GREEN ROOM SESSIONS 2018

Book of Proceedings

Podgorica, Montenegro, 2018

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GREEN ROOM SESSIONS 2018
International GEA (Geo Eco-Eco Agro) Conference
1-3 November 2018, Podgorica, Montenegro
Website: www.greenrooms.me

Book of Proceedings

Publisher: University of Montenegro, Faculty of Philosophy, Geography, Montenegro
Danila Bojovića bb, 81400 Niksic, Montenegro, Web: https://www.ucg.ac.me/ff

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Photo front page: Aleksandar Jaredic / Ribo Raicevic

CIP - Каталогизација у публикацији
Национална библиотека Црне Горе, Цетиње

ISBN 978-9940-694-09-8
COBISS.CG-ID 37143056

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FOREWORD

Green Room Sessions International Conference aims to be platform for international scientific discussion on agriculture in general as well as agriculture in conjunction with economics and ecology, food and nutrition science and technology, rural development, environment and forestry. Green Room Sessions brings together and is connecting research, industry, social concepts and practices. The scientific core is based on applying Eco-Eco (ecological-economical) concepts and principles to optimize interactions between natural, social and built components of the rural environments: plants, animals, soil, water, air, humans and man-made structures. In addition, Green Room Sessions placed social issues at the centre of solutions for a sustainable and fair food system. Green Room Sessions are targeting to multiple benefits to society and the environment, by bringing people together and providing them the opportunity to sit together and exchange ideas and connect the business.

In November 2018, the 1st Green Room Sessions International Conference provided an opportunity for sharing experiences and builds the evidence base on agriculture, forestry, human interactions and built environment, as well as reaching a consensus on the priorities for achieving more sustainable food systems. It also endorsed Institutional roles of National services, Regional and International organisations in supporting further implementation and promotion of Eco-Eco (ecological-economical) concepts and principles.

Dialogue between the participants targeted:

- Enhancing smallholder and family farmers' adaptation and resilience to the impacts of climate change;
- Improving nutrition including through more diversified diets;
- Protecting and enhancing agro-biodiversity in support of ecosystem services;
- Improving livelihoods in rural areas;
- National Food Wealth, the holy trinity: agriculture, economics and ecology (a x e^2);
- Mutual interconnections and how to deal with them and how this mix influence National Food Wealth and National Health.

achieving a transformative change in agricultural practices towards sustainable development.

The Green Room Sessions International Conference synthesized and build on the outcomes of the regional meetings, and provided an opportunity to share and discussed policies that can help scale-up and scale-out agriculture, rural development, agroecology, nutrition in order to achieve the Sustainable Development Goals.

The Symposium also moved the topic of agriculture and rural development from dialogue to activities at the regional and country level by complementing on-going initiatives to integrate biodiversity and ecosystem services in agriculture, identifying opportunities for synergies with National Strategic Programmes and Regional Initiatives, and facilitating regional and International cooperation between the scientists and business.

Green Room Sessions International Conference as a final goal is looking forward to assist people from the rural areas, related business, agriculture and allied sectors to take the advantage of:

- Natural resources, secure access to land and water, and improved natural resource management and conservation practices;
- Improved agricultural technologies and effective production services;
- Linking the interested parties with financial services;
- Transparent and competitive markets for agricultural inputs;
- Opportunities for rural off-farm employment and enterprise development;
- Local and national policy and programming.

We launch this with the aim of unlocking innovative, integrated, multidisciplinary science and technology with activation of all dimensions of sustainable development goals for all the participants.

In this Book of Proceedings we published part of the original scientific full papers presented at the Conference. The other part is provided for publication at the journal Agriculture and Forestry (ISSN 0554-5579, Printed; ISSN 1800-9492, Online), all based on the requests of the authors who participated at the Conference.

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PREDGOVOR

Međunarodna konferencija Green Room Sessions imala je za cilj da bude platforma međunarodne naučne diskusije o poljoprivredi uopšte, poljoprivredi vezano sa pitanjima ekonomije i ekologije, nauci o tehnologiji hrane i prehrane, ruralnim razvojem, životnom sredinom i šumarstvom. Green Room Sessions okupila je i povezivala nauku, istraživanje, industriju, društvene koncepte i prakse.

Naučni principi zasnovani su na primjeni Eko-Eko (ekološko-ekonomskih) koncepata za optimizaciju interakcije između prirodnih, socijalnih i komponenti ruralnih sredina: biljka, životinja, zemljište, voda, vazduh, kao i strukture koje su nastale kao plod rada ljudi. Pored toga, Green Room Sessions je težila da postavi društvena pitanja u centar rješenja održivog i fer sistema proizvodnje hrane. Brojni sastanci održani su tokom Konferencije sa ciljem da imaju višestruke koristi za društvo i sredinu koja nas okružuje, približavajući tokom ovih komunikacija ljude jedne drugima, pružajući im priliku da međusobno komuniciraju na jednom mjestu, razmenjuju ideje i povezuju poslovanja.

U novembru 2018. godine, Green Room Sessions International Conference pružila je mogućnost razmjene iskustava potvrđenih praksi u poljoprivredi, šumarstvu, interakcijama čovjeka i njegovog okruženja, struktura koje su nastale kao plod rada ljudi. Ovo je postignuto organizovanjem susreta naučnika i stručnjaka iz ove oblasti, te razmjenom iskustava, doprinoseći unapređenju održivijeg sistema proizvodnje i prerade. Iskustva drugih koji su gostovali istakli su značaj institucionalne uloge nacionalnih službi, regionalnih i međunarodnih organizacija u podršci i daljoj promociji eko-eko (ekološko-ekonomskih) koncepata i principa.

Dijalog između učesnika bio je usmjeren na:

- Prilagođavanje malih proizvođača i porodičnih farmera i jačanje njihove otpornosti na uticaj klimatskih promjena;
- Zaštitu i unapređenje agro-biodiverziteta, podrške održivosti ekosistema;
- Poboljšanje životnih uslova, životnog standarda u ruralnim područjima;
- „Sveto trojstvo": poljoprivreda, ekonomija i ekologija ($a \times e^2$), njihove međusobne veze i kako se baviti njima, te kako ovaj miks međusobnih relacija utiče na proizvodnju domaće hrane i zdravlje nacije;

- Postizanje tranzicionih promjena u poljoprivrednim praksama u skladu sa principima održivog razvoja.

Konferencija je dijelom uradila sintezu i nadograđivala rezultate regionalnih sastanaka i pružiti priliku da podijeli svoja iskustva sa učesnicima, diskutuje o politikama koje mogu pomoći u povećanju poljoprivredne proizvodnje, ruralnog razvoja, agroekologije, ishrane kako bi se postigli ciljevi održivog razvoja.

Konferencija je takođe inicirala pomjeranje teme poljoprivrede i ruralnog razvoja od dijaloga ka konkretnim aktivnostima na lokalnom i regionalnom nivou, tražeći rješenja očuvanja biodiverziteta u poljoprivredi, identifikujući mogućnosti za sinergiju sa nacionalnim strateškim programima i regionalnim inicijativama, pospješujući regionalnu i međunarodnu saradnju između naučnika i biznisa.

Učesnici na Konferenciji tražili su načine da se pruži pomoć ljudima iz ruralnih područja, njihovim malim biznisima, poljoprivredi i srodnim sektorima da iskoriste prednosti:

- Prirodnih resursa, bezbjednog pristupa zemljištu i vodama, poboljšavajući prakse upravljanja prirodnim resursima i pristupe konzervacije;
- Poboljšane poljoprivredne tehnologije i efikasnijih proizvodnih usluga;
- Povezivanje zainteresovanih strana sa finansijskim servisima;
- Mogućnosti za zapošljavanje i razvoj preduzeća u ruralnim područjima;
- Lokalnih i nacionalnih politika i programiranja.

Ovo inicijativa je pokrenuta sa ciljem otvaranja i susreta sa inovativnom, integrisanom, multidisciplinarnom naukom i tehnologijom uz aktiviranje svih dimenzija ciljeva održivog razvoja za sve učesnike.

U ovom Zborniku radova objavili smo dio originalnih naučnih radova (*Full papers*) predstavljenih na Konferenciji. Drugi dio je proslijeđen za objavljivanje časopisu Poljoprivreda i šumarstvo (ISSN 0554-5579, print; ISSN 1800-9492, online), sve na osnovu zahtjeva autora koji su učestvovali na Konferenciji.

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CONTENT

Plant production, Plant protection and food safety, Genetic resources

Environment protection, natural resources management, Rural development and Forestry

Plant production, Plant protection and food safety, Genetic resources

Original Scientific paper

Alfalfa response to seed pre-inoculation with *Sinorhizobium meliloti*

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Abstract

In the process of legume seed pre-inoculation by bioinoculants, proper inoculant formulations with good stick characteristics and ability to provide long survival of rhizobial bacteria on stored seeds are required. In this study the ability of different liquid rhizobial inoculants to be used for alfalfa seed pre-inoculation was tested and compared to seed inoculation performed at the time of sowing. Two strains effective in nitrogen fixation with alfalfa, *Sinorhizobium meliloti* L4 and L5, and four types of inoculant carriers (yeast mannitol broth-YMB, YMB with the addition of 0.5% agar, 0.5 % sodium-alginate, or 1% polyvinylpyrrolidone) were tested. The alfalfa seeds (cultivar Krusevacka K-28) were inoculated 2 and 1 month before sowing (pre-inoculation) and just prior to sowing (inoculation). The effects of pre-inoculation and inoculation of alfalfa seeds on plant nodulation, plant height, shoot dry weight (SDW) and nitrogen content were evaluated. Results showed that the time of pre-inoculation and inoculant formulation as well as their interaction had highly significant influence ($p<0.001$) on SDW and total nitrogen content of plants, while rhizobial strain influenced total nitrogen content ($p<0.05$). In addition, interaction of time of pre-inoculation, inoculant formulation and strain influenced total nitrogen content ($p<0.01$). Inoculant containing YMB as well as inoculants with the addition of 0.5% agar and 1% polyvinylpyrrolidone provided good nodulation of alfalfa pre-inoculated by both strains 2 months before sowing. Depending on the strain, effective nitrogen fixation in alfalfa was reached by YMB inoculant with L4 strain as well as inoculant with addition of 0.5% agar and L5 strain. Our findings indicated that the application of appropriate combination of medium formulation and strain is successful for alfalfa seed pre-inoculation.

Keywords: alfalfa, *Sinorhizobium meliloti*, pre-inoculation, liquid inoculants, inoculant carrier

Introduction

Natural and ecologically healthy way to increase effectiveness of symbiotic nitrogen fixation (BNF) in agriculture is use of legume inoculation by bioinoculants. Rhizobial bioinoculants consist of a highly effective rhizobial strain that is usually mixed with the solid-based carrier material (such as peat) rich in organic matter (Temprano *et al*., 2002). The role of an appropriate carrier with additives is to provide a microenvironment for the bacterial cell. It is responsible for rhizobial multiplication, growth, long survival (appropriate temperature, oxygen content and humidity), stabilization and protection of bacterial cells during storage of inoculant or inoculated seeds after seed coating. Moreover, good formulation should be cost-effective, easy to handle and apply, as well as, stable during production, distribution, storage and transport (Damasceno *et al*., 2013; Hungria *et al*., 2005; Temprano *et al*., 2002; Xavier et al., 2004).

The practice of adding bioinoculants to leguminous seed is usually done on the day of sawing. In addition, artificial inoculation of seed can be performed several months before sawing by the process of pre-inoculation, in which the inoculants sticking to the seeds was done in seed processing before bagging of seed.

The choice of inoculant forms depends on the available recourses of row materials by countries, the time of seed inoculation (on the day of sowing, some days or months before sowing, at the time of seed germination) and the object of seed inoculation (seed or soil). The inoculants are available as powder, granule and liquid form. One of the most used carriers in the powder or granular inoculant is ground peat due to good physical-chemical characteristic for rhizobial bacteria and low prices as natural row material in the country. Ground peat is "carrier of choice" in the production of rhizobial inoculants world wild (Temprano *et al.*, 2002). In Serbia, peat is used as carrier in powder formulations for almost 70 years, mainly because of low cost, simple production and enables good effectiveness in nitrogen fixation. Some results indicate that the most significant stimulating effect on the growth of *Sinorhizobium meliloti* strains was achieved using peat carrier enriched with some additives (Temprano *et al.*, 2002; Delić *et al.*, 2016).

Liquid inoculants can be more suitable than powder inoculants due to absence of extra carrier sterilisation, little chance for contamination, ease of application, can be cost-effective due to absence of transport connected with the purchase and sterilisation of the powder carriers (Biswas and Bhowmick, 2007). Moreover, liquid bioinoculants are preferred for longer shelf-life (Biswas and Bhowmick, 2007; Goljanian-Tabrizi, 2016).

Alfalfa (Medicago sativa L.) is one of the most important leguminous forage crops with the ability of BNF with specific rhizobia, *Sinorhizobium meliloti* (*Ensifer meliloti*). It is considered that symbiotic association between alfalfa and *S. meliloti* is one of the most efficient interactions between N_2-fixing bacteria and legumes (Zeng *et al.*, 2007; Vasileva and Ilieva, 2012).

The aim of this research was to study the ability of liquid rhizobial inoculants to be used for the pre-inoculation of alfalfa seed by yield and plant quality response to inoculated alfalfa.

Materials and Methods

In our trial, two effective *S. meliloti* (*Ensifer meliloti*) strains from the collection of bacteria of the Institute of Soil Science (L4 and L5) were selected as active agent of rhizobial bioinoculant. Alfalfa cultivar Krusevacka 28 (K-28) from the Institute for Forage Crops, Kruševac was used as host plant. Rhizobial bacteria were cultivated in standard medium, yeast mannitol broth (YMB) (Vincent, 1970). YMB with different concentrations of supplements (0.5% agar, 0.5% Na-alginate and 1% polyvinylpyrrolidone (PVP)) were applied as different liquid medium formulations for seed pre-inoculation. Rhizobial strains L4 and L5 were growing in these medium formulations 48h till late lag-phase and they presented liquid rhizobial inoculants. In the process of pre-inoculation was done in following way: unsterilized alfalfa seeds were inoculated by liquid inoculants (25µl of the inoculant with 0.2 g of seeds) and were stored at room temperature of 22°C (±1°C) during 2 and 1 months before sowing in dark room.

Effectiveness of inoculant formulations were tested in in laboratory conditions in which pre-inoculated seeds were placed into test tubes (2.5 x 25cm) containing N-free Jansen agar (Vincent, 1970). The plants were grown in light chamber under a 16/8h light/dark cycle and 28/20°C day/night temperature. The experimental layout was with 4 inoculated treatments (inoculants with 4 medium formulations) and 2 uninoculated control treatments (Ø control treatment- uninoculated N free control and NØ control treatment-uninoculated full nitrogen control (with full N content of 0.5% KNO3)) with 10 plants per treatment as replications. These 4 treatments were for each strain, L4 and L5 strains, and for both time of seed pre-inoculation as well as for the time of seed inoculation just before sowing. The experiment was completed 45 days after sowing. The following analyses and measurement were performed: nodulation, plant height, SDW (plant were oven dried at 72°C for 24h) and total N content (CNS Elemental Analyzer vario EL III). All data were subject to ANOVA using the statistical analysis system (COSTAT). Means of all treatments were calculated and the differences tested for significance using the Duncan's Test at the 0.5 probability level ($P \leq 0.05$).

Results and Discussion

The ability of different liquid rhizobial inoculants to be used for alfalfa seed pre-inoculation (one and two months before sowing) was tested in this study and compared to seed inoculation at the time of sowing and control treatments without inoculation (Ø and NØ). Formulations of rhizobial liquid inoculants are presented in Table 1.

Table 1. Formulation of rhizobial liquid inoculants.

Ingredients	**YMB**[1]	**0.5% agar**	**0.5% Na-alginate**	**1% PVP**
YMB medium	YMB	YMB	YMB	YMB
Agar (g/l)	/	5	/	/
Na-alginate (g/l)	/	/	5	/
Polyvinylpirolidon (PVP) (g/l)	/	/	/	10

[1] Yeast mannitol broth (YMB) (g/l): 10g mannitol, 0.5g K_2HPO_4, 0.2g $MgSO_4$ H_2O, 0.1g NaCl, yeast extract (100 ml/l), 900 ml H_2O (Vincent, 1970).

The inoculants were consisted of *S. meliloti* strain, L4 or L5, growing in YMB medium as carrier without and with each of following supplements: agar, Na-alginate and PVP. YMB medium is standard medium for rhizobial bacteria cultivation (Vincent, 1970; Somesegaran and Hobs, 1994). YMB without the supplements was proved because rhizobial exopolysaccharides has protective role for rhizobial bacteria. Nevertheless, differences between rhizobial strains on inoculated seeds. It can be connected with seed exudates which can have different influence on strain and compounds of inoculant. Moreover, compounds should be tested for ability to promote survival of rhizobial bacteria (Singleton *et al.*, 2002; Tittabutr *et al.*, 2007). Also, in some studies of inoculant formulations, YMB and PVP as supplement were researched (Singleton *et al.*, 2002). These polymers, such as Na-alginate and PVP are normally used as adhesive when they are applied to seed, where they can protect and provide longer self-life of bacteria (Leo Daniel *et al.*, 2013). In the process of legume seed pre-inoculation by bioinoculants, proper inoculant formulations with good stick characteristics and ability to provide long survival of rhizobial bacteria on stored seeds are required. Liquid inoculants can use broth culture or liquid formulation (water, mineral oil, organic oil, polymer-based products) (Xaviar, 2004; Fernandes Júnior *et al.*, 2012; Sytnikov, 2013). Encapsulation is important method for protection of rhizobial strains during storage period of coated seed by the inoculant and following the introduction of inoculant into soil environment (Young *et al.*, 2006).

Pre-inoculated seeds after 2 and 1 month of seed storage were estimated by visual assessment as suitable for planting. Nodulation response obtained with pre-inoculated stored seeds was similar to non-stored seeds applied at the time of sowing, which is in agreement with results of some authors (Temprano *et al.*, 2002). All plants grown from the pre-inoculated seeds were nodulated. It indicated that liquid inoculants enabled protection and survival of rhizobial strains during 1 and 2 months of storage time. We can suppose that strains L4 and L5 survived because of particular good PVP traits like as mobilisation of toxic compounds from seed exudates during inoculation and seed germination, its high water-binding capacity and ability to have slow drying after application). Mannitol has many potential roles in liquid inoculants because it is the source of carbon supported production of some compounds in the bacterial cell, also protects cell during the rapid drying (Singleton *et al.*, 2002; Tittabutra *et al.*, 2007).

The effect of different inoculant formulations without and with different supplements on plant yield and quality were investigated in laboratory plant experiment. It has been reported that plant SDW is the best parameter to evaluate symbiotic activity of legume-*Rhizobium* associations. Based on three factorial analysis of variance (ANOVA) for SDW and total N content, it was found that the time of seed inoculation and medium with supplement as well as their interaction had highly significant ($P<0.001$) effects on SDW and total N content of alfalfa while, rhizobial strain influenced only total N content ($P<0.05$) (Table 2). Moreover, total N content of alfalfa was influenced also, by interaction of all of 3 factors ($P<0.01$). It is known that rhizobial strain effects total N content (Mytton *et al.*, 1984; Phillips and Theuber, 1985; Rengel, 2002).

Table 2. ANOVA for plant shoot dry weight and total N content of alfalfa inoculatedwith two *Sinorhizobium meliloti* strains placed in YMB medium with 3 supplements

Source of variance	**Shoot dry weight (mg plant^{-1})**	**Total N content (mg plant^{-1})**
Media with supplement	205.43***	272.35***
Time of inoculation	14.04***	15.45***
Strain	1.60^{ns}	4.00*
Interaction		
Media x strain	1.34^{ns}	1.999^{ns}
Media x time	4.98***	4.48***
Strain x time	1.57 ^{ns}	2.48 ^{ns}
Time x strain x carrier	1.64 ^{ns}	2.78**

**,***Significant at $P< 0.01$, 0.001, respectively; ^{ns}-non-significant.

All inoculated plants recorded height between 26.57 and 29.82 cm without significant differences among treatments (data not shown). The plants from pre-inoculated seed treatments together with plants inoculated at the time of sowing had significantly higher average values of alfalfa plant SDW (10.64-13.36 mg plant^{-1}) and plant total N content (0.39-0.49 mg plant^{-1}) in comparison with uninoculated N free control (5.57 mg plant^{-1} for SDW and 0.06 for total N content). The results showed that inoculant formulations provided bacterial survival on alfalfa pre-inoculated seeds to be able to fix atmospheric nitrogen (N_2) (Table 3 and 4).

Among these treatments, the highest average SDW, with both strains and for all three times of seed inoculation, were obtained in treatments with YMB alone, YMB with agar and YMB with PVP; 13.08 mg plant^{-1}, 12.93 mg plant^{-1} and 13.36 mg plant^{-1}, respectively. In correlation with average SDW of these treatments were average total N content: YMB alone (0.48 mg plant^{-1}), YMB with agar (0.49 mg plant^{-1}) and YMB with PVP (0.47 mg plant^{-1}). According to the average SDW and total N content in these treatments, there were no significant differences between these inoculant formulations. Significantly, lower SDW (10.64 mg plant^{-1}) and total N content (0.39 mg plant^{-1}) had plants which were germinated from pre-inoculated seeds by YMB with 1% of Na-alginate. Results showed that the

effects of agar and PVP supplements to SDW and total N content were significantly higher than with alginate supplement.

The results indicates that in any time of seed inoculation SDW was not significantly different by inoculation with the both investigated strains, L4 and L5, 14.52 and 15.22 mg plant^{-1}, respectively (Table 3). However, the values of total N content between inoculants with different strains applied at all inoculation times were significantly different, L4 and L5, 0.52 and 0.57 mg plant^{-1}, respectively (Table 4).

Average SDW (Table 3) and total N content (Table 4) of the plants pre-inoculated 2 months before sowing were the highest, 16.11 mg plant^{-1} and 0.60 mg plant^{-1}, respectively. These values were not significantly different from SDW and total N content realized from the plants inoculated at the time of sowing (15.71 mg plant^{-1} and 0.56 mg plant^{-1}). In comparison with them average SDW (12.80 mg plant^{-1}) and total N content (0.46 mg plant^{-1}) of the plants pre-inoculated 1 month before sowing was significantly lower. We can suppose that the results after 1 month of storage were less good due to stressful influence of the environment and there was need some time for strain adaptation which was seeds after 2 months. Similar influence of storage time was noticed in some research in which correlation between strain survival in the laboratory assay on pre-inoculated seeds and nodulation performance under control conditions improved with storage time (Singleton *et al.*, 2002). Our results showed that inoculants with YMB medium alone and with supplements of 0.5% agar and 1% polyvinyl pyrrolidone were suitable for the survival of both *S. meliloti* strains during 2 months.

Table 3. Shoot dry weight (SDW)* of alfalfa plants from pre-inoculated seeds with *Sinorhizobium meliloti* strains (mg plant^{-1})

Treatments of different inoculant formulations									
		Bacterial medium with different supplements							
The time of seed inoculation	**strain**	**YMB	YMB with 0.5% agar	YMB with 0.5% Na-alginate	YMB with 1% *PVP	Ø	NØ	**Average SDW values** per strain	for the time of seed inoculation
At the time of sowing	L4	12.94	13.54	12.95	16.99	5.57	33.92	15.99 a	15.71 a
	L5	12.44	11.64	11.27	18.66	5.57	33.92	15.58 a	
pre-inoculation 1 month before sowing	L4	10.08	7.00	10.14	9.18	5.57	33.92	12.65 a	12.80 b
	L5	11.87	8.05	8.70	10.11	5.57	33.92	13.04 a	
pre-inoculation2months before sowing	L4	16.57	13.03	9.63	11.95	5.57	33.92	15.11 a	16.11 a
	L5	14.58	24.32	11.15	13.28	5.57	33.92	17.14 a	
Average values for inoculants formulations		13.08 b	12.93 b	10.64 c	13.36 b	5.57 d	33.92 a	L4,14.52a L5,15.22a	
LSD.05				1.8969				1.095	1.3413

SDW*- Mean values of shoot dry weight; **YMB-yeast mannitol broth, PVP-polyvinylpirolidon; Ø (uninoculated N free control); NØ (uninoculated full nitrogen control).

S. meliloti strain L5 was applied in this research as broadly N fixing effective strain with most of alfalfa cultivars (Delić *et al*., 2013a, b). The strain L4 also applied as effective N fixing rhizobial bacteria (Stajković-Srbinović *et al.*, 2012). In addition, according to our previous research, Serbian K-

28 cultivar showed high compatibility in N fixation with strain L5 (Delić *et al.*, 2013a; 2014). These facts provide a presumption that in this study can be carried out effective nitrogen fixation. Based on criteria of Pochon (1954) about a degree of strain efficiency in this research, only strain L4 in YMB medium and strain L5 in YMB medium with 0.5% agar applied on seeds 2 months before sowing were effective in N fixation. In comparison with uninoculated N control, these strains realized significantly lower SDW yield (L4 was about 50% and L5 was 71% SDW of ØNcontrol) (Table 3). These results indicate that strain survival and effectiveness depend on inoculant formulation.

Table 4. Total nitrogen (N) content (mg)* of alfalfa plants from pre-inoculated seeds with *Sinorhizobium meliloti* strains

Treatments of different inoculant formulations									
		Bacterial medium with different supplements						**Average values**	
The time of seed inoculation	**strain**	**YMB	YMB with 0.5% agar	YMB with 0.5% Na-alginate	YMB with 1% *PVP	Ø	ØN	per strain	for the time of seed inoculation
At the time of sowing	L4	0.49	0.49	0.37	0.59	0.06	1.35	0.56	0.56 a
	L5	0.52	0.44	0.40	0.65	0.06	1.35	0.57	
1 month before sowing	L4	0.36	0.27	0.35	0.33	0.06	1.35	0.45	0.46 b
	L5	0.41	0.29	0.34	0.38	0.06	1.35	0.47	
2 monthsbefore sowing	L4	0.60	0.42	0.40	0.44	0.06	1.35	0.55	0.60 a
	L5	0.55	1.02	0.46	0.49	0.06	1.35	0.46	
Average values forinoculants formulations		0.48 b	0.49 b	0.39 c	0.47 b	0.06 d	1.35 a	L4, 0.52 b L5,0.57 a	
LSD.05				0.072				0.041	0.051

*Total N content-Mean values of total N content; **YMB-yeast mannitol broth, PVP-polyvinylpirolidon; Ø (uninoculated N free control);NØ (uninoculated full nitrogen control).

The results of some research showed that there is a degree of interaction between strains of rhizobia and supplements and that is why it is need to choose supplements of liquid inoculants for individual rhizobial species and strains (Tittrabutr *et al*., 2007). Singleton *et al.*, (2002) found that some rhizobial strains influenced the yield of plants more than others in the suitable medium due to the compatible interaction between them. The selection of strain for survival on seeds keeping N fixing effectiveness may be productive way of increasing traits of liquid inoculans which means that the good formulation of inoculant should be created according to the rhizobial strain.

Conclusions

All plants grown from the pre-inoculated seeds were nodulated which indicates that the liquid inoculant enabled the survival of rhizobial strains during 1 and 2 months of storage time. The inoculant formulations provided bacterial survival and protection on alfalfa seeds in the process of pre-inoculation, particularly two months during seed storage. YMB medium without and with the addition ofagar and PVPshowed a good characteristics as the inoculants carriers. The survival of rhizobial strain, as well as their effectiveness depended on the inoculant formulations.

Acknowledgments: The financial support for this investigation given by the Ministry of Education, Science and Technological Development of the Republic of Serbia under the project TR 37006 and TR 31035 is gratefully acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

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Original Scientific paper

Effects of fertilization on production traits of winter wheat

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Abstract

The wheat cultivar used in the experiment was Kruna. This investigation included an untreated seven variants of fertilization: 1-control, 2-80 kg/ha N, 3-60 kg/ha P_2O_5, 4-80 kg/ha N, 60 kg/ha P_2O_5, 5-80 kg/ha N, 100 kg/ha K_2O, 6-80 kg/ha N, 60 kg/ha P_2O_5, 60 kg/ha K_2O and 7-80 kg/ha N, 100 kg/ha P_2O_5, 60 kg/ha K_2O. Total amounts of phosphorus and potassium fertilizers and half the nitrogen rate are regularly applied during pre-sowing cultivation of soil. The trial was set up in a randomized block design with five replications. The crop was harvested at full maturity. Grain yield (t/ha) was harvested and reported at 14% moisture. Three parameters were analyzed: test weight (kg/hl), 1000-grain weight (g) and grain yield (t/ha). Investigation where showed a considerable variation of grain yield which were in dependence from mineral nutrition. The highest grain yields were the highest with mineral fertilizer in the combined $N_{80}P_{100}K_{60}$ (4.738 t/ha). Over the two-year period, all investigated treatments of wheat achieved the highest average 1000-grain weight in the combined $N_{80}P_{60}$ (43.08 g). The highest test weight was the highest with mineral fertilizer in the combined $N_{80}P_{60}K_{60}$ (73.63 kg/hl). Analysis of variance was found highly significant effect of years on 1000 grains weight.

Keywords: fertilization, yield, quality, wheat

Introduction

Mineral nutrition of wheat grown on acid soil reaction is specific. The crucial importance plays equilibred nutrition by nitrogen and phosphorus with higher impact of phosphorus nutrient. Mineral fertilizers play a vital role towards improving crop yields but one of the main constraints in achieving proven crop potential is imbalanced use of nutrients, particularly low use of phosphorus as compared to nitrogen. Đekić *et al*. (2014) and Jelic *et al.* (2015) found it to be absolute mass the grain has a significant influence on the application of mineral fertilizers, was significantly higher in intensified fertilizer treatments, especially nitrogen. The optimum rate of phosphorus application is important in improving yields of most crops (Popović, 2010; 2015; Đekić *et al*. 2013, 2014; Jelić *et al*., 2013, 2014; Terzić *et al*., 2018; Ugrenovic *et al*., 2018).

In Serbia, farmers are using only nitrogen fertilizers for fodder crops while the use of phosphorus fertilizer is negligible. These crops are often grown on marginal lands. Hence, the production is low and quality is poor. The absence of record yields indicates that an answer could be sought in soil, the main substrate for field crop production. Additionally, the major previous crops of small grains also suffer from negative nutrient balance. The use of incomplete production technology in previous decades had definitely affected the potential and actual soil fertility.

Because of appearance of new demanded cultivars at permanent changes in soil fertility level and environmental conditions, still exist need to researches mineral nutrition of wheat, as well as determine optimal rates and balanced nutrition ratios in concrete agro-ecological conditions. The main goal of this research was to investigate the effect of long-term applications of identical amounts and ratios of nitrogen, phosphorus and potassium on the yield components of winter wheat variety „Kruna". The objective of this study was to evaluate the effect of different fertilization systems on the grain yield and quality of wheat grown on a vertisol soil. The study was also aimed at optimizing fertilization for maximum profitability in the future wheat production of Central Serbia.

Materials and Methods

Experimental design and soil conditions

The study was carried out in a stationary field trial involving fertilization over a two years period from 2011/12 to 2012/13. Trials were first set up in the experimental fields of the Small Grains Research Centre in Kragujevac in 1970. Plot size was 50 m^2. The wheat cultivar used in the experiment was Kruna, the dominant cultivar in the production region of Serbia. This investigation included an untreated seven variants of fertilization: C-control, N-80 kg/ha N, P_1-60 kg/ha P_2O_5, NP_1-80 kg/ha N, 60 kg/ha P_2O_5, NP_2-80 kg/ha N, 100 kg/ha K_2O, NP_1K-80 kg/ha N, 60 kg/ha P_2O_5, 60 kg/ha K_2O and NP_2K -80 kg/ha N, 100 kg/ha P_2O_5, 60 kg/ha K_2O. A non-fertilized variant served as a control. Total amounts of phosphorus and potassium fertilizers and half the nitrogen rate are regularly applied during pre-sowing cultivation of soil. The trial was set up in a randomized block design with five replications. Fertilization was regular and followed a long-time scheme.

The crop was harvested at full maturity. Grain yield (t/ha) was harvested and reported at 14% moisture. Three parameters of grain quality, namely test weight (kg/hl) and 1000-grain weight (g) were analyzed. Thousand grain weight was determined using an automatic seed counter.

The trial was set up on a vertisol soil in a process of degradation, with heavy texture and very coarse and unstable structure. The humus content in the surface layer of soil was low (2.22%). Soil pH indicates high acidity (pH in H_2O 5.19; pH in KCl 4.27), nitrogen content in soil is medium (0.11-0.15%), while the content of available phosphorus ranges from very low (1.7-2.9 mg/100 g soil) in the N variant to very high (26.9 mg P_2O_5/100 g soil) in the NPK variants of fertilization. Available potassium contents are high, ranging from 19.5 to 21.0 mg K_2O/100 g soil.

2.2. Agroecologial conditions

This study was conducted over a three-year period in the Šumadija region, Central Serbia, on a Vertisol soil, at Kragujevac location, 173-220 m a. s. l. (**44° 22′ N, 20° 56′ E**), in a temperate continental climate having an average annual temperature of 11.5°C typical of Šumadija districts in Serbia and a rainfall amount of about 550 mm.

The data in Table 1 for the investigated period (2011-2013) clearly indicate that the years in which the researches were conducted differed from the typical multi-year average of Kragujevac region regard the meteorological conditions. The average air temperature in 2011/12 was higher by 0.08°C and 2012/13 was higher by 1.81°C. The sum of rainfall precipitation in 2011/12 was higher by 23.7 mm, where the sum of rainfall in 2012/13 was 24.5 mm higher than the average of many years and with a very uneven distribution of precipitation per months. During the Mart in 2011/12 it was 102.0 mm of rainfall, what was 58.5 mm more compared with the perennial average. During the month of May in 2011/12 it was 87.3 mm of rainfall, what was 22.5 mm and in 2012/13 it was 70.8 mm of rainfall, what was 6.0 mm more compared with the perennial average. In the first year of investigation, higher amounts of rainfall were recorded in April, May and June, which led to water logging lodging of crops and lower yield.

Table 1. Mean monthly air temperature and precipitation (Kragujevac)

Year	Months									Average
	X	XI	XII	I	II	III	IV	V	VI	
Mean monthly air temperature (°C)										
2011/12	10.4	3.1	4.6	0.7	-3.7	8.1	12.9	16.1	23.0	8.4
2012/13	13.5	9.5	1.7	2.0	4.0	6.1	16.5	18.0	19.9	10.13
Average	11.8	5.6	1.9	0.6	2.0	6.2	11.2	16.2	19.4	8.32
The amount of precipitation (mm)										
2011/12	33.3	1.3	43.3	117.2	60.1	5.7	74.5	87.3	57.8	480.5
2012/13	56.2	17.7	16.4	62.4	84.3	102.0	41.2	70.8	30.3	481.3
Average	47.5	50.0	49.5	36.8	33.9	43.5	51.5	64.8	79.3	456.8

Statistical Analysis

On the basis of achieved research results the usual variation 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. Relative dependence was defined through correlation analysis (Pearson's correlation coefficient), and the coefficients that were obtained were tested at the 5% and 1% levels of significance.

Results and Discussions

The grain yield of wheat significantly varied across years, from 0.917 t/ha to 4.603 t/ha in 2011/12, from 1.034 t/ha to 4.873 t/ha in 2012/13. In the first investigation year (2011/12), treatment NP_2K achieved the highest yield of grain (4.603 t/ha), and the second investigation year (2012/13), the grain yield of treatments NP_1K and NP_2K achieved the highest yield of grain (4.622 t/ha and 4.873 t/ha). The study showed that among investigated fertilization variants the highest grain yields were achieved in variant NP_2K with 80 kg/ha nitrogen rate, phosphorus rate of 100 kg/ha P_2O_5 and potassium rate of 60 kg/ha K_2O (4.738 t/ha). Results clearly show that yield components were significantly affected by fertilization (Table 2), the lowest values for grain yield and yield components were obtained in the untreated control. Achieved statistically significantly higher yields in 2012/13 were, primarily, the result of heavy rainfall and their good distribution as well as favorable air temperatures during the vegetation period (Table 1).

The study showed that among investigated fertilization variants the highest grain yields were achieved in variant NP_2K with 80 kg/ha N, 100 kg/ha P_2O_5 and 60 kg/ha K_2O. Usage of fertilizers and certain amendments on extremely acid soils in certain years, particularly those less favorable for production, almost certainly had different effects on grain filling, resulting in diverse relationships between productive and qualitative traits. Presented results confirm the opinion of many authors that the traits analyzed are genetically determined, but strongly modified by the nutrient status and weather conditions (Đekić *et al.*, 2014; Jelić *et al.*, 2013).

Table 2. Grain yield, 1000-grain weight and test weight of winter wheat

Traits	Fertilization	Years						Average		
		2011/12			2012/13					
		$\bar{x}$	S	$S_{\bar{x}}$	$\bar{x}$	S	$S_{\bar{x}}$	$\bar{x}$	S	$S_{\bar{x}}$
Grain yield, (t ha^{-1})	C	0.917	0.253	0.113	1.034	0.218	0.098	0.975	0.231	0.073
	N	2.428	0.574	0.257	2.810	0.586	0.262	2.619	0.583	0.184
	P_1	2.554	0.378	0.169	2.798	0.385	0.172	2.676	0.382	0.121
	NP_1	3.462	0.321	0.144	4.031	0.562	0.251	3.746	0.525	0.166
	NP_2	4.020	0.835	0.374	4.329	0.233	0.104	4.174	0.601	0.190
	NP_1K	4.090	0.054	0.024	4.622	0.477	0.204	4.376	0.430	0.136
	NP_2K	4.603	0.406	0.181	4.873	0.497	0.222	4.738	0.450	0.142
1000 - grain weight, (g)	C	33.22	0.626	0.280	38.30	2.081	0.931	35.76	3.044	0.963
	N	36.30	1.859	0.831	42.32	0.701	0.314	39.31	3.438	1.087
	P_1	37.56	0.940	0.420	41.86	1.144	0.511	39.71	2.472	0.782
	NP_1	40.68	4.068	1.819	45.48	0.890	0.398	43.08	3.756	1.188
	NP_2	40.26	2.663	1.191	45.72	0.356	0.159	42.99	3.390	1.072
	NP_1K	41.66	1.951	0.873	44.46	1.352	0.605	43.06	2.164	0.684
	NP_2K	40.26	0.488	0.218	44.24	0.817	0.365	42.25	2.191	0.693
Test weight (kg/hl)	C	70.77	1.968	0.880	70.97	1.339	0.599	70.87	1.590	0.503
	N	70.85	1.020	0.456	70.69	2.238	1.001	70.77	1.642	0.519
	P_1	71.81	1.486	0.664	71.69	2.022	0.904	71.75	1.674	0.529
	NP_1	72.29	1.187	0.531	72.81	0.297	0.133	72.55	0.860	0.272
	NP_2	71.77	1.092	0.488	71.92	1.179	0.527	71.84	1.074	0.340
	NP_1K	73.01	1.802	0.806	74.25	1.612	0.721	73.63	1.740	0.550
	NP_2K	71.49	1.711	0.765	72.57	1.073	0.480	72.03	1.462	0.462

Thousand grain weight of wheat significantly varied across years, from 33.22 g to 41.66 g in 2011/12, from 38.30 g to 45.72 g in 2012/13. During the first year of investigations, the highest average value of 1000-grain weight achieved the NP_1K (80 kg/ha N, 60 kg/ha P_2O_5 and 60 kg/ha K_2O) and NP_1 (80 kg/ha N, 60 kg/ha P_2O_5) treatments (41.66 and 40.68 g). During the second year of investigations

(2012/13), the highest average value of 1000 grain weight achieved the NP_1 (80 kg/ha N, 60 kg/ha P_2O_5) and NP_2 (80 kg/ha N, 100 kg/ha P_2O_5) treatments (45.48 and 45.72 g). The study showed that among investigated fertilization variants the highest thousand grain weight of wheat were achieved in NP_1 (80 kg/ha N, 60 kg/ha P_2O_5) and NP_1K (80 kg/ha N, 60 kg/ha P_2O_5 and 60 kg/ha K_2O) variants (43.08 and 43.06 g). A number of authors (Đekić *et al*., 2013, 2015; Jelić *et al*., 2013, 2014; Jelic *et al*., 2015; Terzic *et al.*, 2018) underlined that 1000-grain weight is a cultivar-specific trait, with considerably higher variations being observed among genotypes than among treatments or environmental factors.

Table 2 presents average values for grain test weight across years and treatments. All testing fertilization variants had test weight greater than 71 kg/hl, except control and 2 variants (80 kg/ha N). During the first year achieved the highest test weight at NP_1K treatment (73.01 kg/hl), followed by NP_1 (72.29 kg/hl), while the lowest test weight was the control treatment (70.77 kg/hl). During the second year of investigations, the test weight of NP_1K variant (80 kg/ha N, 60 kg/ha P_2O_5 and 60 kg/ha K_2O) was the highest with 74.25 kg/hl, while the slightly lower test weight was realized by control and N variants (70.97 kg/hl and 70.69 kg/hl). The average two-year value of test weight the highest at NP_1K treatment (73.63 kg/hl). In all years, the use of different treatments induced a significant increase in grain test weight.

The analysis of yield variance of 1000-grain weight and test weight of tested winter wheat variants grown at investigated in Kragujevac during two growing seasons 2011/12 and 2012/13, are shown in Table 3.

Table 3. Analysis of variance of the tested parameters (ANOVA)

Effect of years on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(1. 68)	p-level
Grain yield (t ha^{-1})	2.1644	1.710507	1.265	0.264589
1000-grain weight (g)	375.8406	9.476050	39.662**	0.000000
Test weight (kg hl^{-1})	3.0243	2.803649	1.079	0.302666
Effect of fertilization on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(6. 63)	p-level
Grain yield (t ha^{-1})	17.40094	0.223386	77.896**	0.000000
1000-grain weight (g)	76.61600	8.897079	8.611**	0.000001
Test weight (kg hl^{-1})	9.65437	2.154702	4.481**	0.000773
Effect of the year x fertilization interaction on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(6. 56)	p-level
Grain yield (t ha^{-1})	0.071510	0.204996	0.345	0.903678
1000-grain weight (g)	2.805238	2.997214	0.936	0.476750
Test weight (kg hl^{-1})	0.777988	2.286679	0.340	0.912616

Based on the analysis of variance, it can be concluded that are highly significant differences at 1000-grain weight regard the year of investigation (F_{exp}=39.662**). Highly significant differences in grain yield, 1000-grain weight and test weight regard the fertilization of investigation (F_{exp}=77.896**; F_{exp}=8.611** and F_{exp}=4.481**). The present results confirm the opinion of many authors that the traits analyzed are genetically determined but are strongly modified by the nutrient status of the environment and weather conditions (Đekić *et al*., 2014; Jelic *et al*., 2015; Terzić *et al*., 2018).The interaction of the investigated factors (Y x G) exhibited no statistically significant effect 1000-grain weight and test weight (P > 0.05).

Positive correlations were observed (Table 4) between grain yield and 1000-grain weight in all years. Wheat yield in 2011/12 was positively correlated with test weight (0.32) and positively but strong-dependent significantly correlated with 1000-grain weight (0.75**). Test weight was positively correlated with 1000-grain weight (0.28). During the second year of investigation (2012/13), correlations between 1000-grain weight and test weight were statistically significant (0.51*). Wheat grain yield was positively but strong significantly correlated with 1000-grain weight (0.81**) and test weight (0.43*) (Table 4). The results suggest that grain yield and quality formation is affected by both genetic and environmental factors (Đekić *et al*., 2015 and Terzić *et al*., 2018).

Table 4. Correlations between the traits analyzed during 2011-2013

Traits	Correlations in 2011/12			Correlations in 2012/13		
	Grain yield, t/ha	1000-grain weight, g	Test weight, kg/hl	Grain yield, t/ha	1000-grain weight, g	Test weight, kg/hl
Grain yield (t ha^{-1})	1.00	0.75**	0.32^{ns}	1.00	0.81**	0.43*
1000-grain weight (g)		1.00	0.28^{ns}		1.00	0.51*
Test weight (kg hl^{-1})			1.00			1.00

Positive correlations were observed (Table 5) between grain yield and 1000-grain weight in all treatments. Positively and medium correlations were also found between grain yield and 1000-grain weight in the NP_1 (r=0.68**), NP_2 (r=0.70**), NP_1K (r=0.77**) and NP_2K (r=0.74**) treatments. Recorded significant correlations between analyzed traits are in agreement with investigations of other authors (Đekić *et al*., 2014; Jelic *et al*., 2015).

Table 5. Correlation coefficients for the traits analyzed across treatments

Correlations between the traits analyzed in the unfertilized control			
	Grain yield, (t ha^{-1})	1000-grain weight, (g)	Test weight, (kg hl^{-1})
Grain yield (t ha^{-1})	1.00	0.13^{ns}	0.12^{ns}
1000-grain weight (g)		1.00	0.09^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the 80 kg/ha N			
Grain yield (t ha^{-1})	1.00	0.37^{ns}	-0.31^{ns}
1000-grain weight (g)		1.00	-0.04^{ns}
Test weight (kg hl^{-1})			1.00

Correlations between the traits analyzed in the 60 kg/ha P_2O_5			
Grain yield (t ha^{-1})	1.00	0.45^{ns}	0.38^{ns}
1000-grain weight (g)		1.00	0.23^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the 80 kg/ha N and 60 kg/ha P_2O_5			
Grain yield (t ha^{-1})	1.00	0.68**	0.20^{ns}
1000-grain weight (g)		1.00	-0.07^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the 80 kg/ha N and 100 kg/ha P_2O_5			
Grain yield (t ha^{-1})	1.00	0.70**	0.41^{ns}
1000-grain weight (g)		1.00	-0.04^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the 80 kg/ha N, 60 kg/ha P_2O_5, 60 kg/ha K_2O			
Grain yield (t ha^{-1})	1.00	0.77**	0.06^{ns}
1000-grain weight (g)		1.00	0.57^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the 80 kg/ha N, 100 kg/ha P_2O_5, 60 kg/ha K_2O			
Grain yield (t ha^{-1})	1.00	0.74**	0.14^{ns}
1000-grain weight (g)		1.00	0.57^{ns}
Test weight (kg hl^{-1})			1.00

The 1000 grain weight had a significant and positive correlation only with the grain weight per spike (Djuric *et al*., 2018). Iftikhar *et al.* (2012) have established that the 1000 grain weight have a positive and statistically significant correlation with grain yield, and emphasize that the number of grains per spike and 1000 grain weight have a direct effect on yield and that, therefore, they can be used as direct selection criteria. Negative correlation between 1000-grain weight and test weight has been established (Terzić *et al*., 2018). Significant and positive correlation between grain yield and nitrogen levels has been established Đekić *et al*. (2014).

Conclusions

Based on the gain results during two-year investigation on seven treatments fertilization, it can be concluded that the highest yield achieved the treatments of three mineral elements NP_2K (N 80 kg/ha, P_2O_5 100 kg/ha and K_2O 60 kg/ha) 4.738 t/ha and NP_1K (80 kg/ha N, 60 kg/ha P_2O_5 and 60 kg/ha K_2O) 4.376 t/ha have achieved satisfactory results, while the poorest results were achieved by the control (0.975 t/ha).

Statistically were highly significantly different between of year on the 1000-grain weight and highly significantly different between of fertilization on the grain yield, 1000 grain weight and test weight. Significantly positively and strong correlated with yield and 1000 grain weight both in 2011/12 (0.75**, respectively), and 2012/13 (0.81**, respectively). Significant positively and medium correlations were observed between grain yield and 1000-grain weight in NP_1 (0.68**, respectively) and NP_2 (0.70**, respectively), and positively and strong correlations were observed in NP_1K (0.77**, respectively) and NP_2K treatments (0.74**, respectively).

Acknowledgments: Investigations necessary for this paper are part of the project TR 31054 and TR 31057, financed by the Ministry of Education, Science and Technology Development of Republic of Serbia.

Conflicts of Interest: The authors declare no conflict of interest.

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Original scientific paper

Particularities of propagation of the Lonicera caerulea through the cultivation in vitro

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Abstract

Cultivation *in vitro* is an alternative method of plant reproduction and preserving of plant resources. We have studied 14 varieties of *Loniceracae rulea* and found out the particularities of *in vitro* cultivation of this plant. As an initial material, we used sprouts 2-3 centimeters long that have one or two internodes which we obtained by the activation of meristems in the laboratory conditions. The sterilization was carried out due to the scheme: 70%-ethanol (60 c) → 0,1 % $HgCl_2$ (7 min) → distilled water (three times, each 10 min). The influence of growth regulators of auxin, cytokinin, and hybberellin effect on a regeneration of microsprouts from vegetative buds of honeysuckle was studied. The range of agar nutrient media Murashige and Skoog (MS), Halupy, and DKW which we have modified was tested. The grows regulators and aminoacids, such as glycine and glutamine, were added to the nutrient media. For all of the varieties of plants, the highest activation of axillary meristems was observed for the cultures on the MS-nutrient medium with addition of 6-BAP, 4 mg/L. We have established that the most of initial tissues are formed on the DKW medium with the addition of 4 mg/L of 6-BAP and DKW with the addition of 1 mg/L of GA_3. The active process of rhizogenesis of newly formed sprouts of honeysuckle was observed on the MS-medium with half of the concentrations of micro- and macronutrients, vitamins, and IBA in concentration of 1 mg/L. The average length of roots was 2 – 3 centimeters. The rooted sprouts were planted on the soil with the high humidity. The part of survived regenerated plants was 93-95%, depending on the variety of the plant.

Keywords: Lonicera caerulea, explant, nutrient medium, growth regulators, rhisogenesis.

Introduction

The blue-berried honeysuckle is a relatively new and promising variety of berry crops in Ukraine; it has a great potential to health promotion. The results of many investigations have shown that fruits of honeysuckle are valuable and have no analogues in the healing nutrition and dietary. The honeysuckle has the longest lifetime of all berry crops that are cultivated in Ukraine. In the nature, the bush lives to 100 years. The main useful properties of this plant are: the early maturation of fruits, high winter resistance (it can withstand to 50°C), long retaining of productivity (20-25 years), fast maturation of fruits, regular fruiting, high contents of vitamins. The honeysuckle has no quarantine parasites and diseases and it does not need chemical protection (Бутенко 1999; Калашникова 2012).

The honeysuckle propagates by seeds, green cuttings, and sprouts; it forms also sprouts from root system. The most effective method is propagation by green sprouts. However, this method demands much work and expensive equipment. During the propagation by woodened sprouts, the

rooting ranged from 10 to 18%. The survival of green sprouts is 20-30% higher. The propagation by seeds leads to variable posterity and the most valuable in agriculture features and properties of honeysuckle (early and high crops, sugar and vitamins content, and other values) (Кушныр & Сарнацька 2005).

The increase of effectiveness of selection of this valuable plant can be performed by using new modern approaches – methods of biotechnology. Among the different methods of cell engineering, the most widely used method is micro-propagation. This method makes the propagation of a valuable genotype in short time possible and accelerates the implementation of new varieties in industry (Митрофанова et al., 2014; Куклина et al. 2003). Furthermore, methods of micro-propagation, especially the cultivating of meristems, are the basis for foundation of collections of valuable genotypes in vitro. In the last decades, such biotechnological methods of propagation have intensively been developed for fruit and berry crops, decorative and agricultural plants (Murashige T., Skoog 1962; Jones et al. 1976; Скворцов & Куклина 2002; Куклина et al. 2003).

