajlović and Jovanović, 2008), thus changing their histological, morphometric and metabolic characteristics (Đoković i sar, 2011; 2015b).

Due to reduced consumption of dry matter from meals, as well as sudden and increased activity of the mammary gland, there is a negative energy balance, and increased lipid mobilization (Cincović et al., 2012; Đokovic et al., 2014) to

use fats for energy purposes, but cows become prone to developing ketosis and fatty liver.

Increased lipolysis in body depots begins in the last days of pregnancy, that it reached its maximum immediately after calving and was maintained at that high level for the first few weeks of lactation (Drackley et al., 1999, 2001; Đoković, 2010).

The concentration of non-esterified fatty acids (NEFA) and betahydroxybutyrate (BHB) increases in the bloodstream. This phenomenon occurs as a consequence of endocrine and metabolic changes, primarily due to the presence of insulin resistance (Cincović et al., 2014). This mechanism provides glucose savings for milk production.

Due to the increased metabolic load of the dairy cow and the accumulation of fat in the liver cells, the morphological and functional integrity of hepatocytes is disturbed, which results in a decrease in blood concentration of certain indicators of liver function (glucose, total proteins, albumins, globulins, cholesterol, triglycerides, urea). which are synthesized in the liver. Also, the excretory role of hepatocytes decreases, so the concentrations of certain metabolic products in the blood (bilirubin, ammonia, bile acids) are usually increased (Herdt et al., 1983; Sevinc et al., 2003; Bobe 2004; Lubojacka et al., 2005; Djokovic et al., 2007a, 2011, 2019b).

In dairy cows in the peripartum period, the consumption of food is the lowest, which is insufficient to meet the metabolic needs of the organism, especially the mammary glands at the beginning of lactation. The consequence of such a condition is a negative energy and protein balance and the mobilization of fats and proteins from body depots.

Animals exposed to metabolic stress in conditions of negative energy balance use fats for the energy needs of peripheral tissues and glucose for milk production (Cincović, 2016; Cincović and Starič Ed., 2017). High concentrations of NEFA lead to higher production of ketone bodies in the liver when the concentration of beta-hydroxybutyrate (BHB) in the blood increases. Metabolic indicators of poor energy status in cows are: lower glucose concentrations, higher concentrations of NEFA and BHB. This metabolic profile is typical for the periartal period in cows, and in many cows these parameters are significantly outside the reference range (Cincović et al., 2011). NEFA are metabolized in all tissues but the main organ is the liver. The increase in NEFA during the peripartal period affects the cellular immunologic response by changing intracellular signals, gene expressing control, activation of transcriptional factors, apoptosis induction and by modifying mediators of lipid production. Increased proportion of cows with high NEFA and BHB concentrations in the herd can cause reduced milk yield at the end of a standard 305-day lactation. NEFA concentrations can be related to postpartal ovarian activity, especially given that blood NEFA concentrations represent NEFA

concentrations in the ovarian follicular fluid. Cows on farms with lower scores of animal welfare and nutrition have higher concentrations of cortisol, NEFA, BHB, bilirubin, glucose and urea. NEFA and BHB concentrations in early lactation can be used for estimating metabolic adaptation in the first 8 weeks after calving. For the estimation of metabolic adaptation, increased lipolysis has a greater significance than decreased anabolic parameters (Lakić et al., 2018).

Proper carbohydrate metabolism, and especially the process of gluconeogenesis and glycogenesis is of great importance in maintaining the health of dairy cows, especially during periods of high pregnancy and maximum lactation, when there is increased metabolic load in the body and when the need for glucose by the mammary gland is greatest. If gluconeogenesis from glucogeoplastic precursors (propionic acid, glucogeoplastic amino acids, glycerol) cannot meet the needs for glucose, as well as when the glycogen reserves in the liver are depleted, a disorder of carbohydrate metabolism occurs, which very often leads to hypoglycemia, ketonemia and ketonuria (Bell, 1995; Drackley et al., 1999; 2001; Đoković et al., 2003, 2010; Šamanc et al., 2005).

All these changes lead to the redirection of glucose (energy) to the fetus and mammary gland, and fats are intensively used as a source of energy in peripheral tissues. Glycogen reserves are reduced in the liver to compensate for the lack of glucose, and ketone bodies and triglycerides are created and accumulated instead of glycogen due to increased fat metabolism, which all indicates a very significant metabolic stress in dairy cows.

Decreased glucose concentration in cows occurs due to reduced food intake and greater glucose uptake into the mammary gland and pregnant uterus (Bell and Bauman 1997).

In the period around the partus, the glucose concentration decreases intensively. At the moment of the beginning of lactation, the needs for glucose increase. It is believed that dairy cows can meet about 10% of their glucose needs with ingested food, so gluconeogenesis is a key mechanism for maintaining glycemia in cows (Đoković, 2010).

During the peripartum period, propionate, amino acids, lactate and glycerol account for 50–60%, 20–30%, 15–20%, and 2–4% of net glucose production, respectively. Intravenous administration of propionate in healthy cows may cause a large increase in glucose concentration (2–2.5 times) in the first hour after injection. Cows with ketosis, which is associated with endangered liver status, show reduced glucose production, which indicates the importance of the liver in gluconeogenesis (Đoković et al., 2007b).

The mammary gland uses glucose to produce lactose, but also for other energy needs. The use of amino acids creates milk proteins from the blood, and fatty acids affect the production of milk fat. Negative energy balance in cows

leads to increased milk fat concentration (due to increased use of fatty metabolites from the blood) and decreased protein concentration (due to lack of energy and lack of precursors to produce sufficient protein concentration) (Šamanc et al., 2006).

Proteins or amino acids ingested through food or mobilized from body depots are used for the synthesis of milk proteins, as well as glucogeoplastic precursors for glucose synthesis (Drackley et al., 1999, 2001; Đoković, 2010). If glucose were consumed for peripheral tissue, which happens during heat stress in cows, there would be a significant drop in milk production and quality (Belić et al., 2011).

Taking into account metabolic changes in the peripartum period, glucose concentration is inversely proportional to NEFA and BHB concentrations (Husveth et al., 1982). For this reason, the use of propionate can significantly improve metabolism in the peripartum period (Cincović et al., 2010). The use of fatty acids for energy is the most important homeoretic mechanism in early lactation. NEFA in the blood comes from the mobilization of lipids in adipose tissue and fat catabolism caused by many endocrine regulations and reduced food intake. Reduced food intake is not enough to start lipid mobilization (Friggens et al., 2003).

However, NEFA can be a very sensitive indicator of nutrient stocks. Since NEFA has daily variations, their concentrations are highest at night and in the morning (because cows do not consume food during the night). Under heat stress, NEFA is higher in the warmer parts of the day when cows reduce their food intake (Bertoni et al., 2002; Cincović et al., 2010).

Glucose, NEFA and BHB are significantly correlated with a negative energy balance during lactation. Therefore, their concentrations depend on the lactation period and to a lesser extent on the energy balance. Cows fed with lower energy values in different periods of lactation show certain metabolic specificities: energy deficit is highly pronounced in the second trimester of lactation compared to early lactation, but glucose concentration is significantly lower, and NEFA and BHB higher in early lactation (Gross et al., 2011). Negative energy balance and metabolic stress lead to impaired health and reproduction of cows and reduced welfare (Reist et al., 2003; Collard et al., 2000; Nielson 1999).

All problems that occur at the metabolic level in the transition period (period 21 days before and 21 days after parturition) are based on the fact that cows are exposed to a negative energy balance during early lactation, ie that they cannot get the amount of energy they need through food. for maximum milk production. Thus, the organism intensifies its reserves and enters a state of metabolic stress when catabolic processes play a dominant role.

Metabolic stress is accompanied by a number of endocrine changes such as insulin resistance, decreased concentration, decreased concentration of

insulin-like growth factor I IGF-I, decreased thyroid hormone concentration, increased growth hormone concentration, elevated cortisol concentration, increased glucagon concentration and the like (Petrović et al., 2019b).

Due to all the above, in order to assess the condition of the organism, today blood is taken from dairy cows and parameters that reflect the metabolic profile of the animal are determined. Metabolites that are important for the diagnosis of peripartum stress in dairy cows reflect the energy status of cows, protein status of cows, functional status of the liver, mineral status and others.

Conclusions

In the peripartum period, numerous physiological changes take place in the activity of almost all cells in the body, which facilitate the maintenance of homeostasis, and in order to meet the needs of the fetus and mammary gland through the redistribution of nutrients. Partus and the beginning of lactation are the biggest stressors for the body of dairy cows. During the transition period, peripartum stress occurs, when inflammation, insulin resistance and metabolic adaptation begin to develop. Peripartum metabolic stress is decided by negative energy balance, consumption of fat for energy purposes of peripheral tissue and consumption of glucose for udder and milk production. The most important metabolic indicators of poor energy status in cows are: lower concentrations of glucose, higher concentrations of non-esterified fatty acids - NEFA, concentration of beta hydroxybutyrate - BHB. Peripartum metabolic stress can be prevented by taking blood from cows and determining the metabolic profile through routine diagnostics, collecting blood samples from dairy cows in 4 time periods in relation to calving (late pregnancy, early lactation, full lactation and middle lactation) and assessment of selected blood indicators.

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FIRST REPRODUCTIVE CYCLE PARAMETERS OF SIMMENTAL BREED IN RAŠKA AREA

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Abstract: *As the most represented cattle breed in Serbia, Simmental breed take the great part in animal production, and therefore its health and reproductive status have significative meaning. The objective of this research was to evaluate reproductive parameters in first reproductive cycle in Simmental heifers. Data were assembled from Animal breeding service MDS “Pljevljani”, by random sampling conducted for 90 animals, from three multiplicity in Raška area: Novi Pazar, Sjenica and Tutin. Collected data for age at first conception, gestation length, age at first calving, number of liveborn and stillborn calves, gender of calve, length of service period and inter-calving period were analyzed statistically. The results represented statistically significant differences between mean values for age at the first fertilization, as well as at the age of heifers at first calving.*

Key words: *reproductive traits, Simmental cattle breed, heifer, Raška area*

INTRODUCTION

Simmental cattle breed is dominant in cattle breed distribution in Serbia, with 86 % of all cattle breed population. Otherwise, its regional distribution varies and the highest percentage is recorded in Belgrade and Raška area (Republic Institute of Statistic, Serbia). High accommodation capability to macroclimatic and microclimatic environment factors, in different housing system, was the crucial point for achieving a high and permanent contribution rate in animal husbandry. Harmonious body composition, adequate food conversion, fast body gain, longevity, fertility in about 80 % (Perisic et al, 2008) and normal parturition, low mortality in newborns and short inter-calving period are improved Simmental breed traits, marked this breed as highly selected cattle breed in livestock production.