For main decorative plants, fruit and berry crops, these methods are insufficiently developed and have no wide application. This can be explained by poor study of limitation factors and high dependence of morphogenesis on genetics. The data of previous researches, described in the references, are contradictory and do not concern the factors of effectiveness of micro-propagation.

The aim of the study is the analysis of influence of some factors (genotype, nutrient medium) on micropropagation of honeysuckle in vitro.

Materials and Methods

The materials of the investigation were the varieties of honeysuckle with high productivity. The varieties of honeysuckle are of Ukrainian and foreign selection: Karyna, Lidia, Donchanka, Stepna, Ukrainka, Bohdana, Vitaminna, Yuhana, Alisiia, Duet, Nimfa, Sinaiaptitsa, Vostorh.

Time of taking the material of honeysuckle for investigation is the beginning of November. After the fungicide treatment Fundazol 1g/L, the maternal plant was putted into a climate chamber with 16 hour of photoperiod, under the increased level of humidity, the temperature was 22±1°C.

As the initial explants for sterile cultures, the up and axillary buds were used. As an initial material the sprouts 2-3 cm long with one or two internodes were used which we obtained by the activation of meristems in the laboratory conditions. The leaves from the sprouts were deleted without damaging a bud, than they were washed by water with a detergent. The sterilization was carried out in several steps: 70%-ethanol (60 c) → 0,1 % $HgCl_2$ (7 min) → distilled water (three times, each 10 min).

The explants were cultivated on an agar nutrient media of Murashige-Skoog (MS), Halupi, and DKW, modified by the authors, with the addition of 6-benzylaminopurine (6-BAP), indole-3-butyric acid (IBA), 6-benzyladenine (6-BA) and aminoacids glycine and glutamine. As a carbohydrate, the sucrose in the concentration of30 g/L was added.

The cultivation of meristems and sprouts was carried out under the temperature of 24±1°C, the humidity was 70-75 %, illuminance was 3000-4000 lux, and the photoperiod was 16 hours. In the end of each cycle, the number of explants, length of sprouts, number of leaves, and the frequency of multiple sprout formation was established. The experiments were carried out three times, during each experiment 20 or more explants were analyzed. The statistics of the data was calculated due to the programs of MicrosoftOffice (Exel 2016).

Results and Discussion

During our investigations, on the stage of initiating the development of a culture, the effectiveness of the sterilization of explants, taken from green sprouts of plants, was 95,9%. This confirms that the scheme of sterilization that we have proposed is effective.

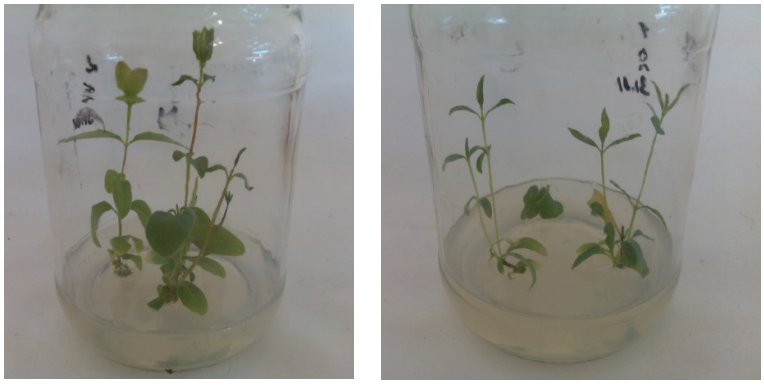
For successful regeneration of plants, the right choice of nutrient media has an especial meaning. After the sterilization, the explants were set on the nutrient medium MS that contained 0,5 mg/L 6-BAP, 0,1 mg/L IBA, 1,5 mg/L glycine and 2 mg/L glutamine; the explants were also planted on the nutrient medium of Halupi.

After 10 – 14 days of cultivation, the activation of axillary buds was observed. After the sterilization, the explants were putted on the MS medium that contained 0,5 mg/L BAP and 0,1 mg/L IBA and on the Halupi medium. After 10 – 14 days of cultivation the activation of axillary buds was observed on the MS medium and not active growth on the Halupi medium as it is shown in Figure 1.

(a) (b) (c)

Figure 1. The growth of explants from the variety of Lidia, the 14th day of cultivation on the nutrient media: **(a)**MS+0,5 мг/л 6-БАП + 0,1 мг/л ІМК + 1,5 мг/л glycine + 2 мг/л glutamine; **(b)**Halupi.

For the induction of the development of axillary buds, the sprouts were cultivated on the nutrient media due to the content of MS with the addition of 6-BAP in the concentrations of 1, 5, 2, 2, 5, 3, 4 mg/L. The concentration of IBA retained the same in all experiments– 0,01 mg/L. When the concentration of cytokinin in the nutrient medium was 4 mg/L, the highest activation of axillary buds was observed; this leads to formation of additional sprouts as shown in Figure 2.



(a) (b)

Figure 2. Multiple formation of sprouts of honeysuckle on the nutrient media MS with addition 4 mg/L 6-BAP: **(a)** The variety Lidiia; **(b)** The variety Vitaminna on the 30th day of the cultivation.

In the experiments with the optimal content of nutrient medium and optimal concentrations of plant

growth regulators, after 30-45 days, the direct morphogenesis was observed during which 3-6 additional sprouts were formed due to the activation of axillary and apical meristems. The additional sprouts had an active growth. The most optimal was the nutrient medium DKW with the addition of 4 mg/L BAP and DKW with the addition of 1 mg/L GA_3. The microsprouts obtained were putted on fresh nutrient media and continued cultivation for increasing of vegetative mass that is shown in Figure 3; the continuation of cultivation increases the propagation coefficient.

(a) (b)

Figure 3. Cutivation of honeysuckle of the variety Donchanka on the nutrient media: **(a)**DKW + 4 mg/L6-BAP; **(b)** - DKW + 1 mg/LGA_3.

The rooting of the microsprouts of honeysuckle were carried out on the media ½MS + 1 mg/LIBAand ½ DKW + 1 mg/LIBA with a half of content of salts and vitamins and 1,5 mg/L glycine. On the 20th day of cultivation, the formation of roots was observed that were 1 cm long, on the 30th day – 2-3 cm, this is shown in Figure 4. All of the varieties were perfectly rooted but the genetic specificity of varieties was observed. The development of sprouts and root system was the most intensive by the varieties of Karyna, Lidia, and Ukrainka.

(a) (b) (c) (d)

Figure 4. Induction of rhizogenesis on the 30th day on cultivating on the medium ½ MS + 1 mg/LIBA + 1,5 mg/Lglycine: **(a)**, **(b)** Karyna;**(c)**,**(d)** Ukrainka.

The formed regenerated plants with developed root system were transferred into the substrate with the composition: turf soil: sand: perlite – 2:1:1. After 27 days the regenerated plants were rooted and on the 100th day the plants were 10-12 cm high and were able to be planted into an

opened soil. The survival of regenerated plants in the soil ranged from 93 to 95% as it is shown in Table 1.

Table 1. The survival rate of regenerated plants of different varieties of honeysuckle in an opened soil.

Genotype (variety)	Planted plants	Survival rate	
		pcs	%
Karyna	46	41	91
Lidia	67	64	95
Donchanka	65	62	95
Skiphska	45	42	94
Stepna	46	43	93
Ukrainka	45	42	93
Bohdana	47	43	91
Vitaminna	45	41	91
Yuhana	48	44	92
Alisiia	46	42	91
Duet	47	44	93
Nimfa	45	41	92
Sinaiaptitsa	40	37	92
Vostorh	38	34	91
$НСР_{05}$		2,2	4,6

The investigations carried out allow to conclude about the particularities of the preparation and sterilization of the plant material of honeysuckle and its propagation *in vitro.*

Conclusions

The results of the researches show particularities of the development of the explants from meristems of 14 varieties of honeysuckle of the Ukrainian and foreign selection: Karyna, Lidia, Donchanka, Stepna, Ukrainka, Bohdana, Vitaminna, Yuhana, Alisiia, Duet, Nimfa, Sinaiaptitsa, Vostorh.

The particularities of the propagation of honeysuckle that we have studied make the using of biotechnological methods for its propagation possible regardless of seasons and promote the economical using of the planting material. The varieties studied have a high percentage of survived plants *ex vivo*.

Conflicts of Interest: The authors declare no conflict of interest.

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Original Scientific paper

Effects of irrigation on production and quality of marigold and basil in different weather conditions

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Abstract

Irrigation improves the water regime of soil creating favorable conditions for growing plants. Frequent oscillations of weather conditions in the region, first of all the amount and distribution of precipitation, are the reason why irrigation is needed in plant production. The aim of the study was to detect the effect of irrigation on the yield and quality of marigold (*Calendula officinalis* L.) and basil (*Ocimum basilicum* L.). The experiments were conducted at the experimental field of the Institute of Field and Vegetable Crops. Irrigation was scheduled on the basis of the water balance method. Daily evapotranspiration (ET_d) was computed from the reference evapotranspiration (ETo) and crop coefficient (kc) in May, June, July and August of 0.5, 0.6, 1.1 and 1.0, respectively. ET_o was calculated using Hargreaves equation. The irrigation depth was restricted to the soil depth of 0.3 m. In other words, irrigation started when readily available water in the soil layer of 0.3 m was completely depleted by plants. The irrigation rate was 30 mm (30 l^{-1} m^{-2}) while the amount of water added by irrigation during the season was 120 mm in 2016 and 300 mm in 2017. According to the results, the yield of fresh herb of all two plants under irrigation was higher compared to non-irrigated, control variant. As well essential oil and carotenoids, the main indicators of the quality of investigation plants were also affected by irrigation.

Key words: Irrigation, dill, marigold, basil, production, quality components

Introduction

Irrigation improves the water regime of soil creating favorable conditions for growing plants. Frequent oscillations of weather conditions in the region, first of all the amount and distribution of precipitation, are the reason why irrigation is needed in plant production. Environmental conditions (weather and soil) have a significant effect on grain yield and quality in sweet basil (Pejić et al. 2017).

Water availability and quality are issues of major concern in reference to irrigation of urban landscapes resulting from the competition with a rapidly growing population that requires vast volumes of good-quality water (Kjelgren et al. 2000). Landscape irrigation accounts for 10% of total volume in the summer rainfall region of the United States, whereas in the arid western regions, irrigation of landscape accounts for nearly 50% (Kjelgren et al. 2000).

In Serbia, basil (*Ocimum basilicum L. Lamiaceae*) has been grown traditionally as a decorative, medicinal, seasoning and ritual herb, and there is a variety of different populations of basil. Basil is considered to have been brought to Serbia in the 12th century by monks returning from their pilgrimages (Picture 1b and c). Essential oils of all tested basil populations were light yellow and had a specific aromatic scent. Basil populations traditionally grown in Serbia have exceptional quality.

They represent an excellent raw material for the production of basil essential oils, for the needs of pharmaceutical, food and chemical industry (Jelačić et al. 2011).

Marigold (*Calendula officinalis* L.), one of the most important ornamental plants, is valued in landscape settings and also as cut flowers (Nau, 1997; Popović, 2010; 2017; 2018). Marigold is grown as an ornamental crop for its flowers (Picture 1a) which are sold in the market as loose flowers in bulk, as specialty cut flowers, or for making garlands. Demand of marigold as cut flower or as a extracted products is high in many countries (Spain, Mexico, UK, United States, Italy, South Korea, Taiwan, Japan). Hence, Exporting of marigold will increase the economic level (Luis et al., 2009).

a. b. c.

Picture 1. Marigold, a, and basil in the field, b and c, Bački Petrovac, Serbia, 2017

Water is becoming an economic scarce resource in many areas of the world, especially in arid and semi-arid regions (Bosma et al. 2003). Agriculture is the largest consumer of global freshwater, accounting for around 70% of withdrawals as irrigation (WWAP, 2009). Increasing the efficiency of water use within agriculture systems is important in order to secure water for agricultural production. Potential water saving strategies has been estimated that, better irrigation scheduling and use of drip irrigation in row crops may save 20% of the water consumption (EU WSPP, 2017).

Proper irrigation scheduling results in increasing water use efficiency (WUE). WUE relates to how much yield is obtained per unit of applied water. Scheduling water application is very critical, as excessive or inadequate irrigations reduce yield, while inadequate irrigation also causes water stress (Sujitha and Shanmugasundaram, 2017).

The aim of the study was to detect the effect of irrigation on the yield and quality of marigold (*Calendula officinalis* L.) and basil (*Ocimum basilicum* L.).

Material and methods

The experiments were conducted at the experimental field of the Bački Petrovac Department, Institute of Field and Vegetable Crops, Novi Sad (N 45°20', E 19°40', 82 msl).Irrigation was scheduled on the basis of the water balance method. Daily evapotranspiration (ETd) was computed from the reference evapotranspiration (ETo) and crop coefficient (kc) in May, June, July and August of 0.5, 0.6, 1.1 and 1.0, respectively.

ETo was calculated using Hargreaves equation. The irrigation depth was restricted to the soil depth of 0.3 m. In other words, irrigation started when readily available water in the soil layer of 0.3 m was completely depleted by plants.

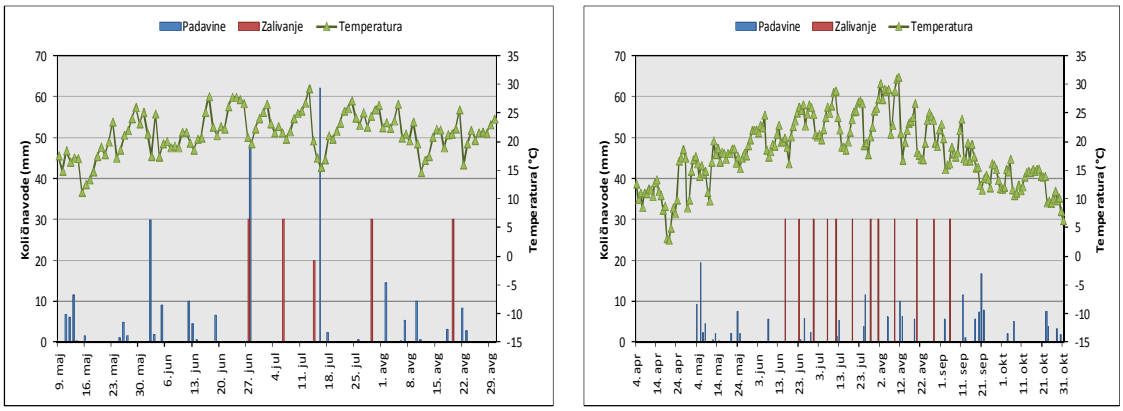
The irrigation rate was 30 mm while the amount of water added by irrigation during the season was 120 mm in 4 watering in 2016 and 300 mm in 10 watering in 2017.

Statistical Analysis

Experiment was set as one factorial split plot method (split-plot), with three replications. Results were interpreted by using a statistical package, statistic 12.

Climatic data

The climatic data for the growing period in Bački Petrovac, near Novi Sad are shown in Figure 1a, b. Irrigation in critical plant development stages is a crucial factor for the successful production. During the vegetation period in 2016 and 2017, there was total precipitation of 440 mm and 219 mm and average temperature of 19.4^{0}C and 21^{0}C, Figure 1a, 1b.



a. b.

Figure 1. Climatic data for the growing period in Bački Petrovac, 2016, a, 2017b, Serbia

Results and Discussion

For all two plant species (marigold and basil) higher yield of biomass was achieved in 2016 compared to arid 2017. Year and irrigation had a statistically significant impact on the yield of biomass for all tested crops, Table 1and Figure 2.

Basil had a significantly higher average biomass yield (27608.50 kg ha^{-1}) compared to marigold (9002 kg ha^{-1}), Table 1 and Figure 2.

The average yield of basil biomass, on average for the study period, varied from 24513 kg ha^{-1} (control) to 30704 kg ha^{-1} (irrigated), Table 1and Figure 2.

The average yield of marigold biomass varied from 7245 kg ha^{-1} (control) to 10759 kg ha^{-1} (irrigated) (Table 1).

Marigold had a higher average carotenoids content in variant with irrigation (0.874 %) compared to control (0.557%), Table 1.

The effect of irrigation on yield it was noticed in both years, rainy 2016 from 8.36% for basil to 8.18% for marigold and in dry 2017, 50.87% at basil to 105.46% at marigold. In irrigation conditions, in both years of production, increased with yields of green biomass and yields of essential oils and carotenoids, as the main indicator quality cultivated crops.

Basil essential oils of all tested basil populations were light yellow and had a specific aromatic scent. The composition of essential oil in the dry herb of the tested populations ranged from 0.87 to 1.84%. The results of gas chromatographic analysis of essential oils in tested basil populations pointed to their complex chemical composition and to the fact that they belong to the most appreciated European chemotype. In total, 33 components have been identified in the essential oils.

The most common fraction of components in all tested oils was terpenoides. The predominant component in all essential oils is monoterpen linalol, ranging from 51.52 to 74.73% (Jelačić et al. 2011).

Table 1. Yield of biomass, kg/ha, essential oil and carotenoids, %, of dill, marigold and basil, 2016-17

Parameter	Year		Data of mowing	Control	Irrigated	Difference	Effect, %	Irrigation norm, mm
Marigold								
Yield of biomass, kg ha^{-1}	2016		30.6-29.8.	8484	9178	694	8.18	120 (4)
	2017		21.6-18.8.	6006	12340	6334	105.46	300 (10)
	Average		-	7245	10759	-	-	-
Carotenoids, %	2017		-	0.5574	0.8738	0.3164	56.76	-
Basil								
Yield of biomass, Kg ha^{-1}	2016	I II X	20.07. 30.08. -	17005 12539 29544	18587 13428 32015	1582 889 2471	9.30 7.09 8.36	120 (4)
	2017	I II X	31.07. 19.09. -	11786 7696 19482	16893 12500 29393	5107 4804 9911	43.33 62.42 50.87	300 (10)
	Average		-	24513	30704	-	-	-
Essential oil content, %	2017			0.632	0.761	0.129	20.41	-

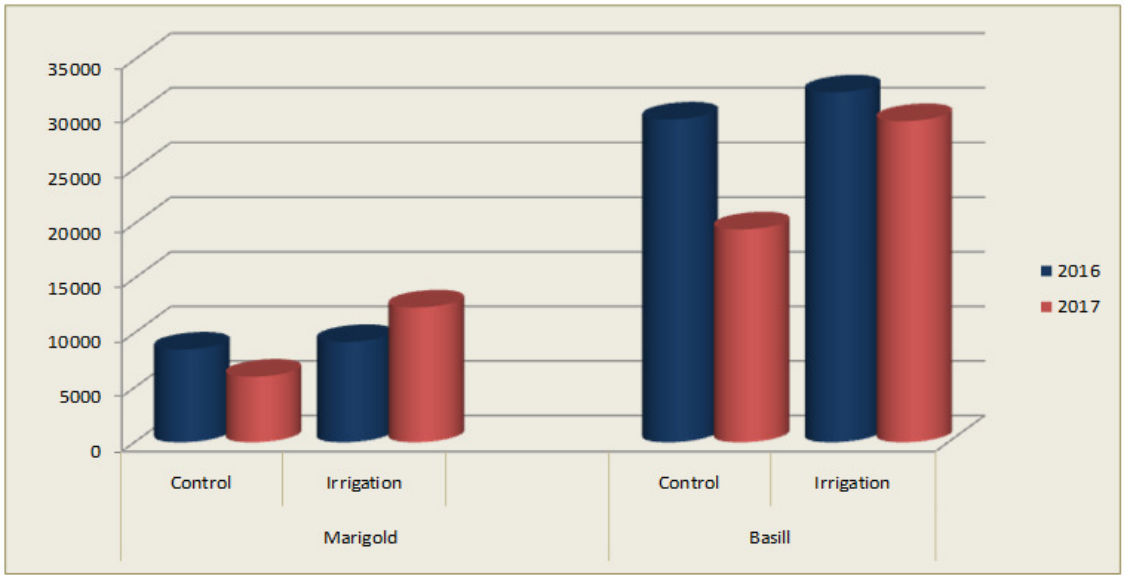


Figure 2. Average yield of biomass in control and irrigated of marigold and basil, 2016-2017

Growth of marigold plants irrigated with water at pH 6.4 significantly decreased as ECw increased; however, the aesthetic value of the plants was not detrimentally affected (Luis et al. 2009). Although, according to literature, dill no great demands on the water, for successful plant production requires a favorable water and air regime in the soil, why it is necessary to irrigate in droughty years, thus achieving high yields of improved quality, and therefore better profitability of growing this spice plants (Maksimovic et al. 2018b). Environmental conditions (weather and soil) have a significant effect on grain yield and quality in sweet basil (Pejić et al. 2017; Maksimović et al. 2018a, 2018b).

Biomass shows a tendency to increase in the years having a higher total amount and better distribution of rainfall during critical plant development stages. Irrigation in these phases is a crucial factor for the successful production of marigold and basil.

Conclussions

According to the results, the yield of fresh herb of all three plants under irrigation was higher compared to non-irrigated, control variant. As well essential oil and carotenoids, the main indicators of the quality of investigation plants were also affected by irrigation.

Environmental conditions have a significant effect on biomass yield and quality in basil and marigold. A more favorable year for the production of biomass was 2016 compared to the drought of 2017.

Basil and marigold populations traditionally grown in Serbia have exceptional quality. They represent an excellent raw material for the production of basil essential oils, for the needs of pharmaceutical, food and chemical industry.

Acknowledgements
Experiment needed for this research is part of the projects TR 31025 and TR 31072 supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Conflicts of Interest: The authors declare no conflict of interest.

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Original Scientific paper

Effects of quantity of nitrogen on maize yield

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Abstract
Maize is one of the leading farm cultures in the world. The primary aim of maize production is to gain high and steady incomes. Varying of the incomes of the grown plants is seen in a great measure both as a consequence of weather conditions and applied quantities of nitrogen fertilizer. In this work, in a two-year period, it is examined how the increasing quantities of nitrogen fertilizer influence the parameters of productivity, that is, morphological features and components of the incomes of Serbian maize hybrid. The examinations were conducted on the locality of Eastern Srem (88 m.s.l.). The examinations covered the following systems of maize fertilization: control (without fertilizer), P_{90} K_{60} N_{30} kg ha^{-1} (basic, phon), P_{90} K_{60} N_{60} kg ha^{-1}, P_{90} K_{60} N_{120} kg ha^{-1}, P_{90} K_{60} N_{180} kg ha^{-1}. Within the morphological features of maize, it is examined the influence of the nitrogen fertilizer on the growth of a plant and the number of leaves on a corncob, and within the components of the income, its influence on the length of a cob, the number of grain rows and the number of grains on a cob. The results of our research have shown that, on the average for the examined factors, the height of a plant was 276.1 cm. On the average for the years, along with the increasing quantities of nitrogen up to 120 kg ha^{-1}, the height of a plant increased, and then it declined. The differences in the height of a plant between treatments were statistically significant and very significant. The number of leaves on a stalk, on the two-year average was 14.0. On the average for the years, along with the increase of nitrogen quantities up to 120 kg ha^{-1}, the number of leaves increased, and then it declined. Statistically significant differences were got between controls and quantities of 60 and 120 kg ha^{-1} N, as well as of phon and 120 kg ha^{-1} of nitrogen. Approximately for the examined factors, the length of the corncob was 20.0 cm. On the average for the years, while the quantities of nitrogen up to 120 kg ha^{-1} increased, the length of a cob grew, and then it stagnated. The shortest length of a cob (18.8 cm) belonged to the controlled plants, and the longest ones (20.6 cm) were those of the plants with the applied of 120 kg/ha^{-1} N. The differences between treatments were statistically very significant. On the average, for the covered factors, the number of grain rows on the cob was 15.2. Between the years of the examinations, some significant difference in the number of grain rows on the cob was not noticed. On the other hand, nitrogen manifested a completely specific influence on this parameter of maize fertility. On the average, within one-year period, with the increase of nitrogen quantity up to 120 kg ha^{-1}, the number of the grain rows increased, and then it stagnated. The differences between treatments were significant and very significant.
Keywords: fertilizer, maize productivity, climate conditions.

Introduction

Maize is one of the leading farm cultures in the world. The primary aim of maize production is to gain high and steady incomes. In order to achieve high incomes of growing plants, it is necessary to

apply agricultural measures in a right way. In that point, in a tecnology of maize production, the choice of hybrids and a proper mineral apply of nitrogen fertilizer has a special importance. (Stojaković et al. 1996; Popović, 2010; 2015; Živanović, 2005; Živanović et al. 2018; Stevanović et al. 2018). Mineral nitrogen nutrition is one of the most significant factors with its influence on productivity and features of plants. Optimal nitrogen nutrition has an influence on a development of the root system and overground biomass as well as on nutritive value of fruits (Glamočlija, 2004; Glamočlija et al. 2015; 2017; Đekić et al. 2014; 2015; Terzić et al. 2018; Božović et al. 2018). Numerous researches here and in the world confirmed that nitrogen mineral fertilizers in a great measure increases the income of maize grains (Brković, 1985; Hojka, 2004; Binder et al. 2000; Šarčević - Todosijević at al. 2016).

On the other hand, increased and uncontroled apply of nitrogen mineral fertilizers, leads to a plenty of phenomena as its consequence which are harmful for plants and the living environment. They include a lengthened vegetation period, a harvest period decrease, an increased sensitivity of plants to patogenic microorganisms and flattening. The apply of nitrogen in the quantities that surpass the needs of plants, leads to the increase of the level of nitrates in soil and their washing off into underground waters. (Schepers at al. 1991; Villar – Mir et al. 2002; Đukić et al. 2007; Glamočlija et al. 2015, 2017).

In this work, in a two-year period, on the locality of Eastern Srem, it is examined how the increasing quantities of nitrogen fertilizer influence of the productivity parameters, that is, morphological features and components of the incomes of Serbian maize hybrid.

Materials and Methods

A microfield testing station was set in Zemun Polje, on the locality of Eastern Srem, on 88 meters altitude. Varying of the incomes of the grown plants is seen in a great measure both as a consequence of weather conditions and applied quantities of nitrogen fertilizer. For undisturbed growth and development of maize, there is a requirement for convenient meteorological conditions, average air temperatures and good regime of rainfall during a vegetation period (Penney et al.; 1996; Schmidt et al., 2002). Eastern Srem is settled in the area of medium continental climate, between two big rivers, the Danube and the Sava. This area is specified by average annual temperature of 11.9^{0}C and the quantity of rainfall is to be 638.3 mm. Winters are with approximate temperatures of 1.6^{0}C and rainfall of 113.4 mm. Springs can be slightly colder (12.1^{0}C) than autumns (12.4^{0}C), but with a higher quantity of rainfall (159.0 mm in relation to 152.1 mm). Average summer temperatures are 21.4^{0}C and rainfall of 213.8 mm. During the research, field micro tests are used, conducted by the split plots method in four goes. The applied technological practices on the examinations was standard, as for regular maize production (Šarčević-Todosijević et al., 2016). The examinations covered the following systems of maize fertilization:

B_1 – Control (without fertilizer),
B_2 – P_{90} K_{60} N_{30} kg ha^{-1} (basis, phon),
B_3 – P_{90} K_{60} N_{60} kg ha^{-1},
B_4 – P_{90} K_{60} N_{120} kg ha^{-1},
B_5 – P_{90} K_{60} N_{180} kg ha^{-1}.

Within the parameters of productivity, it is examined how the increasing quantities of nitrogen fertilizer influence on morphological features and components of the incomes of Serbian maize hybrid. Within the morphological features of maize, it is examined the influence of the nitrogen fertilizer on the growth of a plant and the number of leaves on a maizecob, and within the components of the income, its influence on the length of a cob, the number of grain rows and the number of grains on a cob.

Statistical Analysis

On the basis of achieved research results the usual variation all statistical indicators were calculated: average values. Experimental data were analyzed by descriptive and analytical statistics using the program STATISTICA, 2012. All evaluations of significance were made on the basis of the ANOVA test at 5% and 1% significance levels.

Results and Discussion

The results of our research have shown that the applied quantity of nitrogen had significant impact on maize yield. The differences between treatments were statistically significant and very significant. On the average for the examined factors, the height of a plant was 276.1 cm (table 1).

Table 1. The influence of the nitrogen fertilizer on the growth of a plant, cm

Quantity of nitrogen (B)	Year (A)		Average	Index (%)
	First	Second		
1	273.1	265.1	269.1	100.0
2	279.3	267.8	273.6	101.7
3	280.8	271.1	276.0	102.5
4	285.8	278.3	282.1	104.8
5	287.0	272.4	279.7	103.9
Average	281.2	270.9	276.1	-
Index (%)	100.0	96.3	-	-

LSD	A	B	BxA	AxB
0.05	2.28	2.69	3.80	4.26
0.01	4.19	3.64	5.15	6.53

In the first year of the research, the measured height of a plant was 10.3 cm longer than in the second year of the research. The difference was statistically very significant. On the average for the years, along with the increasing quantities of nitrogen up to 120 kg ha^{-1}, the height of a plant increased, and then it declined. The shortest height (269.1 cm) was measured among the plants on controled variations, while the longest one (282.1 cm) belonged to the plants treated by 120 kg ha^{-1} N. The differences in the height of a plant between treatments were statistically significant and very significant. Observing the years separately, it can be noticed that in the first year of the resreach, with a higher quantity of rainfall, the height of a plant increased to the highest dose of nitrogen (180 kg ha^{-1}), although the interaction was not statistically significant (table 1).

The number of leaves on a stalk, on the two-year average was 14.0 (table 2).

Table 2. The influence of the nitrogen fertilizer on the number of leaves

Quantity of nitrogen (B)	Year (A)		Average	Index (%)
	First	Second		
1	14.1	13.6	13.9	100.0
2	14.2	13.7	14.0	100.7
3	14.3	13.8	14.1	101.4
4	14.5	13.8	14.2	102.2
5	14.3	13.7	14.0	101.1
Average	14.3	13.7	14.0	-
Index (%)	100.0	96.1	-	-

LSD	A	B	BxA	AxB
0.05	0.15	0.11	0.15	0.21
0.01	0.27	0.14	0.20	0.34

Table 3. The influence of the nitrogen fertilizer on the length of a corn cob, cm

Quantity of nitrogen (B)	Year (A)		Average	Index (%)
	First	Second		
1	19.6	18.0	18.8	100.0
2	20.0	18.8	19.4	103.2
3	20.8	19.6	20.2	107.4
4	21.4	20.6	21.0	111.7
5	21.2	19.9	20.6	109.3
Average	20.6	19.4	20.0	-
Index (%)	100.0	94.2	-	-

LSD	A	B	BxA	AxB
0,05	0.89	0.45	0.64	1.11
0,01	1.64	0.61	0.87	1.91

In the first year of the research, with a higher quantity of rainfall and lower temperature, a higher number of leaves for 0.6 was recorded in comparison to the second year of the research, which was drier and warmer. The difference was statistically very significant. On the average for the years, along with the increase of nitrogen quantities up to 120 kg ha^{-1}, the number of leaves increased, and then it declined. The least number of leaves (13.9) was recorded on a variation without fertilizer and the most one (14.2) was recorded at a dose of 120 kg ha^{-1} N. Statistically significant differences were got between controls and quantities of 60 and 120 kg ha^{-1} N, as well as of phon and 120 kg ha^{-1} of nitrogen. In the average as well as per years of the research, a similar tendency of nitrogen influence on the leaves of a stalk was manifested, but without a significant interaction.

Within the components of the maize income, it is examined the influence of the nitrogen fertilizer on the length of a cob, the number of grain rows and the number of grains on a cob.

Approximately for the examined factors, the length of the corn cob was 20.0 cm (table 3).

In the first year of the research, a length of a corn cob was for 1.2 cm longer in comparison to a corn cob in the second year. The difference was statistically significant. On the average for the years, while the quantities of nitrogen up to 120 kg ha^{-1} increased, the length of a cob grew, and then it stagnated. The shortest length of a cob (18.8 cm) belonged to the controlled plants, and the longest ones (20.6 cm) were those of the plants with the applied of 120 kg ha^{-1} N. The differences between treatments were statistically very significant (table 3).

On the average, for the covered factors, the number of grain rows on the cob was 15.2 (table 4).

Table 4. The influence of nitrogen fertilizer on the number of grain rows

Quantity of nitrogen (B)	Year (A)		Average	Index (%)
	First	Second		
1	14.4	14.9	14.7	100.0
2	14.7	15.0	14.9	101.4
3	15.1	15.3	15.2	103.8
4	15.7	15.7	15.7	107.2
5	15.6	15.5	15.6	106.1
Average	15.1	15.2	15.2	-
Index (%)	100.0	100.1	-	-

LSD	A	B	BxA	AxB
0,05	0.29	0.20	0.28	0.41
0,01	0.54	0.27	0.38	0.68

Between the years of the examinations, some significant difference in the number of grain rows on the cob was not noticed. On the other hand, nitrogen manifested a completely specific influence on this parameter of maize fertility. On the average, within one-year period, with the increase of nitrogen quantity up to 120 kg ha^{-1}, the number of the grain rows increased, and then it stagnated. The differences between treatments were significant and very significant.

The number of grains was, on the average for the examined factors, 636.6 (table 5).

Table 5. The influence of nitrogen fertilizer on the number of grains on a cob

Quantity of nitrogen (B)	Year (A)		Average	Index (%)
	First	Second		
1	583.1	585.7	584.4	100.0
2	620.0	622.2	621.1	106.3
3	650.4	654.1	652.3	111.6
4	678.7	659.2	669.0	114.5
5	659.0	653.1	656.1	112.3
Average	638.2	634.8	636.6	-
Index (%)	100.0	99.5	-	-

LSD	A	B	BxA	AxB
0,05	20.68	27.32	38.63	41.71
0,01	37.95	37.02	52.35	62.88

In the first year of the research, the number of grains on a cob was slightly higher than in its second year, but that difference was not justified. Opposite to that, additional nutrition with nitrogen manifested its influence on this component of the income. On the average for the years, along with the increase of nitrogen quantity up to 120 kg ha^{-1}, the number of grains on a cob also increased, and then it mildly stagnated. The least number of grains on a corn cob (584.4) was registered on control, while the highest number (669.0) was registered due to fertilization with 120 kg ha^{-1} N. However, a statistically justified difference was proved only with the use of 60 kg ha^{-1} N. The interaction between the factors, considering the number of grains on a corn cob, was not determined (table 5).

The numerous researchers claim that the effect of the applied mineral fertilizers on the income and the quality of maize grains decreases with the increase of their quantity (Starčević et al, 1994; Blažić, 2006; Šarčević - Todosijević et al. 2016; Janković et al. 2018; Ikanović et al. 2018; Maksimović et al. 2018; Živanović et al. 2018). This noticed pattern was confirmed by the results of our research.

Conclusions

According to the results, there can be drawn the following conclusions.

Applied quantity of nitrogen had significant impact on maize yield. The differences between treatments were statistically significant and very significant.

On the average for the years, along with the increasing quantities of nitrogen up to 120 kg ha^{-1}, the height of a plant, the number of leaves, the length of a cob, the number of the grain rows and the number of grains on a cob increased, and then declined.

According to the conducted research, it can be concluded that on the soil of "černozem" type and in the climate conditions of Eastern Srem, a proper nitrogen nutrition applied on the examined hybrid, implies the use of 120 kg ha^{-1} N, on a phon 90 kg ha^{-1} of phosphorus and 60 kg ha^{-1} of potassium.

Acknowledgments: Experiment needed for this research is part of the projects: TR 31025 supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia and FAO project, Redesigning the exploitation of small grains genetic resources towards increased sustainability of grain-value chain and improved farmers' livelihoods in Serbia and Bulgaria – GRAINEFIT.

Conflicts of Interest: The authors declare no conflict of interest.

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Original Scientific paper

Effects of nutrition on biomass production of Lacy phacelia in organic cropping system

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Abstract

The experiment with *Phacelia tanacetifolia* cultivar NS Priora was conducted during 2018 on experimental plots of the Institute of Field and Vegetable Crops, location in Bački Petrovac, in two variants: control, without nutrition and variant with nutrition, in organic cropping system. Foliar fertilization was applied with Phytograss & clover preparation, by Phytocomplex, two times during the intensive growth of the plants. Phytograss nutrition is a cocktail with micro and macro elements and N (1%), P_2O_5 (0.5%), K_2O (0.05%), S (0.1%), La (0.2 mg kg^{-1}), vitamins, etc. The trial was set up in a randomized block design with three replications. Six parameters were analyzed: biomass yield (t/ha), plant height (cm), length of the leaf (cm), mass of inflorescence (g), length of root (cm) and grain yield per plant (g).

Foliar nutrition had a positive effect on all the tested characteristics. Analysis of variance was found highly significant effect of nutrition on leaf length and yield of biomass. The highest biomass yields were in the variant with nutrition. The yield of green biomass and plant height was higher in a variant with nutrition than the control by 8.9% and 22.18%. Plant height ranged from 70.66 cm in control, up to 86.33 cm in a variant with foliar nutrition. Grain yield per plant ranged from 0.71 g in control, up to 0.96 g in a variant with foliar nutrition. The yield of biomass has a significant positive correlation with grain yield per plant (r=0.77*), length of leaf (r=0.73*), plant height (r=0.66*), mass of inflorescence (r=0.56*), and a higher significant positive correlation with length of root (r=0.83**). Foliar nutrition has shown a significant effect on the production of Lacy phacelia in organic cropping system.

Key words: *Phacelia tanacetifolia,* nutrition, production, organic cropping system

Introduction

Phacelia tanacetifolia Benth. is a annual herb, has a flowering period lasting from 6 to 8 weeks, and which is listed in the top 20 pollen producing flowers for honeybees. Phacelia has been used for seed production and as a forage crops, either on its own or in a mix with peas or vetch to provide forage and honey production as a source of high quality nectar and pollen. Phacelia blooms in the summer months with the blue - purple color blooms which are attracting beneficial pollinators. As a commercial species *Phacelia tanacetifolia* Bent. has long been recognised by beekeepers as a preferred foraging plant for honeybees (Teittinen, 1980; Popovic et al., 2017a) with a high potential for honey yield (Orsi and Bionoi, 1987). Phacelia has also been used as a green manure crop in Europe for a number of years (Anon, 1989). When ploughed as a green manure, increases carbon and nitrogen

content in soil to a depth of over 80 cm (Beckmann, 1977). The crop is also reported to have nematicidal properties (Cazzola, 1987; Anon., 1989; Booker Seeds, 1990). Phacelia has also been used as a forage crop, either on its own (Danial and Zobelt, 1986) or in a mix with peas or vetch to provide forage and honey production (Petkov, 1966; Popovic *et al.*, 2017b). Phacelia has been found to have high energy and protein content, but some questions were raised about possible allelochemical properties of the plant (Danial and Zobelt, 1986). It has a great habit of flowering abundantly and for a long period. This increases beneficial insect numbers and diversity. It's highly attractive to honey bees, bumblebees, etc.

It provides high quantities of nectar, being the second plant after the acacia which gives most nectar. A single flower can give up to 4.5 mg of nectar, with a sugar concentration of 28%. 1ha can produce between 300 and 1000 kg of phacelia honey. It is a sweet and complete flower for the honey bees because it provides both pollen (for protein – needed for egg production) and nectar (for carbohydrates – needed for energy). For humans it is highly important as it provides us with honey in times when other flowers cannot resist the bad weather conditions (Foucault et al., 2013; Popović et al., 2016; 2017a; 2017b; 2018). Phacelia produces relatively abundant biomass. Protein content ranges from 6.7% to 19.8% at the pre-bloom stage (Popović et al., 2017). Phacelia are suitable for the remediation of soils contaminated with heavy metals. Green manure plants absorb nitrogen from the soil, preventing its leaching out of the soil (Foucault et al., 2013).

Picture 1. Lacy phacelia field, Bački Petrovac, 2018 (Photo: Popović, 2018)

The crucial importance play equilibred nutrition by nitrogen and phosphorus with higher impact of phosphorus nutrient. Mineral fertilizers play a vital role towards improving crop yields but one of the main constraints in achieving proven crop potential is imbalanced use of nutrients, particularly low use of phosphorus as compared to nitrogen (Đekić *et al*. 2014). Authors found it to be absolute mass the grain has a significant influence on the application of mineral fertilizers, was significantly higher in intensified fertilizer treatments, especially nitrogen. The optimum rate of phosphorus application is important in improving yields of most crops (Đekić *et al*. 2013, 2014; Glamočlija *et al*., 2015; Terzić *et al*., 2018).

The study was to determined the effect of foliar nutrition on the yield parameters of phacelia cultivar NS Priora.

Materials and Methods

Experimental design and soil conditions

Experiment with phacelia cultivar NS Priora carried out on experimental field of Institute of Field and Vegetable Crops at certified plots in Bački Petrovac, Serbia, in 2018 in two variants: 1. Control; and 2.

Variant with foliar nutrition. The standard technology for growing c. NS Priora was applied during the experiment. Sowing was carried out at the optimum time (10 kg ha^{-1}). Crop cultivation were applied during the vegetation period. Nutrition was aplied in two times before flowering plants. The harvest is carried out manually in technological maturity, after 130 days. Plant height (cm), yield of green biomass (kg ha^{-1}) and seed yield per plant were investigated.

Chemical caracteristic of soil

Chernozem soil at a depth of 30 cm was low in humus (2.32%), slightly alkaline reaction, pH in H_2O was 7.35., medium carbonat soil (4.64%, $CaCO_3$), hight level Al–K_2O and P_2O_5 (37.50 mg/100 g, 35.90 mg/100 g soil).

Climatic data of the experimental area

The climatic data for the growing period 2018 in Bački Petrovac, near Novi Sad, in the Vojvodina region, Serbia, are shown in Fig. 1.

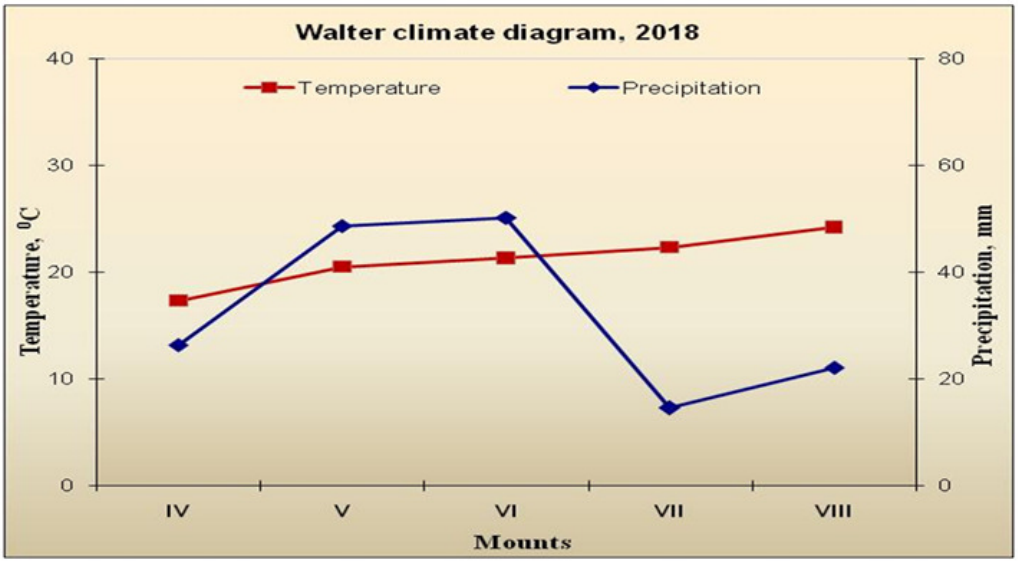

Fig. 1. Mean monthly air temperature and total precipitation, 2018, Bački Petrovac, Serbia

During the vegetation period in 2018, there was total precipitation of 368.20 mm and average temperature of 20.7^{0}C.

Statistical Analysis

Experiment was set as one factorial split plot method (split-plot), with three replications were analyzed with ANOVA by descriptive and analytical statistics. Results were interpreted by using a statistical package Statistics 12. Relative dependence was defined through correlation analysis (Pearson's correlation coefficient), and the coefficients that were obtained were tested at the 5% and 1% levels of significance.

Results and Discussion

For successful production it is necessary to select several stability and adaptability genotypes, most suitable for a certain agro-ecological area. Genotype is just one of the many, but also the most important factor of production, whose effect, fortunately, can be controlled (Popović, 2010; 2015). There are many definitions of stability and adaptability but the following ones prevail. Stability is the

ability of a genotype to have always the uniform yield regardless of environmental effects (Hill et al., 1998; Citate: Becker, 1981). Adaptability is the ability of a variety to provide stable and high yield under different environmental conditions (Hill et al., 1998; Citate: Finly and Wilkinson, 1963). Stability and adaptability of genotypes are best assessed by evaluating the cultivars in different environments and ecological regions (Božovic et al., 2018; Jankovic et al., 2018). Grain yield is one of the most important parameters for estimating cultivar value, in almost all programs of selection and breeding of standard grain quality.

The analysis of variance of tested productivity parameters of cultivar phacelia NS Priora during to 2018 years, are shown in Table 1.

Table 1. Analysis of variance for tested parameters

Effect	SS	Deg.of Freed.	MS	F	p
Yield of crude biomass					
Intercept	3308812	1	33088012	11959.52	0.00000
Variant	58017	1	58017	20.97**	0.00102
Error	11067	4	2767		
Plant height					
Intercept	36973.50	1	36973.50	364.87	0.00004
Variant	368.17	1	368.17	3.6332	0.12933
Error	405.33	4	101.33		
Leaf length					
Intercept	1320.16	1	1320.17	360.05	0.00005
Variant	60.17	1	60.17	16.41**	0.01547
Error	14.67	4	3.67		
Length of root					
Intercept	620.16	1	620.17	61.00	0.00145
Variant	20.17	1	20.17	1.98	0.23178
Error	40.66	4	10.17		
Mass of flowering					
Intercept	55.3281	1	55.3281	20.007	0.01105
Variant	9.3251	1	9.3251	3.372	0.14019
Error	11.0616	4	2.765		
Grain yield per plant					
Intercept	4.2168	1	4.2168	149.09	0.00026
Variant	0.0938	1	0.0938	3.315	0.14278
Error	0.1131	4	0.0283		

Based on the analysis of variance, it can be concluded that are highly significant differences at yield of crude biomass regard the nutrition of investigation (F_{exp}=20.97**). Highly significant differences in leaf length regard the nutrition of investigation (F_{exp}=16.41**), Table 1.

Results clearly show that at yield of crude biomass were significantly affected by foliar nutrition (Table 2). The yield of crude biomass of c. phacelia NS Priora significantly varied between variant, from 2250 kg/ha (in control) to 2446.66 kg/ha, in variant with nutrition, Table 2.

Nutrition was positively effects with tested productivity parameters of c. NS Priora. The plant height of c. phacelia NS Priora varied between variant, from 86.33 cm (in control) to 70.66 cm, in variant with nutrition. Leaf length of c. phacelia NS Priora varied between variant, from 18.00 cm (in control) to 11.67 cm, in variant with nutrition. Length of root of c. phacelia NS Priora varied between variant, from

8.33 cm (in control) to 12.00 cm, in variant with nutrition. Grain yield per plant varied between variant, from 0.72 g (in control) to 0.96 g, in variant with nutrition, Table 2.

Table 2. Descriptive statistics of tested parameters of cultivar NS Priora

Parameter	Factor	No repl.	Mean	Std. dev.	Std. Error	-95.00%	+95.00
Yield of crude biomass, kg/ha							
Total		6	2348.33	177.54	47.98	2224.97	2471.68
Variant	1	3	2250.00	50.00	28.87	2125.79	2374.20
Variant	2	3	2446.66	55.08	31.79	2309.85	2583.48
Plant height, cm							
Total		6	78.50	12.44	5.08	65.45	91.55
Variant	1	3	70.66	10.07	5.82	45.66	95.67
Variant	2	3	86.33	10.06	5.81	61.33	111.33
Leaf length, cm							
Total		6	14.83	3.86	1.58	10.77	18.89
Variant	1	3	11.67	2.52	1.45	5.41	17.92
Variant	2	3	18.00	1.00	0.58	15.52	20.48
Length of root, cm							
Total		6	10.16	3.48	1.42	6,51	13.83
Variant	1	3	8.33	4.04	2.33	1.71	18.37
Variant	2	3	12.00	2.00	1.15	7.03	16.97
Mass of flowering, g							
Total		6	3.04	2.02	0.82	0.91	5.16
Variant	1	3	1.79	0.38	0.22	0.83	2.74
Variant	2	3	4.28	2.32	1.33	1.48	10.05
Grain yield per plant, g							
Total		6	0.84	0.20	0.08	0.63	1.05
Variant	1	3	0.72	0.18	0.11	0.25	1.17
Variant	2	3	0.96	0.14	0.09	0.59	1.33

Figure 2 shows a graphic arrangement and a comparison of nutrition according to the expression of plant height, grain yield/plant and yield of biomass and in Figure 3, a comparison of nutrition according to the expression of leaf length, mass of flowering and length of root.

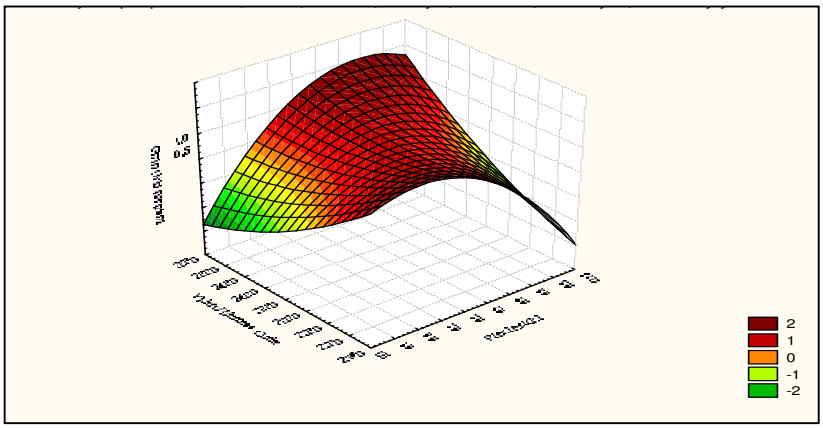

Fig. 2. 3 D Surface Plot for plant height, grain yield/plant and yield of biomass

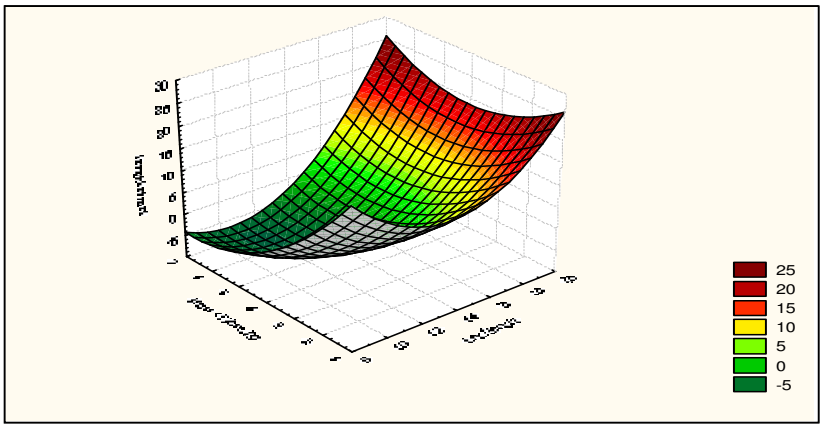

Fig. 3. 3 D Surface Plot for leaf length, mass of flowering and length of root

Correlations of tested parameters

Agro-ecological and agro-technical practices have a significant effect on plant productivity (Popović, 2010; 2015; Kosev *et al.*, 2018). Nutrition was significantly positively and strong correlated with yield of biomass crude and leaf length (r=0.92** and r=0.90**), and significantly positively correlated with plant height, grain yield per plant, mass of flowering and length of root (r=0.69*, r=0.67*, r=0.68* and r=0.58*).

The yield of biomass has a strong significant positive correlation on length of root (r=0.83), and positive correlated of plant height (r=0.66), leaf length and grain yield per plant(r=0.77), Table 3.

Table 3. Correlation coefficients for all tested traits

Parameters	Plant height	Yield of crude biomass	Leaf length	Grain yield per plant	Mass of flowering	Length of root	Nutrition
Plant height	1.00	0,66*	0,76*	0.82**	0.79*	0.31^{ns}	0.69*
Yield of crude biomass	0.66*	1.00	0.73*	0.77*	0.56*	0.83**	0.92**
Leaf length	0.76*	0.73*	1.00	0.57*	0.72*	0.25^{ns}	0.90**
Grain yield per plant	0.82**	0.77*	0.57*	1.00	0.40^{ns}	0.54*	0.67*
Mass of flowering	0.79*	0.56*	0.72*	0.40^{ns}	1.00	0.30^{ns}	0.68*
Length of root	0.31*	0.83**	0.25^{ns}	0.54*	0.30^{ns}	1.00	0.58*
^{ns} – non significant; * and ** significant at 0.1 and 0.5							

Significant and positive correlation between grain yield and nitrogen levels has been established Đekić *et al.* (2014).

NS Priora had high grain yield good quality. Grain yields of NS Priora in 2016 is 902 kg ha^{-1} and flowering plant continues over 60 days (Popović et al, 2017c). Average germination seed, of NS Priora seeds harvested in 2016, was 87% and average germination energy was 77%. The average thousand seeds weight was 1.42 g. NS Priora variety had average nitrogen content is 3.21% and protein content was 20.06% (Popovic et al., 2017c).

Conclusions

Nutrition was positively effects with tested productivity parameters of c. NS Priora. Plant height was average 78.50 cm, and varied at 70.67 cm (control) to 86.33 cm (nutrition). Leaf length was average 14.83 cm, and varied at 11.67 cm (control) to 18.00 cm (nutrition). Grain yield per plant was average 0.84 g, and varied at 0.77 g (control) to 0.96 g (nutrition). Yield of biomass was average 2348 kg/ha, and varied at 2250 kg/ha (control) to 2446 kg/ha (nutrition).

The yield of biomass has a strong significant positive correlation on length of root (r=0.83), and positive correlated of plant height (r=0.66), leaf length and grain yield per plant(r=0.77). Nutrition was significantly positively and strong correlated with yield of biomass crude.

Acknowledgments: Supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia; Projects: TR 31024 and TR 31025.

Conflicts of Interest: The authors declare no conflict of interest.

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Original Scientific paper

Effects of foliar nutrition on production biomass of broomcorn millet (*Panicum miliaceum* L.)

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Abstract

The cultivar Biserka, in the experiment carried out in 2018, in the experimental field of Institute of Field and Vegetable Crops, in Bački Petrovac, has achieved excellent agronomic characteristics. Stems of cultivar Biserka was light green, erect, sometimes branched at the base, and grow 0.9–1.3 m tall. Leaves alternate along the stem were covered with short hair and arching leaves may reach and over 30 cm length. Plants have shallow, fibrous root systems and produce few tillers. Cultivar Biserka has a drooping branched compact inflorescence 20–30 cm long made of many stalked, ovoid spikelet and reproduces by seeds yellow color, 2.5–3 mm long.

Analysis of variance was found highly significant effect of nutrition in all tested characters. Foliar nutrition had the most highly significant influence on plant height. Investigation where showed a considerable variation of biomass yield which was independence from foliar nutrition. The highest biomass yields were in the variant with nutrition. Plant height was statistically significantly higher in the variant with foliar nutrition compared to the control a difference of 17.67 cm was achieved or 18.73%. The yield of green biomass in a variant with nutrition was higher than the control by 8.2%. Seed yield per plant ranged from 6.52 g in control, up to 9.24 g in a variant with foliar nutrition. The yield of biomass has a significant positive correlation on plant height (r=0.79), length of leaf (r=0.99) and grain yield per plant (r=0.93).

Nutrition was significantly positively and strong correlated with plant height and grain yield per plant (r=0.99 and r=0.92), and significantly positively correlated with yield biomass (r=0.74).

Keywords: *millet, foliar nutrition, productivity, biomass yield, correlation*

Introduction

Millets are small-seeded annual cereals grown for food, feed, forage, and fuel. About 20 different species of millet have been cultivated throughout the world at different points in time (Fuller, 2006). Commonly cultivated millet species include proso millet (*Panicum miliaceum* L.), pearl millet (*Pennisetum glaucum* L.R. Br.), finger millet (*Eleusine coracana*), kodo millet (*Paspalum setaceum*), foxtail millet (*Setaria italica* L. Beauv.), little millet (*Panicum sumatrense*), and barnyard millet (*Echinochloa utilis*). Millet ranks sixth among the world's most important cereal grains, sustaining more than one-third of the world's population. Proso millet was likely domesticated in China sometime around 10,000 BP. Asian and African countries are the biggest millet producers (Glamoclija et al., 2015; FAO 2017, Graph. 2). Millet has many nutritional and medical functions. Millets are rich in health-promoting considered functional foods. Consumption of millet and other millets is associated with

reduced risk of type-2 diabetes mellitus because whole grains like millet are a rich source of magnesium. Magnesium acts as a co-factor in a number of enzymatic reactions that regulate the secretion of glucose and insulin. Magnesium can also reduce the frequency of migraine headaches and heart attacks, and is beneficial for people suffering from atherosclerosis and diabetic heart disease. Since millets are high in fiber, antioxidants, and complex carbohydrates, they can be valuable in preventing CVD and cancer (Habiyaremye et al., 2017). It is currently the economic force of Brazil and United States where biomass contributes in three percent of their total energy resources. Millet can be used as a quick growing catch crop planted into corn and sorghum stubble fields. It does well plant in combination with cowpea or soybeans. It has one of the lowest water requirements of any cereal (Lyon et al., 2008), and could be useful in low-input sustainable systems. Earlier summer (June) plantings produce the greatest biomass. It can be terminated by mowing or disking. The aim of the research was tested influence of foliar nutrition on production biomass of millet c. Biserka.

Millet and sorghum are among the oldest of the cereals, and in the ancient world they were common bread grains (Glamoclija et al., 2015; Sikora et al., 2016; Ikanovic et al., 2011; 2017). Sorghum and millet are two of the most basic foods for the poor and rural people in the dry regions that are poor in terms of other grains. When the production regions of these products are reviewed, it is seen that Africa, Central America and South Asia are at the front. Used in various fields such as human food, feed and biofuel; these products are an important food source for the African countries that are especially poor in terms of other grain products. In some parts of Turkey, millet is another name for corn and so most of the time millet is mistaken for corn. However, millet is cultivated around the world for food and feed; it has smaller seeds than corn; it is a really different cereal in comparison to corn in terms of appearance of its plant. Flowers of all millet species stand upright and inclined in shape of dense or sparse bunches. Its seeds do not form a regular sequence around the rachis as it is in wheat or barley. Efficiency and nutrition of millet seeds are much lower than most of the other grains. Liking sandy soil and being drought-tolerant, millet is summer crop which is cultivated in spring. It is warm climate plant which does not need rainfall and can be cultivated even in most arid lands. It is not affected by heavy rainfall. Proso millet and foxtail millet do not like moisture much. Millet seeds sprout in 8-12 ^{0} degree (Anderson, 1949; 2016).

Vast quantities of agricultural and agro-industrial residues that are generated as a result of diverse agricultural process represent one of the most important energy-rich resources. Accumulation of this biomass, in large quantities, results in a huge loss of valuable nutritional materials as potentially useful for man and animals. Biomass contributes about twenty-five percent of the world energy requirement equivalents to twenty million barrels of fuels of oil per day. It is currently the economic force of Brazil and the United States where biomass contributes in three percent of their total energy resources (Bassaria, 2003). Millet can be used as a quick growing catch crop planted into corn and sorghum stubble fields. It does well plant in combination with cowpea or soybeans (Schonbeck & Morse, 2006). It has one of the lowest water requirements of any cereal (Lyon et al., 2008), and could be useful in low-input sustainable systems. Earlier summer (June) plantings produce the greatest biomass (Schonbeck & Morse, 2006). It can be terminated by mowing or disking. Winter wheat has been successfully no-till planted into millet stubble in the fall in the Great Plains (Lyon et al., 2008).

Agro-ecological and agro-technical practices have a significant effect on plant productivity (Popović, 2010; 2015; Đekić et al., 2014; 2015; Maksimovic et al., 2018; Ugrenović et al., 2018;). The small millets are valued by traditional farmers for their nutritional content and health-promoting properties, ability to grow under low input conditions and tolerance to extreme environmental stress, especially drought. In a world facing limiting natural resources and climate change, these crops thus hold tremendous potential as valuable instruments in the toolkit of the New Green Revolution. It is hoped that germplasm resources combined with modern genomic tools can help to accelerate the exploitation of this biodiversity (Goron & Raizada, 2015). Nutrition plays a vital role in improving biomass yields and productivity of millet.

The aim of the study was to determined the effect of foliar nutrition on the yield parameters of millet (*Panicum miliaceum* L.).

Material and methods

This paper analyses the cereals production parameters in the world for the 2016. The data for cereals production in the world were taken from the FAO database (http:// faostat.fao.org/), and data of millet production, c. Biserka, taken from the Institute of Field and Vegetable Crops, Novi Sad, Serbia.

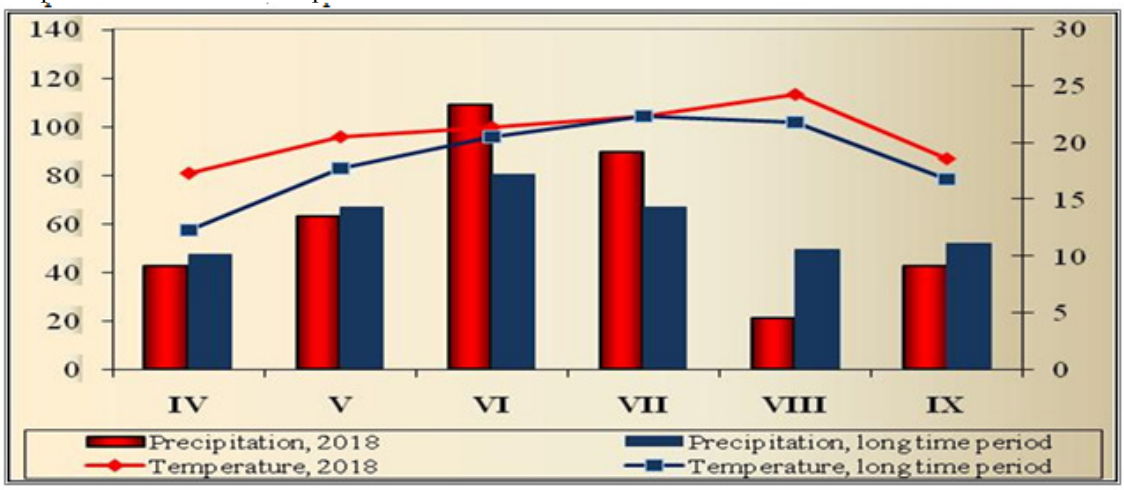
Experiment with millet cultivar Biserka was carried out on experimental field of Institute of Field and Vegetable Crops at certified plots in Bački Petrovac, Serbia, 2018 in two variants: 1. Control; and 2. Variant with foliar nutrition. The millet cultivar Biserka used in the experiment is a cultivar developed by the Institute of Field and Vegetable Crops, from Novi Sad, Serbia. The standard technology for growing c. Biserka was applied during the experiment. Sowing was carried out at the optimum time (50 kg ha^{-1}). Crop cultivation was applied during the vegetation period. This investigation included: foliar nutrition and control without nutrition. Foliar fertilization was applied with Phytocereals preparation, two times during the intensive growth of the plants. Phytocereals preparation is a cocktail with micro and macro elements, vitamins, amino acids, and growth stimulants. The trial was set up in a randomized block design with three replications. Nutrition was applied two times before flowering plants. The crop was harvested at full maturity. The harvest is carried out manually in technological maturity, after 115 days. Four parameters were analyzed: plant height (cm), length of the leaf (cm), biomass yield (t/ha) and grain yield per plant (g).

Statistical Analysis

The experiment was set as one factorial split-plot method, with three replications. Results were interpreted by using a statistical package, Statistic 12. Relative dependence was defined by method of correlation analysis.

Climatic data

The climatic data for the growing period in Bački Petrovac, near Novi Sad, are shown in Graph. 1a, b. During the vegetation period in 2018, there was total precipitation of 368.20 mm and an average temperature of 20.70 ^{0}C, Graph 1.



Figue-. 1. Meteorological data for the growing period of millet in Bački Petrovac, 2018, Serbia

Results and Discussion

World millet production

Of the total world cereals production (2814.98 mil. t) 90.62% were under maize (37.66%), wheat (26.63%) and rice (26.33%). The ten most important cereals in the world sowed at area of 702.40 mil. ha (wheat at 220.11 mil. ha, maize to 187.96 mil. ha, rice to 159.81 mil. ha, barley at 46.92 mil. ha, sorghum to 44.77 mil. ha, millet on 31.71 mil. ha, 4,40 mil. ha at rye, triticale to 4.16 mil. ha, buckwheat on 2.37 mil. ha and quinoa at 0.19 mil. ha, Tab. 1.

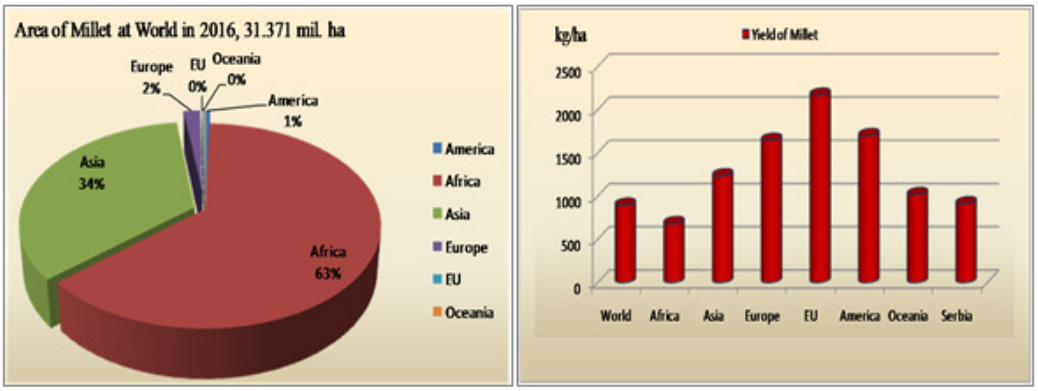
Table 1 Cereals production in the World, 2016

Cereals in World	**Area** **mil. ha**	**Yield** **t ha^{-1}**	**Production** **mil. t**	**Chare of production, % ***
10 Cereals	**702.40**	**2.75**	**2814,79**	**100.00**
Maize	187.96	5.64	1,060.10	37.66
Wheat	220.11	3.41	749.46	26.63
Rice	159.81	4.64	740.96	26.33
Barley	46.92	3.01	141.28	5.02
Sorghum	44.77	1.43	63.93	2.27
Millet	31.71	0.90	28.36	1.01
Triticale	4.16	3.66	15.22	0.54
Rye	4.40	2.94	12.94	0.46
Buckwheat	2.37	1.01	2.39	0.08
Quinoa	0.19	0.80	0.15	0.005
Source: Fao, 2018, http://www.fao.org/faostat/en/#data/QC, *Calculated of authors				

Today millet is an important grain product for the developing countries in Asia and Africa with semi-arid tropical climate. Millet flour is used to make bread and also to make alcohol and boza after fermented. It is used as feed for birds and is one of the most important foodstuff in North African countries. In these regions millet seeds are consumed as mash or flatbread after boiled or milled. In addition, stems and seeds of all kinds of millets are used as animal feed (Anderson, 1949).

According to the data of FAO showing 2016, global harvest area for millet was 31.71 mil. ha and have average yield of 900 kg ha^{-1}, Graph. 2a, 2b.

According to FAO which announced that world millet production amount in the 2016 years is 28.36 million tons, world millet production. According to the estimates of FAO in 2016, the most important producers are Africa and Asia. World's largest consumption in millet used mostly as human food and feed is India that also ranks first in the production. India's millet consumption is slightly higher than the production, Table 1, Graph. 2a and 2b.



a.

b.

Figure 2. Area, ha, and grain yield of millet in world, 2016

Millets are generally among the most suitable crops for sustaining agriculture and food security on marginal lands with low fertility. Millet crops are grown on marginal lands and under low-input agricultural conditions—situations in which major cereal crops often produce low yields (Amadou et al., 2013). Millet can be productive even under harsh growing conditions, especially in regions such as India and Sub-Saharan and West Africa, where average rainfall is often less than 500 mm and soils are sandy and slightly acidic (Changmei and Dorothy, 2014).

Of all the millets cultivated in Africa, 74% are grown in Sub- Saharan and West Africa, accounting for 28% of the world's production (Changmei and Dorothy, 2014). Historically, millets and other warm season crops such as sudan grass and sorghum were grown as forage and grain feed for livestock and birds in different regions of Oregon (Schoth and Rampton, 1939).

The planting time of millet fits well in rotation with winter annual crops such as winter wheat or warm-season broad leaf crops such as sunflower (Herdrich, 2001). Successful millet production in Nebraskai sattributed to the practice of eco - fallow—planting millet in standing wheat stubble in the spring to control weeds and to conserve stored soil moisture (Anderson, 1990).

Effect foliar nutrition of millet plants

Nutrition in critical plant development stages of millet is a crucial factor for the successful production. Nutrition was highly significant effect of all tested morphological characteristics. Total biomass yield was 29.93 t ha^{-1}. The highest biomass yields were in the variant with nutrition. The yield of green biomass in a variant with nutrition was higher than the control by 8.2%, graph. 3. Average plant height was 1.3 m. Foliar nutrition had the most highly significant influence on plant height. Plant height was statistically significantly higher in the variant with foliar nutrition compared to the control a difference of 17.67 cm was achieved or 18.73%.

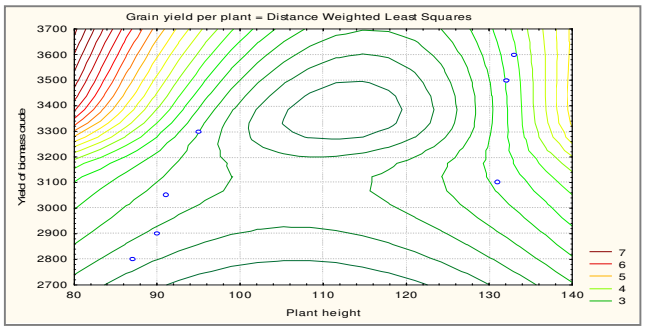


Figure 3. 3D Contour Plot at grain yield/plant, biomass yield and plant height at millet, 2018

Average seed yield per plant was 7.88 g and ranged from 6.52 g in control, up to 9.24 g in a variant with foliar nutrition. Plants of c. Biserka was an average drooping branched compact inflorescence 30 cm long and reproduces by seeds yellow color, 2.5–3 mm long.

Correlation of tested parameters

Agro-ecological and agro-technical practices have a significant effect on plant productivity (Popović, 2010; Đekić et al., 2013; 2015). Nutrition was significantly high positive correlated with plant height and grain yield per plant (r=0.99 and r=0.92), and significantly positively correlated with yield biomass (r=0.74) and with leaf length (r=0.69), Table 2.

Table 2. *Correlation of tested parameters*

Variable	Biomass yield	Grain yield/plant	Plant height	Leaf length	Nutrition
Yield of biomass	-	0.93**	0.79*	0.99**	0.74*
Grain yield/plant	0.93**	-	0.95**	0.91**	0.92**
Plant height	0.79*	0.95**	-	0.75*	0.99**
Leaf length	0.99**	0.91**	0.75*	-	0.69*
Nutrition	0.74*	0.92**	0.99**	0.69*	-
*and ** significant at 0.05 and 0.01					

The yield of biomass has a significant positive correlation on plant height (r=0.79), leaf length (r=0.99) and grain yield per plant (r=0.93), Table 2.

The average protein contents of millet was 12.5%, the carbohydrate was 70.4%, fat 3.4%, fiber was 14.2% and Ca 14%, Table 3.

Table 3. Nutritional composition of millet (*Panicum miliaceum* L.) (in 100 g).

Parameters	Protein, %	Carbo-hydrate, %	Fat, %	Dietaly fiber, %	Mineral mather, %	Ca, %	P, %	Fe, mg
Millet	12.5	70.4	3.1	14.2	1.9	14	206	10
Higher than major cereal sand wheat, adapted from Saha et al. (2016)								

Millets are a major source of energy and protein and have high nutritive value, comparable to major cereals such as wheat, rice, and maize (Amadou *et al.*, 2013; Saleh *et al.*, 2013). Millets are unique among the cereals because of their high calcium, iron, potassium, magnesium, phosphorous, zinc, dietary fiber, polyphenols, and protein content (Hulse et al., 1980; Devi et al., 2014; Gupta et al., 2014).

Millets are gluten-free, ideal for people who are gluten-intolerant, though millet flour cannot be used for raised bread (Hulse et al., 1980; Thompson, 2009; Amadou et al., 2013; Santra, 2013). Millets are easy to digest.

Conclusion

Millet is an important grain product for the countries with semi-arid tropical climate. Millets are valued by traditional farmers for their nutritional content for health, ability to grow under low input conditions and tolerance to extreme environmental stress, especially drought. In a world facing limiting natural resources and climate change, these crops thus hold tremendous potential as valuable instruments in the toolkit of the New Green Revolution.

According to the data of FAO showing 2016, global harvest area for millet was 31.71 mil. ha, production was 28.36 mil. t and was average yield of 900 kg ha^{-1}. The share of the millet in the total world production of cereals was 1.01%.

Foliar nutrition had the most highly significant influence on plant height and yield of biomass of millet. Nutrition plays a vital role at improving biomass yields and productivity of millet.

Acknowledgment
Experiment needed for this research is part of the projects: TR 31025 supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia and FAO project. Redesigning the exploitation of small grains genetic resources towards increased sustainability of grain-value chain and improved farmers' livelihoods in Serbia and Bulgaria – GRAINEFIT.

Conflicts of Interest: The authors declare no conflict of interest.

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Original Scientific paper

Composition Investigation of the Sunflower Seed of the Latest NS Confectionary Hybrids

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Abstract

Because of the favorable amino acid composition of sunflower protein and low content of antinutritive components, the confectionary/non-oil sunflower seed is increasingly gaining in importance. This is why there is a need for increasing the number of this type of hybrids. Breeding new hybrids of different characteristics are obtained. In order to investigate the composition of the seed, three samples of the latest NS hybrids of second filial (F2) generation (NS-H-6792, NS-H-6489, NS-H-6311) were analyzed and compared with the results of the hybrid seed already-used (Cepko). The content of moisture was determined according to SRPS EN ISO 665, 2008, the content of the oil according to SRPS EN ISO 659, 2011, while the mass of 1000 seeds and the content of hull and kernels were made according to Karlović & Andrić, 1996. The moisture content ranged from 5.75±0.00% in the sample NS-H-6792 to 6.57±0.03%, in the sample NS-H-6489. The oil content was in the range of 25.62±0.22%, found in the sample NS-H-6311 to 23.12±0.16%, found in the sample NS-H-6489. The highest content of the hull was found in the NS-H-6792 sample, 46.74±0.80%, while the smallest content of the hull was found in the sample NS-H-6311 and amounted to 41.91±0.71%.

Keywords: non-oil hybrids, oil content, mass of 1000 seeds, hull/kernel ratio, hull/seed ratio

Introduction

Sunflower seed may be a significant source of protein, i.e. significant raw material for protein production (Food Outlook, 2018). Compared to other plant species that represent significant protein sources (eg soybean seed), sunflower seed contains small amounts of antinutritive compounds (eg protease inhibitors, cyanogen, lectins, etc.) (Gassmann, 1983; González-Pérez & Arellano, 2009; González-Pérez, 2015). The amino acid composition of sunflower seed protein, with the exception of a small amount of lysine, is in accordance with the requirements of the FAO/WHO for human nutrition (Gassmann, 1983; Raymond *et al.* 1991). Oil yield, the primary indicator of sunflower productivity, depends on seed yield and seed oil content. High and stable oil yield is a very desirable attribute of sunflower (*Helianthus annuus* L.) hybrids (Škorić et al., 2005; Živanović et al., 2017; Ikanovic et al., 2018).

These are the reasons for hybridization in the direction of increasing the protein content in the seed and the growing presence of confectionary hybrids. Confectionary sunflower hybrid seeds are characterized by a high hull content (from 40 to 50%), a high mass of 1000 seeds (more than 100 g) and an oil content of less than 30% (Jovanović, 2001; Kaya *et al*. 2008; Hladni *et al*. 2011). Although the primary purpose of these hybrids is not the production of oil, since the oil content of the seed is significant, and they could represent a potential raw material for the oil production.

The aim of this study is to characterize sunflower seeds of the latest confectionary NS hybrids by determining the content of oil and moisture in the seed, the weight of 1000 seeds, the content of the hull and the kernels in the seed, and the hull/kernel and hull/seed ratio. The obtained results are compared with the results obtained by the analysis of the seed of confectionary sunflower hybrid (Cepko) that is already in use.

Materials and Methods

Material

Hybrid seeds are a secondary filial (F2) generation. Samples NS-H-6792, NS-H-6489 and NS-H-6311 were grown under conditions of small-plot trials in 2017. The seed was cleaned and 6 months after the harvest, the seed dimensions were examined. The seed of hybrid Cepko is from commercial cultivation in 2017.

Methods

The moisture content was determined according to SRPS EN ISO 665, 2008, the content of oil according to SRPS EN ISO 659, 2011, while the weight of 1000 seeds and the contents of the hull and kernels were made according to Karlović & Andrić, 1996.

Results and Discussion

The highest moisture content was found in the NS-H-6489 sample and amounted to 6.57±0.03% while the smallest content was found in the NS-H-6792 sample and amounted to 5.75±0.00%. The content of the oil in the tested samples ranged from 23.12±0.16%, as found in the NS-H-6489 sample to 25.62±0.22% in the NS-H-6311 sample. The seed of hybrid Cepko has a significantly higher oil content of 40.46±1.84%, while the specific moisture content in this sample was 5.69±0.06% as can be seen in Table 1.

Table 1. Moisture and oil content of the confectionary sunflower hybrid seeds.

Hybrid	Moisture content (%)	Oil content (%)
NS-H-6792	5.75±0.00	23.83±0.31
NS-H-6489	6.57±0.03	23.12±0.16
NS-H-6311	6.15±0.03	25.62±0.22
Cepko	5.69±0.06	40.46±1.84

The content of the hull and kernels in confectionary sunflower hybrid seed is shown in Table 2. The highest content of the hull is determined in the NS-H-6792 sample and amount to 46.74%. The kernels content in this hybrid is 53.26±0.80%. The obtained hull/kernel and hull/seed ratio is the highest in this sample and amounts to 0.88±0.03 and 0.47±0.01, respectively.

The smallest content of the hull of 41.91±0.71% has a hybrid NS-H-6311, the kernel content of this hybrid is 58.09±0.71%. The hull/kernel and hull/seed ratio in this sample is the smallest and is 0.45±0.00 and 0.31±0.01, respectively.

Hybrid Cepko has a significantly lower content of the hull (31.20±0.72%), and therefore the hull/kernel and hull/seed ratio is lower and amount to 0.45 and 0.31, respectively.

Table 2. Hull and kernels content and hull/kernel and hull/seed ratio of the latest sunflower confectionary hybrid seeds.

Hybrid	Hull content (%)	Kernel content (%)	Hull/kernel ratio	Hull/seed ratio
NS-H-6792	46.74±0.80	53.26±0.80	0.88±0.03	0.47±0.01
NS-H-6489	45.38±0.12	54.62±0.12	0.83±0.00	0.45±0.00
NS-H-6311	41.91±0.71	58.09±0.71	0.72±0.02	0.42±0.01
Cepko	31.20±0.72	68.80±0.72	0.45±0.02	0.31±0.01

Table 3. Mass of 1000 seeds of the tested confectionary sunflower hybrids.

Hybrid	Mass of 1000 seeds(g)	Mass of 1000 seeds expressed on dry matter (g)
NS-H-6792	130.72±4.99	123.21±4.71
NS-H-6489	122.25±3.34	114.22±3.12
NS-H-6311	133.79±3.38	125.56±3.17
Cepko	60.80±0.95	57.35±0.90

Based on the data in Tables 1, 2 and 3, cluster analysis was performed in Figure 1. The results of clustering were obtained using the minimum variance method - Ward's method, and clustering is based on Euclidean Distances.

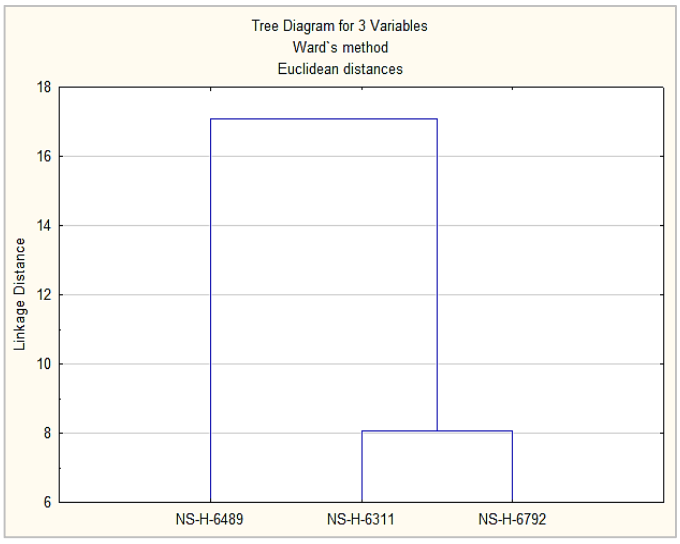

Figure 1. Dendrogram of the hierarchical cluster analysis of the tested samples

The values of the mass of 1000 seeds of the tested hybrids are shown in Table 3. The highest value of mass of 1000 seeds has NS-H-6311 hybrid seed and amounts to 125.56±3.17 g, while the lowest mass of 1000 seeds has NS-H-6489 hybrid seed and amounts to 114.22±3.12 g expressed on dry matter. A significantly lower mass of 1000 seeds of hybrid Cepko (57.35±0.90 g expressed on dry matter) indicats a lower volume of these seeds.

Figure 1 shows that the difference between samples NS-H-6311 and NS-H-6792 is lower and amounts to only 8.1 (expressed as Euclidean distance), while the diversity of NS-H-6311 and NS-H-6792 hybrids and hybrid NS-H-6489 is higher and amounts to 12.5 and 17.1, respectively, expressed as Euclid's distance.

Conclusions

On the basis of the presented results, it is concluded that the latest NS confectionary sunflower hybrids according to the tested parameters differ significantly from the confectionary hybrid Cepko that is already in use. The oil content of the Cepko hybrid seed is 40.46±1.84%, while in the latest hybrids the oil content ranges from 23.12±0.16% to 25.62±0.22%. The content of the hull in the Cepko hybrid seed is 31.20±0.72%, while the content of the hull in the latest hybrids ranges from 41.91±0.71% to 46.74±0.80%. Mass of 1000 seeds of the Cepko hybrid expressed on dry matter is 57.35±3.17 g while in the latest hybrids it moves in the interval from 114.22±3.12 g to 125.56±3.17 g. Diversity in the characteristics of seed of the latest hybrids in relation to the Cepko hybrid can lead to potential problems in further processing of the seed. Therefore, a detailed characterization of the latest hybrids is necessary.

Acknowledgments

This work is the result of research under the projects TR 31014 and TR 31025, financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Conflicts of Interest: The authors declare no conflict of interest.

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Original Scientifuc paper

Study on Dimensions of the Sunflower Seeds of the Latest NS Confectionary Hybrids

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Abstract

The confectionary/non-oil seed of sunflower hybrids is characterized by a higher content of the hull, which is thicker than the oil seed hybrids of sunflower. These seeds can be of different size and shape, with a hull not completely black. The aim of this study is to examine the dimensions of confectionary seeds of the latest NS sunflower hybrids. The results of the analyzed seed samples of the latest hybrids of second filial (F2) generation (NS-H-6792, NS-H-6489, NS-H-6311) are compared with the results of already-used hybrid seeds (Cepko). Equivalent diameter and linear dimensions of seed are characterized by Malik and Saini, 2016. The highest specific value of the equivalent diameter was 8.25±0.60 mm (NS-H-6792) and the smallest was 7.90±0.30 mm (NS-H-6311). Only 24.33% of the measured seeds were between 15.00 and 15.99 mm long, which is the most frequent length interval. The average seed width is from 7.00 to 7.99 mm (44.33%), while the average seed thickness is from 4 to 4.99 mm, which was measured in 46.67% of examined seeds. From the above results of the linear dimensions it can be concluded that the seeds are the most different in length, while the distribution of the results by width and thickness is less.

Keywords: sunflower, non-oil hybrids, size and shape, length, distribution

Introduction

The creation of new hybrids of sunflower expands the assortment of its application. Sunflower breeding in the direction of increasing the protein content leads to the application of this type of sunflower for purposes other than the production of oil, but the production of protein products as well as the consumption kernels. Such hybrids have larger seeds with a thicker hull, most commonly colored (black and white). Hull represents 40-45% of the total seed mass, it is poorly attached to the kernel and is easily removed (Jovanović, 2001; Gonzales-Perez & Vereijken 2007). This seed contains less oil, mostly about 30% (Kaya *et al*. 2008; Hladni *et al.* 2011).

The aim of this study is to examine the seed dimensions of the latest sunflower confectionary/non-oil hybrids. The obtained results are compared with the dimensions of the seeds of the confectionary/non-oil hybrid Cepko, which is already in use.

Materials and Methods

Material: Hybrid seeds are a secondary filial (F2) generation. They were grown under conditions of small-plot trials in 2017. The seed was cleaned and 6 months after the harvest, the seed dimensions were examined. The seed of hybrid Cepko is from commercial cultivation in 2018.

Methods: Length (L), width (W) and seed thickness (T) were measured using a vernier caliper per 100 individual seeds. Equivalent diameter (*De*), Surface area (*S*), Seed volume (*V*) and Sphericity (∅) are calculated according to the following equations (Malik and Saini, 2016):

Equivalent diameter:

$$De = (LWT)^{\frac{1}{3}} \quad (1)$$

Surface area (McCabe *et al*. 1986):

$$S = \pi De^2 \quad (2)$$

Seed volume (Özarslan, 2002):

$$V = \frac{1}{3}\pi De^3 \quad (3)$$

Sphericity (Mohsenin, 1970):

$$\emptyset = \frac{De}{L} \quad (4)$$

Results and Discussion

The values of the length, width and thickness of the seeds of the tested hybrids are shown in Table 1. The seeds of hybrid NS-H-6792 are the longest of the tested hybrids, with the average value of 20.68±1.29 mm, while the shortest seeds are of hybrid NS-H-6489 with an average length of 14.79±0.43 mm. The seeds of this hybrid have the highest value of the width, which is on average 7.99±1.07 mm, while the smallest average width of the seed is measured in the sample NS-H-6792 and is 7.01±0.62 mm. The seed of this hybrid is the thinner with an average thickness of 3.91±0.60 mm, while the NS-H-6489 hybrid seeds have the largest measured average thickness of 4.39±1.00 mm. The NS-H-6311 hybrid seeds have dimensions between these values. Measured dimensions of the seeds of hybrid Cepko are the smallest compared to the latest hybrids and amounts: length 10.65±0.47 mm, width 5.79±0.81 mm and thickness 3.33±0.87 mm.

Table 1. Dimensions of the sunflower seeds of different hybrids

Hybrid	Length (*L*) (mm)	Width (*W*) (mm)	Thickness (*T*) (mm)
NS-H-6792	20.68±1.29	7.01±0.62	3.91±0.60
NS-H-6489	14.79±0.43	7.99±1.07	4.39±1.00
NS-H-6311	16.15±0.83	7.24±0.23	4.24±0.35
Cepko	10.65±0.47	5.79±0.81	3.33±0.87

If we observe the distribution of the seed dimensions shown in Figures 1. (a), (b) and 1 (c), it can be seen that the most frequent distribution of the length is in the interval from 15 to 15,99 mm and amounts to 24.33%. 44.33% of the seeds width of all hybrids is in the interval of 7.00 to 7.99 mm, while the most frequent interval of seed thickness is 4.00 to 4.99 mm and amounts to 46.67%. The seeds of hybrid Cepko are significantly different. The most frequent interval of seeds length is in the range of 10.00 to 10.99 mm and amounts to 57%. 45% of the seeds width is between 5.00 and 5,99 mm, while 45% of the measured seeds thickness is in the range of 3.00 to 3.99 mm (Figure 1. (d)).

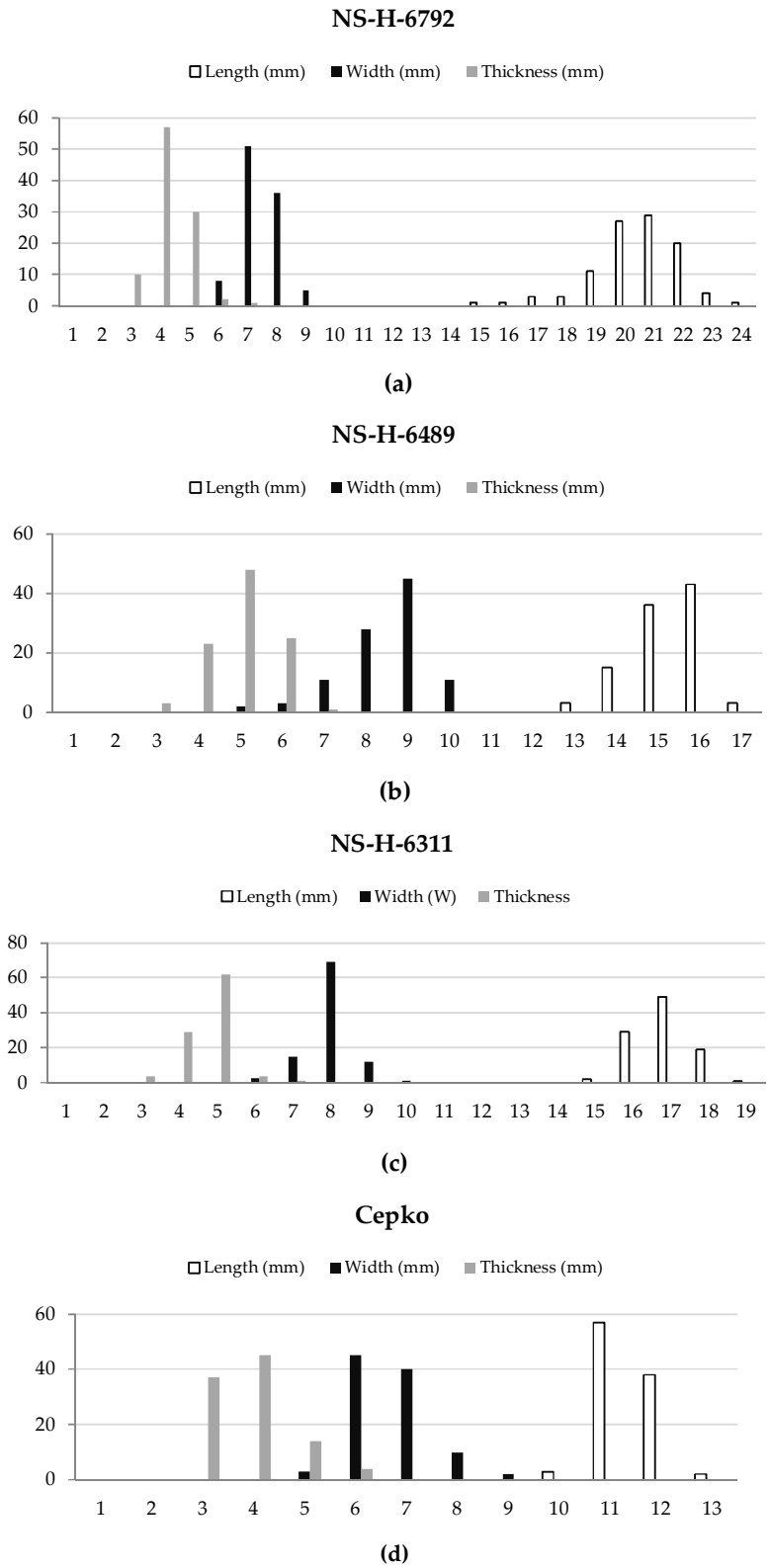

Figure 1. Distribution of the dimensions of the seeds of hybrids: (a) NS-H-6792; (b) NS-H-6489; (c) NS-H-6311 and (d) Cepko

The calculated values of the equivalent diameter, surface area, volume and sphericity of the seed are shown in Table 2. The highest value of equivalent diameter, surface area and volume is determined in the NS-H-6792 hybrid seeds, while the lowest values of these parameters are determined in the NS-H -6311 hybrid seeds. The highest sphericity value is calculated for NS-H-6489 hybrid seeds, while the smallest is for NS-H-6792 hybrid seeds. In the seeds of hybrid Cepko, the calculated sphericity value is the highest, while the other values of the parameters are lower than the other examined hybrids.

Table 2. Equivalent diameter, Surface area, Seed volume and Sphericity of the seeds of the tested hybrids

Hybrid	Equivalent diameter *De* (mm)	Surface area *S* (mm^2)	Seed volume *V* (mm^3)	Sphericity θ (mm^3)
NS-H-6792	8.25±0.60	214.63±31.80	298.15±67.00	0.40±0.02
NS-H-6489	8.00±0.93	203.51±48.70	278.77±102.43	0.54±0.06
NS-H-6311	7.90±0.30	196.27±14.70	259.16±29.17	0.49±0.02
Cepko	5.86±0.77	109.76±29.41	111.01±45.39	0.55±0.06

Differences between seeds of examined hybrids can be clearly seen in Figure 2.

Figure 2. Seeds of hybrids: (a) NS-H-6792; (b) NS-H-6489; (c) NS-H-6311; (d) Cepko

Conclusions

Based on the measured and calculated values, it can be concluded that the highest variability of the seeds of the tested hybrids in the length of the seed of the latest NS hybrids is in the interval from 12.00 to 23.99 mm, while the hybrid Cepko length of the seeds is in the interval from 9.00 to 12.99 mm. The width and thickness of the seeds of all examined hybrids are in narrow intervals, ranging from 4.00 to 9.99 mm (width) and a thickness is in interval from 2.00 to 6.99 mm. The NS-H-6792 hybrid seeds are the longest with the lowest width and thickness of seeds, so the sphericity of these seeds is the lowest 0.40±0.02 mm^3, while NS-H-6489 hybrid seeds are the shortest with the highest values of width and thickness of the seeds, and the sphericity of these seeds compared with other examined hybrids is the largest and amounts 0.54±0.06 mm^3. Between seeds of the latest NS hybrids and hybrid Cepko, a significant difference in dimensions is observed. The dimensions of the hybrid Cepko seeds are smaller than the latest NS hybrids, while the sphericity of this seed is the largest and amounts 0.55±0.06 mm^3.

Acknowledgments: This work is the result of research under the projects TR 31014 and TR 31025, financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Conflicts of Interest: The authors declare no conflict of interest.

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Original scientific paper

Evaluation of genetic diversity of sugar beet (*Beta vulgaris* L.) inbred lines by SSR markers

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Abstract

The purpose of the research was to estimate the polymorphism of sugar beet genotypes based on SSR analysis in order to use the obtained data in breeding for drought tolerance. We studied ten inbred lines of sugar beet created at various experimental breeding stations (EBS) of the Institute of Bioenergy Crops and Sugar Beet, and one inbred line was received from Belarus within the framework of *Betaintercross*. Cluster analysis was carried out to group the genotypes based on SSR analysis data. It was found that the frequency of identified alleles ranged from 0.05 to 0.45, the index of polymorphism on the average for studied markers was 0.80. On the basis of genotype affinity, four clusters were formed. Two inbred lines (line 28145 of Uladivske-Liulyntsi EBS and line 26397 of IvanivskaEBS) were not included to any cluster. Line 26397 of IvanivskaEBS appeared to be the most remote. Line 44504 of BilaTserkva EBS and 27826 of IvanivskaEBS, A-06626/2 of Uman EBS and 07-168MS of Yaltushkiv EBS appeared the most similar genotypes with the value of genetic distances between them equal to 1.73. Therefore, the set of three SSR markers may be used in breeding work: it shows the principle of the inbred lines combination in terms of heterosis, which may be used in breeding for drought tolerance.

Keywords: sugar beer; SSR markers; cluster analysis; genetic polymorphism

Introduction

Being thema in source of sugar production in temperate climates sugar beet (*Beta vulgaris* L.) is one of the most important industrial crops. Molecular markers can be used to increase the efficiency of breeding programs, especially for an estimation of genotypic diversity or for drought tolerance (Sandhu *et al.*, 2016; Kito *et al.*, 2017). In Ukraine, sugar beet (*Beta vulgaris* L.) is one of the most important crops in the sugar industry. To achieve high and stable yields and quality of feedstock is a today's priority in sugar beet growing sector (Ribeiro *et al*., 2016). The main ways of obtaining high yields are the creation of hybrids resistant to abiotic environmental factors, in particular, to drought. Sugar beet is attributed to drought-sensitive crops, which results in a yield shortfall of about 40% (Ribeiro *et al*., 2016). The effect of heterosis is the basis for creating high-yielding sugar beet hybrids. Description of the source material is the primary task of reducing the efforts and costs for the identification of suitable parent matches (Taški-Ajduković *et al*., 2017). One of the approaches to the determination of genetic heterogeneity of the source material is the use of DNA markers (Munns, 2002; Yardanov *et al*., 2003). Among various molecular markers, SSR markers have been widely used owing to their high reproducibility, variability, codominant inheritance, being widespread in the genome, chromosome-specific location (Agarwal *et al*., 2008; Xuand Crouch, 2008; Fedulova et al., 2017a; Fedulova and Fedorin, 2017)and identification by polymerase chain reaction (PCR) (Castillo *et al.*, 2010). For sugar beet, several hundred SSR markers have been developed to use in gene mapping (Laurent *et al*., 2007), gene flow investigation (Arnaud *et al*., 2003; Viard *et al*., 2004) and population

genetic analysis (Fénart *et al*., 2008; Arnaud *et al*., 2009). The Institute of Bioenergy Crops and Sugar Beet of the National Academy of Agricultural Sciences is a major sugar beet breeding centre in Ukraine. It has the considerable material potential for the creation of hybrids competitive in the global market. So far, genetic diversity of the Ukrainian sugar beet source materials has not been studied enough through using DNA markers which allow to significantly accelerate the breeding process aimed at creation of high-yielding hybrids with resistance to abiotic environmental factors (Li *et al*., 2010; Simko *et al*., 2012; Abbasi *et al*., 2014; Shajuk & Roik, 2015; Klyachenko & Prysiazhniuk, 2016; Fedulova *et al*., 2017b). The polymorphism of sugar beet based on DNA was studied using three SSR markers (SB04, SB07 and SB15) (Richards *et al*., 2004; Ćurčić *et al*., 2017). Therefore, the purpose of our research was to evaluate the polymorphism of sugar beet genotypes using SSR markers, which will be used in selection for drought tolerance.

Materials and Methods

The material for the study was ten inbred lines of sugar beet created at various experimental breeding stations (EBS) of the Institute of Bioenergy Crops and Sugar Beet and one inbred line received from Belarus within the framework of the Beta intercross breeding program (Table 1).

Table 1. Sugar beet lines

No.	The number of line	Origin (EBS)
1	44504	BilaTserkva EBS
2	25806	IvanivskaEBS
3	27826	
4	26397	
5	A-2602/04	Uman EBS
6	A-06626/2	
7	28119	Uladivske-Liulyntsi EBS
8	28145	
9	MS2/358 FBC №10 з 8 х ОП1/603 №15 №7*	Belarus
10	07-168MS	Yaltushkiv EBS
11	08–316MS	

*hereafter - MS2/358 FBC No:10

The sampling for the research included 30 genotypes of each line. To isolate DNA from seeds, cationic detergent CTAB (cetyl trimethyl ammonium bromide) was used. Two-step purification was carried out with chloroform-isoamyl alcohol and ethanol solution (Roik *et al*., 2007; Tkachyk, 2015).

Molecular and genetic polymorphism of sugar beet lines was investigated using PCR (polymerase chain reaction) with specific primers by three microsatellite markers SB04, SB07 and SB15 (Table 2), which were selected based on the analysis of studies by Richards *et al*. (2004) and McGrath *et al*. (2007) on the index of polymorphism of the locus.

PCR was carried out on the TC-Y CreaCon (USA) amplifier. The reaction mixture contained 100 ng of total plant DNA, salt buffer (10 mMTris-HCl, pH 9.0, 50 mMKCl, 0.01% Triton X-100), 2.5 mM MgCl2, 250 μM of deoxynucleotide triphosphate mixture (dNTP), 0.6 μM of each primer and one unit of Taq polymerase. The total volume of the mixture was 20 μl.

For each pair of primers, the following temperature and time parameters of PCR were used: step 1 − initial denaturation: (95 °C) 5 min; step 2 − development of specific reaction products:

denaturation (95 °C) 30 s, hybridization of primers (51.0–53.6 °C) 30 s, elongation (72 °C) 30 °C, number of cycles 35; step 3 – (final elongation): (72 °C) 1 min.

The products of amplification reaction were visualized by electrophoresis in a 2% agarose gel in 0.5 × TBE (Tris-borate buffer solution) according to the conventional method with bromide ethidium (Tkachyk, 2015). Electrophoresis has been carried out for 1.5 h at an electric field intensity of 5 V/cm.

The results of the electrophoretic distribution of PCR products were visualized in ultraviolet light. The size of the obtained amplicons was determined in relation to the molecular weight marker using TotalLab v2.01 (trial version).

Table 2. Description of SSR primers of sugar beet markers

Primer name	The nucleotide sequence of primers 5′…3′	Motif	Hybridization temperature(°C)	The expectedsize of amplicons (bp)
SB04 – F*	accgatcaccaattcaccat	(GGA)4, (GTT)8	51.0	172–187
SB04 – R**	gttttgttttgggcgaaatg			
SB07– F	tgtggatgcgctttcttttc	(TC)10	51.0	246–276
SB07 – R	actccacccatccacatcat			
SB15 – F	cacccagcctatctctcgac	(CT)8	53.6	135–165
SB15 – R	gtggtgggcagttttaggaa			

*F – forward primer. **R – revers primer.

To characterize the genetic structure of the sugar beet lines under investigation the frequencies of the detected alleles for each pair of primers were calculated(Roik*et al*., 2007). In order to assess the degree of identifiable variability in the population and the ability of a certain marker to find out the difference between genotypes we calculated PIC (polymorphism information content) using the following formula:

$$PIC = 1 - \sum_{i} p_{li}^{2}$$

where *pli* is the frequency of the *i*allele of *l* locus.