Modern approach in animal husbandry demands permanent

improvement of reproductive indicators, which correlates not only with simple species reproduction, but with an economic input. Cattle breeding is highly subordinate by reproductive efficiency, implying a continuous reproductive cycle and high reproductive performance (Lalović et al, 2016). Female fertility has become a major breeding goal in the dairy cattle industry (Tiezzi et al, 2012). It accelerates genetic progress of required production performances, considering longer and large milk production and higher number of newborn calves, and keeps physiological status in moderate level, prepared to answer the highest performance demands. Reproductive efficiency of a certain individual animal is measured by reproductive indicators such as age at first calving and calving interval (Nilforooshan and Edriss, 2004) and age at first conception, which significantly effects on age at culling and length of productive life (Petrović et al, 2019). Timing of conception in the first breeding season impacts lifetime productivity, reflected in greater weaning weights and earlier calving in subsequential years (Day and Nogueira, 2013). Interval from calving to first estrus, detection of estrus and conception rate determine the reproductive parameters and represent the reproduction targets for dairy cattle industry.

MATERIAL AND METHODS

The aim of this study was to determine average values and variability of reproductive parameters in first reproductive cycle of Simmental heifers in three distinct localities, in Raška area. Investigation was conducted on 90 heifers, by random sampling method, divided into three groups, with 30 animals in group. Each group is formed by the multiplicity in Raška area: I group originated from Novi Pazar multiplicity, II group of heifers originated from Sjenica multiplicity and III from Tutin multiplicity. Following reproductive parameters were recorded: age at first conception, gestation length, age at first parturition, number of liveborn, number of stillborn, calf gender, service period length and inter-calving period. All data were collected from regional breeding service “MDS Pljevljani”, statistically analyzed with data analysis software system Statistica version 12.5 (Statsoft USA). Means of the reproductive parameters were analyzed statistically, by analyses of variance method. The differences between mean values were considered statistically significant if the P value was below 0.05. Results of analysis of traits studied were shown in tables.

RESULTS AND DISCUSSION

Variety of genetic and paragenetic factors conducts successful conception of heifers; breed properties, seasonal influence, nutrition, welfare and biosafety assessment of animals breeding are considering as the most important.

Average value of age at first conception differed between the localities and the highest value was recorded for Novi Pazar's heifers- 509,7 days (Table 1.). Completing the statistical analyses, significant differences was pointed between heifers from Novi Pazar and Sjenica, Sjenica and Tutin ($P \leq 0.05$); and Novi Pazar and Tutin ($P \leq 0.01$). It's considered that the most optimum period for conception is time heifer gains 2/3 of full body development, because sex maturity occurs prior to physiological (Pantelić et al, 2005), or at the age of 15-16 months. Calving at 23 to 25 months is optimal, which requires conception by 15 months (Mohd Nor et al, 2013, Day and Nogueira, 2013, Wathes et al, 2014). Insemination with 50-57 % of body weight may present an economic advantage over developing to 60-65 % of mature body weight, matched with age of approximately 357 days or 320 kg of body weight (Day and Nogueira, 2013), or with live weight of approximate 400 kg and in approximate period for conception in 14 months of age (Krpalkova et al, 2014). Age at first conception can be manipulated by altering growth rates, as lowest age at first conception was achieved with the highest average daily gain. The onset of a heifer's sexual maturity depends more on body weight and fat percentage than age, and can be significantly influenced by the level of nutrition (Krpalkova et al, 2014). Differences between groups in this investigation could be accordingly consequent by different level of nutrition. Djurdjević (2001) investigated genetic background of milk yield and reproduction in Simmental cows and reported 530,66 days as average age at first conception. Lower age at first conception improves lower production costs, shorter service period, birth body weight and increases efficacy of selection (Perisić et al, 2002), but, at the other hand triggers longer service period after the first parturition, because the growth of cows is not yet completed (Petrović et al, 2007). Reduction of the age at which heifers enter into production increases their lifetime productivity and improves economic efficiency (Day and Nogueira, 2013), but it also requires a good standard of management to ensure that potential milk yield of animal is not compromised (Wathes et al, 2014). Rapid growth rate from 3 to 12 months of age led to decrease in milk production, as a result of an increase

in the mammary adipose tissue, indicates the negative effect of high average daily gain during heifer rearing period in milk production in first lactation (Krpalkova et al, 2014).

As gestation is biological constant for certain species, variability in gestation length occurs rarely and those values are not statistically significant, such as in this investigation. Heifers from Novi Pazar multiplicity showed average gestation length of 274,93 days, from Sjenica 281,47 days and Tutin 284,03 days. Even there are some data about effect of paragenetic factors, including nutrition, calf gender and type of parturition, values for gestation length are similar in previous investigation by Petrović et al (2007), which was 284,14 days, or 285,51 days in research by Pantelić (2006). Gestation length is significant parameter of fertility which effects following reproductive traits in one reproductive cycle: age at first parturition and date of following successful conception (Lalović et al, 2016).

Table 1. Reproductive parameters in first reproductive cycle of Simmental heifers

	I group (n=30) ($\bar{X} \pm SD$)	II group (n=30) ($\bar{X} \pm SD$)	III group (n=30) ($\bar{X} \pm SD$)
Age at first conception (day)	509,7±69,89 ^{a, A}	493,3±39,75 ^{a, b}	479,7±24,72 ^{A, b}
Gestation length (day)	274,93±12,26	281,47±15,67	284,03±2,65
Age at first parturition (day)	783,43±78,27 ^A	778,1±34,38 ^a	737,37±19,91 ^{a, A}
No liveborn/stillborn calves	27/3	28/2	28/2
No male/female calves	11/16	13/15	10/18
Service period length (day)	96,13±16,02	98,7±9,68	96,97±7,91
Inter-calving interval (day)	371,27±12,19	371,4±10,001	372,1±8,12

Different lowercase superscripts within lines describe significant differences with $P \leq 0.05$

Different uppercase superscripts within lines describe significant differences with $P \leq 0.01$

Age at first parturition depends on key point of determining the compatible moment for first insemination, considering that earlier moment results in slower body development and potential dystocia, and delay does not justify economic losses (Lalović et al, 2016). Average values for this reproductive parameter were 738,43 days for Novi Pazar's heifers, 778,1 days for Sjenica and 737,37 days for Tutin's heifers. Those results are similar with 778 days in Pantelić (2006), 771,21 days in Nikšić (2017), 780,17 days in Kostić (2014) findings, but lower than 831,49 days, as Djurdjević (2001) reported. Statistically significant differences were found between Novi Pazar and Tutin group ($P \leq 0.01$), and between Sjenica and Tutin group ($P \leq 0.05$), suggested the correlation with the age at first conception. The lower average value for age at first conception and first calving in Tutin area and Sjenica could be related with different climate (elevation approximately and above 1000 m), food quality and nutritional requirements of heifers. Percentage of liveborn calves was 90 % for group in Novi Pazar and 93,3 % in Sjenica and Tutin. Female/male ratio for newborns in percentage was 59,26:40,74 for Novi Pazar's group, 53,57:46,43 for Sjenica's group and 64,28:35,71 for Tutin's group.

Optimal length for service period is about 90 days (Pantelić et al, 2008), which implies the appropriate period for *uterus* involution and reintegration of new reproductive cycle. Average length for service period for group from Novi Pazar was 96.13 days, for Sjenica 98,7 days and Tutin 96,97 days, and these results were not similar with 110,79 days findings (Pantelić 2006). There were not found statistically significant differences for service period values. Previous research data shows that optimum length of calving to calving period is about 390 days. Intercalving period for heifer's group in Novi Pazar was 371.27 days, Sjenica 371,4 days and Tutin 372,1 days, which is within the required value.

Differences in the reproductive parameters of heifers between these three localities could originated in different climate, which may impose temperature and humidity extremes and consequently change in maintenance requirements and appetite. Influence of nutrition on body development, accentually on reproductive system, is unequivocally of fundamental importance. The management of housing condition are influential and can differ considerably, depending on the nutritive value of pasture, according to geographical locality and climate. All these factors may differentially alter health and reproductive status and consequently affect reproductive

performance and productivity. These reasons favour calving animals at younger age and maintenance of regular calving pattern without excessively long calving interval (Wathes et al, 2014).

Considering listed data and data from this study, optimum time for first conception in terms of maximizing future performance and economic return, must be exactly defined, including all aspects of rearing and animal welfare.

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***RURAL DEVELOPMENT AND
AGRO-ECONOMY***

REPUBLIC OF SERBIA POSITIVE LAWS ON COOPERATIVES

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Abstract: *The authors of this paper underline the importance of cooperatives, not only in the field of agriculture, but in other domains of social and economic life. Unfortunately, this particular form of the organization of natural persons (the cooperators) has been neglected for some time from the aspect of normative regulation, but in real life, too. Without the help of the state, which, until now, has rather hindered than helped, it is almost impossible to undertake the reaffirmation and accelerated development of cooperatives. Our country is in the process of transition and it is vital that it supports the revitalization and reawakening of cooperatives in Serbia. Cooperatives are legal entities which by conducting their business on cooperative principles secure their economic, social and cultural interests. It is obvious that we need cooperatives in all spheres of life, yet, it is clear that in Serbia they domineer in the field of agriculture and, therefore, their affirmation is prioritized in the realization of the Republic of Serbia Strategy for the development of cooperatives in Serbia from 2012 and the Law on cooperatives from 2015. The adoption of this law was of crucial importance since it regulates the legal status of cooperatives, their establishment and managing structure, the status of their members, property issues, distribution of profit and covering of losses, their disestablishment, as well as many other issues relevant for their status and work.*

In addition to establishment of cooperative enterprises, other important activities of these entities are the cooperation with other enterprises, if possible in the way it is done in the European Union. The fact that most cooperatives are of small size hinders their affirmation and revitalization, as well as strengthening their position on the market. The cooperative principles represent the basis for developing the normative acts regulating the position and work of cooperatives. In drafting these documents, legislators should pay attention that these regulations should be compatible with other national positive laws, as well as harmonized with major international laws regulating this field.