The ability of the marker set to differentiate the investigated lines was estimated on the basis of cluster analysis using STATISTICA 12 (trial version). Varieties were grouped using the method of un weighted average (Ermantraut *et al*., 2007; Drozdov, 2010; Everitt et al., 2011).

Results

To investigate the molecular and genetic polymorphism of sugar beet lines, three microsatellite loci (SB04, SB07 and SB15) have been analyzed.

3.1. Allele identification

Electrophoretic separation of amplicons resulted in alleles of the expected size. The sampling of each variety under study consisted of 30 individual DNA assays, which allowed evaluating not only inter-linear but also intra-linear polymorphism. Marker SB07 identified the largest number of lines

containing more than one allele: 9 out of 11 lines under study. When using SB04 marker, all lines appeared to be monomorphic.

Shown in Figure 1 are obtained polymorphic and nonpolymorphic alleles for sugar beet lines by SB07 marker for sugar beet line 08-316 MS (Yaltushkiv EBS). Tracks 10, 11 and 14 represent the genotypes in which two alleles of 276 bp and 300 bp were identified. In other genotypes of 316 MS, a single allele of 276 bp was identified. Marker SB15 revealed two alleles per genotype in 7 out of 11 lines. One line, 27826 of Ivanivska EBS, demonstrated the absence of intra-linear polymorphism in all of the markers under study. The intra-linear polymorphism of the investigated sugar beet materials can be explained by the fact that the lines are still at the stage of the breeding process (Fedulova *et al*., 2017b). To describe the marker set used in the research we determined by the frequency of the alleles and the PIC (Table 3, Figure 2).

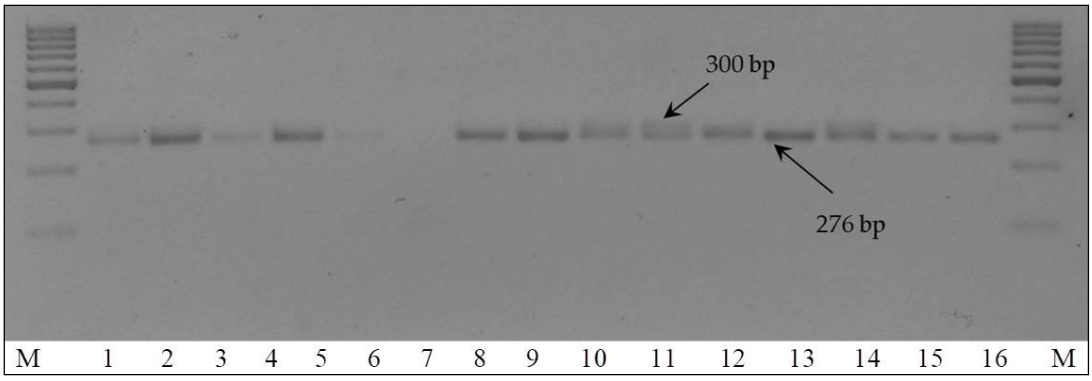

Figure 1. Electrophoretic distribution of alleles by marker SB07 for sugar beet line 08-316MS (Yaltushkiv EBS): M − marker of molecular weight Thermo Scientific O'RangeRuler 100 bp DNA Ladder; 1−16 − amplicons of 16 genotypes of the line.

Table 3. The alleles, identified in sugar beet lines by SSR markers

SSR marker	The number of alleles	Allele size(bp)	PIC
SB04	4	183; 186; 189; 192	0.68
SB07	10	260; 264; 270; 272; 274; 276; 280; 284; 286; 300	0.86
SB15	9	148; 150; 152; 156; 160; 162; 168; 172; 178	0.87

In the lines investigated by SB04 marker, the size of identified alleles ranged from 183 to 192 bp; by SB07 marker from 260 to 300 bp and by SB15 marker from 148 to 178 bp. It is known that the more alleles are identified in a locus and the lower their frequency is, the more valuable that marker is for detecting polymorphism and identifying genotypes (Sanalatiy *et al*., 2006). The index of polymorphism in the locus ranged from 0.68 to 0.87.

The frequencies of the identified alleles in the sugar beet lines ranged by locus from 0.05 to 0.45 (Figure 2 a-c).

The frequency of the allele identified by SB07 marker ranged from 0.05 to 0.27. The largest number of investigated sugar beet lines contained an allele of 276 bp. Alleles of 260, 270, 272 and 274 bp were identified in lines 44504, 25806, 26397 and MS2/358 FBC No. 10, respectively, and they had the lowest frequency. By marker SB15, 156 bp allele had the largest frequency; it was identified in three lines: 44504, 27826 and 26397. The allele of 168 bp identified in line A-2602/04 was unique for the

marker. According to the research data, an allele size 192 bp in line 44504 had the lowest frequency by SB04 marker. Noteworthy, by two out of three markers under study (SB07 and SB04), line 44504 of the BilaTserkva EBS contained unique alleles. This fact can be used for identification of this line.

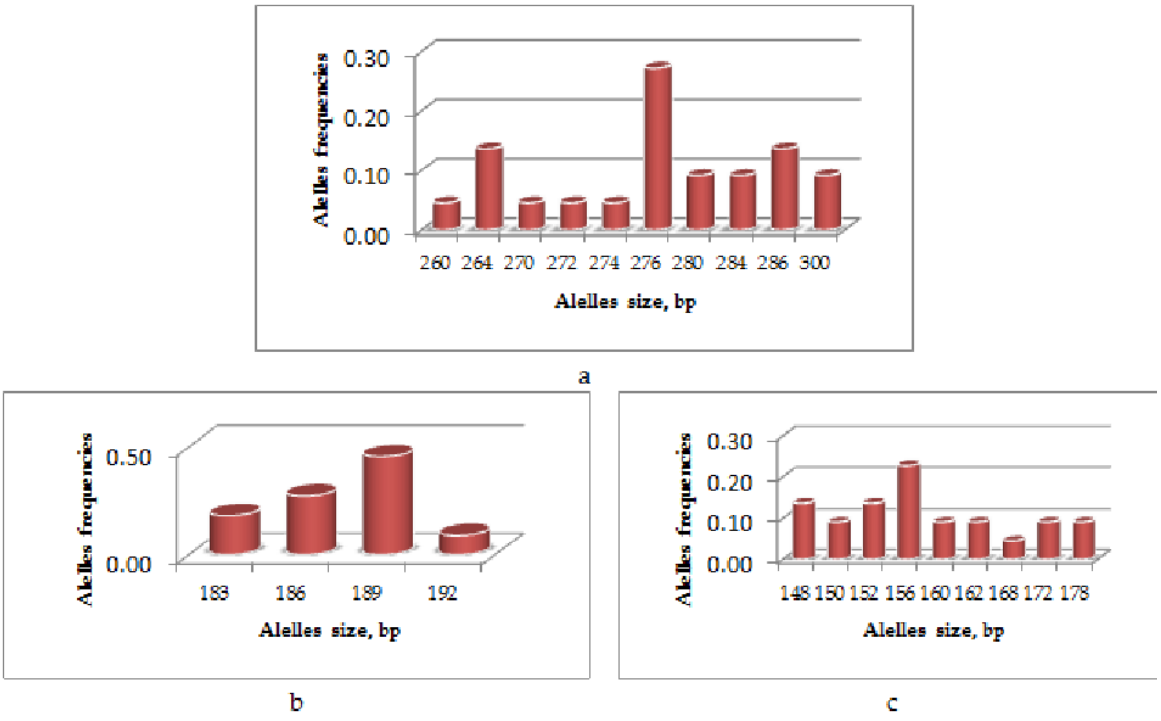

Figure 2. Distribution of allele frequency by SSR markers: (a) SB07; (b) SB04; (c) SB15

Consequently, the high values of the index of polymorphism of the locus (0.68–0.87) suggest that the identified alleles are evenly represented in the sampling of investigated sugar beet lines and that used in the research markers have high resolution for the differentiation of closely related genotypes.

3.2. Statistical analysis

To differentiate sugar beet lines based on the results of PCR analysis, we conducted cluster analysis using three microsatellite markers. Given in Table 4 are the results of the analysis showing the genetic distances between lines.

According to the analysis of genetic distances, the largest distance (3.16) was observed between sugar beet line 26397 of Ivanivska EBS and the lines A-2602/04 of Uman EBS and 07-168MS of Yaltushkiv EBS, as well as between the lines of Yaltushkiv EBS 08-316MS and 07-168MS. As the similarity of the lines increases, their genetic distance shortens. In our research, the closest were the lines with the genetic distance value of 1.73: 27826 of Ivanivska EBS and 44504 of BilaTserkva EBS, 07-168MS of Yaltushkiv EBS and A-06626/2 of Uman EBS. The values of genetic distances between the other lines ranged from 2.45 to 3.00. It is important to point out that the lines obtained from the same experimental breeding station were located at close distances (2.45-2.83). Given that the objects with 0 or close to 0 values of genetic distances are considered absolutely identical, and those with high values considered to be different, we can conclude that the lines under the investigation are different.

The results of the hierarchical classification as a phylogenetic tree are shown in Figure 3.

Table 4. Genetic distances between investigated sugar beet lines by SSR markers (the color reflects genetic distances in descending order where blue (red) denotes small (large) distances, respectively)

Case No.	25806	27826	26397	A-2602/04	A-06626/2	28119	28145	MS2/358F BC No 10	07-168MS	08–316MS
44504	2,83	1,73	2,65	2,65	2,83	2,65	3,00	2,83	3,00	2,65
25806		2,65	2,65	3,00	2,83	3,00	3,00	2,45	2,65	3,00
27826			2,45	2,45	2,65	2,00	2,45	2,24	2,45	2,45
26397				3,16	3,00	2,83	2,83	3,00	3,16	3,16
A-2602/04					2,24	2,83	2,83	3,00	2,83	2,45
A-06626/2						3,00	3,00	2,83	1,73	2,65
28119							2,45	2,65	2,83	2,00
28145								2,65	2,83	2,45
MS2/358FBC No 10									2,24	3,00
07-168MS										3,16

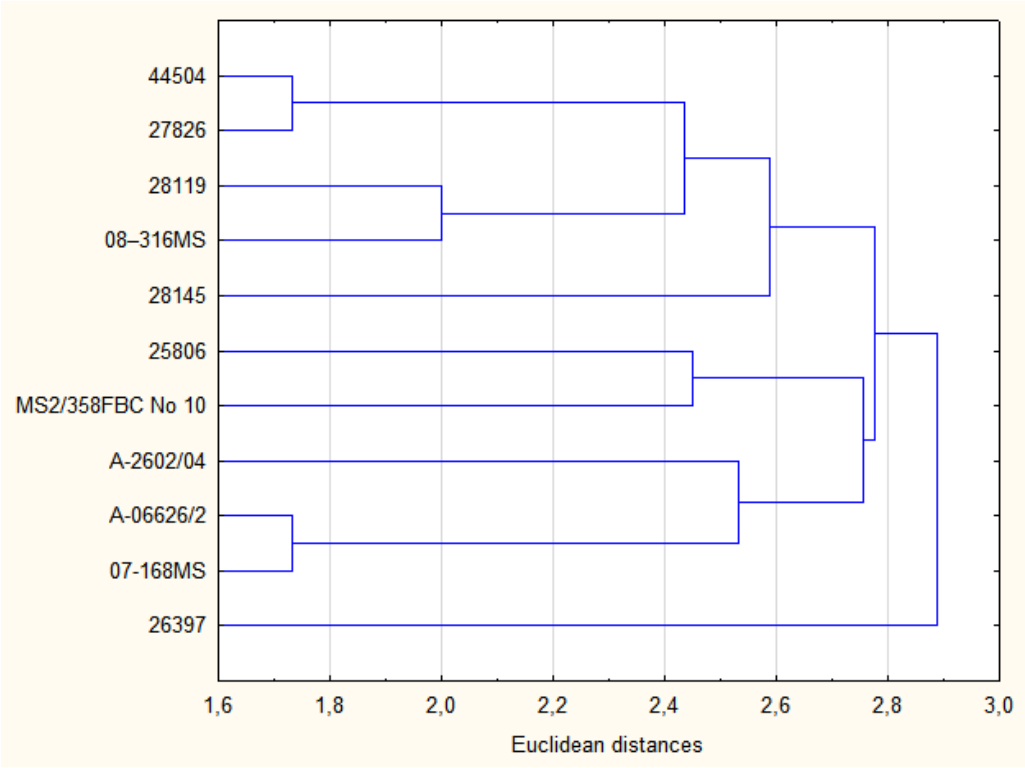

Figure 3. Cluster analysis of sugar beet lines by markers SB04, SB07 and SB015

On the basis of the dendrogram four clusters were identified by our of microsatellite markers SB04, SB07 and SB015. The clusters were formed by the lines 44504 of BilaTserkva EBS and 278226 of Ivanivska EBS, 28119 of Uladivske-Liulyntsi EBS and 08-316MS of Yaltushkiv EBS, 25806 of Ivanivska EBS and MS2/3358FBC No. 10 (Belarus), A-06626/2 of Uman EBS and 07-168MS of Yaltushkiv EBS. We also noted that the lines 28145 of Uladivske-Liulyntsi EBS and 26397 of Ivanivska EBS were not included into any cluster. Line 28145 of Uladivske-Liulyntsi EBS was located next to the cluster formed by lines 28119 of Uladivske-Liulyntsi EBS and 08-316MS of Yaltushkiv EBS with the value of genetic distance equal to 2.45.

Line 26397 of Ivanivska EBS, though not included in any cluster, is most similar to line 27826 of Ivanivska EBS. The most distant lines that entered the same cluster were lines 25806 of Ivanivska EBS and MS2/3358FBC No. 10 (Belarus); the genetic distance between them was 2.45.

Consequently, the sugar beet lines found in different blocks of clusters by markers SB04, SB07 and SB015 are different. Based on the obtained data, a marker system consisting of three SSR markers, namely SB04, SB07 and SB015, allows differentiating 11 lines of sugar beet of different origin.

Discussion

The research related to the selection of parents lines with the highest heterosis potential is important for obtaining high-yielding hybrids (Teklewold and Becker, 2006; Ćurčić *et al*., 2017). In our research, the polymorphism of 11 inbred sugar beet lines has been investigated using three SSR markers and genetic distances between them were identified. The intra-linear polymorphism was found in eight lines by markers SB07 and SB15. Fedulova *et al*., (2017) examined 20 sugar beet breeding numbers with the aid of markers SB04, SB06, SB07, SB09 and SB10 for their use in transgression selection. As a result, they found that 6 out of 20 studied lines were characterized by high heterogeneity. The SB04 marker identified only one amplicon in the lines. This result is consistent with our as we obtained four alleles by SB04 marker.

The number of identified alleles by markers SB07 and SB15 was 10 and 9, respectively. The allele frequencies ranged from 0.05 to 0.45. Svirschevskaya *et al*. (2008) investigated 29 sugar beet samples, of which 17 lines were examined using 7 microsatellite markers (Bmb6, Gcc1, SB6, SB7, SB9, SB11, SB15). According to the results obtained by the authors, the Bmb4 marker identified the largest number of alleles (8). SB7 and SB15 markers had a PIC of 0.52 and 0.57, i.e. lower than our studies (0.86 and 0.87, respectively). However, one should pay attention to the different origin of breeding material and different methods of their creation. The lines studied by Svirschevskaya *et al*. (2008) were obtained through parthenogenesis *in vitro* culture, while our sugar beet lines were produced with the aid of self-pollination *in vivo*.

Richards *et al*. (2004) evaluated the polymorphism of cultural and wild species of sugar beet. In the study, the authors used eight SSR markers of the SB group: SB04, SB06, SB07, SB09, SB010, SB011, SB013 and SB015. According to the markers we used in the research (SB04, SB07 and SB015), the index of polymorphism of the loci ranged from 0.70 to 0.80. The value of PIC obtained by Richards et al. (2004) was higher than in our research. However, there is no doubt that the material used by Richards *et al*. (2004), is more heterogeneous than our breeding materials.

According to our research, markers SB04, SB07 and SB015 allowed to distinguish the lines under study in terms of their genetic distances. Bogachova *et al*. (2017) used five markers to study the genetic variability of parent forms of sugar beet by SSR markers (SB04, SB06, SB07, SB09 and SB10). By means of cluster analysis, breeding materials were grouped according to their types and the feasibility of crossing the distant forms to obtain a high heterozygosity index was shown. A similar research was conducted by Arnaud *et al*. (2010) for the evaluation of the genetic diversity of wild sugar beets species on compatible crops. They determined that the level of genetic diversity represented by allele frequencies and expected heterozygosity were similar to those observed in

conventional beet populations. In addition, the authors identified the similarity of populations from a certain geographic area.

According to the results of the cluster analysis of sugar beet lines in our research, a certain distribution based on the origin of breeding materials was obtained. Thus, the lines obtained from the same experimental breeding station were distinguished by relatively low values of genetic distances. However, it should be noted that certain lines, specifically 44504 of BilaTserkva EBS and 27826DSS of Ivanivska EBS, A-06626/2 of Uman EBS and 07-168 MS of Yaltushkiv EBS are found in separate clusters and they have different origin. This fact can be explained by genetic variability of breeding materials involved in breeding programs of the Institute of Bioenergy Cultures and Sugar Beet.

Ćurčić *et al*., 2017 demonstrated the genetic diversity of various forms of sugar beet using a set of 26 SSR markers. In this study, the effect of heterosis correlated with the genetic distances between sugar beet pollinators and male-sterile lines, but there was no gene linkage found that control productivity characteristics. Therefore, based on the obtained results, it can be stated that the selection of markers associated with the tolerance to drought for the selection of parent components in order to obtain high-yielding drought-tolerant hybrids is promising.

Conclusions

The set of markers consisting of three SSR markers, namely SB04, SB07 and SB15 is efficient for differentiation of 11 sugar beet inbred lines. It was found that the presence of intra-linear polymorphism causes problems for the identification of lines; however, at the same time, it characterizes a high resolution of the marker system. The identified alleles of microsatellite loci are uniformly represented in the sampling, which is proved by the high value of PIC of the locus (0.6−0.87). Unique alleles were identified for the following lines: 44504 of BilaTserkva EBS (260 bp by SB07 and 192 bp by SB04) and A-3202/04 of Uman EBS. This fact can be used for their identification. Cluster analysis, which demonstrates both the common origin of the lines and other factors of genetic variability, allows assessing genetic distances and the degree of proximity of the lines under study. The data obtained will be taken into account when evaluating breeding material for the creation of high-yielding tolerant to drought hybrids.

Conflicts of Interest: The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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Original Scientific paper

Identification of drought-resistant maize lines by DNA markers

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Abstract

DNA marker-assisted selection (MAS) becomes an attractive option for detecting inherited traits in the newly developed cultivars (Jompuk *et al*., 2006). Drought is one of limiting factors to maize production worldwide, as 15–20 % of maize grain yield is lost every year due to drought. Evaluating drought-resistant inbred lines and then developing drought-resistant hybrids are the approach to minimize the impact of abiotic stress (Hao *et al*., 2011; Liu *et al*., 2015). The purpose of the research was to estimate SNP (single sequence repeat) polymorphism of genes *dhn1* and *rsp41* associated with drought resistance in lines of maize. In the research, 113 maize lines selected at Research Institute of Agrarian Business (Dnipro, Ukraine) were studied. The polymorphic SNP sites in the *dhn1* and *rsp41* were determined with PCR using the marker dhnC397 (CCAAAG/CCAAGG) and the marker based of the SNP polymorphism CCAG/CCGG (SNP A/G) of the *rsp41* gene (Liu *et al*., 2015). As a result, a 164-bp segment of the *dhn1* gene was identified using the marker *dhnC397*. For the genotypes carrying the CCAAGG variant (as G genotype), PCR products were digested with *StyI* restriction enzyme and 131- and 33-bp bands were generated. The similar approach was used for identification of rsp41 gene polymorphism. The identified 286-bp segment was digested with *HpaII* restriction enzyme. PCR products from the genotypes carrying the CCGG variant (as G genotype) generated 225- and 61-bp bands. The alleles produced by enzymes are associated with drought-resistant genotypes (Liu *et al*., 2015). Therefore, in regard to dhn1 genes, 100 lines with the allele associated with drought resistance were found. 82 lines were identified with G genotype of the *rsp41* gene. The alleles associated with drought resistance were determined in 68 lines using two markers. These lines can be used for breeding of drought-resistant t maize hybrids.

Keywords: maize (*Zea mays* L.); drought resistance; SNP polymorphism

Introduction

Drought is one of limiting factors to maize production worldwide, as 15–20 % of maize grain yield is lost every year due to drought (Frova *et al*., 1999; Avramova *et al*., 2017). An increase in the air temperature above +35°C during the appearance and flowering of male and female inflorescences in maize reduces the period of pollen viability leading to a steep drop of yield (Dziubetskyi *et al*., 2017). Growing drought-resistant forms and hybrids is a way to overcome the effects of drought on reducing plant productivity. A drought-resistant hybrid of maize is said to be the hybrid ensuring 30% of its potential yield after 6-week stress before and during flowering and grain filling (Lunduka *et al*., 2017).

The evaluation of drought-resistant inbred lines and the subsequent development of drought-resistant hybrids is a promising approach to minimize the effects of abiotic stress (Hao *et al*., 2011a; Liu *et al*., 2015). For a long time, marker-associated selection (MAS) has been used to evaluate breeding material, hybrids and varieties for the purpose of determining the signs of resistance to biotic and abiotic environmental factors (Quarrie *et al*., 1999; Jompuk *et al*., 2006; Zhao *et al*., 2018).

Due to the complexity of the mechanism for the formation of drought resistance, a limited number of functional markers associated with drought resistance are available for use in practical selection (Lübberstedt *et al*., 2005; Xu *et al*., 2009; Yang *et al*., 2012; Babu *et al*., 2013).

Among the functional genes studied, single-nucleotide polymorphism (SNP) PZA01671.1 (A/G) in ctg223 of the 5th maize chromosome and PZA0355.1 (A/G) in ctg283 was detected. It is known that SNP PZA01671.1 is associated with *rsp41* that encodes the splicing factor with rich arginine/serine, and SNP PZA0355.1 is associated with *dhn1* that encodes dehydrin (Hao *et al*., 2011b). Based on these data, Liu *et al*. (2015) developed two functional Cleaved Amplified Polymorphic Sequences (CAPS) markers to identify the allelic status of the *dhn1* and *rsp41* associated with drought resistance in maize. The relationship between these two drought resistance genes in agricultural crops was earlier demonstrated (Rorat, 2006; Palusa *et al*., 2007; Hao *et al*., 2011b). Therefore, the purpose of the research was to estimate SNP (single sequence repeat) polymorphism of genes *dhn1* and *rsp41* associated with drought resistance in lines of maize.

Materials and Methods

In the research, 113 maize lines selected at Research Institute of Agrarian Business (Dnipro, Ukraine) were studied. The work was carried out at the Department of Laboratory Studies on the Qualification Expertise of Plant Varieties (Centre of Certification Tests) of the Ukrainian Institute for Plant Variety Expertise (Kyiv, Ukraine) during the 2017-2018 period.

DNA was isolated from 100 mg of 5-day sprouts using CTAB and dissolved in TE buffer (Velikov, 2013). We used 2 cleaved amplified polymorphic sequences (CAPS) of *dhnC397* and *rspC1090* markers to identify the allele state of SNP A/G polymorphisms in the drought-tolerance genes *dhn1* and *rsp41* (Liu *et al*., 2015).

Polymerase chain reaction (PCR) was carried out on the TC-Y CreaCon (USA) amplifier. A reaction mixture of 20 μl contained: 1×DreamTaq™Greenbuffer, 1 u DreamTaq™ polymerase (ThermoScientific), 200 μM of each dNTP, 30 ng of DNA sample, 0.2 μM of each primer according to the marker (Table 1).

Table 1. Description of primers

Primer name	The nucleotide sequence of primers 5′…3′	Restriction enzymes	Hybridization temperature (°C)	The expected size of amplicons (bp).
*dhnC397*F	ggaggaagaagggaatca	*StyII*	58	164
*dhnC397*R	aggagaagaccaa			
*rspC1090*F	gccggtggcgtaggagccgc	*HpaII*	60	286
*rspC1090*R	gcgttccattccatgcctgtg			
	gatccgtttacctggttttc			

*F – forward primer.

**R– revers primer.

For the primer *dhnC397*, the following parameters of PCR were set: step 1 − initial denaturation: (96°C) 2 min; step 2 − development of specific reaction products: denaturation: (94°C) 1 min, hybridization of primers (58°C) 30 s, elongation (72°C) 1 min, number of cycles 30; step 3 − final elongation: (72°C) 2 min.

For primer *rspC1090*, PCR was conducted according to the following parameters: step 1 − initial denaturation: (95°C) 5 min; step 2 − development of specific reaction products: denaturation (94°C) 1

min, hybridization of primers (58°C) 1 min, elongation (72°C) 1 min, number of cycles 30; step 3 – final elongation: (72°C) 5 min.

The DNA amplification products obtained from maize using markers *dhnC397* and *rspC1090* were treated with *StyII* and *HpaII* restriction enzymes (Thermo Scientific, USA), respectively, according to the manufacturer's protocol.

Electrophoretic separation of amplicons after restriction enzyme treatment was carried out in 2% agarose gel for 1.5 h at an electric field intensity of 5 V/cm (Velikov, 2013). The results of the electrophoretic distribution of the restriction analysis fragments were visualized in ultraviolet light. The size of the obtained amplicons was determined in relation to the molecular weight marker using TotalLab v2.01 (trial version).

Results

As a result of the study, the polymorphism of two key loci of the dhn1 and rsp41 genes which are associated with maize resistance to drought was investigated.

The SNP polymorphism of the *dhn1* (A/G) was determined using the CAPS marker *dhnC397* (PZA03750.2). Resulted from PCR a 164 bp amplicon containing an investigated polymorphism (CCAAAG/CCAAGG) was obtained. According to Liu *et al.* (2015), SNP polymorphism is located near the *Sty1(Eco130I)* restriction site. Therefore, genotypes containing CCAAGG (G) after treatment with the restriction enzyme *Sty1* produced two fragments of 131 bp and 33 bp. CCAAAG (A)-type polymorphism is associated with drought resistance and is manifested in the formation of a 164 bp fragment. Presented in Figure 1 are the results of the electrophoretic separation of fragments after treatment with the restriction enzyme *Sty1 (Eco130I)*.

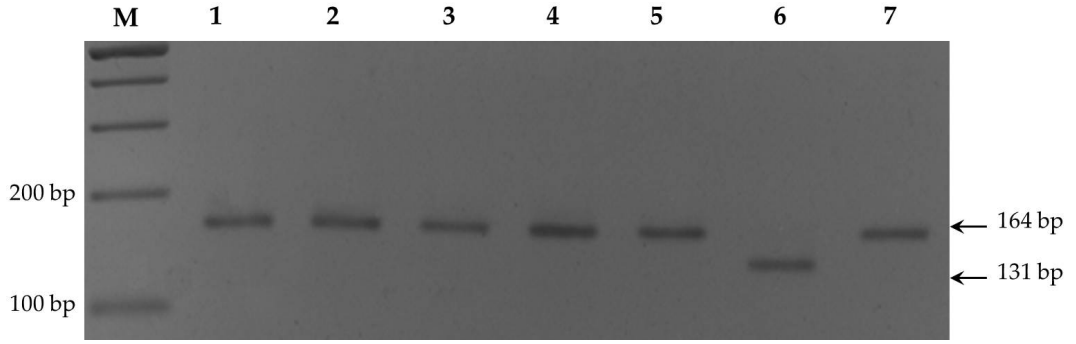

Figure 1. Electrophoresis of the restriction analysis products (primer dhnC397): 1- RL 17; 2 – RL 18; 3 – RL 19; 4 – RL 20; 5 – RL 21; 6 – RL 22; 7 – RL 23; M − marker of molecular weight Thermo Scientific O'RangeRuler 100 bp DNA Ladder.

As a result of the restriction analysis of PCR products by primer *dhnS397*, amplicons of 164 bp and 131 bp were found. Since a fragment of 164 bp indicates the presence of SNP(A), it was found that the lines RL 17, RL 18, RL 19, RL 20, RL 21 and RL 23 have an allele associated with drought resistance. Figure 1 illustrates that the RL 22 line is distinguished by the presence of the *Sty1* restriction site, which resulted in a fragment of 131 bp. This suggests that the RL 22 line has an option of CCAAGG allele (G) by *dhn1* gene marker. Our study identified the presence of a favourable allele of CCAAAG (A) by *dhn1* gene in 100 out of 113 studied maize lines.

To evaluate SNP of *rsp41* gene, the primer *rcpC1090* was used. Since the CCGG(G) polymorphism is associated with the *Hpa II* restriction site (*MspI*), two fragments, namely 225 bp and 61 bp, resulted from hydrolysis. The presence of specific size fragments is associated with drought resistance (Liu *et al.*, 2015). A fragment of 286 bp which is formed in another case

contains CCAG(A) polymorphism, which characterizes the genotypes as drought-sensitive. In our study, lines contained both allele types were found (Fig. 2).

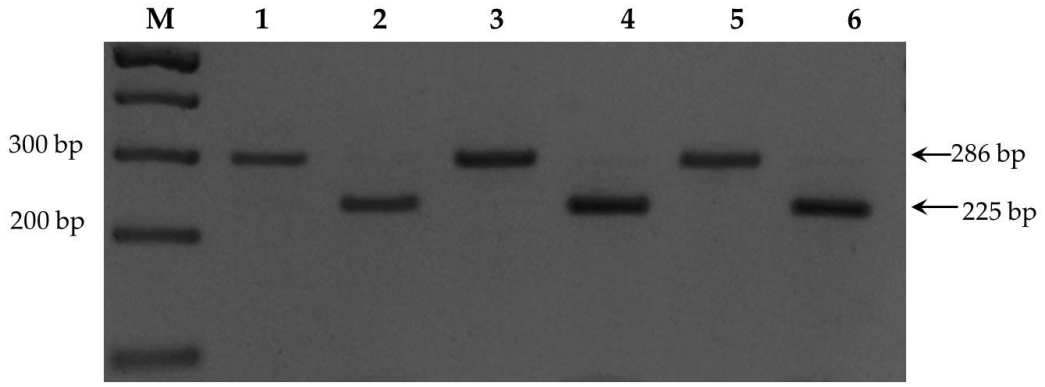

Figure 2. Electrophoresis of the restriction analysis products (primer rcpC1090): 1- RL 24; 2 – RL 25; 3 – RL 26; 4 – RL 27; 5 – RL 28; 6 – RL 1; M – marker of molecular weight Thermo Scientific O'RangeRuler 100 bp DNA Ladder.

As a result of treatment with restriction enzymes *Hpa II (MspI)* amplification products with primer *rcpC1090*, fragments of 286bp and 225 bp were obtained. Figure 2 shows the electrophoretic separation of the restriction analysis products of six maize lines: RL 24, RL 25, RL 26, RL 27, RL 28 and RL 1. The figure shows that a fragment of 225 bp is identified in three lines (RL 25, RL 27 and RL 1). This indicates that these lines have a CCGG(G) allele, which indicates drought-resistance. In other cases, SNP (A) allele was detected in maize lines, which is evidenced by the absence of *Hpa II* restriction site (*MspI*) and the presence of 286 bp fragments in the electrophoregram. Using *rcpC1090* primer the favourable allele associated with drought resistance was identified in 82 out of the 113 maize lines.

In our research, the percentage of genotypes containing SNP(A) of the dhn1 gene totalled 88%. Consequently, 22% of the studied lines are found sensitive to drought. The number of lines characterized by SNP(G) of the *rsp41* gene was somewhat fewer (73%) (Fig. 3).

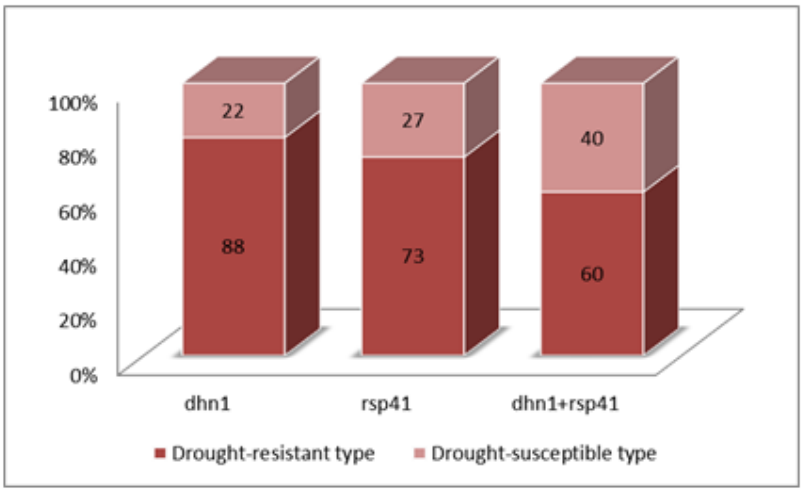

Figure 3. Distribution of maize lines in regard to drought sensitivity

According to the obtained data, the percentage of lines containing CCAG allele of the *rsp41* gene is 27%. By the allele status of both genes dhn1 and rsp4, whose polymorphism is associated with drought tolerance, only 68 (60%) out of 113 maize lines under study contained SNP (A)(G).

Discussion

Liu *et al*. (2015) examined 210 lines of Chinese maize, which differed in their degree of drought resistance. It was found that 141 lines contained a favourable allele by dhn1 gene and 109 lines contained it by *rsp41* gene. As a result of the research, the possibility of using markers *dhnC397* and *rspC1090* as functional markers for further selection for drought resistance was proven. In our studies, a slightly higher frequency of favourable alleles by individual primers was obtained (100 and 82 lines, respectively, out of 113 lines). However, in spite of the fact that Liu *et al*. (2015) assessed phenotypic indicators of drought resistance and determined the index of selectivity, in our research, we plans to involve biochemical and field-based methods for evaluation of promising lines in order to assess the efficiency of using CASP markers and for using in the selection of drought-resistant lines (Cattivelli *et al*., 2008).

Sari-Gorla *et al*. (1999) investigated different approaches to assessment of drought resistance in maize. The researchers confirmed the existence of a positive correlation between the signs of tolerance and the molecular markers for their evaluation based on the linkage map of molecular markers. However, the authors point out that plant tolerance cannot be explained only by a presence of a favourable allele combination in the locus that controls the very sign and based on physiological characteristics not directly related to the control of the characteristics (Tuberosa and Salvi, 2006). Therefore, for an effective MAS process, an integrated approach is required. Similar studies were also carried out by Frova *et al*.(1999).

The researchers investigated the genetic architecture of drought resistance in maize by determining the number and chromosomal location of quantitative traits. The authors identified the presence of several genetic factors associated with tolerance to drought. According to other researchers (Schussler and Westgate, 1991; Satarova *et al*., 2016), hot and dry weather in later stages of organogenesis leads to a significant damage to corn leaves, weakening of the accumulation and outflow of plastic substances in the grain and inhibition of endosperm cells division, which negatively affects the grain filling and causes a decrease in yield (Klimova, 2013). Therefore, as a rule, selection for drought resistance is carried out together with the selection for heat resistance.

The last is also a promising area for our future research. Based on the selection by CASP markers, and with the involvement of field trials and biochemical assessment of the physiological state, we will select lines to create drought-resistant and heat-resistant hybrids.

Conclusions

The research allowed estimating the state of functional corn genes by CASP markers in regards to drought resistance. As a result of the research, favourable alleles by dhn1 gene marker were identified in 100 maize lines and by rsp41 marker in 82 maize lines. The number of lines with favourable allele by the makers of both genes was 68, which makes it possible to continue usage of these lines in selection for drought resistance. The information obtained on these lines can be used in subsequent selection work, and the very lines can be used as donors of favourable drought resistance alleles in the future breeding programs.

Conflicts of Interest: The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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Original Scientific Article

Chemical characteristics of fruit of some genotypes of wild apple (*Malus sylvestris Miller*) grown in conditional of Bijelo Polje

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Abstract

This study described some chemical characteristics of fruits in 9genotypes of wild apple (*Malus sylvestris Miller*)in the ecological conditions of Bijelo Polje (Montenegro) in period 2010 to 2012. The study focused on the chemical characteristics - dry matter, total soluble solids, pH, and total acidity. Dry maters was determined by drying at 105 °C. Total soluble solids was determined by refractometer. The acidity was measured by titration with 0.1 N NaOH. The values for fruit dry mater ranged from 14.6% ± 0.28 to 17.58% ± 0.12, total soluble solid contents ranged from 6.90% ± 0.25 to 8.62% ± 0.32, titrable acid contents ranged from 1.03% ± 0.1 to 1.35 % ± 0.2. The aforementioned data on the chemical composition of the fruits of the studied genotypes of wild apple (*Malus sylvestris Miller*)have a general biological significance. Besides, the data proved that genotypes of wild apples are suitable for the production of apple vinegar and apple cider (local name: vodnjika).

Keywords: wild apple; genotypes; chemical characteristics

Introduction

The centre of diversity of the genus *Malus* is situated in East Asia. Minor to the western provinces of China (Juniper *et al*. 1999). Archaeological evidence for the spread of apple from the central Asia is scanty, but the apple has doubtlessly spread by the travellers on the great trade routes, running from the central Asia to the Danube (Clement, 2005).The diversity of wild and cultivated apples, as a whole, represents a great pool of traits for multiple use. This irretrievable diversity is to be preserved now and in the future by gene banks (Büttner*et al*.2004). The cultivated apple (*Malus* x *domestica*Borkh.) is not a simple taxonomic group and includes all the cultivated biotypes in the genus Malus Mill. In the 1920s, Vavilov(1926) travelled through Central Asia and found large wild stands of M. sieversii in specific localities and proposed that the region could be a centre of origin of the domesticated apple. Another early study based on morphological characteristics suggested that several *Malus* species, including *M. sylvestris Miller, M. prunifolia (Willd.) Borkh., and M. baccata (L.) Borkh.*, were involved in the origin and/or domestication of the cultivated apple (Rehder, 1940).

The capacity of *Rosaceae* species for interspecific hybridization, even beyond genus borders, has been exploited in breeding programmes to incorporate desirable traits of wild populations into breeding gene pools. Hybridization between fruit crops and their wild relatives has probably also occurred 'spontaneously' and individuals with intermediate phenobiotypes are known to occur throughout the European landscape. The importance of (anthropogenic induced) hybridization processes has been underestimated by conservation biologists until recently (Allendorf *et al*.2010). However, it is becoming more and more apparent that hybridization has led to the extinction of many

populations and species and represents a severe threat especially to rare species that come into contact with other, more abundant species (Rhymer and Simberloff 1996).

Apple is the most important temperate fruit crop and has been cultivated in Europe and Asia from antiquity (Janick,2005). Wild apples of Montenegro have never been a subject of a comprehensive research work, neither a subject of collecting and studying processes. Nowadays, the most important issue is how to preserve it, especially since many local populations have vanished during its development or are reduced to a rather limited number of genotypes. Nevertheless, germ-plasma of wild apple in less urban areas, such as the area of Polimlje, is rather preserved.

Materials and Methods

Bijelo Polje is situated between 43° and 43°5′ north latitude and 19°40′ and 19°50′ east longitude. The municipality of Bijelo Polje is situated at the altitude from 520m (estuary of the River Kanjska to River Lim) to 2017m (Komovi Mountain). The terrain of Bijelo Polje municipality is following the direction of River Lim flow, i.e. the north and northwest direction. Larger and better-quality agricultural land is located on the near proximity of the River Lim, and its tributaries (Šebek. 2014). The municipality of Bijelo Polje has average annual temperature of 8.9°C. The warmest period is during the months of June, July and August, with an average temperature from 16.3°C to 18.1°C. The coldest period is during the months of December and January, with an average temperature from 0.1°C to -1.6°C. The lowest daily temperatures on annual level are around -3°C. The winter period is characterized with intense negative temperatures, which can reach up to -27.6°C. The spring period is characterized with low temperatures, which can be more than critical when it comes to fruit production (late spring frosts). Namely, the month of April was recorded in some years with the lowest temperature of up to -8°C. The average minimal temperature during April is -2.8° C for the Bijelo Polje territory.

The annual precipitation level in Bijelo Polje municipality is 893.7 mm/m^2. The maximum precipitation is occurring during the period of October - December, while the period with the lowest precipitation is from June - August. The dominance of the cold and rainy period over the warm and dry one is expressed in relation of 54% vs. 46%.

Nine genotypes of wild apple (*Malus sylvestris Miller*) form the municipality Bjelo Polje were analyzed in this study. Particular individual genotypes, represented as solitary trees in numerous villages of the municipality Bjelo Polje, were selected according to chemical characteristics of fruits. Trees were not trained, having free canopy form, under non-irrigated, extensive cultural practice. During the three year period (2010 - 2012) the chemical characteristics of nine genotypes of wild apple (*Malus sylvestris Miller*) were researched.

The study focused on the chemical traits - dry matter, total soluble solids (TSS), pH, and total acidity. Dry mater was determined by drying at 105 °C. Total soluble solids were determined by refractometer. The acidity was measured by titration with 0.1 N NaOH.

Results and Discussion

The results of chemical characteristics of fruits of nine genotypes of wild apple (*Malus sylvestris Miller*) are shown in Table 1 . Fruit dry mater, total soluble solids, pH and titrable acidity content of nine genotypes of wild apple (*Malus sylvestris Miller*) are shown in Table 1.

The values for fruit dry mater ranged from 14.60 ± 0.28 (Genotypes 5) to 17.58 ± 0.12 (Genotypes 1); total soluble solid contents ranged from 6.90 ±0.25 (Genotypes 5) to 8.62 ±0.32 (Genotypes 1); pH ranged from 2.65 (Genotypes 4 and Genotypes 9) to 2.85 (Genotypes 1); titrable acid contents ranged from 1.03±0.1 (Genotypes 5) to 1.35±0.2 (Genotypes 2).

Table 1. Chemical characteristics of fruit of some genotypes of wild apple (*Malus sylvestris Miller*)

Genotypes	fruit dry mater (%) / total soluble solids(%)(TSS) / pH(0 -14) / total acidity(%)(TA)mean±SDmean±SDmean±SD			
Genotype 1	17.58±0.12		8.62±0.32	2.85	1.15 ±0.1
Genotypes 2	16.95± 0.24		7.84 ± 0.30	2.71	1.35±0.2
Genotypes 3	15.56 ± 0.17		7.45 ± 0.29	2.70	1.25 ±0.2
Genotypes 4	14.65 ± 0.17		8. 40 ± 0.30	2.65	1.10 ±0.1
Genotypes 5	14.60 ±0.28		6.90±0.25	2.70	1.03±0.1
Genotypes 6	15.16 ±0.19		7.16 ± 0.26	2.72	1.24 ±0.2
Genotypes 7	16.88 ± 0.22		7.53 ± 0.26	2.78	1.32 ±0.2
Genotypes 8	17.46±0.16		8.60± 0.31	2.80	1.30 ±0.2
Genotypes 9	16.68 ±0.22		7.63 ± 0.29	2.65	1.18±0.1
LSD 0.05	1.23	1.17	0.25	0.07	
LSD 0.01	2.14	1.54	0.41	0.11	

Discussion

In our investigation, *Malus sylvestris* fruits are characterized by a lower average content of fruit dry mater than those of *Malus baccata* and *Malus manshurica*, in which the fruit dry mater contents were 35,93 and 23,79 to 32,53 percent, respectively (Branke,1935). Compared to the fruit dry mater content of *Malus pumila* fruit, which is between 11,07 and 14,80 % (Kostyk 1950; Dolidze *et al.*1966) fruit dry mater content of *Malus sylvestris* fruit is higher.

According to this characteristics, in our investigation of fruit of some genotypes of wild apple (*Malus sylvestris* Miller) is similar to the fruits of wild apple in southern Kirgizia, which range in fruit dry mater content between 10 and 19 percent (Bezzubov 1949) and in Tajkistan, with 15,91% (Speranskij 1936).

Wild apples are inferior to cultivated apples in the amount of fruit dry mater content. In our investigation, the difference in fruit dry mater content is not great (2.98).

Wild apple acidity is two times higher than that of cultivated apples. The literature reports a higher acidity for wild apple fruits in comparison with cultivated apples (Branke1935; Tserevitinov 1949; Khetagurov 1958; Dzhangaliev 1973).

The low sugar and high acidity of wild apple fruits results in a lowsugar: acid ratio. The ratio for wild apples was less than half that of cultivated apples (Mratinić and Fotrić 2011,2012). Large fluctuations in the sugar and acid contents in the forms of wild apple result in very wide differences in the sugar: acid ratio.

Fruits of wild apples are widely used in the confectionery industry formarmalade production (Sorokin 1947) and sweets filling (Maslovsky and Dombkovskaya 1937; Bezzubov 1949; Lisavenko 1959). They are good for pickling and soaking (Unchiev 1947), cider production (Kilchevsky1933; Savitskij 1938), and wine (Murzaev 1934).

The waste (pomace) ofthe wine industry is utilized for the production of high-quality applepectin used in the confectionery industry (Saburov and Grzhivo 1931;Kilchevsky 1933; Turkin 1935; Sorokin 1947). Fruits of some wild apple forms are used for fresh consumption (Vorobiev 1935; Kirillov 1937; Speranskij1936; Vasilichenko 1963; Zapryagaeva 1964), and almost all wild apples are suitable for dried fruitproduction.

The use of wild fruits in the food industry is brought about not only by a necessity to use natural resources, but also because some cultivated apples do not always meet production requirements for such products as cider. In this connection, some countries with a developed fruit processing industry (France and Spain) deliberately breed specific, wild apple varieties for this purpose (Vecher and

Bukin 1940). The use of wild apple fruits in the fruit processing industry is extensively reported in foreign publications (Mieville 1920; Brimble 1946; Chevalier 1953; Iorga 1964).

Conclusions

Wild apples are inferior to cultivated apples in the amount of fruit dry mater content and total soluble solids**.**

The values for fruit dry mater ranged from 14.60 ± 0.28 (Genotypes 5) to 17.58 ± 0.12 (Genotypes 1); total soluble solid contents ranged from 6.90 ±0.25 (Genotypes 5) to 8.62 ±0.32 (Genotypes 1); pH ranged from 2.65 (Genotypes 4 and Genotypes 9) to 2.85 (Genotypes 1); titrable acid contents ranged from 1.03±0.1 (Genotypes 5) to 1.35±0.2 (Genotypes 2).

The aforementioned data on the chemical composition of the fruits of the studied genotypes of wild apple (*Malus sylvestris* Miller) have a general biological significance.

Besides, the data proved that genotypes of wild apples are suitable for the production of apple vinegar and apple cider (local name: vodnjika).

Conflicts of Interest: The authors declare no conflict of interest.

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Original Scientific paper

Yield components and genetic potential of two-rowed barley

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Abstract

The aim of this study was to determine the significance of the source of the yield variability and components of the two-rowed barley, as well as the varieties and lines based on the investigated properties and the extraction of superior genotypes that exhibit high and stable yields. The average plant height for all examined genotypes of two-rowed barley ranged from 75.70 cm (2009/10) to 77.87 cm (2008/09), while the average plant height was 76.79 cm. The length of spike of the studied genotypes of two-rowed barley ranged from 8.32 cm (2008/09) to 8.50 cm (2009/10), while the average was 8.41 cm. The number of grains per spike of the studied genotypes of two-rowed barley ranged from 22.50 (2009/10) to 22.69 (2008/09), while the average was 22.59. The grain weight per spike of the barley in the area of Zaječar ranged from 1.066 g (2009/10) to 1.122 g (2008/09), while the average was 1.094 g. Correlations between the length of spike and number of grains per spike and grain weight per spike in the examined genotypes of two-rowed barley at the location Zaječar showed a positive value both in the vegetations 2008/09 and 2009/10 of the study.

Keywords: *quality, productivity, barley*

Introduction

Barley is one of the oldest plant species used by human population. It underwent significant genetic changes during the domestication process, which allowed it to be widespread, before the formal beginning of plant breeding in order to create varieties for different purposes. Over the course of thousands of years of barley cultivation as a field crop, there has been a change in its basic purpose, from grain essential to human nutrition to a very important raw material in animal nutrition. Even today, in the northern parts and at high altitudes, barley is the only bread grain (Baik and Ullrich, 2008).

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 beer or forage crop, which can be determined by the testing and directed for a particular purpose (Bratković *et al*., 2014; Đekić *et al*., 2012; Popović *et al*., 2011). The yield per unit area is the result of the action of factors of variety fertility in interaction with environmental factors. The main goal in breeding barley is to create varieties with high yield potential and good grain quality suitable for animal feed or brewing industry (Pržulj and Momčilović, 2002). Improved production technology has contributed to the increase in yields in recent decades, because improved production technologies enable better realization of the potential for yield (Đekić *et al*., 2017). High and stable yield of barley is the main goal of breeding, especially in institutions where it is not possible to adequately test the breeding

material on quality traits (Bratković *et al*., 2014). 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 (Madić *et al*., 2018; Mirosavljević *et al*., 2015; Pržulj *et al*., 2014).

Global climate changes cause ever warmer summers and milder winters, which in the future will shift the dates of sowing and heading as well as the barley farming areas. In Serbia, drought is present almost every year. In some years (1990 and 1992) the lack of precipitation was so pronounced that yields of some cultures were reduced by 70% in comparison to the average. In cases with normal winter precipitation, winter barley mainly ends vegetation before the first spring moisture deficit or, for the end of vegetation it successfully uses moisture accumulated during the winter months (Mladenov and Pržulj, 1999).

Bearing in mind the significance and consequences of the interaction between genotype and the environment in barley breeding, the aim of this paper was to determine superior genotypes based on the yields components, which show minimal interaction, i.e. high stability, and as such can be recommended for expansion in production or as parental components in crossing/breeding.

Materials and Methods

Experimental design, soil conditions and statistical analysis

As a test material, 12 recognized varieties and 8 homozygous lines (F7 and F8 generations) of the two-rowed barley were used in this study. Varieties and lines were originally from the Centre of small Grains in Kragujevac (Jagodinac, Maksa and Rekord) and the Institute of Field and Vegetable Crops Novi Sad (NS-587, NS-293, NS-595, NS-519, NS-565, NS-183, NS-525, NS-589, NS-593, J-110, J-90, J-96, J-82, J-103, J-176, J-81 and J-104). On the basis of the average duration of the vegetation period, early and late genotypes were identified. Early genotypes belong are NS-519, J-96, Maksa, NS-565, NS-525, J-110, and late J-176, Jagodinac, NS-183, J-103 and Rekord.

Field trials were conducted in the two-year period (2008/2009 and 2009/2010) at the Zaječar location (Center for Agricultural Research) under conditions of dry cropping. The trials were set by the random design method in four repetitions. The surface of the basic plot was 5 m² (5m × 1m). The sowing was conducted with the use of a machine with a 12.5 cm intermittent distance. The soil on which the trial was conducted was uniform and well prepared. The quantity of seeds per m² was 400-500 germinating kernels, depending on the characteristics of varieties and lines. During the barley vegetation standard agro-technical measures were applied.

The following characters: stem height, length of spike, number of grains per spike and grain weight per spike were analyzed. The sample for analysis of the height of the stem consisted of 80 plants (20 plants x 4 repetitions) taken directly before harvesting.

The main type of soil on which the trial in Zaječar was carried out was carbonate-free vertisol. The physical properties of this soil are very unfavourable and it belongs to the type of heavy clay soil. According to the analysis, this soil was of a slightly acid reaction (pH_{KCl} = 5.67) and medium level of provided humus (2.52). This soil has the medium level of provided phosphorus (15 mg / 100g of soil), and well-provided of potassium (30.1 mg / 100g of soil).

On the basis of the achieved research results, mean variables were calculated. Statistical analysis was made in the module Analyst Program SAS/STAT (SAS Institute, 2000).

Agroecologial conditions

Zaječar its surroundings belong to a moderate continental climate. The latitude of the meteorological station in Zaječar is 43º 53 ', longitude 22º 17', and the altitude is 144 m. The period from November to February, March and June, the temperatures in the vegetation seasons 2008/2009 and

2009/2010 was below the multiannual average, except from April and May in the 2008/2009 year. Temperature variations on average were higher in the first in relation to the second vegetation season.

The period from November to February, March and June in 2008/2009 and 2009/2010 is characterized by rainfall above average (Table 1). In April, precipitation in the vegetation season 2008/2009 was significantly lower and higher in the vegetation season 2009/2010. In May, the precipitation in the vegetation season 2008/2009 was below the multiannual average. During the ripening phase in June, the precipitations in the vegetation seasons 2008/2009 and 2009/2010 significantly exceeded multiannual values.

Table 1. Values of climate variables in 2008-2010 and long-term data for the location (Zaječar)

Months	Nov-Feb	March	April	May	June
	Temperature variations (°C)				
2008/2009	8.0	10.4	14.0	15.8	13.7
2009/2010	8.0	10.9	11.6	12.3	13.1
1981/2010	9.2	11.8	13.4	14.1	14.6
	Precipitation (mm)				
2008/2009	286.4	58.3	14.4	18.0	76.4
2009/2010	391.5	64.3	73.5	58.9	95.1
1981/2010	184.5	40.6	53.2	52.4	58.1

Temperature, precipitation and a sufficient amount of water in the soil are the three most important reasons for the instability of yield in our area. In ecological conditions of Serbia, high temperatures and water deficit in June lead to reduced yields and deterioration of technological properties of grains and malt, which is why the extension of the total vegetation cannot influence the prolongation of the grain filling period to increase the yield (Pržulj *et al*., 1997). Varieties of shorter vegetation, especially winter beer barley, finish the synthesis of most of the dry matter before the onset of the dry period, but have a lower genetic potential for yield due to the positive correlation between these properties (Pržulj, 2001; Pržulj and Momčilović, 2008; Madić *et al*., 2009; Popović *et al*., 2011; Al-Tabbal, 2012; Carpici and Celim, 2012).

Results and Discussion

The barley with the lowest plant height value in the two-rowed barley was determined in the line J-81 (68.71 cm) and the highest in the variety NS-293 (88.90 cm). More favourable conditions in 2008/2009 resulted in a higher average stem length value (77.87 cm) compared to 2009/2010 (75.70 cm). The line J-96 with the highest average plant height in first year (83.39 cm) and line J-176 in second year (86.08 cm). The highest average stem in all year height in the variety NS-293 (88.63 cm and 89.17 cm). More favourable conditions in the second year resulted in a higher average length of spike value (8.50 cm) compared to the first year (8.32 cm). The average length of spike for all genotypes of two-rowed barley in the area of Zaječar in the all years was 8.41 cm (Table 3). The tested lines were the lowest plant height value respect to the varieties and statistically highly significant differences were determined between the years and varieties of barley ($P<0.01$).

Table 2. Mean values for plant height for genotypes of two-rowed barley in the 2008-2010 years

Genotype		2008/09			2009/10			Average		
		x	Sd	Sx	x	Sd	Sx	x	Sd	Sx
1	Jagodinac	80.00	1.910	0.955	80.17	1.331	0.665	80.08	1.527	0.540
2	Maksa	80.00	1.039	0.520	75.63	0.652	0.326	76.81	1.500	0.530
3	Rekord	71.71	1.876	0.938	70.01	0.857	0.428	70.86	1.625	0.575
4	NS-587	73.50	1.943	0.971	71.14	1.837	0.919	72.32	2.157	0.763
5	NS-293	88.63	1.498	0.749	89.17	1.925	0.962	88.90	1.623	0.574
6	NS-595	76.02	0.984	0.492	75.08	1.124	0.562	75.55	1.100	0.389
7	NS-519	79.86	0.689	0.345	71.73	1.249	0.625	75.80	4.449	1.573
8	NS-565	75.81	1.559	0.779	67.88	0.484	0.242	71.84	4.373	1.546
9	NS-183	79.64	1.668	0.834	81.98	1.294	0.647	80.81	1.866	0.660
10	NS-525	80.46	0.948	0.474	75.04	1.599	0.799	77.75	3.142	1.111
11	NS-589	80.98	1.724	0.862	74.50	1.161	0.580	77.74	3.721	1.316
12	NS-593	74.99	1.678	0.839	72.02	2.071	1.035	73.50	2.359	0.834
13	J-110	75.34	1.609	0.804	71.01	0.797	0.399	73.17	2.597	0.918
14	J-90	75.11	1.329	0.665	74.22	1.514	0.757	74.66	1.402	0.496
15	J-96	83.39	0.729	0.364	80.66	0.873	0.437	82.02	1.637	0.579
16	J-82	81.11	1.021	0.510	79.20	1.162	0.581	80.15	1.438	0.508
17	J-103	76.07	1.201	0.600	72.39	1.325	0.662	74.23	2.287	0.808
18	J-176	80.66	1.404	0.702	86.08	0.920	0.460	83.37	3.097	1.095
19	J-81	69.26	0.446	0.223	68.16	0.815	0.408	68.71	0.849	0.300
20	J-104	76.84	0.674	0.337	78.01	0.148	0.074	77.42	0.770	0.272
Prosek		77.87	4.421	0.494	75.70	5.746	0.642	76.79	5.225	0.413

The average plant height for all genotypes was 76.79 cm (Table 2). The height of the stem in new varieties of barley has been significantly reduced, especially in the case of the two-rowed varieties, and ranges from 60-80 cm (Dodig, 2000). Pržulj (2001) considers that in the further breeding of winter barley the height of the stem should be shortened by about 10-20 cm, and further changes should be directed towards the thickness of the stem and finding the anatomical structure that will provide sufficient strength. With barley, the losses in yield caused by lodging in some years can reach more than 65% (Jezovski *et al*., 2003).

The average length of spike for genotypes of two-rowed barley in the area of Zaječar in the growing period from 2008-2010 years was 8.41 cm. The variety with the lowest average length of spike in all years of testing was Jagodinac (7.91 cm) and the highest in the line J-176 (9.65 cm). More favourable conditions in the second year resulted in a higher average length of spike value (8.50 cm) compared to the first year (8.32 cm). The average length of spike for all genotypes of two-rowed barley in the area of Zaječar in the all years was 8.41 cm (Table 3). The variety NS-593 had the higher average length of spike value in the first year (9.34 cm), and the highest length of spike the variety J-176 (9.12 cm). In 2010 year the variety NS-589 (8.99 cm) had the higher average length of spike value in the first year and the length of spike the variety J-176 (10.18 cm). The tested varieties (8.38 cm) were the lowest length of spike value respect to the lines (8.45) and statistically highly significant differences were determined between the varieties of barley ($P<0.01$) and significant differences were determined between the years ($P<0.05$).

Table 3. Mean values for length of spike for genotypes of two-rowed barley in the 2008-2010 years

Genotype		2008/09			2009/10			Average		
		x	Sd	Sx	x	Sd	Sx	x	Sd	Sx
1	Jagodinac	7.67	0.071	0.035	8.16	0.071	0.035	7.91	0.270	0.095
2	Maksa	8.33	0.037	0.019	8.38	0.036	0.018	8.35	0.043	0.015
3	Rekord	8.13	0.043	0.022	8.53	0.085	0.043	8.33	0.223	0.079
4	NS-587	8.70	0.036	0.018	8.65	0.086	0.043	8.67	0.066	0.024
5	NS-293	8.22	0.088	0.044	8.44	0.085	0.043	8.33	0.142	0.050
6	NS-595	8.44	0.064	0.032	8.68	0.099	0.050	8.56	0.150	0.053
7	NS-519	8.08	0.088	0.044	8.31	0.088	0.044	8.19	0.147	0.052
8	NS-565	8.70	0.085	0.043	7.56	0.072	0.036	8.13	0.614	0.217
9	NS-183	7.50	0.044	0.022	8.64	0.086	0.043	8.07	0.613	0.217
10	NS-525	8.20	0.056	0.028	8.12	0.095	0.048	8.16	0.083	0.029
11	NS-589	8.92	0.053	0.027	8.99	0.071	0.036	8.95	0.069	0.024
12	NS-593	9.34	0.029	0.015	8.40	0.091	0.045	8.87	0.506	0.179
13	J-110	8.77	0.071	0.036	8.52	0.073	0.036	8.64	0.149	0.053
14	J-90	7.26	0.091	0.045	7.78	0.043	0.022	7.52	0.286	0.101
15	J-96	7.82	0.043	0.022	8.02	0.088	0.044	7.92	0.125	0.044
16	J-82	8.07	0.036	0.018	8.50	0.085	0.043	8.28	0.238	0.084
17	J-103	8.50	0.085	0.043	8.78	0.044	0.022	8.64	0.162	0.057
18	J-176	9.12	0.044	0.022	10.18	0.096	0.048	9.65	0.571	0.202
19	J-81	8.20	0.056	0.028	8.90	0.071	0.035	8.55	0.379	0.134
20	J-104	8.37	0.077	0.038	8.47	0.074	0.037	8.42	0.088	0.031
	Prosek	8.32	0.518	0.058	8.50	0.524	0.059	8.41	0.528	0.042

The obtained data on the length of the classes, regardless of the year, showed that there was a significant difference between the genotypes, with the average variety NS-589 and line J-176 (Table 3) on average for all years. Kirchev *et al*. (2012) indicate that the length of spike and the number of grains per spike are largely determined by the genotype and that these characteristics are very influenced by climatic factors.

The number of grains per spike of the studied genotypes of two-rowed barley in the area of Zaječar ranged from 22.69 (2009/10) to 22.50 (2008/09). The average number of grains per spike for genotypes of two-rowed barley in the vegetation periods was 22.59 (Table 4). The variety with the lowest average number of grains per spike in all years of testing was NS-565 (20.06) and line J-96 (20.48) and the highest in the variety NS-593 (25.65) and line J-176 (25.55). The variety NS-593 (27.10) and line J-176 (24.40) had the higher average number of grains per spike in the first year. In the second year the variety NS-593 (24.20) and line J-176 (26.70) had the higher average number of grains per spike value (Table 4). The tested lines were the lowest number of grains per spike value respect to the varieties and statistically highly significant differences were determined between the varieties of barley ($P<0.01$).

Table 4. Mean values for number of grains per spike for genotypes of barley in the area of Zaječar in the growing period from 2008-2010

Genotype		2008/09			2009/10			Average		
		x	Sd	Sx	x	Sd	Sx	x	Sd	Sx
1	Jagodinac	23.80	0.455	0.227	23.10	0.392	0.196	23.45	0.543	0.192
2	Maksa	22.50	0.469	0.234	22.10	0.258	0.130	22.30	0.411	0.145
3	Rekord	23.00	0.508	0.254	22.56	0.330	0.165	22.78	0.460	0.163
4	NS-587	22.40	0.258	0.129	22.20	0.248	0.124	22.30	0.258	0.091
5	NS-293	22.61	0.357	0.178	22.86	0.243	0.121	22.74	0.312	0.110
6	NS-595	24.60	0.258	0.129	23.70	0.416	0.208	24.15	0.578	0.204
7	NS-519	23.10	0.374	0.187	22.50	0.248	0.124	22.80	0.435	0.154
8	NS-565	22.42	0.520	0.260	17.70	0.187	0.093	20.06	2.551	0.902
9	NS-183	20.90	0.187	0.093	23.00	0.108	0.054	21.95	1.131	0.400
10	NS-525	22.20	0.474	0.237	22.30	0.363	0.181	22.25	0.395	0.139
11	NS-589	23.00	0.187	0.093	22.66	0.463	0.231	22.83	0.373	0.132
12	NS-593	27.10	0.469	0.234	24.20	0.316	0.158	25.65	1.594	0.563
13	J-110	22.50	0.389	0.195	20.39	0.374	0.187	21.45	1.181	0.417
14	J-90	20.90	0.147	0.074	22.10	0.147	0.074	21.50	0.656	0.232
15	J-96	20.00	0.248	0.124	20.96	0.461	0.230	20.48	0.618	0.218
16	J-82	20.70	0.258	0.129	21.90	0.420	0.210	21.30	0.718	0.254
17	J-103	23.70	0.303	0.151	23.30	0.474	0.237	23.50	0.426	0.151
18	J-176	24.40	0.147	0.074	26.70	0.508	0.254	25.55	1.277	0.452
19	J-81	21.30	0.294	0.147	23.20	0.294	0.147	22.25	1.051	0.372
20	J-104	22.60	0.248	0.124	22.50	0.248	0.124	22.55	0.236	0.083
	Prosek	22.69	1.599	0.179	22.50	1.688	0.189	22.59	1.642	0.130

The barley with the lowest grain weight per spike value in the two-rowed barley was determined in the variety NS-565 (0.997 g) and line J-82 (1.080 g) and the highest in the variety NS-595 (1.169 g) and line J-176 (1.241 g). More favourable conditions in 2008/2009 resulted in a higher average stem length value (1.122 g) compared to 2009/2010 (1.066 g). The grain weight per spike of two-rowed barley in the area of Zaječar in the vegetation periods was 1.094 g (Table 5). The tested varieties (1.089 g) were the lowest grain weight per spike value respect to the lines (1.101 g) and statistically highly significant differences were determined between the varieties of barley and the years ($P<0.01$).

All this suggests that the selection/breeding in the direction of increasing yields is very uncertain and slow due to the very quantitative nature of yield components and the influence of the external environment factors. Pržulj (2001), states that in our production conditions higher yields are realized by varieties of shorter vegetation because they succeed in forming the largest part of the yield before the occurrence of high temperatures. In these studies, barley was not exposed to extremely high temperatures in both years, so the early ripening did not come to full expression.

Table 5. Mean values for grain weight per spike for genotypes of barley in the area of Zaječar in the growing period from 2008-2010

Genotype		2008/09			2009/10			Average		
		x	Sd	Sx	x	Sd	Sx	x	Sd	Sx
1	Jagodinac	1.052	0.025	0.012	0.970	0.026	0.013	1.011	0.050	0.018
2	Maksa	1.097	0.022	0.011	1.035	0.021	0.010	1.066	0.039	0.014
3	Rekord	1.087	0.027	0.014	1.087	0.022	0.011	1.087	0.023	0.008
4	NS-587	1.150	0.029	0.015	1.102	0.017	0.008	1.126	0.034	0.012
5	NS-293	1.107	0.022	0.011	1.097	0.022	0.011	1.102	0.021	0.007
6	NS-595	1.245	0.021	0.010	1.092	0.022	0.011	1.169	0.084	0.030
7	NS-519	1.132	0.043	0.022	1.065	0.013	0.006	1.099	0.047	0.016
8	NS-565	1.190	0.039	0.019	0.805	0.013	0.006	0.997	0.208	0.073
9	NS-183	1.015	0.029	0.014	1.067	0.017	0.008	1.041	0.036	0.013
10	NS-525	1.117	0.033	0.016	1.050	0.026	0.013	1.084	0.045	0.016
11	NS-589	1.160	0.008	0.004	1.115	0.026	0.013	1.137	0.030	0.011
12	NS-593	1.225	0.013	0.006	1.065	0.013	0.006	1.145	0.086	0.030
13	J-110	1.122	0.026	0.013	0.960	0.022	0.011	1.041	0.090	0.032
14	J-90	1.042	0.035	0.017	1.050	0.018	0.009	1.046	0.026	0.009
15	J-96	1.042	0.017	0.008	1.015	0.035	0.018	1.029	0.029	0.010
16	J-82	1.032	0.027	0.014	1.127	0.022	0.011	1.080	0.056	0.020
17	J-103	1.185	0.031	0.015	1.167	0.024	0.012	1.176	0.027	0.010
18	J-176	1.207	0.010	0.005	1.275	0.026	0.013	1.241	0.040	0.014
19	J-81	1.090	0.018	0.009	1.097	0.017	0.008	1.093	0.017	0.006
20	J-104	1.132	0.017	0.008	1.077	0.017	0.008	1.105	0.033	0.012
	Prosek	1.122	0.069	0.008	1.066	0.091	0.010	1.094	0.085	0.007

The analysis of plant height(cm), length of spike (cm), number of grains per spike and grain weight per spike of tested two rowed barley varieties grown at Zaječar during two growing seasons 2008/2009 and 2009/2010, are shown in Table 6.

Analysis of variance in two-rowed barley genotypes tested showed statistically highly significant differences in plant height and grain weight per spike in relation to the growing season ($P<0.01$). Also, the impact of the genotypes on plant height, length of spike, number of grains per spike and grain weight per spike of the tested two-rowed winter barley genotypes was statistically highly significant. It was observed that the number of grains per spike showed no significant difference in the growing seasons. Statistically highly significant difference in plant height, length of spike, number of grains per spike and grain weight per spike is determined under the influence of the interaction year x location x variety.

Table 6. Analysis of variance of the tested parameters (ANOVA)

Effect of year on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(1. 158)	p-level
Plant height (cm)	187.5323	26.28431	7.13476**	0.008352
Length of spike (cm)	1.3487	0.27165	4.96503*	0.027277
Number of grains per spike	1.4402	2.70310	0.53280	0.466514
Grain weight per spike (g)	0.1238	0.00656	18.87812**	0.000025
Effect of cultivar on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(19. 140)	p-level
Plant height (cm)	185.3408	5.849849	31.68300**	0.000000
Length of spike (cm)	1.6326	0.094632	17.25252**	0.000000
Number of grains per spike	16.0392	0.884181	18.14018**	0.000000
Grain weight per spike (g)	0.0291	0.004331	6.72367**	0.000000
Effect of the year x cultivar interaction				
Traits	Mean sqr Effect	Mean sqr Error	F(19. 120)	p-level
Plant height (cm)	22.35860	1.721944	12.9845**	0.000000
Length of spike (cm)	0.59402	0.005112	116.1935**	0.000000
Number of grains per spike	5.68224	0.119855	47.4095**	0.000000
Grain weight per spike (g)	0.02172	0.000582	37.3024**	0.000000

*Statisticaly significant difference ($P<0.05$) **Statisticaly high significant difference ($P<0.01$)

The average values of the Pearson coefficient of correlation (*r*) of examined properties in two-rowed barley are shown in Table 7. Correlations between plant height and length of spike (r=-0.12), number of grains per spike (r=-0.12) and grain weight per spike (r=–0.15) in the examined genotypes of two-rowed barley at the location Zaječar showed a negative value both in the vegetation 2008/09. This shows that the plant height in the two-rowed genotypes has a negative effect on the yield components, which can be explained as a result of the lower resistance to lodging of higher genotypes. The weaker negative correlation between the yield and the height of the stem in the two-rowed and multiple-rowed barley was obtained by Jui *et al*. (1997).

Table 7. Correlation coefficients of the examined properties in two-rowed barley

2008/09 / 2009/10	Plant height	Length of spike	Number of grains per spike	Grain weight per spike
Plant height	-	-0,12	-0,12	-0,15
Length of spike	0.31*	-	0.67*	0.79*
Number of grains per spike	0.42*	0.76*	-	0.77*
Grain weight per spike	0.37*	0.80**	0.85**	-

Positive correlations in the second year of the study between plant height and length of spike, number of grains per spike and grain weight per spike. Most authors point out the weak to very positive effect of grain yield on both types of barley (Akdeniz *et al*., 2004; Bhuta *et al*., 2005). Pržulj *et al*. (1996) 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 weight of 1000 grains and increasing resistance to lodging.

Conclusions

The investigated genotypes of two-rowed barley had significantly lower plant height value in the second year of research (75.70 cm) compared to the first year (77.87 cm). The average height of the stem for all examined genotypes of two-rowed barley was 76.79 cm. The length of spike of the studied genotypes of two-rowed barley while the average was 8.41 cm, number of grains per spike 22.59 and grain weight per spike 1.094 g.

Statistically highly significantly different between of year on the plant height and grain weight per spike and highly significantly different between of genotypes on the all tested parameters. Significantly positively and strong correlated with grain weight per spike and length of spike and number of grains per spike in 2009/10 (0.80** and 0.85**, respectively) and positively and strong correlated in 2008/09 (0.79* and 0.77*, respectively).

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.

Acknowledgments: Investigations necessary for this paper are part of the project TP 31054 and TP 31057, financed by the Ministry of Education, Science and Technology Development of Republic of Serbia.

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Environment protection, natural resources management, Rural development and Forestry

Original scientific paper

Calculation of Soil erosion intensity and Runoff in the S7-8-int basin of the Shirindareh Watershed in Iran

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Abstract

Soil resources are valuable for the livelihoods of human beings, but also vulnerable. Sustainable use of land depends on the conservation and potential use of soil and water resources. Increasing population livelihoods depend on limited natural resources. Landslides, mudslides, the collapse of man-made terraces, soil loss from steep slopes, and the decline of forest/pasture areas are the main reasons for land resource degradation. Soil erosion is a global environmental threat for the natural environment and agriculture. Soil erosion by water is one of the most dangerous processe of land degradation. It is a process of detachment, transportation of surface soil particles from its original location and accumulation of it in a new depositional area. In Iran is one of the prime concerns of the nation. This paper presents modelling of soil erosion processes using a "River Basin" model for prediction of erosion potential, assessing the impacts of area's physical-geographical conditions, climate and land use. Calculations have been made for the S7-8-int basin of the Shirindareh Watershed in Iran. Calculations processed by model shown that the Coefficient of the river basin form, A, is 0.77; Coefficient of the watershed development, m, is 0.38. (A)symmetry coefficient indicates that there is a possibility for large flood waves to appear in the river basin. The value of Density of the river network of the basin, G, of 1.91, indicates that there is high density of the hydrographic network. Production of erosion material in the river basin, W year, is 5443 m^3 $year^{-1}$. Calculating this result with the coefficient of the deposit retention, Ru, that is 0.234, the model shown that the Real soil losses, G year, are 1276 m^3 year-1, that is 74 m^3 km^{-2} $year^{-1}$, per square kilometer. The value of 73.95 m^3 km^{-2} $year^{-1}$ indicates, according to Gavrilovic classification, that the river basin belongs to the region of very weak erosion. The results of the research and earlier application of the "River Basin" model in the studied area of the Shirindareh River Basin in Iran shown that this is a good tool for rapid assessment of erosion risk to support decision-making and policy development.

Keywords: *River basin model, soil erosion, water erosion, runoff, watershed.*

Introduction

Soil erosion is a very complex and is a result of complicated interactions among many existing factors in a watershed system. Elucidating soil erosion phenomenon and magnitude can therefore represents balance level in the watershed systems and provides a useful index of land degradation, soil erosion severity and trends (Sadeghi, 2017). Accurate estimates of sediment yield and its temporal variation are needed for various purposes, including the design of impoundments and erosion control structures, river morphological computations, and evaluation studies of the effects of various land use management practices (Zakerinejad and Maerker, 2015).

Understanding of soil erosion process in the watershed is a basic necessity in achieving integrated land management and soil and water conservation (Khaledi Darvishan et al., 2014). Direct measurements of erosion in a watershed are possible with multi-years measurement of solid transport in the closing-section (Behzadfar et al., 2014a and Behzadfar et al., 2014b). The water and sediment sampling in given intervals need a lot of time and is costly (Khaledi Darvishan et al., 2010), assessment of sediment yield using soil erosion models have been used more and more (Spalevic et al., 2013a, 2013b, 2013c, 2013d; Vujacic et al, 2017; Vujacic et al, 2015). The modelling of the erosion process has progressed rapidly, and a variety of models have been developed to predict both runoff and soil loss.

For this exercise we used "River Basin" model of Spalevic (Spalevic et al., 2000; Spalevic, 1999) for prediction of soil erosion intensity from the watershed area.

Material and Method

The study was conducted in the area of the S7-8-int basin of the Shirindareh Watershed (Surface, F, 17 km^2, perimeter, O, 23 km) from the north eastern parts of Iran, close to the Caspian Sea. The shortest distance between the fountainhead and the mouth, lv, is 5.6 km; and the total length of the main watercourse with tributaries is 33 km.

The average slope gradient in the river basin, Isr, is calculated on 8.24 % what indicates that in the river basin prevailing mild slopes. The average river basin altitude Hsr, is calculated on 1110 m; the average elevation difference D, on 151 m.

Basic climatological data: The volume of the torrent rain; Average annual air temperature; Average annual precipitation; needed for calculation of the soil erosion intensity and runoff from the River Basin were received from the meteorological stations located in North Khorasan province of Iran. The climate of the studied region is continental, with the absolute maximum temperature of 34.6°C and the negative of -24.4°C, respectively. Average annual air temperature, t0, is 9.1°C and the Temperature coefficient of the region, T, is calculated on 1; the amount of torrential rain, hb, on 34.68 mm. The average annual precipitation, Hyr, is 317 mm (Source: Data from the North Khorasan Meteorological stations of Iran).

The geological analyses (geological formations of North Khorasan province, including those in the study area of the S1-2 watershed) were based on the research of the National Geological Survey Organization (NGS) of Bolourchi (1987).

We used morphometric methods to determine the slope, the specific lengths, the exposition and form of the slopes, the depth of the erosion base and the density of erosion rills (led by Behzadfar).

For calculation of soil erosion intensity we used a River basin model (Spalevic *et al*, 2000) for quantification of erosion processes by estimation of erosion intensity, sediment production and transportation of erosion sediment by river network, based on the Erosion Potential Method (Gavrilovic, 1972).

This method was based on the Method for the Quantitative Classification of Erosion (MQCE), formally developed in 1954, which later became a part/segment of today`s version of the Gavrilovic

method (Dragicevic, 2016). Extensions of this method were directed towards the quantification of erosion processes by assessing the sediment transported downstream that reaches the control profiles (Amini *et al*., 2010).

Equations and description of the parameters for the Gavrilovic method are presented in the table 1 (de Vente and Poesen, 2005; Gavrilovic, 1972).

Table 1. Equations and description of the parameters for the Gavrilovic method

$W_a = T * P_a * \pi * \sqrt{Z^3} * F$	(1)	W_a	Total annual volume of detached soil [m^3/year]
		T	Temperature coefficient [-]
		P_a	Average annual precipitation [mm]
$T = \sqrt{\frac{T_0}{10} + 0.1}$	(2)	Z	Erosion coefficient [-]
		F	Study area [km^2]
		T_0	Average annual temperature [°C]
$Z = Y * X_a * (\phi + \sqrt{J_a})$	(3)	Y	Soil erodibility coefficient [-]
		X_a	Soil protection coefficient [-]
$\xi = \frac{\sqrt{O * z}}{(l_p + 10)} * D_d$	(4)	ϕ	Coefficient of type and extent of erosion [-]
		J_a	Average slope of the study area [%]
		ξ	Sediment delivery ratio [-]
$D_{d.original} = \frac{1}{0.25} = 4$	(5)*	O	Perimeter of the watershed [km]
		z	Mean difference in elevation of the watershed [km]
$D_{d.modified} = \frac{l_p + l_a}{F} = \frac{L}{F}$	(6)**	D_d	Drainage density [km/km^2]
		l_p	Length of the principal waterway [km]
$G_y = \xi * W_a$	(7)	l_a	Cumulated length of the secondary waterways [km]
		L	Cumulated length of the principal and the secondary waterways [km]
		G_y	Actual sediment yield [m^3/year]

* Originally set as a constant value, continues to be applied in various research

** Modification of the method made by Lazarević (Tosic and Dragicevic, 2012), applied today in various studies

According to de Vente (de Vente, 2009; de Vente and Poesen, 2005), this method can be characterised as a semi-quantitative method because it is based on a combination of descriptive and quantitative procedures.

Results and Discussion

The studied area belongs to the Middle-East of the Kope-Dagh geographical region.

River basin planning, coefficient of the river basin planning, Xa, is calculated to be 0.61.

Coefficient of the vegetation cover, S2, is calculated to be 0.90.

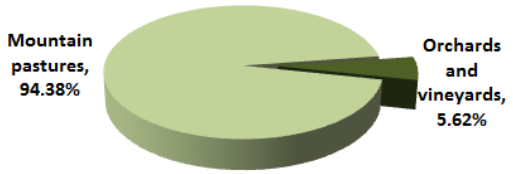

Figure 1. Land us structure in the S7-8-int basin of the Shirindareh Watershed

The dominant erosion form in this area is surface erosion and is the most pronounced on the steep slopes without vegetation cover. Calculation of Sediment yield of S7-8-int basin of the Shirindareh Watershed is presented at the "River Basin" Report 1.

Table 1: River basin report for the S7-8-int basin of the Shirindareh Watershed

Input data:			
River basin area	F	17.26	km²
The length of the watershed	O	22.24	km
Natural length of the main watercourse	Lv	5.64	km
The shortest distance between the drainage divide and mouth	Lm	4.8	km
Main watercourse with tributaries of class I and II	ΣL	33.05	km
River basin length measured by a series of parallel lines	Lb	1.57	km
The area of the larger river basin	Fv	9.6	km²
The area of the smaller river basin	Fm	7.66	km²
Altitude of the first contour line	h0	1000	m
Equidistance	Δh	100	m
The lowest river basin elevation	Hmin	959	m
The highest river basin elevation	Hmax	1345	m
Portion of the basin with very permeable rocks	fp	0.1	
Portion of the basin with medium permeable rocks	fpp	0.67	
Portion of the basin with low permeability rocks	fo	0.23	
Portion of the river basin which is forested	Fs	0	
Portion which is grass, meadows, pastures and orchards	Ft	1	
Portion of plough-land, ground without grass vegetation	Fg	0	
The volume of the torrent rain	hb	32.12	mm
Incidence	Up	100	years
Average annual air temperature	t0	12.9	°C
Average annual precipitation	Hyear	286.1	mm
Types of soil and related types	Y	1	
Coefficient of the river basin planning	Xa	0.61	
Numeral equivalents of visible erosion process	Φ	0.43	
Results:			
Coefficient of the river basin form	A	0.77	
Coefficient of watershed development	m	0.38	
Average river basin width	B	10.99	km
(A)symmetry of the river basin	a	0.22	
Density of the river network within the basin	G	1.91	
Coefficient of the river basin tortuousness	K	1.18	
Average river basin altitude	Hsr	1110	m
Average elevation difference of the river basin	D	151.16	m
Average river basin decline	Isr	8.24	%
The height of the local erosion base of the river basin	Hleb	386	m
Coefficient of the erosion energy of the river basin's relief	Er	60.28	
Coefficient of the region's permeability	S1	0.74	
Coefficient of the vegetation cover	S2	0.8	
Analytical presentation of the water retention inflow	W	0.4362	m
Energetic potential of water flow during torrent rains	2gDF^½	226.25	m km s
Peak discharge from the river basin	Qmax	44.87	$m^3 s^{-1}$
Temperature coefficient of the region	T	1.18	
Coefficient of the river basin erosion	Z	0.446	
Production of erosion material in the river basin	Wyear	5443.541	$m^3 year^{-1}$
Coefficient of the deposit retention	Ru	0.234	
Real soil losses	Gyear	1276.34	$m^3 year^{-1}$
Real soil losses per km²	Gyear	73.95	$m^3 km^{-2}$

Figure 2. From the filed, Shirindareh Watershed in Iran (Photo: Morteza Behzadfar, Velibor Spalevic)

In the rsults of table (1) generated by the River basin is stated that the Coefficient of the river basin form, A, is 0.77; Coefficient of the watershed development, m, is 0.38; Average river basin width, B, is 10.99 km. (A)symmetry of the river basin, a, is 0.22 and indicates that there is a possibility for large flood waves to appear in the river basin.

Production of erosion material in the river basin, W year, is 5443 m^3 god^{-1}. Calculating this result with the coefficient of the deposit retention, Ru, that is 0.234, he model shown that the Real soil losses, G year, is 1276 m^3 year-1, that is per 74 m^3 km^{-2} $year^{-1}$, per square kilometer. The value of 73.95 m^3 km^{-2} $year^{-1}$ indicates, according to Gavrilovic classification, that the river basin belongs to the region of very weak erosion.

The results of the research and earlier application of the "River Basin" model in the studied area of the Shirindareh River Basin in Iran shown that this is a good tool for rapid assessment of erosion risk to support decision-making and policy development.

The provided methodology have been successfully used in Iran in the regions of Chamgardalan, Kasilian, Kermanshah, Razavi Khorasan (Spalevic *et al*., 2016; Draganic *et al*., 2015a; Draganic *et al*., 2015b; Behzadfar *et al*., 2015; Barovic & Spalevic, 2015) and other regions.

Conclusions

This research was an attempt to present the status of soil erosion processes in the S7-8-int basin of the Shirindareh Watershed in the North East Iran. Calculations processed by the River basin software shown that the Production of erosion material in the river basin, W year, is 5443 m^3year^{-1}. Calculating this result with the coefficient of the deposit retention, Ru, that is 0.234, the model shown that the Real soil losses, G year, is 1276 m^3 $year^{-1}$, that is 74 m^3 km^{-2} $year^{-1}$, per square kilometer. The value of 73.95 m^3 km^{-2} $year^{-1}$ indicates, according to Gavrilovic classification, that the river basin belongs to the region of very weak erosion.

The results of the research and earlier application of the "River Basin" model in the studied area of the Shirindareh River Basin in Iran shown that this is a good tool for rapid assessment of erosion risk to support decision-making and policy development.

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Original scientific paper

Floods on the river Beljanica in May 2014

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Abstract

Catastrophic flooding caused by a prolonged cyclonic activity in May, 2014, affected a wide area of Serbia, Bosnia and Herzegovina and Croatia. A cyclone activity supported by stormy winds, created the conditions in this area for a vast amount of precipitation, on average of 150 - 250 mm and more. The period preceding the catastrophic floods was characterized by prolonged low rainfall intensity, so that the soil was completely saturated with water. On such a saturated soil subsequent enormous rainfalls caused a sudden swelling and concentration of water in the small and large watercourses. The coincidence of high waters in all basins in the wider area was very pronounced. In this paper has been presented the flood wave on the river Beljanica, the right-side tributary of the Kolubara River, central Serbia. The aim of this study was to analyze the flows of high water in the riverbeds of the medium-sized watercourses, of the catchment area up to 1000 km^2. Based on the registered markings of large waters during the floods and after the cessation of the flood wave, the reconstruction of high waters was done in order to review the scope and size of the floods.

Keywords: *flash floods, small river basins, water flow, sediment transport, flood wave.*

Introduction

Floods in Serbia, in May 2014, occurred after three days of heavy rainfall and strong cyclonic activity that swept through the central part of the Balkan Peninsula. Precipitation from 13 to 15 May, 2014, engulfed the whole of the Balkans. On smaller watercourses in the Kolubara basin the flood peak took place from 14th - 16th May, and since that date the flood wave began to recede gradually. The cyclone was called "Tamara" and affected a huge area of the Central and Southeast Europe. It began to form on 12th May and from 13th May, 2014, registered its strongest activity. It spread out on a large horizontal surface, with its vertical thickness of up to 100 *km* throughout the troposphere (RHMZ-Serbia, 2014).

Humidity saturation of the air mass during the course of cyclone activity was close to 100%, and the moisture content kept increasing owing to the warm air from the south and east (Kondolf, G.M. et al. 2002).

In support of the development of such a low pressure area was also contributed the physical geographical specificity of the Balkan Peninsula, a continental mountainous area. The centre of the cyclonic area was over Serbia and Bosnia and Herzegovina, where between 13. and 15. May fell a large amount of rainfall. The largest ever recorded since the beginning of making meteorological measurements and observations. (RHMZ- Serbia, 2014).

On the basis of data measurement and calculation, it was found that the incidence of rainfall of greater intensity and the likelihood of its occurrence is within the limits shown in *Table* 1. Precipitation phenomena of 0.01% the order of magnitude 190 mm, *Table 1*. In this period, from 13. to 14. May, in Stepojevac fell 293.50 mm, in Ljig 229.50 mm, which indicates that there occurred the disaster of a less frequent probability. Statistically, less than once in 10,000 years. The disasters caused

widespread destructions and flooding, and will have far-reaching consequences for the affected region. The weakening and disappearance of the cyclone started during 16. May. (*2014*).

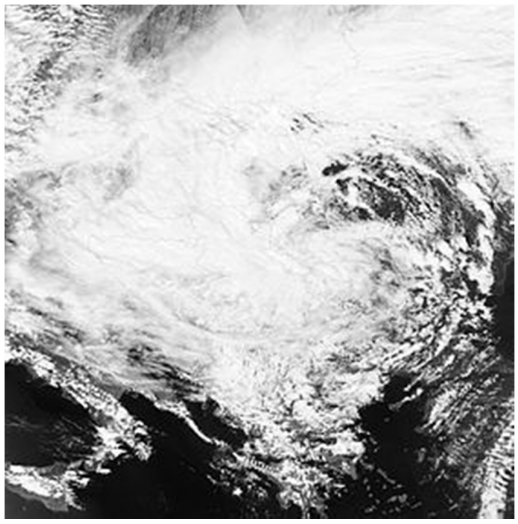

Figure 1. The satellite image of the cyclone activity in the Balkans (source: www.google.com)

Obrenovac was hardest hit by the floods. It is estimated that 90% of the settlement was submerged. From the city and suburbs were evacuated about 8,700 residents. In close proximity of Obrenovac is Nikola Tesla thermal power plant (A and B), the largest in Serbia, sending nearly 50% of the electricity into the electric power system of Serbia. Thermal power plants were not flooded thanks to good and urgent interventions.

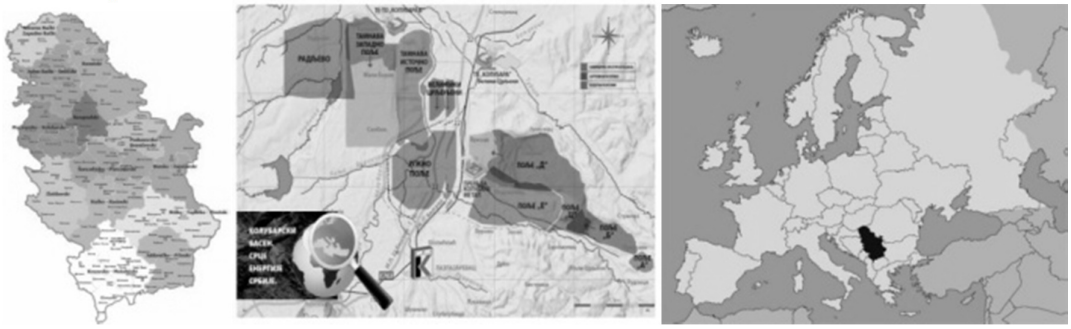

Figure 2. Overview map of the vulnerable areas Vreoci, in the a river basin

Figure 3. Flooding of Beljanica in the area of May 15., 2014 (photo. A. Anđelković)

The open pit mine Tamnava East and West field, were flooded with water of the Beljanica, Pestan and Kolubara rivers. The city centres of the following towns were also flooded: Obrenovac, Lazarevac, Smederevo, Paraćin, Jagodina, Petrovac on Mlava river, Svilajnac, Smederevska Palanka, Ljig, Preljina near Čačak, Krupanj and many other places were flooded with small streams, with the catchment area of less than 1000km^2. Beljanica River with its largest tributary Turija in the area of the settlement Vreoci and Stepojevac swelled out of its main watercourse and flooded the entire river valley Fig.3.

Basic characteristics of the Beljanica river basin

The catchment area of the river Beljanica with its largest tributary Turija is 454,10 km^2. Its spring is at 445m in the mountain of Kosmaj, under the name of Velika Tresija, merges with the river Mala Tresija and from there it flows as the Turin river. Among the major tributaries below the village Mirosaljci at km 35 + 450 on the left side into it flows the Turin stream. At km 20 + 500 on its right side into it flows Sibnička river and near the village of Sibnica the Turija flows into the Beljanica. From this point the Beljanica flows to the mouth along the regulated watercourse without tributaries. Direct tributaries of the river Beljanica in the source area, are: at km 13 + 400 from the confluence with the Turija near the village Beljina, it flows into Stojnička and Guberevačka river. At km 11 + 400 it on its right side it receives the stream Buljkovac, at km 7 + 880 into it flows the Barajevska river, at km 3 + 360 on the right side into it flows the river Oparna and at km 1 + 780 on the left side into it flows the Seona. (Đeković, V., et al 2015 / b) (Notaro, V. 2014)

It is estimated that into the two surface coal mines poured into the "Zapadno polje" 187.30 million m^3 of water and in the open coal mine "Veliki Crljeni" 27.5 million m^3 of water.

The office report of the open coal mine "Tamnava" states that the surface of the water mirror at "Tamnava Zapadno polje" is 7.44 km^2 and "Veliki Crljeni" 2.27 *km*^{2}. The maximum width of the larger lake is 3.2 km and its length is about 5.0 km, while the smaller lake has width 1.1 km and its length is 2.60 *km*. The conveyors, excavators which conducted excavations, utility machines, workshops and equipment were also submerged as. Fig.4 i.5

Direct damage caused by the devastating floods that have recently befallen Serbia amounted to 810.1 million euros, while the indirect losses amount to 661.9 million euros, showed the official report on the assessment of flood damages adopted by the Government of Serbia which was presented at a donor conference in Brussels on 16. July 2014.

On Fig.4 can be seen the excavator "Glodar" from whose huge metal structures over 40 meters high now could be seen above water only its top parts. Under the water, which in some places is over 70 meters deep, there is a real small town. Underwater remained over 20 km of conveyor belts, Fig.5. automated stations, one mobile workshop for excavator repair, a number of machines and various mining equipment. Under water remained "Zapadno Polje", the water surface mirror is P = 7,44km^2, volume of that poured into this surface pit is W = 187.30 million m^3 and the maximum depth is h = 66.4 m, a surface mining "Veliki Crljeni" after water is P = 2.27 km^2, with a volume of water W = 27.50 million m^3, and a maximum water depth h = 28.90 m. (Zarić, M., 2014).

Figure 4. Immersed excavator in the open pit "Tamnava"

Figure.5. Conveyors "Tamnava East Field" (Photo. V. Đeković)

For all the tributaries of the river Beljanica is characteristic that in the lower water courses and the flood-prone areas they have spread out of river valleys, and along their banks has developed intensive agricultural production and village settlements. Their cross water courses are relatively small meandering river courses, changing the course of their flow. Beljanica River from the mouth of Turija River to the confluence into the river Kolubara has a length of 13, 4 km, with an average fall of the riverbed around 3.91 ‰. (Đeković, V., et al., 2015 /a)

In the lower watercourse of the river Beljnica in the experimental section, due to frequent changes in morphology, the fall of the regulated river bed is fractured Fig.6. The causes of the fall of the regulated river bed are in the uneven geo-mechanical structure of the material at the bottom and along the banks of the riverbed, which is why there are variable dimensions of the flow profiles. (Đeković, V., et al. 2015 / b) 5. (Dolgopolova E. N. 2003)The effects of geo-mechanical structure of the material from the flow profile of the watercourse indicate evidence of deformation at the bottom and banks during flooding. The particle coarseness of sediment fractions is in the range of 0.001 to 1.0 *mm* (*Diagram* 1). This process is recorded during the floods of 2014.

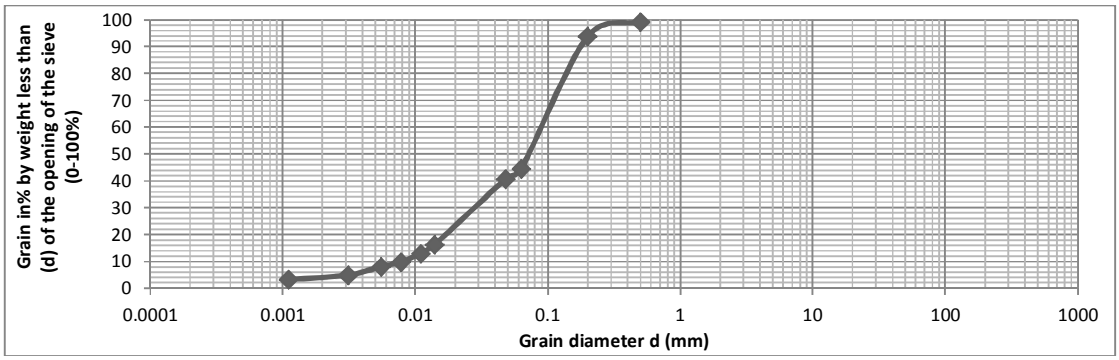

Diagram 1. Granulometric composition of material from the banks of the river bed at the measurement point at km + 1 500km. (Original source)

When the waters are concentrated into the flow profile while forming and when the flood wave is formed, Figure 10, then the traction force of flow, regardless of the geo-mechanical characteristics of the material in the flow profile, has a key role in the initiation of sediment transportation (movement) from the banks and the bottom of the river bed (Braunović Sonja et al. 2016) (Lane, S.N. 2005). In such conditions, river flow moves large amounts of erosion material and transports it through a hydrographic network of a watercourse, creating a deformation of the flow profile of the watercourse. Fig. No.8, 9. Morphological changes are a result of the "erosion and deposition of sediments" which the watercourse periodically moves, carries or disposes in a flow, regulated profile or a natural river bed.

The process of erosion begins at the stage when atmospheric precipitations begin to pour down the river basin slopes and at the time when the first concerted flows along the lines of the biggest drop begin, subsequently leading to the process of fluvial (river) erosion. Then, as a condition of further erosion phenomena occurs the energy of formed river courses which destroys the structural land aggregates, undermines the bank, and the resulting sediment is moved by the turbulent force of the flow along the formed river courses. The peak areas of catchments, flooding in May 2014, possessed their destructive force and caused damage to communications (Yannopoulos S. et al.2013),(Chin, A., and Gregory, K.J. 2005)

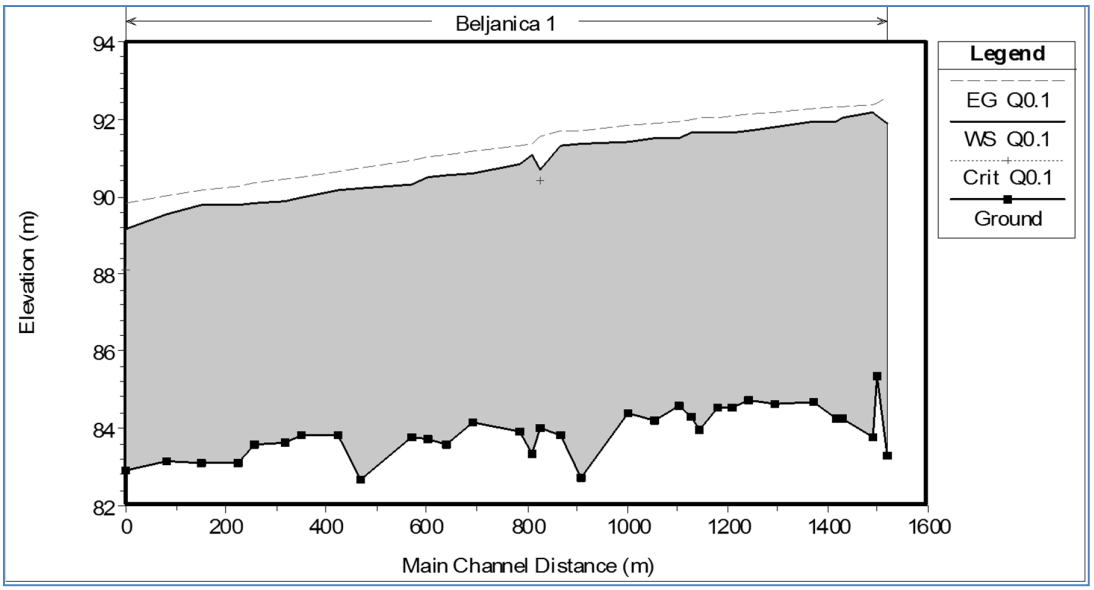

Figure 6. The longitudinal profile at the studied sector of the Beljanica sector watercourse "Hec-Ras"

Figure 7. The profile of the Beljanica River after floods (photo. A.Anđelković)

Figure 8. and 9. The formation of the flood wave in the source tributaries of Beljanica and Damaged communications (photo. A. Anđelković)

Materials and Methods

To study the influence of cyclonic activity from 15 to 16 May, 2014, and subsequent occurrence of heavy rainfall leading to widespread flooding in Serbia, a methodology of experimental river basins was used. In the lower watercourse of the river Beljanica were registered extreme water flows in a confined space and estimation was also made about the impact on the occurrence of floods in the downstream watercourse as well as their implementation on the occurrence of global floods in Serbia.

Material for writing this paper are the data of field research papers of the authors during the floods and immediately after the passage of the flood wave.

The aim of this work is the reconstruction of the flood wave from 14th to 16th May, 2014, during the period of extreme rainfalls. The combined method of reconstruction based on consequences of high waters and tared computational throughput of the water course via the "Hec-Ras" program was applied. For the model to be applicable are necessary extensive field measurements and recordings both during the flood period and subsequently after the passage of the flood wave. We applied the following methods:

Experimental research on the water course and in the Beljanica river catchment, divided into two phases:

1. Direct recording of water levels at flow profiles of the watercourse in the flood period, 15 May, 2014, and summarized, in the entire section of the watercourse;

2. Locating of the measured profiles (33 in total were located on the section of the length of 1 + 500 km.).

Field reconnaissance of the basin, specifying the conditions for the occurrence of surface erosion processes and high water concentration in the main river course and its tributaries.

For further analysis the input data include the results of the direct surveying of markings of the flood wave and the morphology of the river course flow profiles in the zone of markings of high waters. Assessment of the impact of the river course resistance coefficient to reduce velocity and river course drag force and to prevent fluvial erosion processes as well.

In this paper are shown the results of reconstruction of the flood wave that occurred in the lower water course of the Beljanica river by using a differential equation of non-uniform flow in a deformable flow profile through the elaborate computer program "Hec-Ras," with extensive surveying on the section of the 1 + 500 km and 33 measured profilesof the watercourse.

Results and Discussion

Reconstruction of the flood wave

Hydraulic calculation of the high waters flow in the flood on 15 May, 2014, and the calculation of other hydraulic parameters were carried out by means of the differential equation of non-uniform flow. The basic parameters of water course used in the calculations are morphological parameters of the flow profile along the experimental section, and predetermined points of large water levels as well as the route of the water course with a drag coefficient upon the contour of the wetted watercourse perimeter. The river bed drop is defined by the longitudinal profile of the watercourse, measurement points and mileage markings. Calculations were carried out through "Hec-Ras" and a differential equation (1)

$$\Delta Z = \frac{Q^2}{K^2} \cdot \Delta L + \frac{(V_u^2 - V_n^2)}{2g} \qquad [1]$$

Where:

DZ – denivelation of the water level on the total section of the watercourse from one profile to another one;

K - mean value of the flow module;

V^2_u and V^2_n - flow velocities in the upstream and downstream profile (from profile to profile).

The hydraulic analysis included the local section of the watercourse with a total length of 1 + 500 km where there were precisely registered marks of high water. The application of "Hec-Ras" program was performed, tarring of the watercourse flow capacity up to the registered high water mark.