Key words: *Republic of Serbia, cooperatives, legislation on cooperatives, European Union, revitalization of cooperatives, Constitution, Law, Strategy.*

Introduction

Before we start with a broader analysis of the positive and legal regulations of the cooperative legislation of the Republic of Serbia, we will point out the fact that, according to the nature of the law itself, we distinguish two most important types: 1) natural (eternal, unchangeable) and 2) positive (changeable, created by people). The first is given by human nature itself, and the second is an existing right set by humans. Therefore, throughout history, positive law has changed and, according to certain phenomena, institutes, organizations and etc, different positions have been taken, which are a consequence of certain understandings and ideologies, which are all reflected in the relevant legal regulations. So it was with the cooperative, as a very important and specific form of organizing cooperatives, through which they pursue their various interests - economic, social, cultural and others. Namely, in the previous period, in our country, not enough attention was paid to cooperatives and cooperative legislation. The Faculty of Agriculture of the University of Belgrade is the only higher education institution in Serbia in whose curricula starting from 1919 until today, "Cooperatives" is a compulsory subject. The cooperative movement in Serbia is registered today as an association in the Agency for Business Registers (APR) and it gathers scientifically and professionally competent cooperative representatives.

The very important thing is that, only recently, much more attention has been paid to this way of organizing and gathering individuals. Therefore, professors whose focus is cooperatives, are increasingly involved in reviving the cooperative movement from the Faculty of Agriculture in Belgrade, the Faculty of Agriculture in Novi Sad, the Faculty of Economics in Belgrade, the Faculty of Economics in Subotica, the Faculty of Economics in Nis, the Faculty of Economics in Kragujevac, Faculty of Law in Belgrade, Faculty of Law in Kragujevac, Faculty of Philosophy in Novi Sad and other faculties of social sciences and humanities. Also, a significant contribution is given by members of the academic community, who have received their PhD's and master's degrees in the field of cooperatives, authors of textbooks, monographs, other publications and papers, who deal with this matter.

History of cooperatives in Serbia

The cooperative has a long history, since it was first founded in 1844 in England (Rogel), but its development flourished in the second half of the 19th century, at the time of the Industrial Revolution. Given that the cooperative sector, in addition to the private and public (state), is one of the basic forms of business, today the cooperative movement in Europe includes about 92 million people, who pursue their economic interests through various types of

cooperatives. At the global level, it is much more, because the cooperative movement in the world consists of about 800 million people from almost 100 countries. In the 19th century, Serbia was a distinctly peasant country and sparsely populated (according to archival data, in 1815, it was divided into 18 nahijas, counties with about 400,000 inhabitants²⁸). However, it should be said that it was not at all far behind Europe at the time, although it was still under Turkish rule. Namely, the first Serbian cooperative was founded in Bački Petrovac in 1846 (the so-called rule of the constitutionalists from 1842 to 1858), immediately after the founding of the first cooperatives in European countries. The first cooperative in the city center, the so-called the carpentry and locksmith company was founded in Belgrade in 1870, and in 1871 other cooperatives were founded, such as tailors, shoemakers, weavers and wheelwrights. The first credit agricultural cooperative in Serbia was founded in 1894 in the village of Vranovo, near Smederevo. Cooperatives in Serbia were founded out of a real and growing need to defend poor peasants from greengrocers and trade speculators, so that the following year (1895) the Main Union of Serbian Agricultural Cooperatives was founded, whose headquarters were in Smederevo, when the first Law on Agricultural and Craft Cooperatives was adopted. Only after three years (1898) the head office of this Alliance was moved to Belgrade (1898), and in the same year it participated in the founding of the International Co-operative Alliance (ICA) in London²⁹. Until 1900s there were over 650 cooperatives in Serbia! They sprouted like mushrooms after the rain, as a medicine and defense against greens and the impoverishment of the rural population on the transition from the nineteenth to the twentieth century.

As we stated at the beginning of this chapter, cooperatives in Serbia have a long history, but from its inception until today, it has had many ups and downs, depending on the social and economic and political conditions, which were characteristic of individual stage. Thus, before the Second World War, the Cooperative Movement in Serbia enjoyed a much better status, but after the liberation, relatively little attention was paid to cooperatives, both in the literature and in the legal aspect of cooperative activities³⁰.

²⁸With the immigration of people from Montenegro, Bosnia and Herzegovina and Macedonia and the large natural increase, the number of inhabitants in Serbia increased to almost 1,400,000 in six decades (1875), and in 1900 that number amounted to over 2,500,000 inhabitants..

²⁹The importance of the cooperative sector can be seen from the fact that until 1930, in the First Yugoslav State (from 1918 to the Kingdom of SCS, and from 1929 to the Kingdom of Yugoslavia) it carried out the largest consolidation of cooperative property in all of Southeast Europe. .

³⁰At that time, the Cooperative Movement was regulated by numerous regulations of an ideological nature..

The Law on Economic Cooperatives of the Kingdom of Yugoslavia was passed in 1937, so that the Constitution of the Federal People's Republic of Yugoslavia from 1946 emphasized the role of general and peasant labor cooperatives (SRZ), which were based by a model of Soviet collective farms that is by simple unification of peasant farms. However, very quickly, with the intervention of the state, trust in cooperatives was reduced, whose work was characterized by a gradual departure from cooperative principles.

Only four years after the liberation (1949), the Basic Law on Agricultural Cooperatives was passed, which defined the cooperative as: "an economic organization in which the working peasantry unites to improve agricultural production, raise their living standards and build socialism in the countryside." However, this period after the Second World War was characterized by the fact that cooperative values and principles were subordinated to the norms of the socialist social and economic system, and this led to the formal abolition of certain forms of cooperatives (for example, savings and credit). All this led to the fact that our legislation was in a long-term lag and discrepancy with the European cooperative legislation. A partial reorganization of peasant labor cooperatives was carried out in 1953, when general agricultural cooperatives gained importance, so that the period from 1957 to 1965 could be considered a period of relatively successful development of cooperatives. The Constitution of the SFRY from 1974 was the basis for the enactment of the Law on Associated Labor in 1976 (ZUR), with which cooperatives were abolished and became the basic organizations of associated labor (OOURs) within labor organizations, that is at economic conglomerates and complex organizations of associated labor (SOURs)³¹. At the same time, this law confiscated their property, and only the Federal Law on Cooperatives from 1990 prescribed that the cooperative property was to be returned to the previous users from whom it was confiscated, which was confirmed by the Law in 1996.

After 1992 that is with the creation of the Third Yugoslav State (Federal Republic of Yugoslavia), cooperatives were established and operated in accordance with legal regulations, which respected international cooperative regulations much more than before. The end of the XX and the beginning of the XXI century was marked by numerous social and economic changes, which created numerous changes in the cooperative sector, opened the ways for new research, re-examined the meaning and identity of cooperatives, basic cooperative principles and values and environment in which cooperatives had operated. All this influenced the significant transformation of cooperative legislation, as well as the revitalization of the cooperative movement, to which the activities of relevant international organizations significantly contributed.

³¹Томић, С., Гулан, Б., Костов, Р., *Водич кроз задругарство Србије— практични приручник*, Институт за економику пољопривреде Београд, Београд, 2017, стр. 18-21.

However, for the past quarter of a century, the gap in this branch of legislation could not be filled, so the process of revitalization of the Cooperative Movement in Serbia was very intense (better said, demands for revitalization of this movement today have been numerous and comprehensive), but for its success it was very important to harmonize the domestic cooperative legislation with the valid regulations in this area, which have been applied for many years in the European Union and in the countries around us.

Legal framework of Serbian cooperatives (term and characteristics of cooperatives)

After the so-called 5th October revolution in 2000, several draft laws were prepared in the field of cooperatives, but it took a long time to pass the current Law on Cooperatives, which came into force in 2016³². Its adoption aimed to encourage the establishment of clear and simple rules for the establishment, management and operation of cooperatives, to offer a sustainable model of meeting the general interests of the community, the fight against poverty and social reintegration of vulnerable groups, to encourage healthy competition in industries in which (in our conditions and circumstances) dominant positions of large companies were traditionally represented (housing, consumption, agriculture) and to revive local economic development. Act 1 of this Law provided everything that was necessary for the establishment and normal functioning of cooperatives, primarily their legal position, management and most important bodies, acquisition and termination of the status of cooperatives, property and business, profit distribution and loss coverage, cooperative associations, cooperative registration, cooperative audit, termination of the cooperative, as well as other issues important for the position and operation of the cooperative.

When it comes to the revitalization of cooperatives and cooperatives in Serbia, it should be emphasized that this is an unavoidable activity of Professor Sevarlic, who has been trying for many years to organize cooperatives in Serbia and restore their reputation and place. For a modern cooperative, it is very important to determine the basic features of its identity, so, in that sense, it is necessary, first of all, to present the three most important components: 1) define the cooperative, 2) determine cooperative values and 3) determine cooperative principles.