To determine the probability of water flow during the floods from 14 to 16 May, 2014, on the ground were registered marks of water and the water levels elevation at profiles Fig.7. (RHMZ-RS. 2014)

According to the main project for the regulation of Beljanica river flow the occurrence of once in 100 years flow is $Q_{1\%} = 228\, m^3 \cdot s^{-1}$ while the water flow probability of once in 1,000 years $Q_{0,1\%} = 370\, m^3$ Based on the registered marks of high waters in profiles during flooding on the river Beljanica and detailed surveying recordings of the cross sections, after the passage of the flood a series of the watercourse throughput capacity was calculated, until the exact marks of the water level were obtained. The flow rates $Q_{1\%} = 228\, m^3 \cdot s^{-1}$ and $Q_{0,1\%} = 370\, m^3 \cdot s^{-1}$ do not outflow from the watercourse flow profile of the watercourse, while the flow of Q =400 $m^3 \cdot s^{-1}$ is at the registered elevation marks in the watercourse flow profile. Millennial high waters, according to the main project for the regulation of Beljanica river in this sector are $Q_{0,1\%} = 370\, m^3 \cdot s^{-1}$ The analysis found that these waters do not outflow from the basic watercourse. Partial outflows occur only for outflow of $Q = 400\, m^3 \cdot s^{-1}$which significantly exceeds $QQ_{0,1\%}$ so that it can be concluded that the flood wave on 15 May, 2014, formed a flood wave with the flow exceeding $Q_{0,1\%}$.

This paper presents only some flow profiles with water level marks and line of energy flow and the final analysis of the flow of indicating the scope and size of this natural disaster. Based on Table 1. rainfall probability of once in 10,000 years amounted to $H_{0,01\%}$=194 *mm.* During the cyclone "Tamara" at the meteorological station Stepojevac was registered H=293,50 *mm Tab. br. 2.* (RHMZ-Srbije 2014)

Table 1. Applicable computational rains for the calculation of high waters for the analyzed area (source of origin)

Applicable computational rains for the calculation of high waters			
N⁰	**Probability %**	**Recurrence period**	**H_d (p)**
1.	0,01	10 000	194,00 (*mm*)
2.	0,1	1000	137,00 (*mm*)
3.	1	100	93,80 (*mm)*
4.	2	50	82,00 (*mm*)
Parameters of distribution: P=1,599 C=0,086 C=0,527			

Tab. 2. Data on measured rainfalls in the close proximity of Beljanica river basin (source: RHMZ RS)

N⁰	Area GMS	Station name	Data on precipitations in *(mm)*			
			14.5.2014	15.5.2014	16.5.2014	Total for three days
1.	Beograd	Pinosava	16.20	129.00	72.20	217.20
2.	Beograd	Makiš	31.10	100.80	46.20	178.10
3.	Beograd	Jainci	21.20	148.20	48.20	217.60
4.	Beograd	Cerak	25.20	104.00	47.20	176.40
5.	Valjevo	Stepojevac	23.60	185.10	84.80	293.50
6.	Valjevo	Ub	61.20	114.80	51.20	227.20
7.	Valjevo	Ljig	31.00	125.00	73.50	229.50

Based on the probability of extreme rainfalls in Tab. 1. and really registered precipitations during 14 - 16 May, 2014,in Fig.2 it could be preliminarily concluded that the floods are of catastrophic proportions and therefore, in the final analysis data was used on the occurrence of high waters >0.1%, $Q = 400\ m^3 \cdot s^{-1}$. Parameters of theoretical distribution of high intensity rainfalls before recent peak rainfalls in May, 2014, max. daily sum, Log-Pearson-III, as well as the results of the calculations are shown in *Tab. 1.*

Figure 10. The outflow of water from the main Beljanica watercourse.

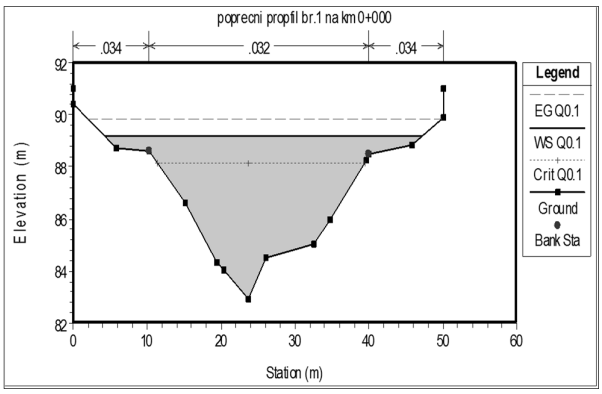

Figure 11. The initial flow profile in Hec-Ras

Model Tarring and display of a section of cross sections along the watercourse

At the selected profile no. 7 km 0 + 350.37 is observed an overflow of high water on the right side bank with a water depth of 0.50 m, the same phenomenon has been registered at several profiles along the experimental watercourse section and on the presented profiles no. 28 and 32. Also along the watercourse occur fractures in the drop of river bed levels Fig.6.

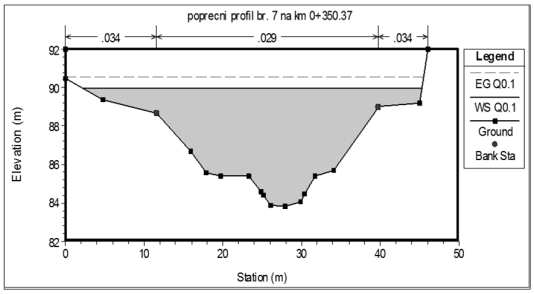

Figure 12. Profile no. 7 at km. 0 + 350.37

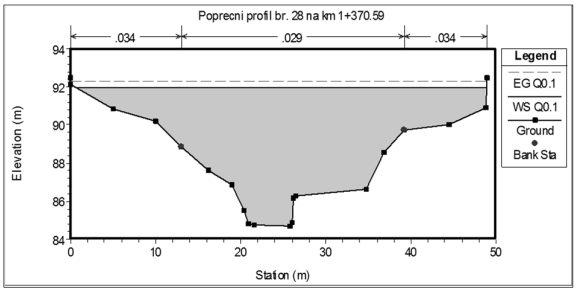

Figure 13. Profile no. 28 at km. 1 + 370.59

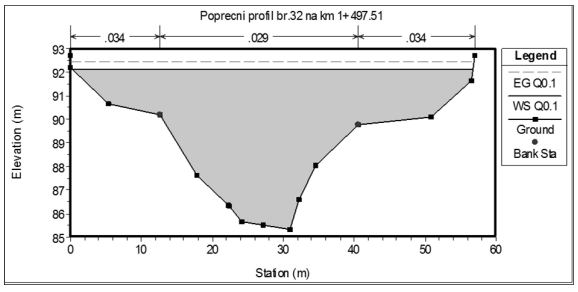

Figure 14. Flow profile no. 32 at km 1 + 497.51

Table 3. Presents the results of the hydraulic analysis, and there can be noted that the flow regime in the experimental section is still ($F_r < 1$), while the medium flow rate is in the range of $Q_{sr} = V2{,}58 - 3{,}64m^1 \cdot s^{-1}$, along the profiles the depth of waters at the same time vary within the range of BAM 6,14 - 8.56 meter while the tangential stress along the bottom (river bed) of the flow is($\tau_0 = 0.0438 - 0{,}2478kN \cdot m^{-2}$)*Table 3* The value of the critical tangential stress with the tarred Shields constant is "0.0478" and medium-sized granular sediment (d_{sr}) from the watercourse bed is in the range of d ($\tau_{cr} = 0.0478 \cdot g(\rho_s - \rho)d_{sr} = 0.04149 - 0.0826 \cdot kN \cdot m^{-2}$). Based on this, it can be concluded that deformations of the flow profile are mainly located in the upper sector of the experimental section. In spite of the calm flow regime in the bed of the watercourse occurred vast morphological changes due to increased tangential stress, *Table 3., Fig. 10.* (Letić, LJ., Et al 2008)(Frantar, P. 2013)(Dan D., 2016)

Table 3. The presentation of hydraulic parameters "Hec-Ras" for flow Q_{max} =400 $m^3.s^{-1}$ (source original)

Profile	Min.Ch.El	W.S.Elev	Wat. depth	E.G.Elev	E.G.Slope	Vel Chnl	Flow Area	Top Width	Fraud	Tang. Tens
N⁰	*m*	*m*	*m*	*m*	*m/m*	*m/s*	*m²*	*m*		*kN.m⁻²*
33	88.30	91.89	8.56	92.55	0.001926	3.64	115.52	34.05	0.53	0.1886
32	85.34	92.11	6.77	92.42	0.000779	2.61	179.32	56.36	0.38	0.1926
31	83.75	92.17	8.42	92.38	0.000402	2.15	210.33	47.86	0.27	0.2010
30	84.23	92.02	7.75	92.34	0.000736	2.60	172.50	46.61	0.36	0.2019
29	84.26	91.96	7.70	92.33	0.000811	2.90	168.70	45.58	0.38	0.1886
28	84.68	91.95	7.27	92.28	0.000769	2.65	171.32	48.33	0.37	0.0448
27	84.63	91.79	7.16	92.20	0.001026	2.94	150.68	43.40	0.43	0.0438
26	84.71	91.70	6.99	92.14	0.001206	3.04	145.32	45.75	0.46	0.0455
25	84.51	91.67	7.16	92.11	0.000991	3.09	152.55	46.46	0.43	0.0542
24	84.53	91.67	7.13	92.06	0.000939	2.89	157.92	45.87	0.41	0.0468
23	83.95	91.65	7.70	92.03	0.000952	2.77	156.81	46.26	0.41	0.0712
22	84.28	91.67	7.39	91.99	0.000812	2.58	169.69	49.41	0.37	0.0728
21	84.57	91.54	6.97	91.96	0.001054	3.02	153.06	47.49	0.44	0.0677
20	84.22	91.52	7.30	91.90	0.000959	2.85	157.92	46.98	0.41	0.0723
19	84.40	91.40	7.00	91.84	0.001088	3.05	149.14	45.24	0.44	0.0813
18	82.70	91.39	8.69	91.72	0.000772	2.61	167.92	45.54	0.35	0.0705
17	83.81	91.30	7.49	91.69	0.000898	2.82	158.31	46.58	0.40	0.0885
16	83.99	90.71	6.72	91.57	0.003550	4.23	105.24	48.44	0.75	0.1027
15	83.33	91.07	7.74	91.39	0.000770	2.54	168.56	48.99	0.37	0.0827
14	83.95	90.82	6.89	91.34	0.001402	3.25	135.72	46.64	0.49	0.0863
13	84.14	90.59	6.45	91.18	0.001782	3.51	126.69	46.55	0.55	0.0824
12	83.60	90.54	6.94	91.08	0.001562	3.33	132.21	45.71	0.51	0.0874
11	83.74	90.52	6.78	91.02	0.001407	3.18	136.22	48.08	0.49	0.0910
10	83.76	90.32	6.56	90.95	0.001922	3.57	120.88	45.12	0.57	0.0843
9	82.65	90.25	7.60	90.73	0.001613	3.10	135.64	47.74	0.51	0.0847
8	83.80	90.18	6.38	90.65	0.001526	3.06	135.60	47.98	0.51	0.0827
7	83.82	89.96	6.14	90.53	0.001656	3.39	126.50	43.12	0.54	0.2073
6	83.62	89.91	6.29	90.47	0.001610	3.37	129.26	46.35	0.53	0.2105
5	83.57	89.83	6.26	90.35	0.001825	3.23	129.41	46.30	0.55	0.2229
4	83.10	89.80	6.70	90.29	0.001613	3.12	134.95	47.03	0.52	0.2244
3	83.10	89.77	6.67	90.16	0.001152	2.79	151.49	48.28	0.44	0.2438
2	83.15	89.54	6.39	90.05	0.001684	3.24	134.45	46.15	0.49	0.1960
1	82.92	89.18	6.26	89.86	0.002765	3.67	113.38	42.88	0.62	0.2478

The erosion material launched from the watercourse profile and the upstream sections acted as a coagulant and carrier of many pollutions along the watercourse, so that after the cessation Table no. 3. Presents the results of the hydraulic analysis, and there can be noted that the flow regime in the experimental section is still ($F_r < 1$), while the medium flow rate is in the range of $Q_{sr} = V2{,}58 - 3{,}64m^1 \cdot s^{-1}$, along the profiles the depth of waters at the same time vary within the range of BAM 6,14 - 8.56 meter while the tangential stress along the bottom (river bed) of the flow is($\tau_0 = 0.0438 - 0{,}2478kN \cdot m^{-2}$)*Table 3* The value of the critical tangential stress with the tarred Shields constant is "0.0478" and medium-sized granular sediment (d_{sr}) from the watercourse bed is in the range of d ($\tau_{cr} = 0.0478 \cdot g(\rho_s - \rho)d_{sr} = 0.04149 - 0.0826 \cdot kN \cdot m^{-2}$). Based on this, it can be concluded that deformations of the flow profile are mainly located in the upper sector of the experimental section. In spite of the calm flow regime in the bed of the watercourse occurred vast morphological changes due to increased tangential stress, *Table 3., Fig. 10.* (Letić, LJ., Et al 2008)(Frantar, P. 2013)(Dan D., 2016)

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The erosion material launched from the watercourse profile and the upstream sections acted as a coagulant and carrier of many pollutions along the watercourse, so that after the cessation of the flood wave all flooded buildings must be disinfected to prevent epidemics.

Figure 15. The view after dropping of riverbed after the flood;

Conclusions

The featured section of the watercourse in the lower course of Beljanica river illustrates quite well the scope and extent of the catastrophic floods in May,2014. Precipitations during the cyclone activity and preliminary precipitations, of less intensity led to the saturation of soil and outer watercourses with water leading to a high coefficient of outflow. The occurrence of floods in all tributaries and their coincidence caused floods in the lower course of the river. The coincidence of high waters on all tributaries of Kolubara river brought the conditions for the submerging of the surface pit "Tamnava Zapadno polje" and "Veliki Crljeni". In order to re-launch the exploitation of coal from surface mines it is necessary to eliminate the water from the mines, the total volume of water in the pits is W = 214.88 million cubic meters. If we take into account the permit of the public company "SRBIJA VODE" no. 23 / 05.2014. stating that in the profile Kolubara only $Q = 11.0m^3 \cdot s^{-1}$ should be put in, then it is easy to infer how long the open pits will be unused.

Given that the amount of rainfall from14th to 16th of May, 2014, according to the RHMZ report - Table 2. in Serbia amounted to well above, precipitation of 0.01%,Table 1.,At Stepojevac meteorological station in three days it was measured a total of 293.50 mm, which exceeds the value of the probability of 0.01%, and it was expected that any water flows in this basin would manifest themselves according to the established criteria. In order to define the probability of high waters flow in flood from 14 to 16 May, 2014, the recordings and measurements of markings of high waters were made in the field for the duration of the flood. Surveying of the flow profiles were carried out after the passage of the flood wave according to the trigonometric benchmarks in the field. Along the experimental section were surveyed 33 profiles, as well as the changes of the morphological characteristics of the watercourse, and also the changes of the roughness coefficient along the watercourse.

Based on the equation 1 after which the computer program was developed for hydraulic computations (Hec-Ras), the taring of throughput capacity of the river bed was carried out according to the given morphological parameters and the coefficient of roughness until a given level of high water was obtained on the ground for the duration the flood. Since there were predefined markings of the water level along the watercourse, cross-sections and longitudinal profile, by analysis was obtained the Froude number and the velocity of flow in the profiles. Like many other parameters on the basis of which conclusions could be drawn about the stability of the watercourse flow profile, the flow regime and the degree of probability of high waters flow, Table 3. The analysis used high water that has left its mark on flow profiles, following the withdrawal and termination of flooding, and by reconstruction was established that during the flood along the watercourse flow was$Q = 400m^3 \cdot s^{-1}$.

Based on this analysis we can conclude that the flow of high waters in the lower watercourse of the river Beljanica in the period from15 to 16 May, 2014,was of the order of magnitude between 0.1% and 0.01%. Given that the project documentation defined the level of protection of riverbank from high waters of the occurrence of Q2%, and that after the regulation repair works a steady change took place in the morphology of the flow profiles with a tendency to undermining and removing of sediment from the bottom and the banks along the watercourse. In the meantime, the dimensions of flow profiles increased multiple times, so that today the watercourse flow profile provides the flow of water above $Q_{0,1\%}$. All this led to massive destructions and flooding in the lower river Beljanica watercourse, and in coincidence with other watercourses of Peštan and Kolubara it also led to flooding of open pits "Tamnava", and the town of Obrenovac.

Acknowledgments

This paper was written in cooperation with the University of Belgrade, Faculty of Forestry, Belgrade. ** Within the project "The new bio-ecological materials for the protection of land and waters" (TR 37002) and the project "Investigation of climate changes on the environment: monitoring the impact of

adaptation and mitigation" (III 43007), financed by the Ministry of Education, Science and Technological Development of Republic of Serbia.

Conflicts of Interest. The authors declare no conflict of interest.

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Original Scientific paper

Content of the trace elements in corn grown on the territory of the municipality of Mali Zvornik in the Republic of Serbia

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Abstract

In the area of Mali Zvornik municipality, during 2017 on plots of agricultural land 12 soil samples to the depth of 30 cm depth and plant material was collected. The survey included observations was carried out on five types of soil and one plant species (the analysis was carried out on corn grains). The content of Pb, Ni, Cr and Cd was determined in the soil and plant material. At five sites, the trace element content in the soil was above the maximum permissible levels (MPL). At two sites, the content of lead (Pb) was above of MPL, on one site nickel (Ni) content was above of MPL, on one content of nickel (Ni) and lead (Pb), and on one site content of chromium (Cr), nickel (Ni) and lead (Pb) was above the MPL.

The content of the examined elements in the samples of plant material (corn grain) at sites with the content of trace elements above the MPL was either below the detection limit or within the limits of the normal values for this plant species, except in one sample where the lead content was above the allowed values for human consumption. Despite the value of the trace elements above the MPL for soil, the value of the examined elements in the analyzed plant samples was not above the toxic values (TV), which indicates that the translocation is affected by the set of physical-chemical properties of the soil, the place of accumulation and the physiology of the plant species. The assumption is that the increased lead content in one corn grain sample is due to air pollution.

Keywords: Trace elements, translocation, corn grain

Introduction

Presence of heavy metals in agricultural soils is of major environmental concern and a great threat to life on the earth (Sarwar *et al.,* 2017). Since heavy metals are non-biodegradable, they accumulate in the environment and subsequently contaminate the food chain (Ali *et al.,* 2013). Human exposure to contaminated food is a worldwide health concern and a food safety issue that threatens agricultural trade (Corguinha *et al.,* 2015). The distributions of heavy metals in the soil and their availability to plants are regulated by several factors, including soil characteristics, plant species, fertilization and irrigation characteristics, and the interrelationships between these factors (Lu *et al.,* 2015). From the chemical and physical properties of the soil, three factors are in particular distinguished: soil reaction, content of organic matter and colloidal clay in the soil.

In addition, other factors can influence the mobility of trace elements, like, mechanical composition, humidity, $CaCO_3$ content, hydrated oxides of Fe and Al (Džamić and Stevanović, 2007). The health risks arising from heavy metal pollution (HMP) in agricultural soils have attracted global attention, and research on the accumulation of heavy metals in soil-plant systems is the basis for human health risk assessments (Wang *et al.,* 2017). Results of the study by the Dutch Environment

Ministry (2009) indicates that the mobility of Cr and Ni does not affect the content of organic matter in the soil, while the mobility of Pb equally affect the content of clay and organic matter. Also, it has been found that Cd mobility has different effects of clay and organic matter content. The transfer of trace elements from soil to plant can be monitored through the bio-concentration factor of soil - plant (Soil-to-plant bio-concentration factor - Biological Concentration Factor - BCF). According to Ashraf *et al.* (2011) BCF represents the ratio of metal concentration dry weight (DW) in the plant to the metal concentration DW in the soil. BCF is an indication of the magnification of contaminants from a lower to a higher trophic level. For plants, the BCF has been used as a measure of the metal accumulation efficiency, whereby value greater than 1 is an indication of plants potential to phyto extract. Contrary to the previous author, (Marchiol *et al.,* 2010) have been established that the mobility of the heavy metals from the polluted substrate into the roots of the plants, and the ability to translocate the metals from roots to the harvestable aerial part, were evaluated respectively by means of the bio concentration factor (BCF) and the translocation factor (TF). BCF is defined as the ratio of metal concentration in the roots to that in the soil while TF is the ratio of metal concentration in the shoots to the roots. According to Versluijs *et al*., (1998) with increasing content of the clay, organic matter and pH value in soil, BCF for Cd, Cr, Pb and Ni decreases. For the pH value <5.5, 5.5-6.5 and >6.55, then, for the clay content <15%, 15-40% and >40%, and for the organic carbon (OC) content <1%, 1-3% and >3%, the highest BCF decrease is for Ni (80 times) and the least is for Cd (16 times).

In the study of heavy metal accumulation in maize organs and determining the possibility of accumulation and excluder abilities of maize for four metals (Lu et *al*.,2015) found that Cr, Pb, and Ni mainly accumulated in the maize roots, and Zn mainly accumulated in the maize fruit. Because TF < 1 and BCF ≥ 0.2, maize was considered as both an excluder plant for Pb, Ni, and Zn and a potential accumulator plant.

The mobility of heavy metals in soil-plant systems is also affected by the way heavy metals enter the plants. Chemical elements are primarily uptake by roots from the soil or by leaves from the atmosphere. Pb is a typical heavy metal that mainly enters the plant through the leaves (Wang et *al.,* 2017). Cr is slightly available to plants and not easily translocated within plants, thus it is concentrated mainly in roots (Kabata-Pendias and Mukherjee, 2007). Cd concentrations are greater in roots, tubers or leaves of plants, so that leafy and root vegetables generally have higher Cd concentrations than fruits or grains (Stacey *et al*., 2010). Ni is easily mobile in plants, usually all parts of plants show a high concentration of this element (Dinić *et al.,* 2018).

Description of the field of research and methodology of sampling

Area of study and sampling

The research included the territory of Mali Zvornik municpality in Serbia on 12 locations (Figure 1). The study was conducted on 5 types of soil (Table 1). Soil sampling was performed in a disturbed state from the depth of 0-30 cm. Maize grain samples were taken after harvest from each location.

A sample locations where it was investigated the content of trace elements (Pb, Cr, Cd and Ni) in the soil and their translocation in the test plant material - maize, is based on previous studies where on certain locations was determined the contents of trace elements above the maximum permissible levels (MPL) (Institute of soil science, 2016).

Soil samples analysis

The soil is after sampling, dried to an air dry state and penetrated through a 2 mm mesh sieve. The following physical and chemical parameters were analyzed in soil: granulometric composition was analyzed by determination of particle size distribution in mineral soil material, using the method by sieving and sedimentation - International pipettes modified method B (Hadžić *et al.,* 1997); according to granulometric composition the texture class was determined by ISSS (Baize, 1993); soil acidity (pH in H_2O and 1M KCl) was analyzed potentiometrically, using glass electrode (SRPS ISO 10390: 2007);

$CaCO_3$ was analyzed volumetrically, using the Scheibler method (SRPS ISO 10693:2005); soil organic matter (SOM) content was determined using the Kotzman method (Jakovljević *et al.*, 1985); available phosphorus (P_2O_5) and potassium (K_2O) were analyzed by AL-method according to Egner-Riehm (Riehm, 1958), where potassium was determined by flame emission photometry and phosphorus by spectrophotometer; determination of the water content in the form of a mass fraction was determined using gravimetric method (SRPS ISO 11465:2002); determination of the pseudo-total trace elements forms (Pb, Ni, Cr and Cd) was done by inductively coupled plasma-atomic emission spectrometry - THERMO iCAP 6300 Duo (radial/axial view versions) ICP-OES, after the digestion of the samples with aqua regia (ISO 11466:1995; ISO 22036:2008).

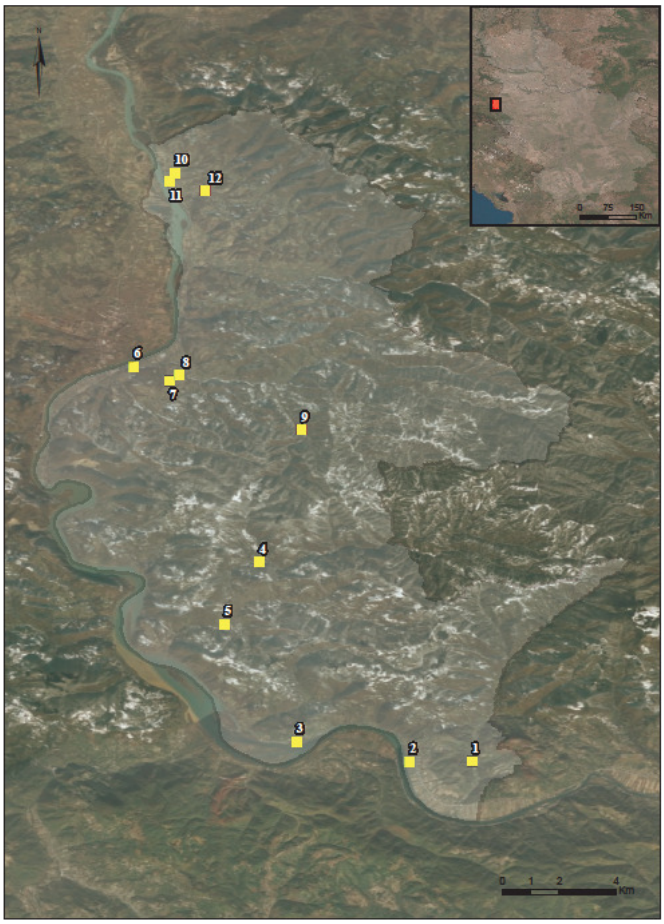

Figure 1. Soil and plant sampling spots

Plant samples analysis

Maize grains after sampling were air dried, milled in an analytical mill and dried on 105 °C. The analysis of the plant material included the digestion of the plant samples with concentrated HNO_3 and redox reaction with 30% H_2O_2 for total forms extraction and determination of Pb, Ni, Cr and Cd using THERMO iCAP 6300 Duo (radial/axial view versions) ICP-OES (Kalra,1998).

For the interpretation of the obtained results of the research of certain content of maize trace elements, the allowed maximum concentrations were defined by national regulations and published literature data. For the interpretation of the contents of Cd and Pb, the Regulation on the maximum permissible levels of residues of pesticides in food and feed additives (Official Gazette, No. 22/2018) was applied, and for Cr and Ni, available literature data (Kisić, 2012; Adams 1975; Kloke *et al.*, 1984) have been used for comparisons.

Results and discussion

Data on the type of soil and the examined physical characteristics are shown in Table 1. The survey covered six types of soil. The content of the clay fraction (<0.002 mm) ranged from 0.4% to 27.1%. According to the texture class, it was determined that the examined soil samples belonged to class loam to class clay loam. The basic parameters of fertility in the test soil samples are shown in Table 2. The reaction of the soil solution of the tested soil samples was in a wide range. The potassium value ranged from pH 4.51 to pH 7.66, ie from acid to alkaline environment (Table 2). According to the content of the carbonate, the tested soils were from carbonate to medium carbonate (BLMD - 6.17%) and with medium to high content of organic matter (2.27% - 6.14%) (Table 2).

According to the content of alkaline phosphorus and potassium, the tested soil has a very low to very high content of both elements (P_2O_5 of 2.2 mg 100^{-1} g^{-1} to > 40.0 mg 100^{-1} g^{-1}; K_2O of 4.4 mg 100^{-1} g^{-1} to > 40.0 mg 100^{-1} g^{-1}).

Table 1. Texture class and types of soil samples

Study site	Clay (<0.002 mm) %	Soil texture	Soil type WRB classification, 2015.
1	12.7	L	Lithic leptosol (dystric) - Hyprskeletic leptosol (dystric)
2	18.5	CL	Haplic fluvisol (eutric arenic)
3	0.4	L	Haplic fluvisol (eutric arenic)
4	19.2	CL	Haplic cambisol (dystric skeletic)
5	20.7	CL	Haplic cambisol (dystric skeletic)
6	21.3	CL	Haplic cambisol (eutric, leptic)
7	27.1	LC	Haplic cambisol (dystric skeletic)
8	17.8	SCL	Haplic cambisol (dystric skeletic)
9	25.0	SCL	Haplic cambisol (dystric, skeletic)
10	23.8	CL	Haplic fluvisol (eutric arenic)
11	23.3	CL	Haplic fluvisol (eutric arenic)
12	21.3	CL	Stagnosol

L-Loam; CL-Clay Loam; LC-Light Clay; SCL-Silty Clay Loam.

Table 2. Main chemical parameters of the tested soil samples

Study site	pH		$CaCO_3$	SOM	P_2O_5	K_2O
	H_2O	1M KCl	(%)		(mg 100^{-1} g^{-1})	
1	7.01	6.19	BLMD	3.86	6.6	4.4
2	8.26	7.33	0.85	3.86	9.0	9.0
3	8.64	7.57	6.17	6.14	14.2	11.3
4	7.53	6.54	BLMD	5.46	6.4	14.9
5	8.37	7.37	1.92	5.98	>40,0	29.4
6	5.49	4.51	BLMD	4.90	7.6	13.5
7	8.20	7.18	2.34	4.10	2.2	6.4
8	8.51	7.66	3.84	4.50	32.6	>40,0
9	7.01	6.00	BLMD	3.10	2.4	6.6
10	8.07	7.19	0.83	5.40	>40,0	>40,0
11	8.54	7.51	5.42	5.29	15.6	8.2
12	8.23	7.28	0.25	2.27	10.8	9.9

SOM – soil organic matter; BLMD – below the limit of method detection.

The results of the content of the total trace elements in the soil samples are shown in Table 3. From the trace elements tested, Cd was at all locations below the Maximum permissible levels (MPL) for agricultural soils (BLMD-1.81 mg kg-1). Cr at site 4 was significantly above MPL 291.7 mg kg^{-1}, while in other locations it was below MPL for agricultural soils (16.6 mg kg^{-1} - 57.9 mg kg^{-1}). Ni was at three sites above MPL, from 73.7 mg kg^{-1} to 420.9 mg kg^{-1}, while in other locations it was below MPL for agricultural soils (27.4 mg kg^{-1} - 49.1 mg kg^{-1}). Pb at 4 sites was above MPL, from 120.9 mg kg^{-1} to 285.9 mg kg^{-1} while at tested samples from other sites was below MPL (18.3 mg kg^{-1} - 67.4 mg kg^{-1}). Soil sample from the site no. 4, out of 4 investigated trace elements, 3 (Pb, Ni, and Cr) are above MPL and their value is significantly higher than the content at other sites.

Table 3. The content trace elements in soil samples.

Study site	Cd	Cr	Ni	Pb
		(mg·kg^{-1})		
1	0.74	26.06	30.12	120.91
2	0.49	44.33	73.72	67.41
3	1.81	48.87	87.57	169.31
4	0.44	291.73	420.87	285.86
5	0.38	16.61	29.37	56.76
6	0.06	57.88	43.86	31.65
7	BLMD	39.66	31.02	18.33
8	0.05	35.78	31.23	30.52
9	BLMD	34.40	30.68	25.85
10	0.07	29.87	27.42	33.54
11	0.21	35.22	43.28	148.01
12	0.08	47.11	49.08	30.33
MPL	3	100	50	100

BLMD - below the limit of the method detection; MPL - maximum permissible levels

Table 4. The content trace elements in maize grain

Study site	Cd	Cr	Ni	Pb
		(mg ·kg^{-1})		
1	BLMD	0.110	0.285	BLMD
2	BLMD	0.145	0.435	BLMD
3	BLMD	0.155	0.295	0.155
4	BLMD	0.190	0.910	BLMD
5	BLMD	0.170	0.240	1.235
6	BLMD	0.155	0.370	BLMD
7	BLMD	0.120	0.280	BLMD
8	BLMD	0.230	0.435	BLMD
9	BLMD	0.120	0.275	BLMD
10	BLMD	0.105	0.180	BLMD
11	BLMD	0.090	0.350	BLMD
12	BLMD	0.095	0.105	BLMD
MPL/TV	0.1	1	5	0.2

BLMD - below the limit of the method detection; MPL - maximum permissible levels; TV- toxic value

The concentration of trace elements in maize grains from the investigated sites is shown in Table 4. In samples of plant material (maize grain) from all sites, Cd was below BLMD. The content of Cr, ranged from 0.09 mg kg^{-1} to 0.23 mg kg^{-1}, which is below the limit of toxic values for the test species. Ni content in the samples ranged from 0.11 mg kg^{-1} to 0.91 mg kg^{-1}, also below the limit of toxic values for the test species.. The content of Pb was at 11 sites below MPL (BLMD - 0.16 mg kg^{-1}) while at site no. 5 was 1.24 mg kg^{-1} which is above MPL for maize grain.

The results of the study of the content of trace elements in soil and maize grain sites where the content above the MPL in soil was recorded (sites 1,2,3,4 and 11) showed that there was no translocation into the plant material (maize grain) in quantities greater than MPL and TV for the test species. Analyzing the main factors affecting the mobility of trace elements, it can be concluded that their impact is different. The pH value (1M KCl) was at the range from 6.19 to 7.57, indicating a reduced mobility of trace elements if only that parameter is observed. The content of soil organic matter was from 3.86% to 6.14%, which is less than the maximum values defined in the The Dutch Environment Ministry (2009) of 10%, indicating that the Cd and Pb mobility in these locations increased in relation to the SOM. Also, the content of the clay fraction (0.4% to 23.3%) is less than the maximum values defined in the mentioned literary source, of 25%, which also indicates the increased mobility of all investigated elements. At site 5, the content of Pb in maize grain was above MPL (1.235 $mg \cdot kg^{-1}$), while the content of the pseudo-total Pb at this site is below MPL for the soil. This indicates that the value of Pb above MPL is due to contamination by air pollution or the use of pesticides, as indicated by the results of other authors' research (Ibrahim *et al*., 2015). The content of Pb in maize grains was 6.32 $mg \cdot kg^{-1}$, 12.18 $mg \cdot kg^{-1}$ and 22.32 $mg \cdot kg^{-1}$, which is above the maximum level prescribed by the legislation, although the quantities of the investigated elements in the soil were below the tolerable maximum limits for potentially toxic elements (PTE). Based on the above, the assumption is that the resulting value is due to use of pesticides and fertilizers at the given sites. Research Li *et al.* (2014), which included the investigation of soil contamination Pb and the examination of its content in maize grains, showed that Pb content (350 $mg \cdot kg^{-1}$) of Pb grain content in the grain is concentrated to a concentration of 0.8 mg kg^{-1}. The concentration of Pb in the grain at the control sites were below 0.2 $mg \cdot kg^{-1}$. The soil pH and SOM were the most important factors that can control Pb uptake in corn grains, is the conclusion of their research.

Conclusions

The values of the trace elements tested in maize grains at 11 sites are below MPL or TV. At location No. 5 Pb concentration is above the MPL for the test species and cannot be used in the production of health-safe food. At five sites, the trace element content in the soil was above the MPL (Table 3.). At two sites, the content of lead (Pb) was above of MPL, on one site nickel (Ni) content was above of MPL, on one content of nickel (Ni) and lead (Pb), and on one site content of chromium (Cr), nickel (Ni) and lead (Pb) was above the MPL.

pH values indicate reduced mobility of trace elements at these sites, while SOM content indicates increased mobility of Cd and Pb. The clay content indicates an increased mobility of the trace elements tested. Regardless of the value of the factors affecting the mobility of the tested elements and their translocation in the investigated plant species, the trace content of maize grains is below MPL and TV, indicating that it is possible to obtain a healthy safe corn at sites with increased content of Cd, Cr, Pb and Ni above MPL for soil. On mobility and accumulation of trace elements from the soil into the plant, it is evident that several factors influence the physical and chemical properties of soil, as demonstrated by the results of many studies. For more detailed research it is necessary to include and examine additional factors. At sites with trace element values above MPL in soil, it is necessary to regularly monitor their content in maize grain or other cultivated species in order to obtain information on the health safety of such products.

Acknowledgment: This research was financially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Project TR 37006 and TR 31018).

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Original scientific paper

Water quality assessment for irrigation from the South Morava river

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Abstract

During 2013, in July, August and September, samples were collected from 37 locations from the South Morava River, used for irrigation of agricultural soil directly along the river. The suitability of water for irrigation is assessed by the Stebler, Nejgebauer, US Salynity Laboratory, FAO and RSC classifications. The obtained results indicated certain limitations in the quality of the samples of water samples from South Morava at certain locations during August (area Pavlovac and Zlatokop) and in September (area: Bujanovac, Srpska kuća, Pavlovac, Donji Neredovac, Zlatokop and Suvi Dol). The content of microelements and heavy metals in all tested water samples was below the maximum permissible levels in relation to the legal regulations of the Republic of Serbia. Based on the obtained results, it is estimated that South Morava river water can be used for irrigation with restrictions at places where limitations were recorded during the summer months, in order to protect agricultural soil from further degradation.

Keywords: Classifications, irrigation, water

Introduction

Irrigation is a hydro-reclamation measure that aims to improve the physical properties of the soil by adding water to achieve optimum moisture during the growing season and thus achieve optimal yield.

It aims to meet the ever-growing needs for food due to population growth (Aleme & Desta 2017). Since in many areas of the world (Letei *et al.*, 2011) without irrigation, agricultural production would not be possible, it is necessary to pay special attention to the available water resources both on quantity and on their quality. Quality of irrigation water can significantly reduce the expected yield of agricultural production (Caper *et al.*, 2016; Tovfivul Islam *et al.*, 2017). Water quality is a term used to describe the physical, chemical and biological parameters of water characteristics and defines the suitability for a particular purpose (Diersing & Nanci, 2009). Water of inadequate quality can affect the salinization, alkalization and deterioration of water-physical properties of the soil (Dragović *et al.*, 2006, Belanović, 2012). In addition, slow growth of plants, deformation of the fruit, and in some cases even complete absence of plant growth may occur. To overcome this, specific indicators for assessing the quality of water for irrigation have been adopted. Theere are a set of parameters that are widely accepted and used in the decision-making process (Ferhad *et al.*, 2017). It is very important to estimate the risk of screening irrigation sources for any agricultural area in order to maximize yields of cultivated crops (Shammi *et al.*, 2016). There are traditional and modern methods and classifications for assessing the quality of water for irrigation. None of them can be considered absolutely applicable to all conditions in plant production (Pivić *et al.*, 2018). Classification of irrigation water is mainly based on the determination of the total amounts of salt in water, the ratio of Na^+ ion content to ion content Ca^{2+} and Mg^{2+}, the presence of Cl and B salts and electrical conductivity.

Description of the field of research and methodology of sampling

Water sampling locations for irrigation at 37 points from the South Morava Stream are shown in Figure 1, and the corresponding coordinates in Table 1. Water from the river flow was sampled during July, August and September 2013. The sampling, transport and storage process was carried out using the methodology described in the available literature sources. Water samples for chemical analyzes were sampled wherever possible from the middle of the river at a depth of 0.20-0.50 m with specialized equipment, with a lath with a water catching container, while the container was oriented towards the water stream. If the envisaged sampling point was near the mouth of a river, it was sampled at about 500 meters downstream from the mouth. About 2 liters of water were sampled with addition of 4 drops of concentrated HNO_3. Transportation to the laboratory was carried out in hand-held refrigerators with the storage temperature below 4 °C.

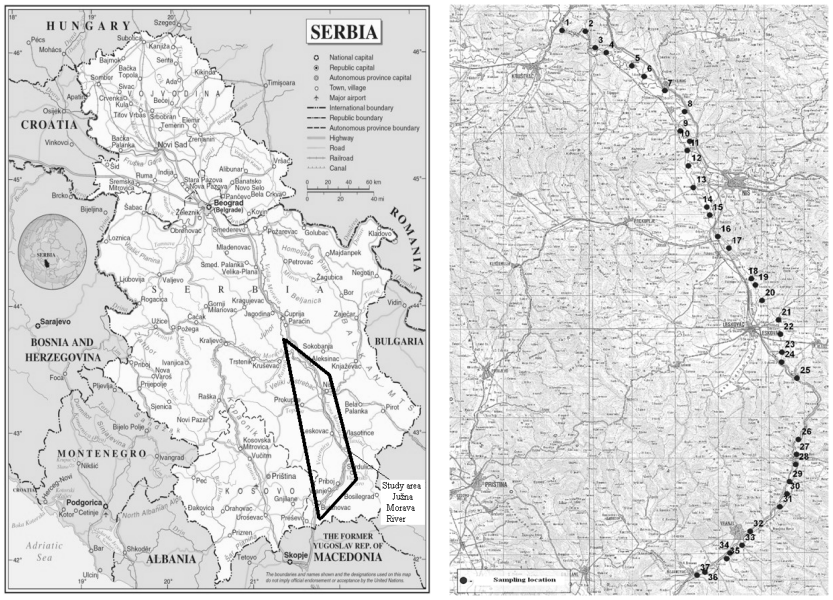


Figure 1. Location emap of Južna Morava valley with selected sample sites

Table 1. Sampling points along the Južna Morava River

Sampling point	Coordinate E	Coordinate N	Sampling point	Coordinate E	Coordinate N
1	756507	470127	20	757768	477421
2	756687	470200	21	757246	478172
3	757192	470535	22	756985	478456
4	757270	470673	23	756800	478987
5	757550	470866	24	756732	479184
6	757743	471207	25	756418	479664
7	758429	471811	26	756304	480196
8	758597	472127	27	756276	480575
9	758653	472431	28	756336	480800
10	758800	472852	29	756116	481052
11	759702	473326	30	756217	481532
12	758862	473467	31	755756	482046
13	758831	474977	32	755276	482396
14	758471	475371	33	754993	482657
15	758476	475613	34	754398	482984
16	758447	476057	35	754138	483101
17	758400	476411	36	753910	483506
18	758017	476886	37	753364	483529
19	757861	477269			

Research methods

In the irrigation water samples, the following parameters are determined: pH-potentiometric (SRPS H.Z1.111: 1987), electrical conductivity (EC) - (SRPS EN 27888: 1993) - electrometric; the total dissolved solids content (TDS) - gravimetric (Greenberg et al., 1998); CO_3^{2-}; HCO_3^-; Cl-volumetric, K^+; Na^+ - plamenfotometric (APHA,1992). The content of heavy metals and other toxic elements (As, B, Cd, Cr, Cu, Fe, Ni, Pb, Zn) and SO_4^{2-}; Ca^{2+}; Mg^{2+} are determined by EPA method 200.7, on the ICAP 6300 ICP optical emission spectrometer (ICP-OES); (SAR) - calculating (Rhoads *et al.*, 1992).

Results and Discussion

Various methods for assessing the suitability of irrigation water are available in the literature, all mainly based on determining the total amount of salt in water, the ratio of Na^+ content to the Ca_2^+ and Mg_2^+ content, the presence of Cl, boron salt, ECw and total soluble solids (TDS). Recommendation (FAO 1985; DoE, 1997) is that water for irrigation is in the pH range of 6.5 to 8.5. Preferably, it contains 1.1-1.7 g l^{-1} of various salts.

Water for irrigation with pH values above those values can affect irregularities in plant nutrition or affect the increased availability of toxic ions. The water samples from the South Morava River had a pH value from neutral to slightly alkaline which was satisfactory.

Electrical conductivity (ECw) is a measure of the ability of an aqueous solution to conduct electricity and dependent on the presence of dissolved salts, usually sodium chloride, sodium sulfate, calcium chloride, calcium sulfate, magnesium chloride, etc. Absolutely demineralized water does not conduct electricity, but even with low concentrations of dissolved salts it becomes a good conductor. If the measured value is 1 μS per liter of water, this means that about 0.7 g of salt is dissolved in it (Dragović *et al.*, 1994). The presence of some water-soluble elements is useful, but if their concentration becomes high, they can become dangerous for undisturbed growth and development of cultivated plants, and may also adversely affect the structure of the irrigated land.

Total Soluble Solids (TDS), represent the total concentration of soluble salts in water. Dissolved solids in water are all inorganic salts, silicon dioxide and soluble organic matter (Atekvana *et al.*, 2004; Ahipathi & Puttaiah, 2006).

Sodium adsorption coefficient (SAR) represents the ratio between the soluble Na^+ and the soluble bivalent cations (Ca^{2+} and Mg^{2+}) (Alrajhi *et al.*, 2015). It is a measure of the sodicity of the soil determined through quantitative chemical analysis of water in contact with it (Shammi *et al.*, 2016). The values of the analyzed parameters are shown in Figures 2-5 per series, months of sampling water for irrigation from the South Morava watercourse.

The estimate of the irrigation coefficient (K) obtained from the concentration of the corresponding ions Na^+, Cl^-, SO_4^{2-} was determined on the basis of the Stebler classification that the samples analyzed in July and August were in the category of good waters K> 18 that can be used without special measures to prevent the accumulation of harmful salts in the soil, while in September 78.4% of the samples were assessed as good water and 21.6% of the samples were unsatisfactory, K (5.9-1.2).

The water quality assessment for irrigation was also carried out on the basis of the Neigeberger's classification, 1949, which takes into account estimation of the total amount of salt in irrigation water in interaction with Na^+ concentration with Ca^{2+} and Mg^{2+}. Compared to the above classification, the tested samples in July 40.6% belong to the Ia class, in which the dry residue is less than 700 mg l^{-1}, and the ratio (Ca + Mg): (Na + K) is greater than 3; 54.0%, class Ib, where the dry residue is less than 700 mg l^{-1}, and the ratio (Ca + Mg) is greater than 3. These are impeccable water with ameliorative characteristics of flushing salt marsh. 5.4% of analyzed samples in the given period belongs to class IIa where the dry residue is less than 700 mg l^{-1}, and the ratio (Ca + Mg): More than 1, and these are good irrigation water.

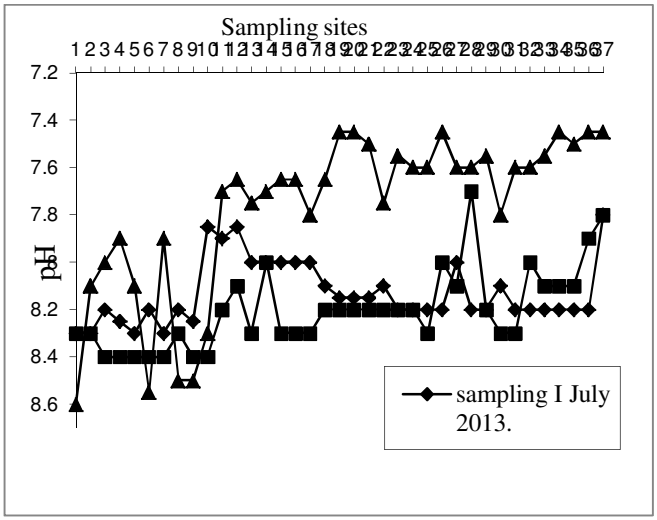


Figure 2. pH value of the tested water samples in batches of monitoring

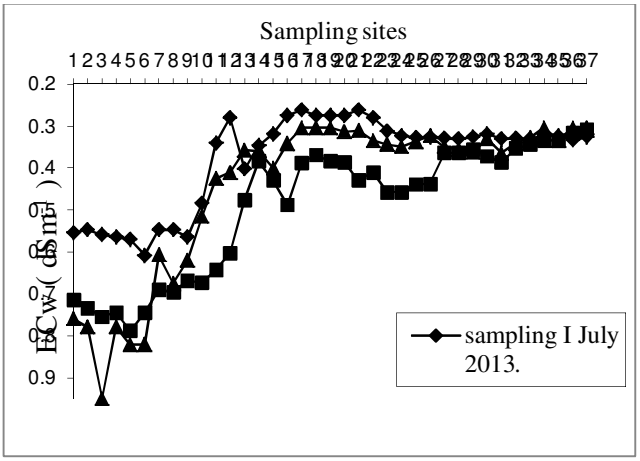


Figure 3. ECw value of the tested water samples in batches of monitoring

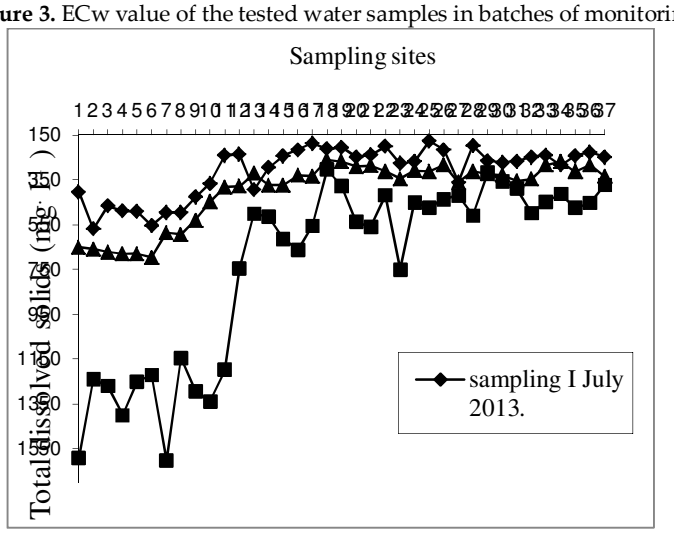


Figure 4. TDS value of the tested water samples in batches of monitoring

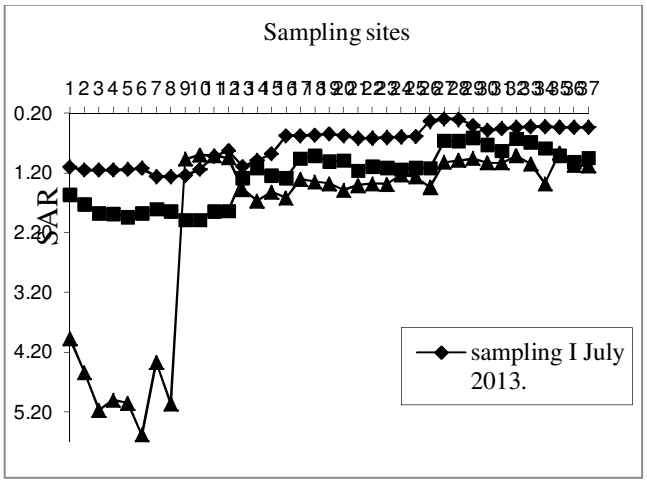


Figure 5. SAR value of the tested water samples in batches of monitoring

In August, 18.9% of the samples were Ia class, 18.9% of the class Ib, 27% of Class IIa and 35% of Class IIIb in which the dry residue was 700-3000 mg l^{-1}, the ratio (Ca + Mg): Na greater than 1, which is distinguished by water whose irrigability for irrigation in our circumstances needs to be further examined. In September, 35.1% of water samples were class Ib, while 64.9% were class IIa.

The largest contribution to the study of water quality and its classification in relation to the benefits of irrigation of agricultural crops was given by the experts of the Laboratory for Saline soil University Riverside, USA. The method that they defined as "US Salinity Laboratory Classification" is applied worldwide. The basis for assessing this method is the value of ECw and SAR. In the tested irrigation water samples, in July, 100.0% of the samples belong to the class of water of the class C2-S1, in which the ECw values range from 0.250 to 0.750 dS m^{-1} and they can be used for irrigation of plants with medium tolerance towards salt. During August, 91.9%, or in September, 83.8% of the tested samples belong to the mentioned C2-S1 class. The remaining 8.1% in August, ie 16.2% in the September samples tested, belongs to the C3-S1 class, in which the ECw values range from 0.750 to 2.250 dS m^{-1}, and their use requires the application of special measures in the prevention of soil depletion.