In order to give a definition of a cooperative, which is the first component of its identity, it should be said that it is most often defined as "an autonomous association of persons voluntarily united in order to meet common

³²*Law on Cooperatives*, "Official Gazette of RS", no. 112/2015.

economic, social and cultural needs and aspirations, through jointly owned and democratically controlled company "³³. From the above definition of a cooperative, many of its characteristics can be derived, the most important of which are the following: 1) the cooperative is an independent and autonomous organization; 2) membership in the cooperative is voluntary (members can join, but also leave the cooperative at will); 3) the basic role of the cooperative is to enable its members to meet their various needs; 4) the operations of the cooperative are carried out in a democratic manner (decisions are made freely and the cooperatives are not under the control of government institutions). It should be said that cooperatives and cooperatives in general in Serbia, unfortunately, in the past decades presented a much neglected area, which was finally regulated by the Law on Cooperatives. This Law regulates the legal position of cooperatives, their establishment, management, acquisition and termination of the status of cooperatives, property, operations and bodies of cooperatives, provision of services and other issues important for the position and labor of cooperatives. A cooperative is a legal entity, and it represents a special form of organization of individuals (cooperatives), who operate on cooperative principles and, in that way, realize their economic, social, cultural and other interests. Hence, a cooperative member can be said to be an individual who, as a member of the cooperative, in whole or in part, operates through the cooperative using its services. Namely, with the help of the cooperative, he ensures the sale of his products or the service, purchases products, uses services necessary for the performance of its activity or, on the other hand, directly participates in the performance of the activity for which the cooperative has been established.

We need the above definition to determine the identity of the cooperative, which can't be fully formulated, if we don't mention the cooperative values and principles, citing the provisions of the current Law on Cooperatives of the Republic of Serbia ("Official Gazette of RS", No. 112/2015). The members of the cooperative believe in the ethical values of honesty and responsibility, which contain elements of Christianity, and consist, for example, of: 1) self-help (cooperative members can help the cooperative in situations of a crisis); 2) self-responsibilities (members have multiple responsibilities towards their cooperative); 3) democracy (the right of members to participate in the work of the cooperative, to be informed and involved in the decision-making process); 4) equality (members of the cooperative have the same rights and opportunities); 5) equity (all cooperative members have equal treatment) and 6) solidarity (cooperatives are a manifestation of collective strength and joint responsibility)³⁴.

³³Николић, М., *Еволуција задружног законодавства у Европи*, Друштво аграрних економиста Србије, Београд, 2009, стр. 11.

³⁴Law on Cooperatives, Act 4, paragraph 2

Finally, the third component of the identity of a modern cooperative is represented by cooperative principles, principles of cooperatives, as guides (guidelines) through which cooperatives incorporate their values into practice, because the establishment and operation of cooperatives is based, precisely, on these principles, which have become international in the last few decades³⁵. Thus, according to the current Law on Cooperatives (Act 4, paragraph 3, point 1-7), the most important cooperative principles are: 1) voluntariness and open membership (cooperatives are voluntary organizations, open to all people who want to use its services and who wants to accept the responsibility of membership); 2) exercising control over the work of the cooperative by the cooperative members themselves, who actively participate in decision-making and formulation of its business and development policy; 3) economic participation of cooperative members and democratic control of the cooperatives' capital; 4) autonomy and independence of the cooperative (independence, self-help and mutual control); 5) education, training and informing members by the cooperative; 6) inter-cooperative cooperation (joint work of cooperatives through local, regional, national and international structures); 7) concern for the community (work through a policy approved by their members).

Therefore, the cooperative is based on voluntary and open membership, which means that it is a voluntary organization, open to all people who can and want to use its services³⁶, that cooperative members have equal voting rights in the management and control of the cooperative³⁷, which is autonomous and independent organization, as well as to inform young people about cooperatives, benefits and services it provides. As we have seen, these are some of the main international principles on which cooperatives are founded and on which they operate, it certainly includes inter-cooperative collaboration, which indicates that cooperatives most effectively help their members, they provide services and work together through different structures.

As it can be concluded, the stated principles of cooperatives represent the basis from which the cooperative legislation derives. They represent a certain philosophical support on which the cooperative movement is built. Therefore, when defining and drafting cooperative laws, it is necessary to point out the cooperative principles, in order to avoid certain deviations, namely, deviations of cooperative legislation. The role of law is very important,

³⁵The Cooperative Principles of 1937 (15th Congress) were changed (modified) in 1966, at the 23rd Congress of the International Cooperative Union in Vienna, but were not substantially changed.

³⁶For membership in a cooperative, it is important that people who want to be members of the cooperative are ready to accept responsibility of membership, regardless of gender, social, racial, political and religious differences.

³⁷The principle "one cooperative member - one vote" is applied here.

considering the fact that it encompasses legal norms, which represent rules defined in order to regulate human behavior. Thus, the current Law on Cooperatives transforms external elements into legal provisions and represents much more than just a group of specific rules that apply to cooperatives. At the same time, it is part of both the national and international legal system, so when it was adopted, ensuring that its solutions were compatible with solutions in other positive, that is current laws, was taken into account.

Having in mind the importance of the cooperative sector for the economy of every country, it should be said that the participation of this sector in the reform of cooperative legislation is very important. Because of that it is important to emphasize that during the creation of the mentioned Law, the participation of all interested parties was necessary, especially cooperative unions. That is nothing new for Western European countries and the countries of North America and Japan, because they are, in fact cooperative alliances, that is representatives of the cooperative sector, most often, the initiators of changes, considering the fact that they are in a situation where they first see the need to reform the cooperative legislation.

Types (classification) of cooperatives

Analyzing the business of the cooperative, we will point out the fact that it can perform only the activities permitted by law, and the prevailing activity of the cooperative³⁸ determines its type. When it comes to the types of cooperatives, it should be said that they can be established as agricultural, housing, consumer, craft, labor, student-youth, social, health, as well as other types of cooperatives for production, trade, services and other activities in accordance with the said Law, while the establishment and operation of the so-called. "student" cooperatives are regulated by regulations in the field of primary and secondary education and upbringing. Of course, this is not the end of the classification of cooperatives, given that the mentioned cooperatives can be further divided into subtypes, depending on what specific jobs and services they are engaged in. Thus, for example, farming or agricultural cooperatives can be general and specialized, while the latter can be classified into field, fruit, vegetable, vineyard, livestock, beekeeping and others. The activities of these cooperatives (agricultural or agricultural) are very diverse and consist of providing numerous services, such as: taking over, buying and selling agricultural, food and other products of their cooperatives, as well as their supply of reproductive material, energy, means of production, parts for agricultural machinery and other goods. Also, this includes trade in goods and services of cooperatives and for cooperatives, providing services to households

³⁸The prevailing activity is the activity, which as such is determined by the founding act and written in the register (Law on Cooperatives, Article 9, paragraph 3).

of farmers in the organization and development of rural tourism, as well as in all other activities of interest to the business of the cooperative. Specialized farming cooperatives have a similar function, whose main activity consists, in addition to organizing the production of certain products and their processing, in the products placement on the market .

In addition to the above, there are numerous other cooperatives, of which we would, in the first place, analyze *housing cooperatives*³⁹, which organize the construction and maintenance, build and maintain apartments, apartment buildings, garages and business premises for cooperatives. They do that by hiring funds and work of cooperatives and other individuals and legal entities⁴⁰. There is more talk, both in the media and in real life, about *consumer cooperatives*, which are gaining more importance today. Namely, they provide joint procurement of services and goods for their members, while *craft cooperatives* perform craft services and supply cooperatives with materials and means of production, in addition to making and selling their craft products and home-made products. *Workers' cooperatives* produce and sell their products and provide services, and they do so by performing their activities through associations of cooperative members (they are, as a rule, also employees), in accordance with the regulations governing the field of work in the cooperative (which is their employer).

However, at this point we would especially emphasize the existence of *student-youth cooperatives*, whose main role is to provide, in an organized manner, members of cooperatives to perform temporary and occasional jobs with companies, in order to acquire additional funds for education and basic social, cultural and other personal and the common needs of students and youth. In addition to these, we also list the so-called *social cooperatives*, which perform various activities in order to achieve social, economic and labor inclusion of members of vulnerable social groups⁴¹. These cooperatives perform these activities in order to meet other related needs of the same groups, to meet the general interests within the local community, as well as to provide services to members of vulnerable social groups. Social cooperatives have their set social goals, which are determined more closely by the cooperative rules, provided that they are obliged to invest at least half of the realized profit (surplus value over expenses), which they realize by performing their activities, in improving and achieving set social goals. Finally, we have *health*

³⁹These cooperatives are contractors and investors.

⁴⁰This engagement of funds and personal work of cooperative members is regulated by an individual contract between the housing cooperative and its members in accordance with positive regulations and cooperative rules.

⁴¹When it comes to members of vulnerable social groups, it should be said that, in terms of the Law on Cooperatives, they mean people who belong to social groups who are in a state of social need, in accordance with the law governing social protection and social security and the law governing the prohibition of discrimination.

cooperatives, whose main purpose of existence is to provide assistance to cooperative members and their family members, through assistance for voluntary health insurance, procurement of medicines, financing of treatment costs and other types of assistance. Of course, it should be emphasized that cooperatives can be established to perform other activities that are not prohibited by law, and due to their importance and performance of specific activities, such as production, trade, services of various kinds, etc., cooperatives enjoy special protection of the Republic of Serbia, provinces and units of local self-government, in performing their prevailing activities. This special protection is reflected in the encouragement of cooperatives through economic, agricultural and housing policies, as well as other development policies⁴².

Reaffirmation of the cooperative movement

Having in mind the importance of the cooperative movement for overcoming the existing world crisis, today many experts and connoisseurs of this issue point out the necessity of reaffirming the said movement, primarily due to the fact that it was proclaimed a "movement for all times"⁴³. This was done due to the fact that the cooperative movement, during its centuries of existence, encountered numerous problems and severe crises, but it always found a way out and thus survived⁴⁴. Hence, cooperatives represent a special form of business with a long tradition and rich history, not only at a national, regional or other level, but at a global level. In the very term (name) "zadruga" (cooperative in Serbian), the existence of cooperation is indicated, so that this term is increasingly being replaced by the term "cooperative" in our country as well⁴⁵ which throughout history has not had a single understanding, considering the fact that there have been many definitions of its essence. It was only in Manchester in 1995, at the Congress of the International Co-operative Alliance (ICA), that the concept of modern cooperation was finally established, so that Europe has been the starting point of development of the cooperative union since ancient times. When it comes to the level of development of the

⁴²Here, first of all, we mean the provision of appropriate facilities and benefits (which are determined by special regulations), as well as the possibility of establishing special funds, ie. a foundation for the development of cooperatives by a unit of local self-government or an autonomous province, or by providing the necessary funds in the budget of the Republic of Serbia, an autonomous province or a unit of local self-government.

⁴³Закић, З., *Задругарство и рурални развој: повезане теме у процесу евроинтеграције Србије*, у Шеварлић, М., Козенко, З. (уредници), Тематски зборник "Стање и перспективе задругарства 3", Београд, 2013, стр. 38.

⁴⁴Вујатовић-Закић, З., *Коопменаџмент*, Dunav-Preving, Београд, 2000.

⁴⁵The prefix "coop" is used in almost all world languages to indicate the existence of cooperation.

cooperative sector in the European Union, several countries stand out, such as France (has about 21,000 registered cooperatives with 23 million citizens - members of one or more cooperatives), Germany (has over 8,000 registered cooperatives with 20 million members), Italy (has over 70,000 cooperatives, which employ about a million people), followed by Belgium, Great Britain, Finland and others⁴⁶. Adding all these (listed and unlisted) figures, according to the data obtained from the relevant institutions, we come to the data that in the European Union there are over 100 million people in cooperative organizing, while tens of millions have found employment there.

When it comes to the development of cooperatives (cooperative movement) in Serbia, it is very important to note that it went through different phases, from the rise, which was harmonized with this movement in Europe, to the real collapse, and through the Yugoslav self-government system, as a great experiment. Today, our country is going through very difficult and painful changes, considering the fact that we live in turbulent and uncertain times, which further requires radical changes and reforms in the field of cooperatives. It is an indisputable fact that cooperatives are multifunctional and a lot of attention is paid to that through the organization of numerous scientific gatherings and the publication of scientific papers. In our country, today, as before, numerous consulting teams are used to help strategy development of certain types of cooperatives in Serbia, and within these teams are, very often, some authors who deal with this issue and who are globally affirmed in the field of cooperatives. However, if we are talking about the cooperative union of the Republic of Serbia, which is in the process of European integration, we will point to the fact that in our country the cooperative movement is being reaffirmed, so that today we can boast of a total of 2124 cooperatives, in which about 123,000 people are enrolled, and they are members of 16 alliances.

Speaking of the attempt to reaffirm the cooperative movement throughout the world, we must point to the fact that the crisis of this movement was caused by the global financial crisis, which culminated at the beginning of the 21st century, with the collapse of the liberal market economy. It was the precise reason why we set out on the path of reaffirmation of the cooperative movement in our country (as well as in the whole world), which had a significant foothold in the support of the United Nations⁴⁷, so that the resolutions of the Economic and Social Council called for close cooperation between the United Nations and the International Cooperative Union. As it was mentioned above, since the jubilee Congress of the International Cooperative Union in 1995, the International Cooperative Day has been celebrated every year, while in 2009 the Resolution of the United Nations General Assembly

⁴⁶Of the former Yugoslav countries, Slovenia has a very developed cooperative sector.

⁴⁷From the very beginning of its existence, the United Nations has understood the importance of the cooperative movement in the world.

was adopted, which was called *Cooperatives in Social Development*. The year 2012 was declared the International Year of Cooperatives, so that many organizations in the field of cooperatives around the world, during that year, focused on the reaffirmation of the cooperative movement.

Based on the above, it can be concluded that Serbia's European integration is a very important and serious topic, within which, in the coming period, we must emphasize agro-rural policy, because, unfortunately, it has not been sufficiently recognized by decision makers so far. What is even more dangerous, even today there are numerous obstacles on this European path, but of all these problems that prevent a more effective renaissance of the cooperative movement, we can list a few most important: 1) ignorant attitude of the state towards cooperatives (failure to recognize the importance of cooperative entrepreneurship); 2) insufficient engagement of the academic public; 3) neglect in the education system; 4) lack of an appropriate institutional framework for the reaffirmation of the cooperative movement and others. Hence, the adoption of a fundamental strategy for the development of cooperatives in Serbia is inevitable, and it must be based on a good knowledge of current trends in the new European cooperative model, in which the so-called the cooperative path is treated as one of the ways out of the global crisis.

CONCLUSION

Based on all the above, the role of cooperatives of different types can be seen in general, in providing services, increasing efficiency, reducing costs, saving the time needed to perform certain tasks, avoiding unnecessary administrative tasks, reducing bureaucracy, finding seasonal and other jobs, merging employers and cooperative members and the like. What is especially important to emphasize in the final remarks of this paper is the fact that a major step has been made in the legal regulation of cooperatives, but it is indisputable that some types of cooperatives (areas) remained unregulated and did not get their rightful place in the current Law on Cooperatives, although they have existed for more than a century. Therefore, we believe that it is necessary to continue informing the public about the importance of providing services by cooperatives and especially to intensify activities on the introduction into the legal framework of individual cooperatives, which have undeservedly remained outside the scope of our legislation.

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EXPERTISE AS A METHOD OF DETERMINING THE COMPENSATION FOR EXPROPRIATED LAND

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ABSTRACT

Abstract: *Compensation paid to property owners for land expropriation is often not accepted by property owners, and on the other hand, there is often no willingness of expropriation beneficiaries to offer and pay a fair price. Therefore, the issue of a fair market price is very controversial and creates a gap between the participants in the expropriation process. The non-acceptance of the price offered by the landowner in the procedure of expropriation of agricultural and construction land on the route of construction of the future highway Moravski koridor, in the municipality of Krusevac, did not stop the process of highway construction, but the expropriation process was not completed, and some cases have ended in court. In these cases, the court requires the determination of compensation for expropriated land by an expert witness opinion authorized by court or a commission of expert witnesses. The paper will consider several factors that affect the assessment of the average market value of land, and with a comparative analysis of similar locations and land of similar or the same quality.*

Key words: *expropriation, market price of land, compensation, agricultural land, court experts*

INTRODUCTION

The expropriation of real estate is regulated by the Expropriation Law, which provides that real estate may be expropriated only in the public interest. The government may determine the public interest in expropriation if the expropriation of real estate is necessary for the construction of facilities in the field of education, health, social protection, culture, water management, sports, transport, energy and communal infrastructure, exploitation of mineral resources, environmental protection and protection from natural disasters, (Expropriation Law, Article 20). The relationship between the state and private

property is one of the central issues of an organized society. Within the domestic legislation, rules are established and they refer to the interference of the state in property rights in the form of expropriation and regulation of property relations. (Praduroux, 2019)

Compensation for expropriated agricultural land and construction land is determined in cash according to the market price of such land. The assessment of the market price is performed by the body responsible for determining the tax on the transfer of absolute rights to real estate. (Expropriation Law, Article 42). Local self-government units are relatively generous in reducing the average prices of real estate that contributes less to property tax revenues (forest and construction land), which is not the case with real estate that provides the lion's share of tax revenues (apartments and houses). (Vasiljevic, 2015)

The fee cannot be lower than the market price. When determining the fee, a higher amount than the market price may be determined, taking into account the material and other personal and family circumstances of the previous owner, if these circumstances are important for his existence (number of household members, number of household members capable of earning, i.e. who are employed, health status of household members, monthly household income, etc.). (Expropriation Law, Article 51)

Most developed countries in the world have accepted the concept of calculating property tax (ownership) based on real estate values. In order to be able to determine the value of several real estates at the same time, a model of mass real estate valuation was developed. Developing a model of mass real estate valuation is a topic that preoccupies a large number of experts in almost every country. The value of real estate is calculated based on the characteristics of real estate, so the biggest challenge is the choice of parameters that affect the value of real estate, that is, not taking into account those characteristics of the real estate which turn out not to have an impact on the value. (Božić, Milićević, 2012)

In case of expropriation of agricultural land for the construction of line infrastructure facilities, the compensation for that land is determined by giving ownership to the previous owner of another appropriate agricultural land of the same culture and class or appropriate value in the same place or immediate surroundings. (Expropriation Law, Article 15). Compensation for an expropriated vineyard or orchard that bears fruit is determined by determining compensation for the land, adding to that amount the market price of non-depreciated investments made for the construction and maintenance of such vineyard or orchard and the amount of net yield that vineyard or orchard would give, given its age and fertility, in as many years as it takes for a new vineyard or orchard to be raised and come into full fruition. Compensation for an expropriated young vineyard or orchard that does not bear fruit is determined

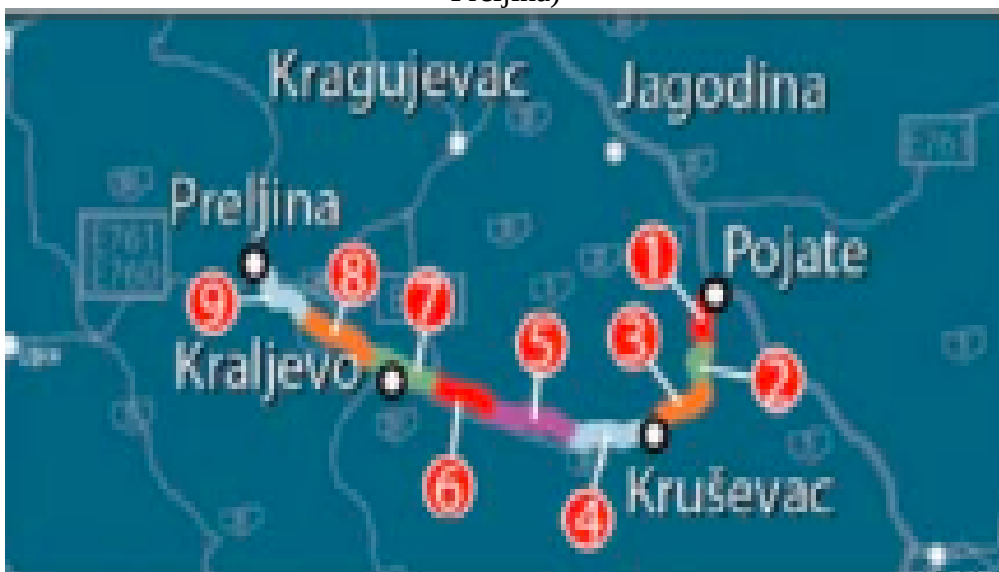
by determining the compensation for the land, adding to that the value of investments made for its raising and the amount of net yield that would be realized for as many years as that vineyard or orchard is up to the moment of expropriation. (Expropriation Law, Article 45)

The Moravian Corridor project is located in the West Morava region of Serbia, which is approximately 112.4 km long and connects Preljina near Čačak and Pojate on the A1 motorway (north-south motorway in central Serbia) via Kruševac. Image 1 shows the location of the Project.