Modified FAO classification (Aiers & Westcot, 1994) thoroughly analyzes the effect of dissolved salt in irrigation water and its impact on the water-physical properties of the soil, primarily on infiltration. It takes into account the risk of saturation, based on the amount of electrical conductivity (ECw) and the salt concentration in the test sample (TDS).

It was found that 67.8% of South Morava water samples tested from samples sampled in July belongs to the class of unsaturated drinking and irrigation water (PN) where ECw <0.7 dS m^{-1}, TDS <500 mg l^{-1} and 5.4% for irrigation (N) where ECw: 07-2 dS m^{-1}; TDS 500-1500 mg l^{-1}, while 27% belongs to the class of medium-slaughtered water-primary drainage (VAT), where Ew: 2-10 dS.m^{-1}; TDS 1500-7000 mg l^{-1}. In August, 2.7% of the samples were in class (VAT); 51.35% in the class (N) and 45.9% (PN). In September, 83.8% of the water samples tested were in class (PN), and 27.1% of the samples in the class (N). An additional estimate using the possible influence of some elements dissolved in irrigation water, analyzing Na^+ effects through different relationships with other tested substances (Na_2CO_3) can be determined using the RSC-Residual Sodium Carbonate classification (Joshi *et al*., 2009). Based on this classification, in July and September, 100% of samples were assessed as good water and in August 97.3% of the tested samples (RSC <1.25) and 2.7% of the water class at the usability limit (RSC = 1.25-2.50).

The obtained values of the content of the studied microelements and heavy metals are shown in Table 2, and interpretation was carried out on the basis of the limit values in the Ordinance on the

allowed quantities of hazardous and harmful substances in soil and water for irrigation (Official Gazette of Republic of Serbia, 23/94, 1994) and the literature data (Ayers & Westcot, 1994 *).

Table 2. Maximum permitted amount of hazardous and harmful substances in irrigation water

Element	As	B	Cd	Cr	Cu	Fe*	Ni	Pb	Zn
					(mg l^{-1})				
MPL	to 0.05	to 1.0	to 0.01	to 0.5	to 0.1	to 5	to 0.1	to 0.1	to 1.0

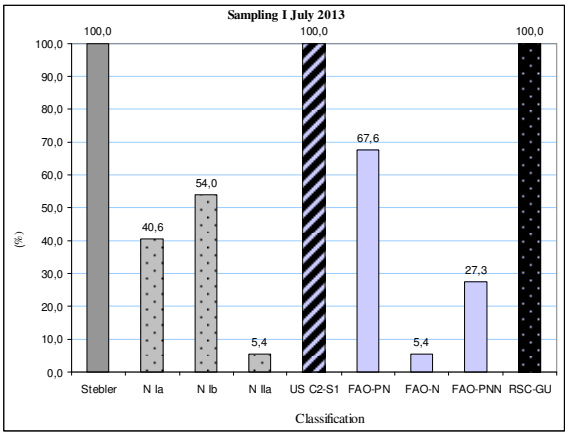
MPL- maximum permissible levels

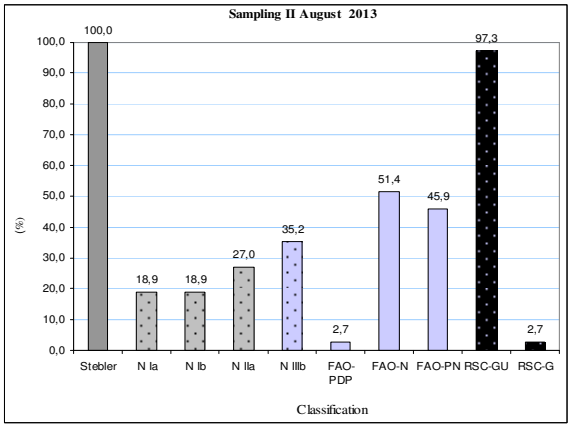
The content of trace elements and heavy metals in irrigation water samples in all tested irrigation water samples was below the maximum permissible concentration (MPL), Table 2.

Results and suggestions

Figure 6, shows the percentage assessment of the classification of irrigation water samples analyzed in three cycles monitoring related parameters.

Based on the obtained and analyzed results of the irrigation water quality test, it can be concluded that water from the sampling site can be used with certain restrictions for irrigation of cultivated crops during the summer months, and that its use may in part have negative consequences on the structure of the soil on which applies. An analysis of water for irrigation from the South Morava river flow should be carried out regularly and with limited use in summer months in order to prevent crust formation and deterioration of soil structure on which irrigation is applied.





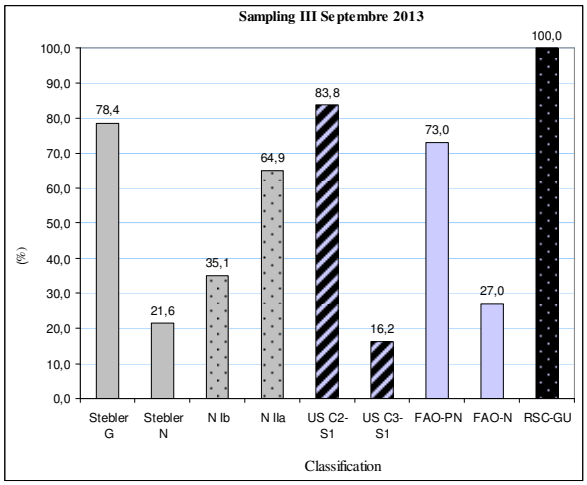


Figure 6. Percentage representation of applied water classification for irrigation through monitoring cycles

Acknowledgment: This research was financially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Project TR 37006)

Conflicts of Interest: The authors declare no conflict of interest.

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Review Article

The importance of amelioration on soil and water resources in the rural areas of Turkey

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Abstract

The main problems on soil and water sources in Turkey are over irrigation, over grazing, erosion in the inclined terrains and irrigated areas, run off and soil losses. The reasons of all these are climate, topography and mismanagement of natural resources. Erosion frequently occurs because of topography, geological structure, geographical location, land and climatic conditions and mismanagement of natural resources. Considering all these, the purposes of small scaled investments on soil and water resources in rural areas are (i) to provide sustainable economic development, (ii) to increase in overall welfare level, (iii) to decrease in unemployment, (iv) to protect and improve the environment. Some regional and local small projects on development and improvement on soil and water resources in Turkey have been implemented. A wind erosion control project in Karapınar is one of good examples in the world. A catchment improvement project resulted in increasing household incomes in the target micro-catchments on average 53 % between 2005 and 2012, soil fertility on sloping lands increased by more than 20 %, vegetative cover increased in the project area by 77 % above baseline over the seven years of the project, over 30 % of farmers adopted environmentally friendly agricultural practices and 60 % of farmers adopted improved manure management. On the other hand, with the control of erosion, reforestation, the rehabilitation of degraded forest lands, pasture rehabilitation, improvements in irrigation systems technologies in agricultural lands and the ongoing rehabilitations, the transported soil has been decreased from about 500 to about 178 million tonnes/year.

Keywords: amelioration**,** land management, natural resources, rural area, soil erosion, Turkey

Introduction

The agricultural sector in Turkey has been still considerably taking part in national economy. Almost 20 % of the population in Turkey live in rural areas and work in the agricultural sector. From a total land area of 77.8 million hectares, Turkey has currently about 24 million hectares under cultivated farming (TUIK, 2018). Within this, more than 6 million hectares have been developed for irrigation (DSI, 2018).

Thus, Turkey's overall social and economic prosperity still depends upon the interplay of population, agricultural production and rural environment. Consequently, rural areas are in need of significant investment to ensure the development of sustainable agricultural production, social infrastructure and education (Hallett *et al.,* 2003).

Considering the topographical conditions of Turkey, more than 50 % of the country is above an altitude of 1000 m. In fact the mean altitude (1131 m) is substantially higher than the means of 330 m and 1050 m for Europe and Asia respectively. The mean annual precipitation is 574 mm (MGM, 2018), but the mean annual total ranges from 250 mm in the South East to 2500 mm in the North East next to the Black Sea coast. About 64% of Turkey has a slope greater than 12%, and 67% of the land has a soil

depth shallower than 50 cm. The topographical conditions of Turkey is to be required either permanent crop or tree cover or structural soil conservation works such as graded banks and waterways to minimize soil degradation (Kük and Burgess, 2010).

Soil loss is mainly dependent on topography, soil characteristics, improper agricultural cultivation and the lack of soil conservation precautions. It is estimated that on average about 500-616 million tons of soil is lost from Turkey annually; equivalent to a mean of 6.5-8.0 t ha-1 (Haktanir, 1999). Stopping erosion in areas suitable for agriculture, preventing damage from flood waters, preserving water in the soil and maintaining productivity, rendering suitable for agriculture, lands in private or state tenure that are degraded because of acidity, salinity or alkalinization, providing services for irrigated agriculture, including land-leveling, on farm irrigation and drainage (Hallett *et al.*, 2003).

On the other hand, 1.5 million hectares of the Turkish arable land resource suffer from production limitations due to salinity, with a further 2.8 million hectares suffering from waterlogging in the irrigated lands. The condition of much of the available arable land has deteriorated over recent decades due to water and wind erosion. Some 63% of the total erosion recorded is categorized as high or very high, with 20% classed as moderate and 14% slight or negligible erosion (KHGM, 1987).

In this article, it is reviewed problems on soil and water resources and it is given some information on improvements and ameliorations of soils, lands and catchments.

Problems on Use of Soil and Water Resources in Turkey

Turkey is very sensitive to erosion because of its geographical location, topography, geological structure, climatic and soil conditions. Erosion increases with the improper or excessive use of nature. The slope class for all lands in Turkey are given in Table 1. In General, the lands of Turkey show slopy characteristics and this conditions cause soil losses by water erosion. In addition, this situation is required to be more efficiently and sustainable management of these lands.

Table 1. Slope classes of lands in Turkey (Anonymous, 2018a)

Slope class (%)	All lands (ha)	Rate (%)	Agricultural Lands (ha)	Rate (%)
0-2	20.998.409	26.9	12.640.104	46.2
2-6	16.518.075	21.2	6.673.373	26.0
6-12	16.733.024	17.1	2.327.644	16.0
12-20	13.362.912	9.7	910.724	3.1
20-30	7.570.594	3.3	234.407	0.8
30-45	2.604.204	0.3	10.043	0.03
45+	212.452		26.692.469	
Total	77.999.669		26.692.469	

The erosion levels in all the lands of Turkey has been estimated as slightly in the area of 5.6 million ha, moderate in the area of 15.6 million ha, high in the area of 28.3 million ha and very high in the area of 17.4 million ha. In addition, 59 % of the cultivated agricultural lands are under the erosoion treat (TEMA, 2018). Thus, it is estimated that losses are significantly higher amounts. The wrong use of lands creates negative effects on extant flora and animal along with agriculture production. Connecting this issue, an increase in technologically based food production practices and poor land management has lead to wind and water erosion (Ozden and Sonmez, 1998; Taysun et al., 1998), waterlogging, sodicity and salination of the soil resource (Cullu *et al*., 1998).

In addition, irrigation is inevitable to meet food requirement of human being for increasing population. Erosion in some irrigated lands in Turkey is the main problem. The reasons of this are

no technical practices depending on the irrigation methods, no achieving land leveling; applying surface irrigation in the lands has higher slopes and use of the excessive irrigation water. This results infertility soils and increasing of amount of sediment in the rivers and dams and it cause, thus, some environmental problems. Runoff and mass sediment losses in the some irrigation districts in the study area ranged from 28.0–42.4% and 98.4–4503.6 tons for one irrigation season, respectively (Çetin et al., 2015) .

On the other hand, the cultivated agricultural area were approximately 28 millions ha in 1980s in Turkey. However, it is currently about 24 millions ha (TUIK, 2018). The main reasons in decreasing agricultural lands might be attributed to the industrialization, urbanization and new settlements, tourism, transportation and mining.

As a result, the main reasons of the soil degradation in Turkey are soil erosion, overgrazing, deforestation, unprotected agriculture, uncertain rainfall, river flooding, depletion of surface water and depletion of ground water. Figure 1 shows the soil erosion occurred by irrigation events and natural rainfall conditions. Figure 2 shows deforestation and over grazing on land degradation.

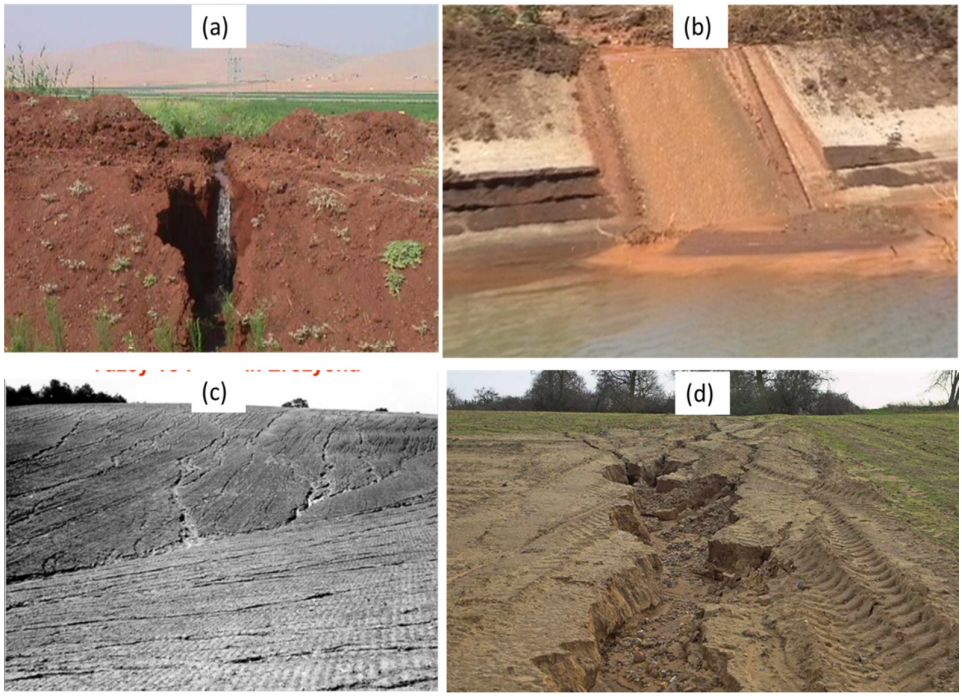

Figure 1. Soil erosion occurred by irrigation events (a and b) and natural rainfall (c and d).

Figure 2. Deforestation (a) and over grazing (b) on land degradation (Anonymous, 2016).

Amelioration Works on Soil and Water Resources in Rural Areas

Although there are different organizations and institutions on management of soil and water resources in Turkey, the main responsible public institution is Ministry of Agriculture and Forestry (MAF). The institutions of MAF protects mountainous and upper basins as the origin of streams and undertakes the development of projects to protect such areas (i.e., afforestation; rangeland rehabilitation; erosion control, utilization and protection of in-forest streams, rehabilitation lakes and creation of reservoirs, etc.) (Baris and Karadag, 2007). In addition, MAF is responsible for the development of villages, the development of agriculture, assistance in the development of water and soil resources, carrying out investigations and research, planning programs and projects to meet the needs for protection and development of water and soil.

State Hydraulic Works under the MAF also ensures performing basic investigations such as, flow gauging, soil classification, water quality monitoring, preparation of river basin development plans and formulation of proposals for constructions financing and subsequent operation of these works (Baris and Karadag, 2007).

Non Governmental Organizations (NGOs) such as TEMA (Turkish Foundation For Combation Erosion For Reforestation and the Protection of Natural Habibats) have been realizing environmental protection activities and public consciousness about the scarcity of water resources, soil erosion and afforestation. Thus, many NGOs play a considerably roles at the national and regional level in the field of the environment in Turkey. The main national NGOs are actively involved in many water and environmental problems in order to create public awareness and to encourage public participation. They propose efficient solutions and act as pressure groups in the decision-making process (Baris and Karadag, 2007).

Considering all forestry lands, the total forestry land of 9.1 million ha on the afforestation and cautions on prevention of erosion need to be treated. These cautions are afforestation, erosion control, restoration for forestry trees (Anonymous, 2016).

Some regional and local small projects on development and improvement on soil and water resources in Turkey have been implemented. All these projects are supported by MAF, Agricultural and Rural Development Support Institutions, Development Agencies, European Union and Worldbank Projects (Figure 3).

For instance, combating and preventing of wind erosion in Karapınar, Konya in Turkey is one of the successful implementation projects. Wind erosion and dune shift has reached an intolerable level from the viewpoints of agriculture and life quality in early 1960s when an experimental station was implemented to halt and reverse this degradation process. The Project Area of Karapınar covers 13.000 ha which is sub-divided to different problem areas taking into consideration the nature of problems and the types of measures to be taken 4300 ha of sand dunes (Afforestation), 4000 ha of dune shadows (pasture), 1500 ha of bazaltic rocks and 3200 ha of dry farming area, 2000 ha of strip-cropping, 1000 ha of farmer s area and 200 ha of irrigation area (vineyard and seedling production area). The project area, presently, is a national park; it is a recreation area for the amusement, holiday and hunting pleasure of the peoples (Çarkacı, 2018).

The "Anatolia Watershed Rehabilitation Project" was supported by World Bank. The target of this project is to develop sustainable natural resource management and poverty reduction on a watershed. This project was focused on promoting a participatory approach to natural resource management and improved agricultural production on a watershed basis, and resulted in that household incomes in the target micro-catchments increased on average 53% between 2005 and 2012, soil fertility on sloping lands increased by more than 20%, vegetative cover increased in the project area by 77% at the end of this project for 7 years. In addition, over 30% of farmers adopted environmentally friendly agricultural practices and 60% of farmers adopted improved manure management, reducing the nutrient load in the water sources draining into the Black Sea (Anonymous, 2013).

On the other hand, with the control of erosion, reforestation, the rehabilitation of degraded forest lands, pasture rehabilitation, improvements in irrigation systems technologies in agricultural lands and the ongoing rehabilitations (Figure 3), the transported soil amount became as about 178 million tonnes/year from 500 million tonnes/year (Anonymous, 2018b).

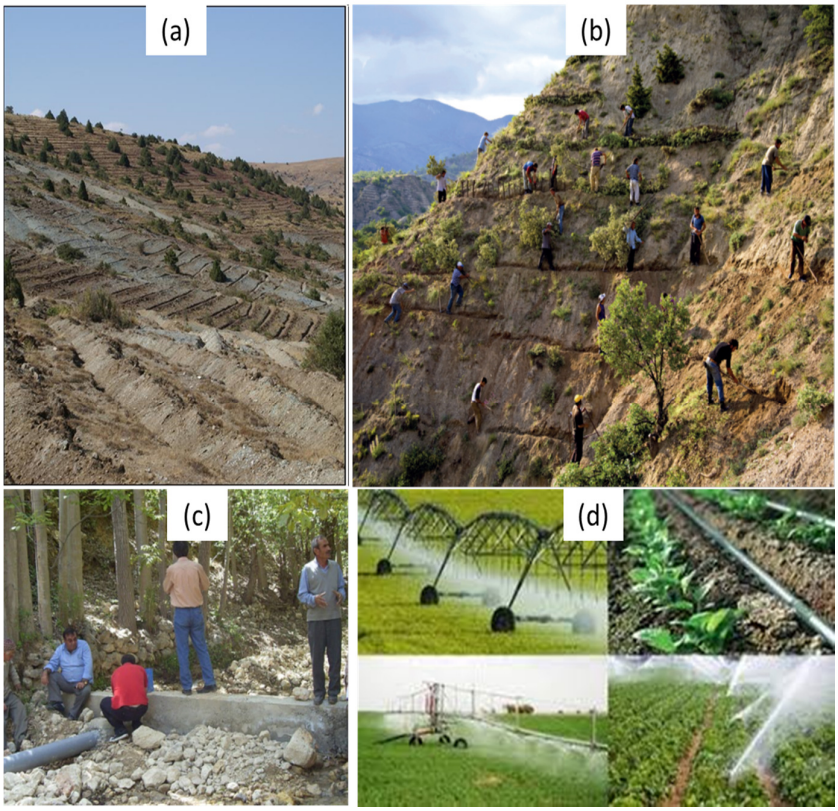

Figure 3. Improvements on prevention of soil degredation;
terracing and afforestation (a and b), flood control and small scaled
irrigation (c), use of modern irrigation systems

Conclusions

Turkey is vulnerable to erosion due to climate conditions, topography and soil structure. Therefore, soil conservation and watershed rehabilitation works are priority to prevent land degradation. The scope of erosion control activities include; planting forests in degraded forest lands exposed to erosion, and sloping lands with destroyed vegetation, transforming degraded forest lands to productive forest lands, rehabilitation of existing vegetation cover, and constructing plants in upper river basins to regulate water flow to restore natural balance.

Unfortunately, problems originating from human such as mismanagement of natural resources are significantly level also beside the environmental problems occurred since topographical and climate conditions in Turkey. Most significant effects of land use include urban sprawl, soil erosion, soil degradation, salinization and desertification. Land use change is one of the sources of carbon dioxide that is a dominant greenhouse gas.

Protection and the development of agricultural systems that are sustainable and environmentally sensitive. The soil and water resources of Turkey are in a deteriorating state. Land and water

management should be integrated and management should be on basin. Farm basin management is also important. Farmers and farmers' trainers and consultants should be Transition to drip irrigation systems in rural areas has accelerated

On the other hand, agriculture areas are generally used for housing, tourism, industry and infrastructure, but guiding these areas differently will increase efficiency. At the same time, mismanagement on agriculture areas have decreasing agricultural efficiency.

Watershed management is a mechanism to save natural resources such as soil, water and forests and to increase incomes and welfare for generally poor farmers. Sustainable management of soil and water resources requires a holistic and integrated approach involving engineering socio-economic and environmental aspects. Fortunately, agricultural policies have increasingly focused on food safety, environmental issues since 2000. Although the problems on use of soil and water resources in Turkey has been continued, there are the successful projects implementations on those. Overall combating and integrated implementations in the cathments require for conservation and sustainability on soil and water resources.

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Original Scientific paper

Optimization of indicators for assessment of agro-ecosystems condition under the DPSIR approach

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Abstract

In April 2013, the European Commission issued the "Mapping and Assessment of Ecosystems and their Services" report related to the European Union Biodiversity Strategy by 2020, according to which every Member State, including Bulgaria needs to assess and map the state of the ecosystems condition on its territory as well as the ecosystem services (the benefits and goods derived from ecosystems) they provide. One of the world-wide approved approaches for selection of ecological indicators that can be used for assessment of the ecosystems condition and ecosystem services is the DPSIR approach (driving forces - pressure - state - impact - responses). During the implementation of this method, networks made up of causal links are created, analysis of which leads to selection of the relevant indicators. These networks also provide an opportunity to define the significance of each indicator. On the basis of a detailed analysis of the indicators selection, a method for establishment of reduction coefficient is proposed. It is aimed at indicators optimizing and increasing the accuracy in the final assessment of the biophysical condition of the agro-ecosystems.

Keywords: agro-ecosystems; ecosystem services; DPSIR; ecological indicators; ecosystems biophysical condition assessment; ecosystem services assessment

Introduction

The extraction of tangible and intangible benefits from nature has been practiced from the earliest times, but the scientific research of this process became popular as late as the 1970s of the 20th century when the term "environmental services" appeared for the first time in the interdisciplinary examination " Study of Critical Environmental Problems''. The main reason for the heightened interest in this area is the increasing nowadays direct and indirect anthropogenic pressure on natural and semi-natural ecosystems, which in turn aggravates their condition and respectively compromises the flow of ecosystem services (Sh. Lele, Oliver Springate-Bi. *et al*., 2013; Tr. Patterson, 2011; Er. Gómez-Baggethuna, Rudolf de Gr., *et al.*, 2010).

Since then, a number of significant reports in that field have been issued worldwide, including the „Millennium Ecosystem Assessment“ (Millennium Ecosystem Assessment, 2005a), mainly focused on the assessment of the effects of environmental changes on human well-being and answering questions like: ''How have ecosystems changed?'' ; ''How have ecosystem services and their use changed?''; "How do ecosystem changes affect human well-being and poverty reduction?"; "What are the most important factors causing changes in ecosystems?" etc (Millennium Ecosystem Assessment, 2005b).

At European level, there is also a significant increase in interest in the concept of ecosystem services and as a proof, this is the report issued in 2013: „Mapping and Assessment of Ecosystems and their Services" related to the European Union biodiversity strategy by 2020 (MAES, 2013). The main goal of the Union is the assessment and the mapping of the condition of all ecosystems and

ecosystem services they generate (MAES, 2014). In order to achieve this aim, in the next few years the report has been republished several times (MAES, 2016a; Maes, 2016b), last in 2018 (MAES, 2018), as each subsequent edition amplifies the previous one and adds new proposals for development of the topic by taking into account the level of progress achieved in this direction. The report sets out a clear conceptual framework for performance of the assessment and the mapping, which should be achieved by using environmental indicators selected through the globally recognized approach DPSIR.

As a member state of the European Union, Bulgaria does not make any exception in terms of goals already set and it should also assess and map its territory. Of particular interest to Bulgaria are the agroecosystems existing and functioning in the agricultural areas, as they not only occupy the largest part of the country's territory, but also play an important role in the economic, social and cultural development of Bulgaria (Yordanov Yav., M. Damyan, *et al.*, 2017).

For the solution of this kind of issues, it is necessary to optimize the set of indicators and during that procedure it should be highlighted the most significant and less significant among them. It is required also to introduce a model of reduction coefficients, which will inevitably lead to an improvement in the accuracy of the final assessment of the biophysical condition of the ecosystems.

Materials and Methods

By definition of EOCD[1], the indicators represent parameters or values derived from the parameters for a particular period of time (stage, point), provide information about the state characterization of a phenomenon / environment/ space and have a significance extending beyond that directly associated with any given parametric value.

When using the DPSIR indicator selection approach (Figure 1), the indicators are classified into five groups: Driving forces, Presure, State, Impact and Response. The logic on which the method is based is that the ***Driving forces*** defined as socio-economic sectors that satisfy human needs, such as: food, water, shelter, health, safety and culture, function by human activity, indicate conscious or not, direct or indirect pressure on the environment and change its current state. At the onset of the changes, which in most cases are negative and aggravate the condition of the ecosystems, it can be considered the impact that modify the flow of ecosystem services. ***The Response*** represents the actions and the measures implemented to limit or completely eliminate the negative pressures on the condition of ecosystems and the stabilization of the flow of ecosystem services. Often, in practice, the construction of the network occurs in a certain sequence in which firstly, the pressure, the state and the impact indicators, then those of the driving forces, are determined. And finally, after detailed analysis, the response indicators are identified (L. Maxim, Joachim H. Sp., M. O'Connor, 2009).

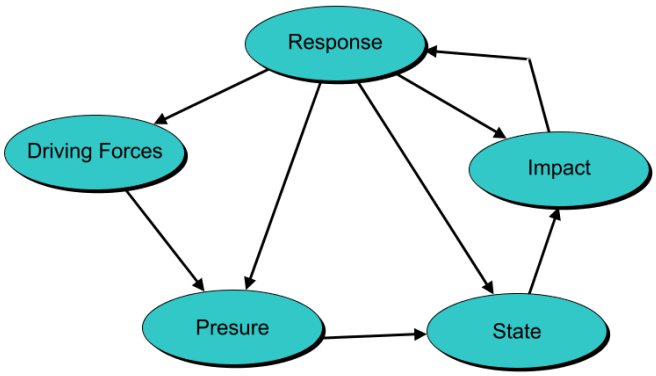

Figure 1. The concept of the DPSIR approach.

[1] EOCD - Organization for Economic Co-operation and Development

The implementation of the approach is based on the construction of cause-and-effect networks (Figure 2) composed of cause-and-effect links of the cause-and-effect type. The cause-and-effect links describe the consequences of the action of one phenomenon on another and their application in the selection of indicators is a reliable way of tracking all possible scenarios obtained by the action of some element of the network. Usually, when working with the DPSIR approach, the maximum number of indicators is introduced and after a detailed analysis of the cause-and-effect network, they are reduced to a number sufficient to allow reliable assessment. The applied analysis includes both the consideration of each link individually and its relation to the entire network. This is possible by defining the key nodes divided by root (DFi), central (Pi) and nodes at the end of the network (Ii). The root nodes are represented by those ones characterized only by outgoing connections and have a definite impact on the central nodes. The central nodes are essential for analyzing the network, as they provide information not only about the impact on them, but are also a source of impact on the nodes at the end of the network, which are characterized only by incoming connections (D. Neimeijer, R. de. S. Groot, 2006).

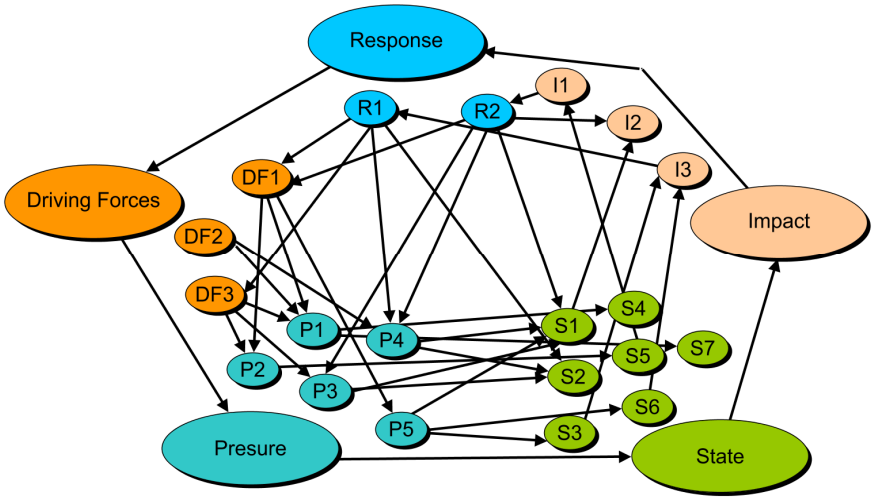

Figure 2. Illustration of a cause-and-effect network of the DPSIR approach (cause-and-effect links between the different groups of indicators)

The subjects of the study are agroecosystems and their condition to a particular period.

Results

For the purposes of the study, only part of the cause-and-effect network has been analyzed. That part includes a limited number of pressure indicators, the condition of the agroecosystems and the impact on the flow of ecosystem services. The relations between them have been also explored (Figure 3).

Pressure indicators

In most cases, the nature of the pressure is anthropogenic and is caused by the aspiration of the people artificially to increase the productivity of agroecosystems with the purpose of increasing the flow of material ecosystem services to satisfy human needs. Examples of such indicators are bad farming practices that are widely applied worldwide, although they are undesirable, because they can permanently damage the agricultural areas (M. Önder, Ercan C., Al. Kahraman. 2011). Such practices can lead to intensification of degradation processes, change of soil properties and dicrease in the natural productivity of the ecosystem as a whole.

Another anthropogenic activity that has a negative impact on the condition of the agroecosystems is the use of pesticides that are often harmful to the natural vegetation in agricultural areas or kill bees and other pollinators and can also damage the yield or impair its quality (Ph. Jeanneret, Graham B., *et al.* 2016; Md. Wasim Aktar, Dwaipayan S., Ashim C. 2009; K. F. Isherwood.

2000). Pressure on the condition of the agroecosystems should also be the presence of invasive species (plants or animals) (D. M. Huber, Martin E. Hugh-J., et al., 2002). Very often the appearance of these species is due to human error, by carrying them unintentionally, and the damage is equal to the total agricultural production (C. A. Carter, James A. Ch., R. E. Goodhue., 2004).

State indicators

After defining the main factors and processes that put pressure on ecosystems, it is required the present condition to be studied as well. When talking about agroecosystems, it shoud highlight two main factors that are determining in their functioning (Swift, M. J., Vandermeer, J., Ramakrishnan, et al., (1996). Firstly, the soil (edaphic factor) should be marked as an indispensable component of the ecotope of this type of ecosystems, as it provides the necessary environment for the development of species of plants (New, 2005). The assessment of its condition is possible through a large number of indicators, among which: ***soil topsoil organic matter content, pH, soil productivity expressed in a bonity index, soil water holding capacity, soil degradation processes, etc***.

Secondly, it is necessary to assess the climatic conditions in the explored territory as they are crucial in the selection of food crops (Olawale Emm. Ol., Is. Kow Tetteh, Labode P., 2016; Mazurczyk W., Lutomirska B., *et al*., 2003). Among the widely used indicators in this category are: ***average temperature during the vegetation period, average annual precipitation, differences between precipitation and evapotranspiration etc.***

When examining the condition of the agroecosystems, it is mandatory to assess the land use in agricultural areas (B. Feledyn-Szewczyk, Jan K., J. Stalenga, *et al*., 2009). For this purpose information is required for: ***arable land area, irrigation area, agricultural area considered as a protected area or zone under NATURA 2000.***

Impact indicators

When exploring agricultural areas, it is possible to reveal a large number of indicators related to the impact on the flow of ecosystem services they generate (Maes J., F. Nina, Zulian G. et al., 2015. A well-known fact is that agroecosystems are mainly considered as a source of food production, but they are source of non material benefits like ***erosion prevention, climate regulation, etc*** (Garbach, K., Jeffrey C. Milder et al., 2014)**.** For the purposes of the current study two indicators have been selected: ***yield impact and pollination.***

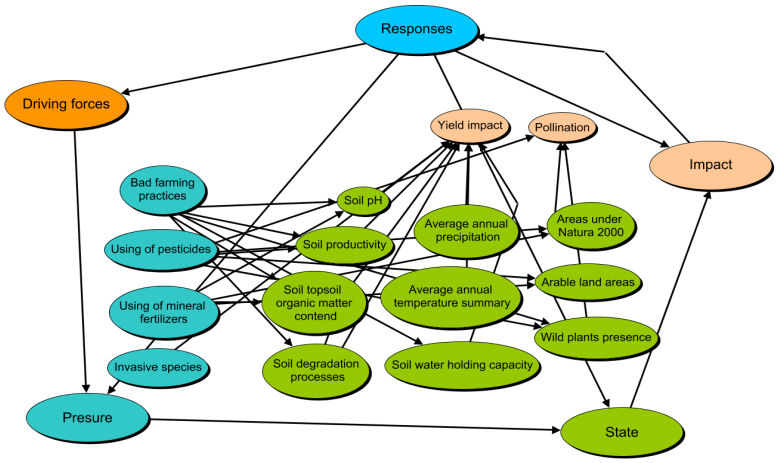

Figure 3. A cause-and-effect network for assessment of the bio-physical [2]condition of an agroecosystem (complete package / set of indicators).

[2] Assessment of the bio-physical state includes assessment of indicators of biotic and abiotic heterogeneity and the processes that occur in it.

Discussion

Once the set of indicators has been selected and accepted, each of them should be assessed by significance, which will lead to an increase in the accuracy of the final assessment of the bio-physical state of the agroecosystems and the ecosystem services they provide. For this purpose, it is proposed to analyze the number of connections that exist between the particular indicators (Table 1) as well as their direction.

Table 1. Assessment of the number of incoming and outgoing links of the indicators participating in the DPSIR network.

Indicator №	Indicator name	Links In	Links Out
1	Bad farming practices	0	6
2	Using of mineral fertilizers	0	4
3	Using of pesticides	0	5
4	Invasive species	0	1
5	Soil pH	2	1
6	Soil productivity	2	1
7	Soil topsoil organic matter contend	2	0
8	Soil degradation processes	1	2
9	Average annual precipitation	0	1
10	Average annual temperature summary	0	1
11	Soil water holding capacity	1	1
12	Areas under Natura 2000	2	1
13	Arable land areas	2	0
14	Wild plants presence	2	1
15	Yield impact	8	0
16	Pollination	3	0

It is logical to assume that the most important indicators are those that are involved in the largest number of cause-and-effect links.

From Table 1 it is easy to see that the pressure indicators: ***bad farming practices***, ***using of mineral fertilizers*** and ***using of pesticides***, are characterized by the largest number of outgoing connections, which means that they are the biggest source of changes in the condition of the agro-ecosystems.

When examining the soil indicators, it becomes also clear that they occupy an important place in the cause-and-effect network as they are key nodes in it. By changing their condition due to the

applied pressure, each of them has a definite repercussion on the ecosystem services and a more particular yield impact. The prioritization of the indicators also includes climate indicators that are critical for generating services from the agroecosystems (***yield impact).***

Analogical, after defining this set of indicators, the most significant ones should be identified. As mentioned in point 4, this will be performed by analyzing the number of connections in which each indicator is involved.

Two indicators have the largest number of connections: ***„Bad farming practices"*** (a total of 6) and ***„Yield impact"*** (a total of 8) (table 1). The first indicator is a representative of the pressure group and logically has the largest number of outgoing links showing the pressure put on the state, while the last one is part of the impact group and has the largest number of incoming links. The other indicators of that sample of the cause-and-effect network are characterized by a variable number of participations (between 1 and 5 connections).

The biophysical condition of the ecosystems reflects their potential to provide ecosystem services and is usually calculated as the average of the values of all indicators. However, as already observed, some of them are more significant than others, they have a more serious impact than others, and therefore, it is necessary that they take their place in the final assessment with greater weight. It is important to mention that when assessing the biophysical condition of the ecosystems, it is required that the assessment is not made separately, but to be related to the ecosystem services they generate. For example, if some important features of the flow of agroecosystem services are not taken into account, it is possible that several indicators will not to be properly assessed by significance.

For this purpose, besides examining the results of the application of the DPSIR approach, a detailed analysis is needed in order to observe which of the state indicators are also important for ecosystem services. Soil indicators stand out as the most significant according to the method used, while climatic ones are the least involved in the network and if only the logic of approach is followed, then they are the most irrelevant. In practice, however, the climate has a leading role in agriculture and this fact should be taken into account. On the other hand, the indicators „Arable land areas" and „Areas under Natura 2000" provide general information on agroecosystems, but are not determinant in forming the assessment of their condition.

Based on the analysis of the number of outgoing and incoming connections, has been made the proposal to introduce correction coefficients through which to recalculate the final assessment of the condition of the agroecosystems (Table 2). The proposed coefficients are consistent with the above-mentioned considerations and analysis. As the most important, these soil indicators that participate three times in the network will be multiplied by 1.3.

For objective reasons, climate Indicators will be ranked second in importance and they will participate in the final assessment of the condition with coefficient of 1.2. On third place, with correction coefficient of 1.1 remain those soil indicators that have two participations in the cause-and-effect network. Other indicators are assessed as having the least relevance to ecosystem services and will be reduced to a coefficient of 0.5.

By definition, the lowest value of the assessment is "1" (very bad evaluation) and higher is "5" (very good evaluation) regardless of the introduced correction coefficients.

Table 2. Correction coefficients for agroecosystems condition indicators is based on the number of outgoing and ingoing connections.

Indicator №	Indicator name	Number of participations in indicators network	Correction coefficient
1	Soil pH	3	1.2
2	Soil productivity	3	1.2
3	Soil topsoil organic matter contend	2	1
4	Soil degradation processes	3	1.2
5	Average annual precipitation	1	0.7
6	Average annual temperature summary	1	0.7
7	Soil water holding capacity	2	1
8	Areas under Natura 2000	3	1.1
9	Arable land areas	2	0.8
10	Wild plants presence	3	1.1

In order to justify the elaborated proposal, the study was conducted in which two scenarios with indicative assessment of the indicators of the condition are reviewed. Option 1 examines the case when less important indicators receive a higher evaluation, while the significant ones are worse rated. Option 2 examines the opposite situation where significant indicators receive a higher evaluation, while the less important are worse rated.

Table 3. Results of the final assessment of the bio-physical state of agroecosystems before and after introducing correction coefficient.

			Option 1		**Option 2**	
Indicator №	Indicator name	Correction coefficient	Indicator score without correction	Indicator score with correction	Indicator score without correction	Indicator score with correction
1	Soil pH	1.3	1	1	4	5
2	Soil productivity	1.3	1	1	4	5
3	Soil topsoil organic matter contend	1.1	1	1	4	4
4	Soil degradation processes	1.3	1	1	4	5
5	Average annual precipitation	1.2	1	1	4	5
6	Average annual temperature summary	1.2	1	1	4	5
7	Soil water holding capacity	1.1	1	1	4	4
8	Areas under Natura 2000	0.5	4	2	1	1
9	Arable land areas	0.5	4	2	1	1
10	Wild plants presence	0.5	4	2	1	1
		Final score:	2	1	3	4

An analysis of the results of the two scenarios shows that after the introduction of correction coefficients the final assessment of the status of the agroecosystems changes. For Option 1, the final estimate after correction is lower due to the fact that the less important indicators have high estimates but are multiplied by a factor of 0.5 which reduces their value. For Option 2, the results are different and the final score is increased because the essential indicators are multiplied by the coefficients 1.1, 1.2 and 1.3, which increases their value after correction.

Conclusions

Agro-ecosystems are an indispensable part of peoples live since ancient times and their sustainable use is the basis of their potential for generating ecosystem services conservation for future generations. It is therefore required to explore in detail their condition, which is crucial for the generation of a sustainable flow of ecosystem services for a long time.

The DPSIR approach is a sufficiently reliable tool for selecting indicators and its use for analyzing the individual processes in any cause-and-effect network can provide information about the reliability of each indicator. The assessment of the condition of the agroecosystems should not be made independently, but it is necessary to refer it to the flow of ecosystem services and to the peculiarities of the ecosystems that generate it. The application of correction coefficients aims to improve the accuracy of the final assessment of the condition of the agroecosystems, which will inevitably lead to more correct results in its mapping and this in turn will contribute to its more precise visualization in space.

Conflicts of Interest: The authors declare no conflict of interest.

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Original scientific paper

The frequency occurrence of the drought in Montenegro from the 1981-2017, vulnerability and impacts

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Abstract

The purpose of the article is to analyze the drought frequency in Montenegro in the period from 1981-2017 due to the climate change. The analysis is based on statistical methods, climate projections, available remote sensing data and drought impact analysis. Analysis show that drought has become more frequent since the beginning of the 21st century, and in particular, 2000, 2003, 2007, 2008, 2011, 2012, 2017 and 2018. Such situation is in line with national projections and IPCC projections for the region of Southeast Europe. According to the investigated historical effects of droughts on economy, environment and society in Montenegro, it has been confirmed that in recent decades its consequences have been increasing more than in the 20th century, followed by higher temperature than in the last century. Considering that the risk is high, the sectors of agriculture, forestry and water supply should be in the center of attention.

Keywords: climate change; drought more frequent; risk, attention, Montenegro.

Introduction

Institute of Hydrometeorology and Seismology, hereafter IHMS till 2010 did not have permanent drought monitoring. There were documentations about analyses of droughts mainly from meteorological point of view, published in public journals or scientific papers.

Before the project "Drought Management Centre in South East Europe" (DMCSEE), sparse information about the droughts could be found in IHMS's archive, such as: drought reports for specific towns, meteorological outputs that define onset and duration of the drought made for the purpose of Conferences, Media and customer's requests, outputs from the project "Extreme Atmospheric Conditions in Montenegro - AEN" based on the aridity index of De Morton.

Detailed vulnerability assessment did not exist before the project DMCSEE. An initiative in 2003 to calculate SPI index was unsuccessful as it was evident the lack of the staff and a great demand of disposable experts to be trained.

Those very important actions were implemented in IHMS through the project DMCSEE since 2010 and ongoing project DriDanube[3] since 2017. The innovative approaches, tools, uniform data

[3] DriDanube – Drought risk in the Danube region

collection, risk assessment, online GIS and dissemination to end beneficiaries brought drought monitoring and reporting to a new level than before.

Materials and Methods

Based on electronic archive of newspapers, web sites of state institutions, local governments, enterprises as well as statistical yearbooks, data on the drought effects since 2000 were classified per years and selected in three categories: economic, environmental and social, with emphasis on those which according to available material most frequently affected society. Some of the consequences could be classified in more than one category (e.g. forest fires can be classified in each of these categories). Special attention was dedicated to the drought in 2000, 2003, 2007 and 2011 when its significant impact occurred. Similar results are stated by Maksimovic et al. (2018).

Following set of indicators that are mostly in use to monitor and characterize drought magnitude in IHMS are presented in the table 1:

Table 1. Set of indicators that are mostly use to characterize drought magnitude are as follows:

Elements	Indicators
Temperature	Anomalies in ^{0}C / percentiles, HWD[4], number of days with temperature over 90th percentile
Water	Percentiles, SPI, CDD, water level, outlooks for accumulated water balance relative to percentile classes
Soil	Near real time soil water state in areas of agriculture, forest, viticulture, fruits and olives, SWDI, SWI[5]
Remote sensing data	FVC, LAI, NDVI,
Socioeconomic	Air quality assessment, losses in agriculture, losses in electro-distribution, yield prediction

From the aspect of data availability it should be noted that the density of the stations in Montenegro was 6.88/1000 km2 up to 2010. The network was consisted of 94 active stations. From them 9 are main, 18 were climatological while 67 were rainfall stations. From the 2011 rapid decrease of precipitation stations is evident mainly due to financial problems. About 20 precipitation stations are currently in use. It is important to stress that in nowadays IHMS faces the problems of rapid

[4] HWD - Heat Wave Duration
[5] SWI – Soil Water Index

reduce of precipitation stations with decrease in density less than 6 /1000 km² what will reduce the quality of analysis timely and spatially as well. The table 2 represent the meteorological stations till 2011 categorized by their quality.

Table 2. The number of all active stations till 2011 sorted by altitude and assessed by categories from bad to very good

Altitude categories	Number of all active meteorological stations	Category (very good, good, acceptable, bad)
0-200	22	Very good
200-500	4	Acceptable
500-1000	45	Good
1000-1500	24	Acceptable

Network of hydrological stations has 51 stations that measures water level. Certain number of them are automatic for the main rivers in the Adriatic and Black Sea catchment. Data are available on-line over the web site of IHMS (www.meteo.co.me).

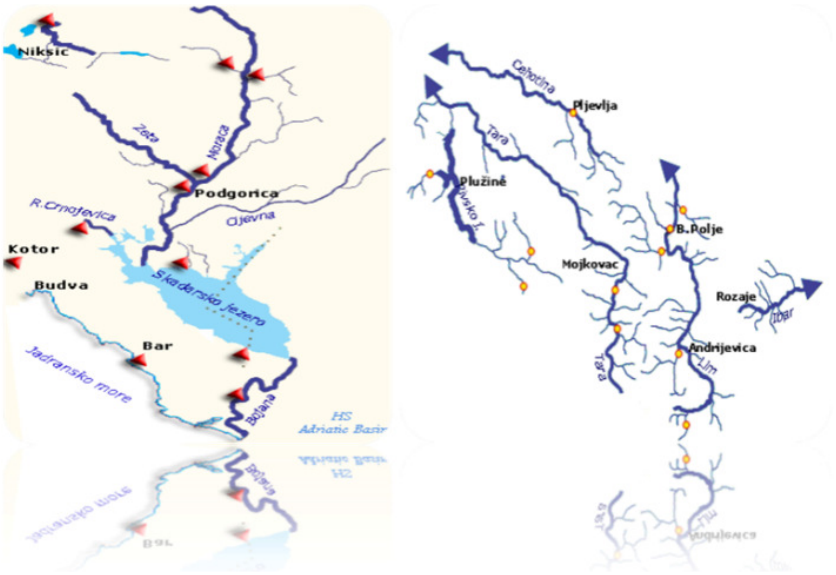


Figure 1. Network of automatic hydrological stations on Adriatic (a) and Black Sea (b)

Results

The monitoring of the drought is presented in several figures below. Two projects of EU were crucial that IHMS increased capacity and became able to monitor the drought permanently. That was IPA DMCESEE (Drought Management Centre for South East hosted by ARSO in Ljubljana, Slovenia), and ongoing project INTERREG DriDanube (Drought risk in the Danube Region).

Figure 2 presents the drought intensity in 2017 and 2018, while the figure 3 present the state of the soil water index and NDVI on the 11th August 2018. Both are in brownish color emphasizing soil water deficit and dry vegetation.

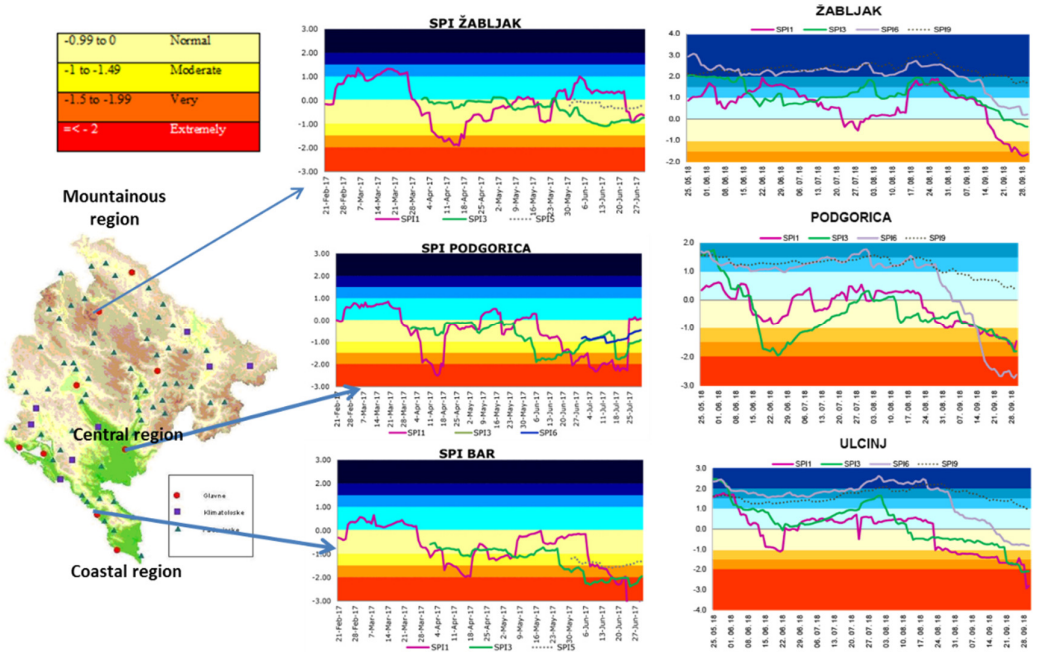


Figure 2. Network of meteorological stations till 2010 and the examples of moderate(a) agricultural and hydrological drought in 2017 monitoring by SPI3 and SPI 6 index and moderate to extreme **(b)** hydrological drought in 2018 in Ulcinj and Podgorica respectively (Source: IHMS)

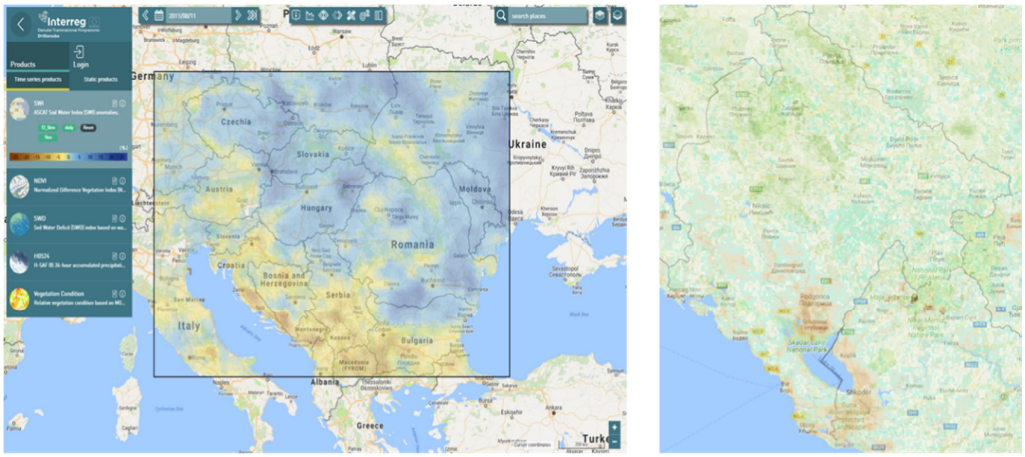
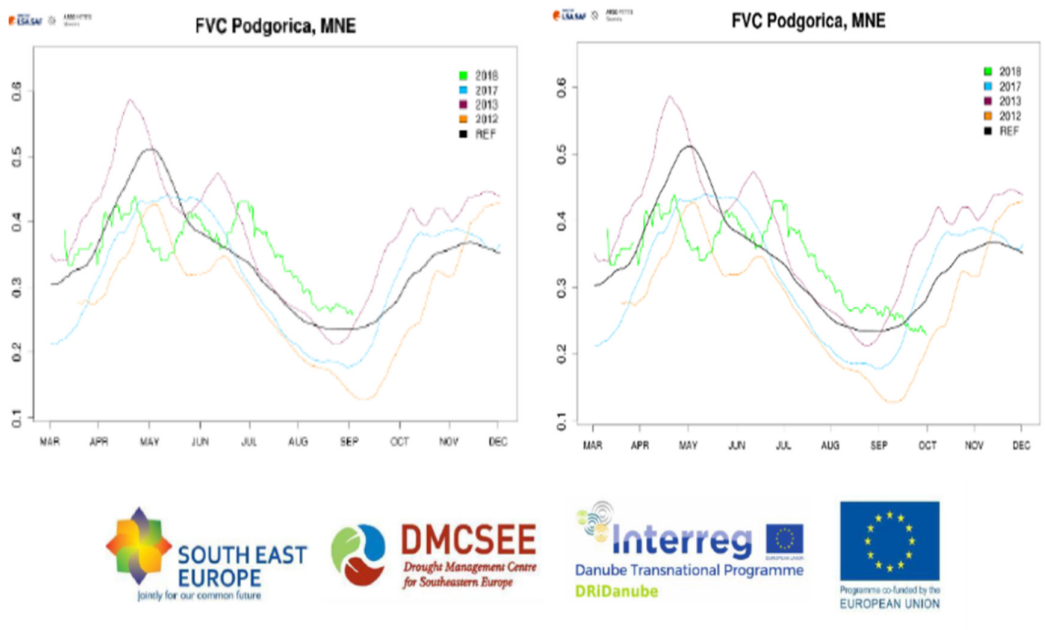


Figure 3. The state of the soil water index (a) and NDVI (b) on the 11th August 2018

Comparison of satellite indices for the 2012, 2013, 2017 and 2018 relative to the referent period 2007-2013is presented on the figure 4. The development of FVC index in 2018 during the Autumn is presented till September on the picture (a) and October (b).