The expropriation zone on the route of the future highway passes through three sections: (Highway project)

- The first section includes 17 cadastral municipalities, within the borders of three municipalities (Kruševac, Čičevac and Varvarin).
- The second section of the highway route passes through the municipalities of Kraljevo, Vrnjačka Banja, and Trstenik. This Section covers 42 cadastral municipalities.
- The third section of the highway route that passes through the municipality of Čačak. This section includes 17 cadastral municipalities

Image 1. Location of the Project – Moravian Corridor (Pojate (motorway A1) – Preljina)



Source:

[http://koridorisrbije.rs/site/content/media/img/files/Morava%20SEP%20Rev_10%20\(14_01_2020\)_Serbian_\(1\).pdf](http://koridorisrbije.rs/site/content/media/img/files/Morava%20SEP%20Rev_10%20(14_01_2020)_Serbian_(1).pdf)

The project requires temporary confiscation of land for construction works, for setting up facilities and camps, temporary disposal of excavated material (land, felled trees), and there will be a permanent loss of private construction and agricultural land, which will be expropriated, which will make landowners feel loss of the main or partial source of income of their household. Besides that, the remaining parts of the expropriated plots of agricultural land, after expropriation, will most likely not be suitable for cultivation.

The owners, whose plots are located on the route of the future highway, the Moravian Corridor, in the first section, started signing contracts on land expropriation in 2019. These are plots in settlements along the part of the highway from Pojate to the Kruševac West, it is 27.8 kilometers long. The Tax Authorities determined the market price of agricultural land of 200 dinars per square meter, based on certain criteria, including the prices of expropriation of local self-governments in the area. More than 2,000 expropriation decisions have been issued in Kruševac over the past two years.

All property owners who are not satisfied with the estimated amount have the right to refuse the expropriation and in that case the court appoints experts who will re-determine the value. However, these appeals do not delay the expropriation process.

This paper analyzes the determination of the market price of real estate in Kruševac, especially in KO Lazarica, which is located on the northern section of the Moravian Corridor route. In cases when the previous owners of agricultural land did not agree with the offered price of the tax authorities, the court requested the determination of the market price by the procedure of expertise of authorized expert witnesses or a commission of experts, i.e. the relevant institutions. It is mainly agricultural land, first class fields, of exceptional quality, suitable for tillage and cultivation of various field and vegetable crops, exceptional locations (have directly exit on the regional road Pojate-Kraljevo). Private lands make up 83% of the land, public land makes up 17% of the total land, of which state land makes up 14% of the land and municipal land 3%.

MATERIALS AND METHODS

Compensation paid to property owners for land expropriation is always a controversial topic, due to difficulties in determining the market price, as well as "willingness of property owners to accept the offered price, and willingness of expropriation beneficiaries to pay the asking price", which creates a gap between expropriation participants. (Zhongyu, Asami, 2014)

The assessment used a comparative approach to assessing the value of land (Chart 1).

Chart 1. Comparative analysis of traded land in KO Lazarica and neighboring KO Bivolje - agricultural and construction land

Location	Area in m ²	Date of turnover	Type of land	Sales (exchange rate NBS 117,58 on 10.05.21.)	Price per m2, in RSD - agr.land	Price per m2, in RSD – constr.land ⁴⁸
KO Lazarica	272	12.12.2012.	Agr.	726.880 RSD	2.672	
KO Lazarica	4745	15.12.2019.	Agr.	2.850 EUR	71	
KO Lazarica	2213	26.10.2018.	Agr.	2.219 RSD	118	
KO Lazarica	582	11.04.2017.	Agr.	300 EUR	61	
KO Lazarica	1512	18.09.2017.	Agr.	48.000 RSD	32	
KO Lazarica	5758	29.06.2018.	Agr.	4.600 EUR	94	
KO Lazarica	581	15.06.2016.	Agr.	7.777 EUR	1.573	
KO Lazarica	1501	30.08.2012.	Agr.	15.100 EUR	1.182	
KO Lazarica	2407	29.06.2004.	Agr.	17.812 EUR (contract)	870	
KO Lazarica	1445	31.05.2018.	Agr.	150.000 RSD	104	
KO Bivolje	4020	01.01.2017.	Agr.	3.000 EUR	88	
KO Bivolje	3841	29.07.2019.	Agr.	5.000 EUR	153	
KO Bivolje	248	26.01.2018.	Const.	300.000 RSD		1.210
KO Bivolje	2528	27.08.2019.	Const.	1.000.000 RSD		396
KO Lazarica	1256	18.05.2016.	Const.	1.900.000 RSD		1.513
KO Lazarica	2640	24.07.2019.	Const.	2.474.850 RSD		937
KO Lazarica	1000	21.12.2018.	Const.	12.000 EUR		1.410

⁴⁸ Building land outside the construction area

KO Lazarica	4805	23.09.2020.	Const .	36.300 EUR		888
KO Lazarica	400	12.04.2019.	Const .	600.000 RSD		1.500
KO Lazarica	3909	21.09.2020.	Const .	36.000 EUR		1.082
KO Lazarica	404	25.12.2018.	Const .	4.000 EUR		1.164
Decision of the Assembly of the City of Kruševac for 2021					99,7	2.593
Average land price per m2 - according to the type of land					440	1.269
Correction					194	507
Average					350,5	

Source: <https://katastar.rgz.gov.rs/RegistarCenaNepokretnosti/> (date of access 12.05.2021).

Based on the Rulebook Code of Ethics Appraisers, the market value of land is defined as: "The estimated amount for which land could be exchanged on the valuation date between a willing buyer and a willing seller, in a transaction between independent and unrelated parties, with adequate marketing, both sides possessed sufficient knowledge, acted prudently and were not coerced. " The terms willing buyer and willing seller are interpreted as follows:

- "... between a willing customer" - "It implies the existence of a hypothetical, not a real customer. It is a person who is motivated, but not forced, to buy the real estate concerned. That buyer is neither too eager nor determined to buy it at any price. That buyer is also a person who buys in accordance with the real situation on the existing market and with expectations arising from the situation on the existing market, and not on an imaginary or hypothetical market whose existence cannot be proven or assumed. That person would not pay a price higher than what the market demands of him. Among the persons who make up the market is the current owner of the real estate concerned. "

- "... and a willing seller..." - "This implies a hypothetical seller, not the actual owner. It is assumed that he is neither too eager to sell nor forced to do so at any price, nor is he considered willing to delay the sale in order to achieve a price that is not considered reasonable in the existing market. The interested seller is motivated to sell the real estate under market conditions for the best price that can be achieved on the free market after proper marketing, whatever the price. The specific circumstances in which the beneficial owner finds himself are not taken into account since the "willing seller" is in fact a hypothetical owner. "

When defining the market value of real estate, the following is taken into account:

- Data of the Republic Geodetic Authority (RGA), eCadastré - database of real estate cadastre in the Republic of Serbia (<https://katastar.rgz.gov.rs/eKatastarPublic>)
- Market Real Estate Prices from the Digital Platform (<https://a3.geosrbija.rs/>)
- Real Estate Price Register of the Republic Geodetic Authority
- Preliminary estimates of the revenue administration of the branch office in Kruševac
- Interview with the owner (owners) of the land and persons who performed land transactions and who sell the land in the observed area
- Realized sales contracts
- Market opportunities (supply and demand)
- Decisions of the Assembly of the City of Kruševac on determining the average prices per square meter of real estate for determining property tax for 2019, 2020 and 2021 on the territory of the city of Kruševac
- Data on the purpose of the land concerned
- Functional significance of that land for the area, owner and possibility of placing in the same property position without the plot concerned
- Crops grown on the plot and possibilities for growing crops on the plot concerned
- Alternative solution for the owner to buy another land of the same use value
- Water-air and physical properties of the land, which were determined on the basis of field inspection of the mechanical composition.

On the basis of all these parameters, an analysis of the production and economic possibilities of the agricultural production realized so far and the market price of the land was performed.

RESULTS AND DISCUSSION

1. Location and appearance of plots (micro and macro)

The real estates on which the expertise was determined are in private ownership, they are registered in the eCadastré of real estate and the list of real estate of KO Lazarica. The land is located next to the main road (highway Pojate-Kraljevo), from where the plot can be accessed directly. The plots concerned can also be accessed by field roads located between them, which can be accessed directly from the regional road Pojate-Kraljevo. Apart from the

direct exit to the main road, there are no other infrastructural facilities (water, electricity, sewerage, storage space ...).

Image 2. Macro location land in KO Lazarica



Source:

(<https://katastar.rgz.gov.rs/eKatastarPublic/NepokretnostProperties.aspx?nepID=Le5rIcEStsY1PdWeCvR9YQ==>)(date of access 20.05.2021.)

2. Culture and quality of the plot concerned

The plots concerned are registered as agricultural land, in the eCadastre of Real Estate of the RGA of the Republic of Serbia. By the decision of the Assembly of the city of Kruševac on determining the zones and the most equipped zone on the territory of the city of Kruševac, this land is classified in zone III.

By the decision of the Assembly of the City of Kruševac from 2016, the purpose of the land was changed from agricultural to construction, based on the Decree on determining the Spatial Plan of the special purpose area of the infrastructure corridor. This created the conditions for the realization of the line infrastructure facility - highway and any other construction facility cannot be built on these plots. Previous landowners have never paid compensation for the transferred land from agricultural to construction, they have not contributed anything to this change, they cannot get a building permit, nor gain any material benefit. This conversion of land from agricultural to construction has led to unrealistic expectations of previous landowners that in the process of determining the compensation for expropriated land, they expect it in the amount of the market price of construction land.

3. Shape, configuration, purpose and equipment of the plots

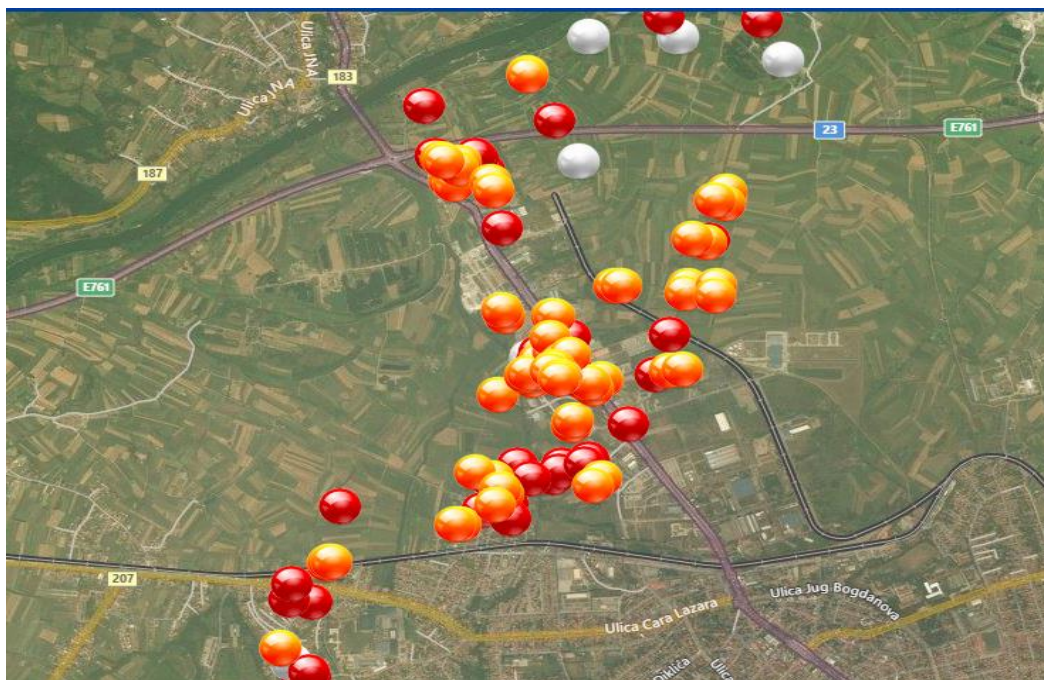
The land is flat terrain and it is located at about 150 m above sea level, clayey mechanical composition, crumbly structure, good throughput, good physical properties and favorable water-air regime and it is suitable for processing.

Having in mind the fertility of the land and the accompanying infrastructure, it is possible to organize field and vegetable agricultural production in the open field.

4. Market price of agricultural and construction land

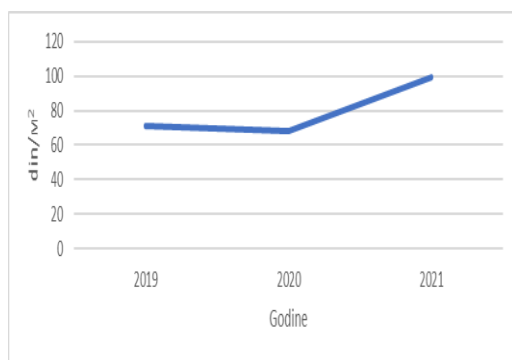
Based on the data from Chart 1, having insight into the realized sales contracts from the real estate price register of the Republic Geodetic Authority (Image3) and interviews with people who bought land in this area, we conclude that there is a large variation in the price of land between the market values of real estate.

Image 3. Traffic construction and agricultural land in KO Lazarica

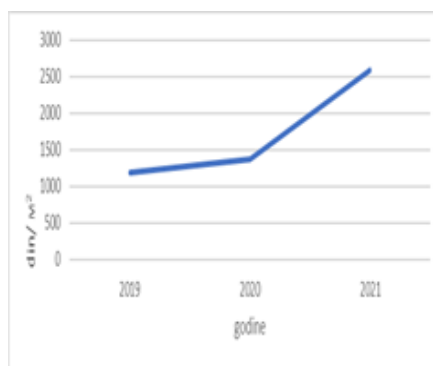


Source: <https://katastar.rgz.gov.rs/RegistarCenaNepokretnosti/> (date of access 20.05.2021.)

The stated average prices per square meter of real estate for determining the property tax for 2020 on the territory of the city of Krusevac in the third zone range from 68.4 dinars for agricultural land to 1,368.00 dinars for construction land (Decision of the Assembly of the City of Krusevac on determining average price per square meter of real estate for determining the property tax for 2020 on the territory of the city of Kruševac from November 19th, 2019).



Graph 1. Movement of the average price of agricultural land in din/m² in the third zone



Graph 2. Movement of the average price of construction land in din/m² in the third zone

Source: Authors, based on the decisions of the City Assembly on determining the average prices per square meter of real estate for determining the property tax, for 2019, 2020 and 2021

By the decision of the Assembly of the City of Kruševac on determining the average prices per square meter of real estate for determining the property tax for 2021 on the territory of the City of Kruševac from November 19th, 2020. year, the average price per square meter of real estate for determining the property tax for 2021 in the territory of the City of Krusevac in the third zone are 99.7 dinars for agricultural land and 2,593.00 dinars for construction land. Based on this assessment of the City of Kruševac, we can see that there was an increase in the average price compared to 2020 by 46% for agricultural land and 90% for construction land, which is an average increase of 67%. Based on the same decisions of the City Assembly on determining the average prices per square meter of real estate for determining the property tax in the period from 2019 to 2021 (Graphss 1 and 2), it can be determined that prices in 2021 compared to 2019 were increased on average by

79.1% (for agriculture they were increased by 39.4% and 118.8% for construction land).

It is necessary to take into account a number of criteria and include qualitative analyzes in assessing the market value of real estate, i.e. land.

The basic elements of the comparison are:

- location (macro and micro)
- size and area
- distance from the asphalt road
- shape and configuration
- purpose of the land
- culture, quality and infrastructural equipment
- functionality and orientation
- market opportunities (supply and demand)
- other elements related to the specificity of the plot concerned

The assessment of the market value of the land on the plot concerned, obtained by interviews with people who bought and sold land in KO Lazarica and comparable prices of land in the neighboring KO Bivolje, ranged from 32 to 2,672 dinars per m² for agricultural land, and 396 to 1,513 dinars per m² for building land outside the construction area.

Having insight into the real estate price register of the Republic Geodetic Authority (Chart 1) by comparative analysis of traded land in KO Lazarica and neighboring KO Bivolje, in addition to large variations in prices of agricultural and construction land, we see situations in which agricultural land was sold at higher prices than construction land and vice versa. By comparative analysis of similar locations, it can be concluded that the price at the locations concerned is around 350 dinars/m².

CONCLUSION

The formation of the market price of land is influenced by a large number of qualitative parameters, so the market price is adjusted in relation to them. Starting from the register of real estate prices of the Republic Geodetic

Authority, and a comparative analysis of land, in KO Lazarica and neighboring KO Bivolje, which are located on the route of construction of the highway Moravian Corridor and by correcting qualitative parameters that affect the formation of market price, we come to the price of 350 dinars per m². This price is in accordance with the Decisions of the Assembly of the City of Kruševac on determining the average prices per square meter of real estate for determining the property tax. Based on these decisions, in the period from 2019 to 2021. year, there was an increase in average prices per square meter of real estate by 79%, with the prices of agricultural land increased by almost 40%, and construction land by slightly less than 119%.

By the decision of the Assembly of the City of Kruševac from 2016, the purpose of the land was changed from agricultural to construction, based on the Decree on determining the Spatial Plan of the area of special purpose of the infrastructure corridor. This created the conditions for the realization of the line infrastructure facility - the highway. Landowners have not contributed to this change in any way, they cannot get a building permit or gain any material benefit, which is why it is unrealistic for them to expect the same in the process of determining the compensation for expropriated land in the amount of the market price of construction land.

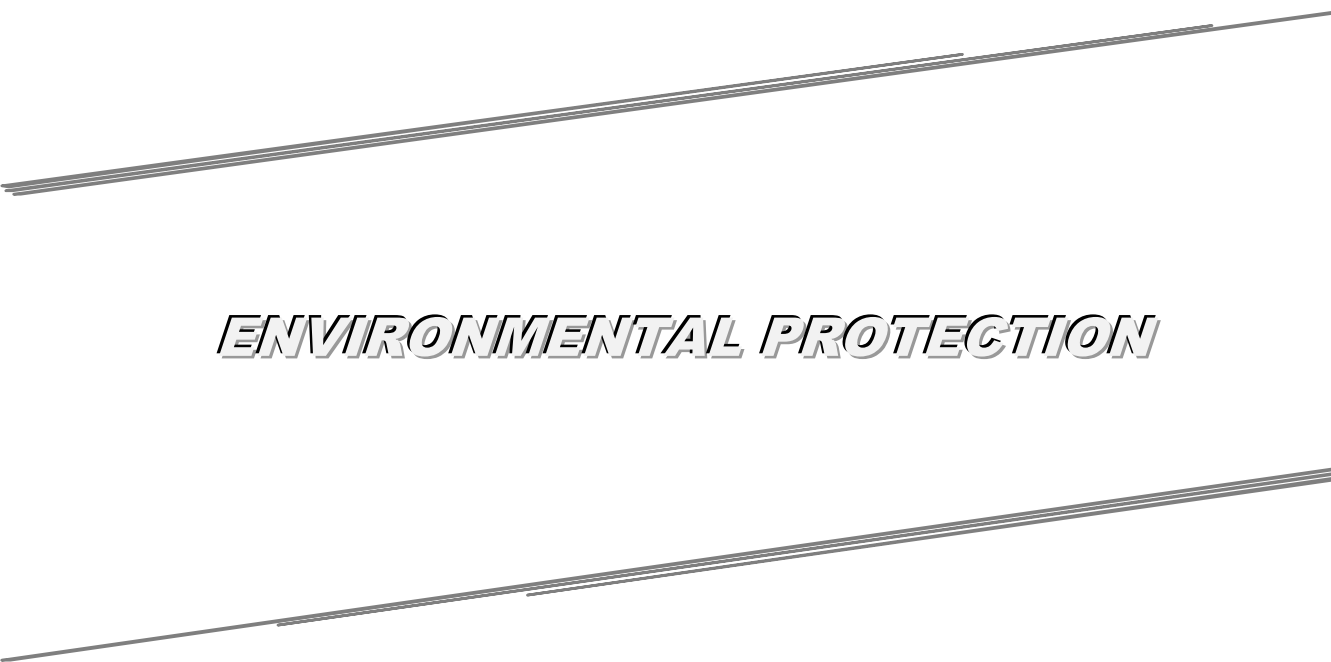
ACKNOWLEDGEMENTS

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ENVIRONMENTAL PROTECTION

NEW INVASIVE SPECIES IN THE ZAPADNA MORAVA RIVER

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Abstract: *The Zapadna Morava River is the central component of the Serbian hydrosystem. In the last decade, the Zapadna Morava ecosystems (Međuvrške and Ovčar Banja reservoirs and fast-flowing sections of the river) dramatically changed due to different human interventions. The combined effects of water quality degradation, river regulation, overfishing and other impacts are responsible for the generally deteriorated hydroecological conditions. The resulting changes include, among others, the appearance of the invasive aquatic plants (macrophytes) *Trapa natans* and *Najas minor*, the fish *Neogobius fluviatilis* and the turtle *Trachemys scripta*. The increase in the numbers of these and other invasive species can endanger the survival of indigenous species and contribute to the degradation of living conditions in this watercourse.*

Keywords: *the Zapadna Morava River, invasive species, biota.*

INTRODUCTION

The hydrographic network of the Republic of Serbia comprises about 66,000 km of watercourses and 150 reservoirs (Karadžić and Mijović, 2007). The Zapadna Morava River (308km in length) is a major tributary of the Danube River in Serbia. The immediate course of the river is torrential in the upper part (rocky-gravelly-sandy bottom), whereas the middle and lower parts are characterised by alternating fast- and slow-moving waters (gravelly-sandy-muddy substrate).

To meet the needs of the electric power industry in the 1950s, two

reservoirs were built on the Zapadna Morava River (Međuvršje and Ovčar Banja). These two artificial lakes are exposed to high sediment deposition levels (more than 70% of the original volume) and intense eutrophication (Lenhardt et al., 2009).

The highly diverse hydroecological conditions have brought about differences in existing habitats and their biocenoses.

The watercourse is a recipient of large amounts of municipal, industrial and agricultural wastewaters from Čačak, Kraljevo, Kruševac and other urban and rural areas (Čakić et al., 2018). In recent years, the river water has been polluted due to increased levels of heavy metals, ammonium ions and mineral oils, bacterial contamination, and other factors (Pantelić et al., 2021).

Anthropogenic activities in this watercourse have adverse effects, such as the appearance of some invasive plant and animal species.

MATERIAL AND METHODS

Periodic hydrobiological studies on the upper and middle course of the Zapadna Morava River were conducted in the period 2010–2020. The composition and structure of aquatic biocenoses of the Međuvršje reservoir and some river sections were analysed.

Biological material was sampled using various types of equipment and identified by standard procedures and methods (Josifović et al., 1970; Simonović, 2006). In addition to the authors' own research findings, the available literature data were used.

RESULTS AND DISCUSSION

Invasive macrophytes

Macrophytes (macroscopic aquatic plants) are important components of freshwater ecosystems. These heterogeneous plant species have diverse roles.

Apart from being major producers of organic matter and oxygen in water habitats, they absorb toxic substances, mineralise substances, perform phytofiltration, and provide food and habitat for fish and other hydrobionts (aquatic organisms).

However, the massive development and decomposition of dead macrophytes lead to the production of potentially harmful substances, increase oxygen consumption, and accelerate eutrophication processes.

The most favourable conditions for the development of macrophytic vegetation are provided by warm standing or slow-flowing waters, shallow

depths and muddy-sandy bottoms (Wetzel, 2001). Such ecological conditions exist in the Međuvršje reservoir on the Zapadna Morava River.

Previous botanical research showed the presence of 19 species of macrophytes from 13 families in this eutrophic ecosystem, with representative species of the Poaceae and Potamogetonaceae families dominating (Đelić and Vićentijević-Marković, 2002). Recent studies have reported the presence of two new species – *Trapa natans* and *Najas minor*.

Trapa natans

The water chestnut (*Trapa natans* L., Trapaceae) is a floating macrophyte widely distributed in freshwater ecosystems. *T. natans* is indigenous to Eurasia, and has been introduced to North America and Australia. It is a thermophilic species favoured by high water temperatures and constant nutrient inputs. In some European countries it is a protected rare species, whereas in the USA and other world regions it has the status of an invasive water garden plant (Hummel and Kiviat 2004).

In Serbia, *T. natans* is widespread in stagnant and slow-flowing waters of the Vojvodina Province (Borojević, 2009). In Central Serbian watercourses, *T. natans* was recorded for the first time in the Međuvršje reservoir on the Zapadna Morava River in 2011 (Markovic et al., 2015).

T. natans, together with *Typha latifolia*, *Myriophyllum spicatum* and *Potamogeton fluitans*, became a dominant member of the macrophytic community in the Međuvršje reservoir in the last decade. In some parts of the Međuvršje reservoir this species covers over 50% of the water area.

The massive development of this invasive plant threatens the survival of other macrophytes due to competing root systems and the development of the leaf rosette, leading to shading, which interferes with the photosynthetic activity of native macrophytes. The uncontrolled propagation of this species can reduce the overall primary production of the ecosystem.

In recent years, the occurrence of *T. natans* has been observed in swamps and slow-flowing sections of the Zapadna Morava River. These facts confirm the high degree of invasiveness of this species.

Appropriate measures to limit the propagation of *T. natans*, including constant monitoring and mechanical removal (eradication) of this species from some habitats, are recommended.

Najas minor

The brittle naiad (*Najas minor* All., Hydrocharitace) is an annual submersed freshwater macrophyte. It is widespread in calm waters (ponds, reservoirs and natural lakes) in Europe, Asia and North America. In Serbia, this species is frequently found in many reservoirs.

In the Međuvršje reservoir, its presence was recorded for the first time in 2012 (Topuzovic et al., 2015).

N.minor is an invasive species since it grows in large groups covering large areas and enveloping native macrophytic vegetation. This species is difficult to control as it can reproduce not only from seeds but also from stem fragments. The use of herbicides is not reliable since they destroy the vegetative parts, but not the seeds. Mechanical removal can lead to the fragmentation of the stem into smaller parts that allow vegetative propagation. Recent research has indicated that biomanipulation using herbivorous fish, such as grass carps (*Ctenopharyngodon idella*), which feed on macrophytes is a possible factor in suppressing and limiting the propagation of this species (Anonymous, 2018).

N.minor is present in the Međuvršje reservoir, but – unlike *Trapa natans* – no expansion of this species has been observed. Constant monitoring is needed due to its propagation potential, which has been favoured by increasing water temperatures in recent years.



Water chestnut (*Trapa natans*)



Brittle naiad (*Najas minor*)

Invasive animals

Neogobius fluviatilis

The monkey goby (*Neogobius fluviatilis*, Pallas, Gobiidae) is a small benthophagous fish that inhabits sandy-muddy bottoms into which it often buries itself. It is of minor sport-fishing and economic importance (Simonović, 2006).

N.fluviatilis is one of the 5 species of the alien genus *Neogobius* (native to the Ponto-Caspian region) in Serbian watercourses. The species inhabits the entire course of the Danube through Serbia and its major tributaries (Marković et al., 2015).

The first record of *N.fluviatilis* in the Zapadna Morava River was in

2012. Two specimens were caught near the town of Čačak (Đikanović et al., 2013). The reports of this species in later investigations and increases in its population numbers confirm good acclimatisation of the species to new habitats. Further propagation of the species is expected to lead to the aggravation of competitive relationships with native fish species in this ecosystem.

Trachemys scripta

The pond slider (*Trachemys scripta*, Thunberg in Schoepff, Emydidae) is a medium-sized turtle. This species is a popular pet around the world. In a natural environment, it has negative ecological effects. In addition to competing with the European pond slider (*Emys orbicularis*) (native turtle), this species preys on amphibian larvae, destroys nests and young birds, and transmits certain pathogenic bacteria (*Salmonella* sp.) to native wildlife and humans (Polo-Cavia et al., 2010).

It is therefore included in the list of the world's 100 most invasive species (Scalera, 2006).

The species consists of three subspecies, the red-eared slider (*Trachemys scripta elegans*) being the most common in Serbia (Urošević et al., 2019). Due to great danger from the negative impact of the increase in their numbers in Serbia, trade in all three subspecies of *T.scripta* is banned and their import prohibited (Official Gazette of the Republic of Serbia issue No. 99/2009).

It was first recorded in the Zapadna Morava River in 2015 near the town of Čačak and the Međuvršje reservoir (Urošević et al., 2019). The species has great potential for breeding and massive population growth.

To limit the population expansion of this invasive species, measures such as the collection and eradication of *T.scripta* specimens from natural habitats and the formation of turtle sanctuaries are recommended.



Monkey goby (*Neogobius fluviatilis*)



Red-eared slider (*Trachemys scripta elegans*)

CONCLUSIONS

The Zapadna Morava River (308km in length) is an important component of the Serbian hydrosystem. The watercourse flows mostly through urban areas, which causes pronounced anthropogenic impacts, such as wastewater discharges, river regulation, excessive extraction of river bed materials, and overfishing.

In addition to these changes, the emergence of some invasive plant and animal species has been observed. They may threaten the survival of native flora and fauna.

Very high expansion potential has been exhibited by the macrophyte water chestnut (*Trapa natans*).

Constant monitoring and other measures are recommended to eliminate or limit the spread of these species in this ecosystem.

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S A D R Ź A J

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