(a) (b)

Figure 4. Comparison of satellite indices for the 2012, 2013, 2017 and 2018 relative to the referent period 2007-2013. Development of FVC till September 2018 (a) and October 2018 (b) is marked in green

It could be concluded that in the 2012 the drought was lasted almost whole year with all 4 phases well developed: meteorological, agicultural, hydrological and socioeconomic. The values of FVC index were low in April indicated that vegetation was very dry, while in July and August was unusually high what means that in those months was enough moisture in the soil. The situation is changing in September and October when the values of the FVC index started to sharply decrease. That coincides with the development of hydrological drought based on SPI indexes and hydrological monitoring.

Identification and categorization of the drought impacts in Montenegro show that the drought affected mostly economy with direct (e.g crop yield reduction) or indirect consequences in agriculture (e.g. increase price for crops on the market), livestock, forestry and water supply.

Focusing on water resources shortage and their impacts, the most vulnerable sectors in economy are:

- agriculture, food and milk production
- water supply
- electricity production
- environment.

The most vulnerable groups of the society are small farmers (wheat, ray, barley, oats and maize producers), producers of fruits and vegetables (olives, figs, citrus, raspberries and grapes, potatoes, cabbage and pepper), ranchers and milk producers.

Regarding the public health, the most vulnerable groups are those with respiratory problems, heart disease as well as children who suffer the most effects of forest fires smoke. There is insufficient data and information on drought and damages caused by drought in previous years. Drought in

Montenegro were not permanently analyzed and monitored in the past. There is no archive on the damages dedicated particularly to the drought.

Drought impact archive was created during the project DMCSEE and updated in 2018 within the project DriDanube. Collected data on economic impact of the drought on annual basis show that during the dry years maximum losses in electricity production are in range from 3 – 3.5 million Euros. Total hydropower potential of Montenegro is about 9900GWh annually. In the period 1999-2008. deficit was 14121GWh or 620million EUR.

Collected data on water supply show that in Podgorica, capital town of Montenegro, water supply uses about 2.000lit/s, with a daily injection into water supply system of about 130.000m3 . The daily loss of profit during the drought ranges from 15.000EUR to 85.000 EUR.

Impact of the drought in December 2011 reflected in unprecedented lowest water level in Montenegro. In agricultural sector during the, crop production was suffered the greatest damage (the damages are estimated from 30 to 60 percent of expected yield). Throughout the crop production great impact was on livestock.

Milk production was second ranked as extremely affected by the drought. An urgent measures such as subsidies for the import of cattle's food necessary for production of milk, was sought by the Agriculture Union of Montenegro. They addressed to the Government with the strong need for support of the milk production.

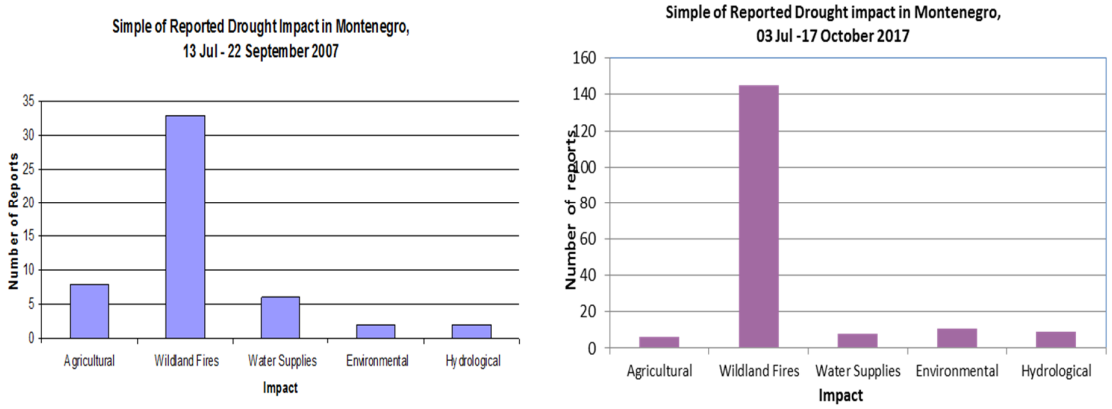


Figure 5. Comparison between the number of reports on the effects of drought in July to September and July to October 2017 (source: Montenegrin newspaper archive)

North parts of Montenegro were exposed to below normal height of snow cover in 2017 in January and February, and to seasonal deficit in precipitation from April to June. Although January was extremely high, from February to June was warm, very warm and even extremely warm. The consequences of such climate situation was: minimum water level 2 months earlier than usual (i.e. in August instead in October), accumulations close to minimum, suitable conditions for forest fires, one month earlier grape harvest (i.e. in September instead in October), less honey and tomatoes, but excellent conditions for the olives. The government promised to support the construction of the irrigation system as of the adaptation measures (http://www.privredniportal.me/portal/poljoprivrednici-pretrpjeli-ogromnu-stetu-zbog-suse-rod-gotovo-prepolovljen/).

Discussion

The increase trend of temperature, more frequent extreme events such as heat waves, droughts and heavy rainfall, affect Montenegro too. Decade 2001-2010 is the hottest decade since the beginning of the measurement what coincide with the warmest decade in the world (WMO, 2013). The biggest

changes in the temperature anomalies was detected on the north (e.g. in Zabljak + 1.4 0C in that period).

Followed by higher temperature, drought has become more frequent since the beginning of the 21st century, and in particular, 2000, 2003, 2007, 2008, 2011, 2012, 2017 and 2018. Such a situation is in line with national projections and IPCC projections as well for the region of Southeast Europe.

According to national climate projections, the biggest changes can be expected in the northern regions in winter, spring and summer, by + 0.9C, + 1.1C and + 1.3C respectively in the period from 2001-2030 for the A1B scenario. Then, decrease in precipitation for 10% in the winter and spring in all parts of Montenegro, the increase in intensity of extreme events such as heavy precipitation, droughts, heat waves and less days with frosts.

The establishment of an archive with identified and categorized consequences of the drought was the basis for further research. Therefore, in 2018 within the project DriDanube, the networks of reporter were established for weekly reporting from the field about the condition of soil moisture agricultural crops and forest vegetation. Reporters' network consists of agricultural producers, employees of NP Durmitor, Biogradska Gora and Lovćen, meteorological technicians, as well as agricultural engineers (the focus of interest is on agriculture and forestry).

Starting with information on the current condition of soil and plant moisture, weekly reports are basis for several types of maps for the territory of Montenegro: a map of the current state of soil moisture (per week), the state of the soil moisture status (after weeks) and the impact maps (current and backward after the weeks) based on the methodology implemented within the INTERREG DriDanube project.

According to specific needs, the same maps can be made especially for forest, crop and fruit crops. The significance of the existence of the reporter network in the field, its reporting and the mapping of soil moisture and the effects of drought, is reflected in the improvement of the existing monitoring and early warning of drought and its consequences in Montenegro.

Conclusions

Based on aforementioned results and considering observations and projections of climate change in on national, regional and global level, there is an urgent need for skills in drought management both on individual and institutional levels, establishment of drought authority and organized drought management, implementation of irrigation scheduling system (e.g. WINISAREG which was applied in IPA DMCSEE resulted in Montenegro as an efficient and very precise tool in agricultural water management).

Regarding the irrigation, higher portions of water required each year, comparing to the past. The intensity of this problem is evident not only in southern part of Montenegro but also in the hilly and mountainous region under cold continental climate where irrigation traditionally was not used in the past.

In Montenegro does not exist a national policy or strategy related to the drought. There are only a few strategic documents such as: Montenegro Spatial Plan until 2020 and Montenegro Water Law, 2007).

Montenegro Water Law from 2007 is an important document in combat the drought and its mitigation (http://www.gov.me/files/1246958897.pdf). This Law regulates the water management.

Montenegro ratified in 2007 the EU Convention to Combat Desertification (UNCCD) (http://www.ncsa-montenegro.com/index.php?jezik=0&opcija=0&id=5). By approaching to UNCCD and adopting obligations, it is expected to produce own national strategies directly involved in combating the droughts. Ratifying and approaching the Convention Montenegro has the obligation of Development and implementation of programs for sustainable irrigation, like necessary condition for agricultural development in rural and arid areas. Program has to be a part of existing and future agro-ecological programs on local and national level. Realization of this plan is expected to have

numerous positive effects on agricultural production, in combating the drought and drought mitigation.

Conflicts of Interest: The authors declare no conflict of interest.

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Review paper

The Energy-Smart Public Building in the City of Novi Sad

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Abstract

Nowadays, the building sector is responsible for 40% of global energy consumption and 30% of GHG emissions. In order to minimize these negative impacts, worldwide solutions are being developed to use RES and environmental protection.

The aim of this work was to present one project energy-smart public building in the city of Novi Sad, whose construction implemented on a global scale would be significantly reduced energy consumption and GHG emissions. The basic advantages of this smart building are reflected in the energy sense (savings in heating, cooling and lighting of the building) and ecological (significantly reduced CO_2 emissions in the atmosphere, decrease in radiation intensity, better rainwater retention, etc.).

Keywords: energy, smart public building, RES, GHG emissions

Introduction

Houses of our ancestors were energy sustainable, as being made from natural materials, such as wood, mud, cane, soil and similar materials. But, after the Second World War, in the former Republic of Yugoslavia, the quantity, and not the quality and comfort of the buildings, was taken into account, which soon led to the large influx of rural population to the cities *(Ašonja, 2017)*.

Today, the largest number of residential buildings in the Republic of Serbia is in energy class "G", which means that they consume more than 175 kWh/m^2 per year for heating. Here we should also add that maintenance costs rise over the years due to the age of buildings and their installations. The average energy required to heat buildings in Serbia is about 2.5 times higher than in EU countries *(Discuss energy 2014; Ašonja andRajković 2017)*.

Today in world buildings are responsible for more than 40% of primary energy usage and 70% of overall electricity usage *(Ašonja andRajković 2017; U.S. Energy Information Administration 2008)*. The energy consumption of buildings is responsible for 38% of CO_2 emissions to the atmosphere, 52% of SO_2 emissions, and 20% of NOx emissions *(Mehdi, et al.,2017; Wang, et al., 2005).* Transition to sustainable energy buildings requires the reduction of energy consumption to a minimum, while keeping comfort at the same level. In order to make appropriate energy-efficient building projects, one should take care of the isolation, the way of lighting, the orientation of the building itself, and other factors that can affect the energy consumption. Nowadays, there are various studies dealing with the use of different sensors in order to reduce energy consumption. One such example of a energy-smart public building is shown in the paper.

The European Commission has proposed a five-point action plan for European energy security and solidarity through *(Franjić, 2016):*

- construction of infrastructure and the diversification of energy supplies;

- international energy relations;
- creation of oil and gas reserves and the mechanisms of response to emergencies;
- energy efficiency; and,
- best use of domestic resources within the EU.

Reducing imported energy and energy productsdependency is the goal of EU energy policy that promotes energy efficiency measures and renewable energy sources (RES) integration*(Ivanović, 2015; Šerman et al., 2017).*

Materials and Methods

After 31 December 2020, all new buildings in the EU will have to spend energy in the amount of "close to zero" and energy could significantly should be covered from renewable sources. Today, across Europe are building buildings that produce up to 5 times more energy than they consume. One such project of highly efficient buildings (future building of the Energy Agency of the City of Novi Sad) was done by academician Veljko Miljkovic.

The aim of the paper was to present the first energy-smart object in the City of Novi Sad and the entire Republic of Serbia. Namely, this is a project of a smart, highly efficient energy building, fig. 1 (future buildings of the Energy Agency of the City of Novi Sad).The facility is intended to be a demonstrative / educational center for RES and energy efficiency, both for the needs of the City of Novi Sad and the Autonomus Province Vojvodina.

Figure 1. The first designed energy efficient object in the City of Novi Sad

Results and Discussion

The project of energy-smart public building in Novi Sad

The aim of presenting this project in the publication is to familiarize the general public with one of the most successful domestic projects in the building, which has been designed in full accordance with nature. Among other things, the project called *"Novi Sad Energy - Haus"* has won several awards for quality and innovation in the field of ecology and energy.

In addition to the minimum CO_2 emissions in the atmosphere, the ecological dimension of the object also gives green surfaces, whose surface is larger than the surface area of the soil. In addition to horizontal green surfaces, the object also has vertical green surfaces.

The basic characteristics of the energy-smart building are:

- *name of the object:*"Novi Sad Energy - Haus"
- *the surface of the building basically amounts to:* 600 m² (P+1, P+2),
- *plot area:* 2.000 m²,
- *position of the building: facing south*,
- *way of managing an object: automatic (smart building),*
- *characteristics of the building: sustainable construction, eco-innovations, high energy efficiency, passive solar architecture, RES application, vertical greening, etc.*

The main advantages of this energy-smart building are *(Construction Project of Novi Sad Energy House, 2012)*:

- savings in heating 85%,
- savings in cooling 100%,
- savings in lighting 30%,
- savings in building materials 10%,
- significantly reduced CO_2 emissions into the atmosphere.

Namely, this type of construction of a self-heating eco building contributes to the independence of conventional energy sources. In particular, the object depends only on the Sun. At the object itself, reflecting surfaces up to 2.5 times can increase the level of light and heat in the building,fig.2. From the RES, the facility includes solar energy (panels and collectors), heat pump, biomass boiler, and other sustainable solutions such as wastewater treatment plants, fig.3 and 4, *(Construction Project of Novi Sad Energy House, 2012)*. The management of these systems is independent of man, which places this object in smart buildings. Further, the facility is intended to use LED lighting and other machines and appliances that have minimal energy consumption. In front of the building, it is planned to install a power plant that all times publicly displays data on the production and consumption of energy, achieved CO_2 savings due to its reduced emissions, and the similar, fig.5.

Above the parking lot, it is planned to install solar panels (cells) that would produce and store electricity, which would be used by electric cars, fig.6. At the same time, these panels would protect cars flying from high temperatures, and in winter in the snow.

The consequences of the occupation of green spaces due to urbanization in the city are reflected in the growth of GWP, the inability to receive water into the ground, and the like. However, the presented object in this work has chartered the green surface (green roof and green wall), which were made in accordance with the European norms that provide 15 m²/apartment of green areas within urban areas.

Figure 2. Reflective surfaces increase the input of light and heat into the object

Figure 3. The dominant energy source is solar energy - panels and collectors on the building

Figure 4. Boiler room - heat pump and biomass boiler

Figure 5. Energomonitor on the object

a)

b)

Figure 6. Polar station (canopy) for charging an electric car

With the construction of the presented green roofs, it is possible to reduce the temperature in such parts of the city by several degrees. Also, with strong rainfall slowing down the flow of water that reduces the risk of flooding, and the possible overload of the sewage system is delayed for more than half an hour which is quite enough to avoid flooding floods.

In many cities, the plant uses clean water from the water supply system, although for many processes this is not necessary, which affects the consumption of water per capita or, to the utmost energy consumption. This project envisions the construction of an underground rainwater collection tank. So collected rainwater provides technical water that will be used for watering lawns, washing cars and so on.

In Fig. 7, the advantages of green are compared to the traditional roof.

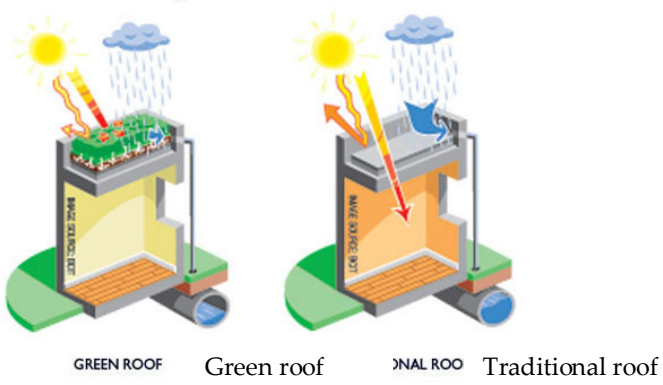

Figure 7. Green and traditional roof

In addition to contributing to a healthy environment, the main advantages of such projected green roofs are also reflected in *(Ašonja and Ćirilović 2017)*:

- The growth of real estate prices;
- Reduced energy consumption;
- Improved isolation of the building from noise;
- Long service life of the roof;
- Reducing the intensity of radiation;
- Better retention of rainwater;
- Possibility of installing and installing RES;
- Possible organization of agricultural production;
- Aesthetically comfortable and naturally suitable space for people's rest and recreation;
- Increasing air humidity;
- A healthy environment.

Conclusions

Project energy-smart public building in the city of Novi Sad significantly reduced energy consumption and GHG emissions. The basic advantages of this smart building are reflected in the energy sense and ecological (significantly reduced CO_2 emissions in the atmosphere, decrease in radiation intensity, etc.).

Conflicts of Interest: Declare conflicts of interest or state.

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Original Scientific paper

Dynamics of precipitation's acidity at Montenegro area

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Abstract

Institute of Hydrometeorology and Seismology from Podgorica has carried out of annual program of the precipitation quality measurement at entire state area, by sampling and chemical analysing of 24h-precipitation's samples at the monitoring network in Montenegro. The main ionic species, including pH and conductivity, are implied by physical-chemical analysis of the precipitation composition. The most important wearers of precipitation acidity, like pH, content of sulphates and nitrates, are included by analysis, which is carried out in the paper. Data about precipitation's acidity in 2000-2017 periods are processed, and the review of available data on precipitation acidity in the previous period, is also made.

Analysis of occurrence of the acidity is done on the base of content of the acidity wearers, the frequency of the "acid rains" and spatial distribution of them. Statistical analysis of the data consistency and mutual correlation of them is carried out. Temporal trends of data series and spatial distribution of "acid rains" analysis related to impact to the ecosystems, are done.

Results obtained shown that the occurrence of the acid rains was the most frequent at the forest area in the north. Acidity of the precipitations was of the middle range, with the exception of several cases of strong acidity. Frequency and spatial distribution of the acid rains were variable during the measuring period.

Key words: Precipitation composition, acidity, statistical analysis, temporal and spatial distribution

Introduction

Precipitations are very efficient way of cleaning of the troposphere. The cleaning effects have the biggest contribution for the dissolution and deposition of atmospheric gases, likewise small particles. Removal of gases and aerosols is more efficient by wet, then by dry deposition [Pacyna, 1995].

The atmospheric emission of acid gases was dominant among all anthropogenic emission. Major part of this emission is emission of SO2. Another acidification matters are nitrogen-oxides, than some organic acids. Natural emission sources of SO2 are dimethyl-sulfide from sea water, volcano eruptions etc. Anthropogenic sources are fuel combustion, domestic heating, traffic, industry etc. The sources of nitrogen oxides are fuel combustion, agriculture, industry. Anthropogenic emission can reach natural.

SO2 is oxidized in presence of atmospheric water, to sulfate ion, which is main carrier of acidity of the precipitations. This shown the strong correlation between SO2(g) and SO42- [Avila et all, 2002]. Similar to that, nitrogen oxides with cloudy waters give nitrates ion. Also, composition of the precipitation depends of many factors, as a method of sampling [Staelensa et all 2005].

The contents of S and oxidized N in the atmosphere are decreased in recent years by political measures of their emission reduction. The deposition of S and N decreased in Europe and its maximal

release moved from Central to Eastern Europe [Fagerli et all 2006]. During 1990-2009 decrease of SO2 in air amounts 75%, SO42- in aerosols 56% and in precipitations 64% [Tørseth et all 2012].

Anyhow, acid rains are global issue, which is more characteristic in big industrial areas, but in unpolluted areas too, because the transfer of polluted precipitations by air masses. Acid rains can have very damaging consequences on terrestrial ecosystems: drying of forests, acidification of waters, mobilization of toxic metals in waters and soil etc. Global deposition of S and N exceeds critical values for ecosystems, by what it endangered a 7%-17% of global natural ecosystem`s surface [Bouwman et all, 2002].

The regular precipitation`s monitoring shown that the acid rains are usual occurrence at whole Montenegro area [Đurašković i sar., 2003]. Correlation of pH and quantity of precipitations is negative and moderate. There is a similar situation about quantity of precipitations and SO42- and NO3- [Đurašković, 2016]. But, the investigations showed that there was no measurable influence of acid rains to SO42- content in the rivers in Montenegro [Đurašković, 1995].

Results of the processing and research of the data series of rain`s parameters in the 8-years period, available from regular precipitation`s monitoring program, are presented in this paper.

Materials and Methods

Data series of pH, SO42- and NO3- in precipitations at measuring network of Institute of Hydrometeorology and Seismology, are processed. Measuring data are collected by regular monitoring program. The sampling method was "bulk" and sampling period is 24h by UTC. The obtained period was 2010-2017. All stations were represented with data in the whole period, except station Tivat, were the data collected only for 2016-2017. Particularly, deposition samples in Podgorica, wet and dry, are taken in each day. Dry deposition samples are taken after washing of the sedimentation funnel with 0,5ml of deionized water.

Measuring parameters analyzed by reference analytical methods: pH by ion selective glass electrode and ions by UV-VIS spectrophotometry.

Data series processed by standard statistical methods (Excel statistical package).

Results and Discussion

Annual average of pH was predominatly between 6 and 7, except in Pljevljma, B. Polje and Tivat, where they were a little over 7 (Tables 1-4). Minimum of average values was in Golubovcima (6,27), but maximum in B. Polje (7,13).

Table 1.: Measures of central tendency of data series

Stat.	Stations	Žabljak			Pljevlja			B. Polje			Berane		
	param.	pH	NO_3^-	SO_4^{2-}	pH	NO_3^-	SO_4^{2-}	pH	NO_3^-	SO_4^{2-}	pH	NO_3^-	SO_4^{2-}
	m.unit		*mgL^{-1}*	*mgL^{-1}*		*mgL^{-1}*	*mgL^{-1}*		*mgL^{-1}*	*mgL^{-1}*		*mgL^{-1}*	*mgL^{-1}*
A		6,63	2,31	6,37	7,08	3,47	14,10	7,13	3,04	10,18	6,58	3,01	14,19
G		6,61	1,28	3,71	7,07	1,86	8,83	7,11	2,10	6,22	6,57	1,83	7,11
MEDIAN		6,60	1,41	3,70	7,10	2,03	8,64	7,14	2,16	6,66	6,52	1,88	7,25
C5		5,79	0,17	0,62	6,35	0,28	2,21	6,50	0,40	1,02	5,97	0,36	0,88
MIN		4,39	0,01	0,03	5,24	0,01	0,08	5,13	0,04	0,07	4,85	0,02	0,04
C95		7,61	6,59	20,02	7,76	11,13	39,40	7,72	8,38	32,12	7,43	8,72	52,98
MAX		9,06	70,52	95,22	8,24	63,60	393,46	8,64	20,75	72,96	8,10	35,00	97,68
SDEV		0,58	4,06	8,97	0,44	5,72	23,12	0,42	2,80	11,04	0,44	3,94	17,31

Arithmetic and geometric averages stressed the homogeneous and central distribution of the data of pH. Extreme values stood out in Žabljak, Nikšić, Podgorica and Bar, what confirmed by differences of the values of percentile C95 and Max. On the other side, the homogeneity of data are confirmed in pH high values zone, by small difference of the C95 and Max, in Kolašin, Cetinje, than in coastal region except Bar. Maximal extreme values were measured in Bar, with C95=8,10 and Max=10,31, but the minimal in Cetinje (C95=7,24).

Table 2. : Measures of central tendency of data series

Stat.	Stations	Kolašin			Nikšić			Cetinje			Podgorica		
	param.	pH	NO_3^-	SO_4^{2-}	pH	NO_3^-	SO_4^{2-}	pH	NO_3^-	SO_4^{2-}	pH	NO_3^-	SO_4^{2-}
	m.unit		*mgL⁻¹*	*mgL⁻¹*		*mgL⁻¹*	*mgL⁻¹*		*mgL⁻¹*	*mgL⁻¹*		*mgL⁻¹*	*mgL⁻¹*
A		6,40	2,35	7,72	6,99	2,60	6,21	6,46	2,38	6,78	6,58	1,39	3,21
G		6,37	1,44	3,52	6,97	1,52	4,13	6,44	1,50	3,60		1,47	1,43
MEDIAN		6,39	1,55	3,18	6,99	1,56	4,03	6,47	1,57	3,40	6,56	0,89	1,50
C5		5,51	0,22	0,54	6,19	0,22	1,01	5,65	0,3	0,78	5,73	0,04	0,14
MIN		4,50	0,01	0,04	5,94	0,01	0,04	4,83	0,01	0,01	4,31	0,01	0,01
C95		7,34	6,71	32,66	7,71	7,47	18,83	7,24	7,22	26,66	7,45	4,75	10,42
MAX		7,89	33,40	98,75	9,88	40,96	92,10	8,02	32,76	89,14	9,02	28,34	99,90
SDEV		0,56	2,92	12,51	0,45	3,68	7,47	0,51	3,10	10,71	0,56	1,77	6,94

Table 3.: Measures of central tendency of data series

Stat.	Stations	Golubovci			H.Novi			Tivat			Budva		
	param.	pH	NO_3^-	SO_4^{2-}	pH	NO_3^-	SO_4^{2-}	pH	NO_3^-	SO_4^{2-}	pH	NO_3^-	SO_4^{2-}
	m.unit		*mgL⁻¹*	*mgL⁻¹*		*mgL⁻¹*	*mgL⁻¹*		*mgL⁻¹*	*mgL⁻¹*		*mgL⁻¹*	*mgL⁻¹*
A		6,27	5,10	7,51	6,70	4,13	10,74	7,06	2,44	8,27	6,87	3,36	4,87
G		6,24	2,52	4,84	6,68	2,49	6,10	7,05	1,91	4,20	6,86	2,51	3,78
MEDIAN		6,25	2,38	4,66	6,71	2,27	6,46	7,05	1,95	4,17	6,83	2,41	4,05
C5		5,32	0,35	1,23	5,89	0,59	1,44	6,49	0,69	0,54	6,27	0,69	1,13
MIN		3,63	0,03	0,40	4,11	0,14	0,10	6,34	0,45	0,12	5,92	0,14	0,26
C95		7,33	19,70	23,15	7,48	14,48	40,00	7,87	6,27	26,53	7,47	9,29	12,38
MAX		7,97	76,72	72,30	7,92	70,64	96,96	8,20	14,65	121,35	7,95	20,10	29,60
SDEV		0,64	7,79	9,29	0,52	5,89	14,68	0,42	2,19	15,59	0,38	2,83	3,70

Table 4.: Measures of central tendency of data series

Stat.	Stations	Bar			Ulcinj		
	param.	pH	NO_3^-	SO_4^{2-}	pH	NO_3^-	SO_4^{2-}
	m.unit		*mgL⁻¹*	*mgL⁻¹*		*mgL⁻¹*	*mgL⁻¹*
A		6,83	3,18	5,94	6,52	5,72	10,85
G		6,80	1,70	4,30	6,48	2,94	7,40
MEDIAN		6,80	1,73	4,28	6,59	2,62	7,32
C5		6,02	0,22	1,34	5,02	0,64	1,89
MIN		5,25	0,01	0,05	4,03	0,06	0,10
C95		8,10	12,36	16,08	7,46	24,31	28,22
MAX		10,31	30,16	59,50	8,47	84,80	99,50
SDEV		0,67	4,17	5,93	0,72	8,47	12,75

Minimums are different from percentil C5. Percentil C5 belongs to the acidity zone in Kolašin, Cetinje, Golubovci and Ulcinj. Extreme minimal values in Žabljak, Berane, Podgorica, H. Novi belongs to the same zone too, what means that the higest acidity, is a rare and random occurrence. The differences between Min and C5 for SO42- and NO3- are even bigger, what underline the random occurrence of extreme acidity.

Standard deviation of pH data series was the highest in Ulcinj (0,72), Golubovci, H.Novi, Kolašin and Podgorica, but bellow 0,5 in all other statons. Skattering of data of SO42- and NO3- is much more higher, more for SO42- than for NO3-. Data deviate from central tendency`s distribution. The highest standard deviation of SO42- was for northern region, but the lowest for coastal region (Budva).

The most frequent acid rains were occured in Kolašin, extended Podgorica area and Ulcinj. The moderate frequency of acid rains was in Cetinje and Žabljak. Acid rains can affect forests at Kolašin and Žabljak area, as well as the soil of agruculture area around Golubovci. Acid rains were not occured at Nikšić, Tivat and Budva area, but at B. Polje only once.

The strong acidity occured in Golubovci (pH=3,63) and Ulcinj (pH=4,03). At all other stations the acid rains were of a small to moderate acidity (pH=5-5,6).

The highest SO42- content in precipitations, according C95, was in the north highland area, as it is usual in continental precipitations [Anatolaki et all, 2009; Herut et all, 1995]. Higher values of NO3- are evidented in the agricultural area of Golubovci, than in Pljevlja, where it is a consequence of termopower plant influence, the same as in the case of SO42-. The highest values of NO3- were in southern (Bar, Ulcinj) and northern (H.Novi) coastal area, where the stations are under direct influence of sea water, withdrawn by the wind of south direction. On the other side, minimal NO3- are in the central region. The important difference of NO3- values, as C95, in Podgorica urban area (4,75mgL-1) and Golubovci (19,70mgL=1), which places are at the short distance, highlihts the impact of agriculture in precipitation composition (washout effect). Of course, it shold be considered the neutralization effect of the joint processing of dry and wet deposition in Podgorica, unlike in Golubovci (where is only wet deposition).

Table 5.: Temporal occurrence of the acid rains, by number of cases

Stations	Years	2010	2011	2012	2013	2014	2015	2016	2017	
Žabljak	N	3		7	1	4	4	3	5	22
Pljevlja	N				2		2			4
B.Polje	N								3	3
Berane	N	1				1		1		3
Kolašin	N	13	2	6	22		8	5	13	69
Nikšić	N									0
Cetinje	N	7	2	1	3	10	11	1	4	39
Podgorica	N	14	9	3	3		17	8	7	61
Golubovci	N	18	12	9	2	1	15	6	4	67
H.Novi	N	7			2	2		2	1	14
Tivat	N									0
Budva	N									0
Bar	N					1	1	2		4
Ulcinj	N	5	9	3	2	15	17	10		61
	Total N	68	34	29	37	39	75	38	37	

Along the period, the number of occurred acid rains was variable (Table 5.). The biggest number of acid rains is evidenced in 2015, than 2010, but in other years, these numbers were uniform. The most frequent occurrence was in Kolašin, than in Golubovci, Podgorica and Ulcinj. Golubovci and Cetinje had the acid rains in each year along the period. Žabljak, Podgorica and Ulcinj had not

acid rains only in one year within the period. The linear trend of acid rains distribution is falling (Figure 1.).

The rains of extreme acidity were evidented in the same years, where the acid rains were most frequent occurrence, 2010 and 2015 (Table 6.). Acid rains with minimal pH bellow 5, are occured in all other years, except in 2012. The distribution of minimal pH during the period, is presented in Figure 3.

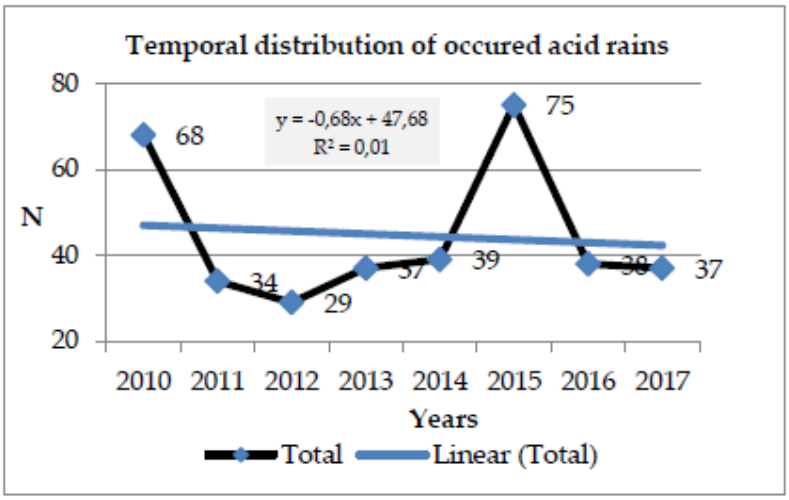

Figure 1.: Temporal distribution of occured acid rains (by pH) in IHMS network stations

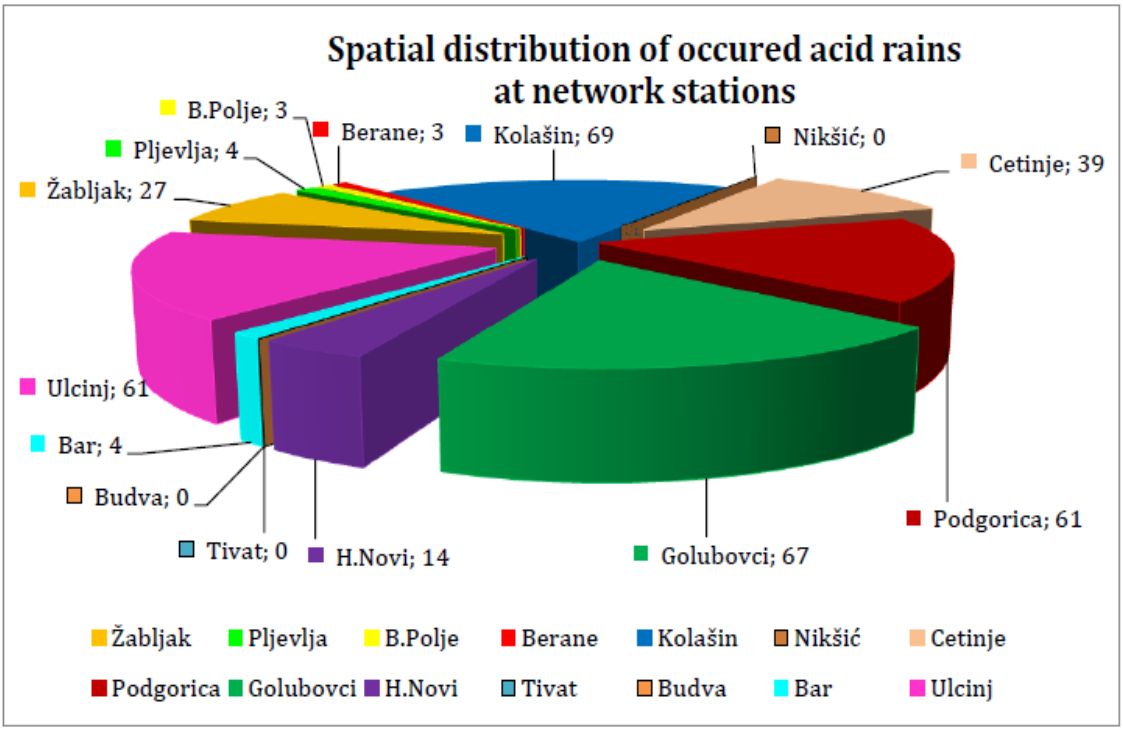

Figure 2.: Spatial distribution of acid rains occurred, by number of occurrence, during the period, in IHMS network stations

Spatial distribution of occurred acid rains is presented in Figure 2. Each station is presented with number of occurred acid rains with pH less than 5,6.

Correlation coefficient is calculated for pairs of data series pH-SO42-, pH-NO3- and SO42- -NO3-, for all stations (Table 7.). Correlation is moderate for pH-SO42- for stations Žabljak, Tivat, than Berane, Cetinje and Pljevlja, and poor for stations Bar, Golubovci, Kolašin and H. Novi. There is not the correlation of the pairs at all other stations. For B. Polje the correlation is poor and negative. Poor correlation for Podgorica maybe is consequence of use of the total data for wet and dray deposition, unlike the other stations where the data series consist of wet deposition only. Correlation for pair pH-NO3- was poor, except in Cetinje (R=0,25). Correlation was negative in Golubovci, Bar and Ulcinj, where the values were moderate (Ulcinj) to poor. This significant negative correlation refers to influence of present ammonia, and forming of base NH4NO3. Correlation of SO42--NO3- was much more higher than for other pairs. The coefficient values ranging from moderate to strong.

Table 6.: Magnitude of extreme acidity by minimal pH

Stations	Years	2010	2011	2012	2013	2014	2015	2016	2017
Žabljak	min pH	4,55		5,20	5,57	4,39	5,45	5,36	4,63
Pljevlja	min pH				5,24		5,43		
B.Polje	min pH								5,13
Berane	min pH	5,45				5,56		4,85	
Kolašin	min pH	4,87	5,34	5,08	4,86		4,50	5.44	4,56
Nikšić	min pH								
Cetinje	min pH	4,57	4,91	5,58	5,11	5,13	4,83	5,47	4,96
Podgorica	min pH	4,31	4,72	5,09	5,09		5,01	5,48	5,52
Golubovci	min pH	3,63	5,38	5,06	5,20	5,59	5,00	5,35	5,32
H.Novi	min pH	4,11			4,99	5,36		5,39	5,59
Tivat	min pH								
Budva	min pH								
Bar	min pH					5,44	5,43	5,25	
Ulcinj	min pH	4,95	4,46	5,08	5,37	4,50	4,35	4,03	
Total	min pH	3,63	4.46	5.06	4.86	4.39	4.35	4.85	4.56

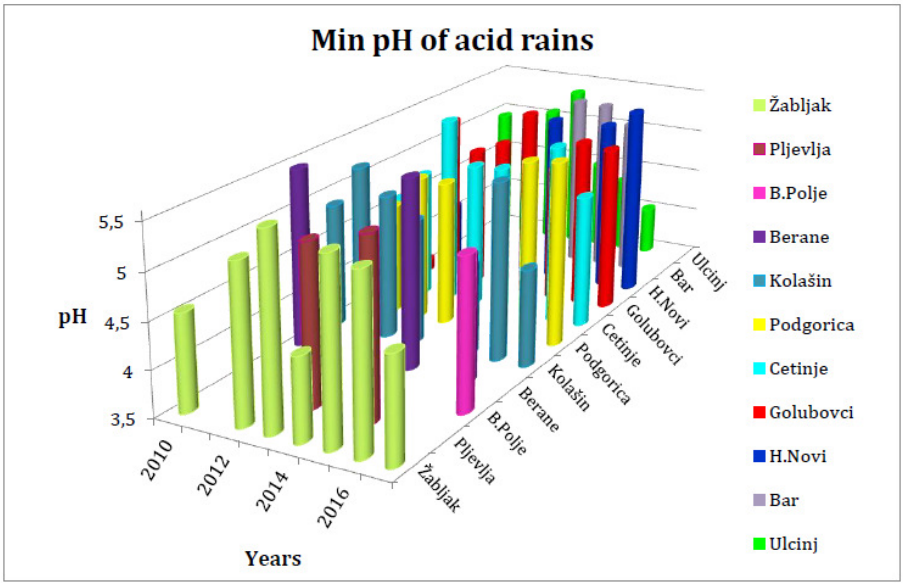

Figure 3.: Minimal annual pH in acid rains during the period, in IHMS network stations

Data series for H.Novi and Žabljak selected for correlation analysis, which precipitations belongs to the same cloudy masses, due to investigation of eventual changes of their chemical composition during air masses movement above the land, from the seaside to the mountin. The other pair Nikšić-Pljevlja is selected for analysis, because both ot these sites are an industrial areas, but with different pluviografic regime, in Nikšić maritime and in Pljevlja continental.

Table 7.: Coefficient of correlation (R) among stations

R	pH-SO4	pH-NO3	SO4-NO3
Žabljak (N*=1020)	0,34	0,04	0,28
Pljevlja (N=640)	0,22	0,02	0,22
B. Polje (N=486)	-0,01	0,15	0,34
Berane (N=425)	0,27	0,01	0,08
Kolašin (N=998)	0,14	0,12	0,26
Nikšić (N=797)	0,12	0,01	0,42
Cetinje (N=820)	0,25	0,25	0,21
Podgorica (N=2128)	0,02	0,03	0,31
Golubovci (N=459)	0,20	-0,12	0,30
H. Novi (N=620)	0,16	0,09	0,36
Tivat (N=67)	0,37	0,09	0,25
Budva (N=427)	0,03	0,03	0,55
Bar (N=394)	0,20	-0,16	0,30
Ulcinj (N=602)	0,05	-0,23	0,46

N - number of pairs of data*

Table 8.: Correlation coefficient of pairs H. Novi- Žabljak

Pairs	pH-pH	SO_4^{2-} - SO_4^{2-}	NO_3^- - NO_3^-
R	0,31	0,04	0,23
N	476	400	473

Table 9.: Correlation coefficient of pairs Nikšić - Pljevlja

Pairs	pH-pH	SO_4^{2-} - SO_4^{2-}	NO_3^- - NO_3^-
R	0,11	0,17	0,30
N	377	377	371

The correlation between pairs pH and NO3- in H. Novi and Žabljak was relatively significant, what show that pH, in overall sense, and NO3- are changed a little on the way above the land. There is no correlation of SO42-. This ion is changed because many atmospheric chemistry processes during the movement above land. The correlation for the pairs in Nikšić and Pljevlja exists but it was small. The stronger correlation was only for NO3-. This level of correlation hardly can be caused by the similar cloudy masses above related areas, considering different pluviographic regime. It's more likely that this is about similar influence of local air pollution to the precipitation composition of this ion.

Conclusions

Data series of pH, content of SO42- and NO3- in precipitations for 2010-2017, as an indicator of their acidity, are processed. These data series are treated by basic statistical analysis, measures of central tendency and correlation of selected parameters and sites. Results are presented by tables and graphics.

Annual averages of pH were predominantly between 6 and 7, except in Pljevlja, B. Polje and Tivat, where were above 7. The strong acidity was evidenced in Golubovci (pH=3,63) and Ulcinj (pH=4,03), but, the major acid rains had a small to moderate acidity (pH=5-5,6).

The highest SO42- content was in the northern area (nss-SO42-). The highest NO3- content was in Golubovci (influence of agriculture), Pljevlja (termo power plant influence) and coastal region (direct influence of sea water – ssSO42-).

The most frequent occurrence of acid rains was in Kolašin, than in extended Podgorica area and Ulcinj, with the biggest number in 2015 and 2010. Acid rains were occurred in each year in Golubovci and Cetinje, but the absence of them only in one year was in Žabljak, Podgorica and Ulcinj. Acid rains are not occurred in Nikšić, Tivat and Budva, but in B. Polje only once.

Homogenous and central distribution is determined for pH data series, with sporadic Max extremes in Žabljak, Nikšić, Podgorica and Bar. The rains of strong acidity are a rare and random occurrences. Percentil C5 belongs to ``acid rains`` for stations Kolašin, Cetinje, Golubovci and Ulcinj. Standard deviation of pH data series was the lowest, in Pljevlja, B. Polje, Berane, Nikšić, Tivat and Budva, but the highest in Golubovci, H.Novi, Bar and Ulcinj. Scattering of data for SO42- and NO3- is much more higher than for pH, more for SO42- than NO3-, the highest in northern region, the lowest in coastal region.

Correlation coefficient for pH-SO42- goes from moderate (Žabljak, Tivat, than Berane, Cetinje and Pljevlja) to poor (Bar, Golubovci, Kolašin and H. Novi). Correlation for pH-NO3- was poor, except in Cetinje (R=0,25). Correlation, poor to moderate, was negative in Golubovci, Bar and Ulcinj. Correlation for SO42--NO3- was moderate to stronger.

Correlation of selected data series for H.Novi (coastal) and Žabljak (mountains on the north), stronger for pH pairs, poorer for NO3-, identificates the same air masses, which relatively change own chemical composition, during their movement above the land. The similar analysis, performed for industrial areas Nikšić and Pljevlja, sites with different pluviografic regime, shown the significantive correlation only for NO3-, probably as a consequence of the local pollution influence.

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Original Scientific paper

Assessment of accuracy of agricultural land for the Southern Ukrainian organic land

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Abstract

Organic farming in Ukraine is dynamically developing. Over the past ten years, the area under the production of organic products has increased almost threefold. The subjects of the market of organic products in the Kherson region are 20 agricultural enterprises that process more than 11,3 thousand hectares of land.

In the structure of exports of organic products, 68% are grains, 18% technical, 9% fruit and vegetable and 5% others. The main countries of consumers of organic products are Germany, Austria, Poland, Italy, France, Holland, Denmark, Switzerland, Canada, USA. Despite the rather high agro-resource potential of the Kherson region, areas under organic farming occupy only 0.64% of the total area of arable land.

Most of the agricultural lands of the Kherson region are southern chernozems (41,8%) and dark chestnut soils (21,0%) with heavy texture, 70,8% of the soils have medium and high humus content, an average content of mobile phosphorus and a high content of exchangeable potassium. Less e are provided with easily hydrolyzable nitrogen.

The content of heavy metals in the soils of the Kherson region does not exceed the maximum permissible concentrations. Residual amounts of organochlorine compounds, 2,4-D and other pesticides have not been identified. The content of radionuclide's (cesium and strontium) in the soils of the region is stably low and does not exceed the maximum permissible concentration.

Taking into account that the Kherson region has the status of an ecologically clean zone, in conditions of increasing demand for organic products - it has prospects for developing export potential while simultaneously addressing economic and social problems. To do this, it is necessary: to formulate the appropriate regulatory and legal framework, to introduce certification and labeling of organic products, to provide state support to farms during the transition period, to develop innovative technologies for growing organic crop production, to intensify information and advisory work

Keywords: organic production, agricultural land, soil, suitability.

Introduction

The agrarian sector of the economy with the potential of production, which far exceeds the needs of the domestic market, may contribute to the development of the economy of the Kherson region. Organic production and territory of the Kherson region, as well as throughout Ukraine, are rapidly and dynamically developing, due to a number of obvious environmental, economic and social benefits inherent in this area of activity.

The aim of the study:

- to determine the current state of development of organic production in the Kherson region;

- provide an assessment of the suitability of the agricultural land of the Kherson region (Ukraine) for the cultivation of organic products.

Materials and Methods

Methods: analysis and synthesis, system analysis, soil research. Used materials of the Department of Agricultural Development of the Kherson Regional State Administration, Institute of Soil Conservation of Ukraine, materials of their own research.

Results and Discussion

As of 2017, 38 organic producers are certified on the territory of the Kherson region and are one of the leaders in organic production in Ukraine (Table 1, Fig. 1, 2).

Table 1. Total area of organic and transitional agricultural land in Ukraine

Administrative region Адміністративна область	Total number of operators (organic and transitional)	Total area of agricultural land (organic and transitional), ha	Including the area of agricultural land with organic status, ha
Odessa	38	102238	88888
Kherson	**38**	**75866**	**46148**
Dnipropetrovsk	23	42290	37750
Zhytomyr	27	31576	22708
Chernihiv	16	24777	23534
Other areas	…	…	….
Total for Ukraine	**426**	**381173**	**289551**

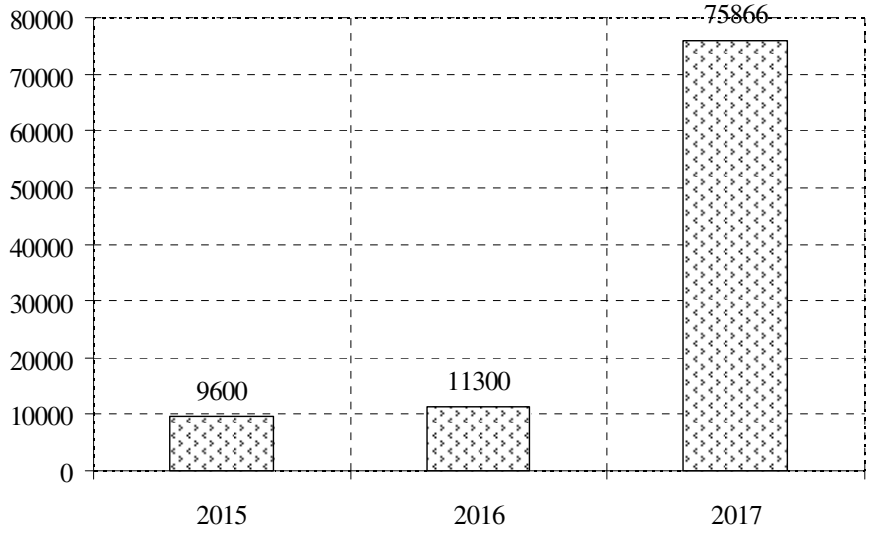

Figure 1. Squares of organic land in Kherson region, Ukraine (ha)

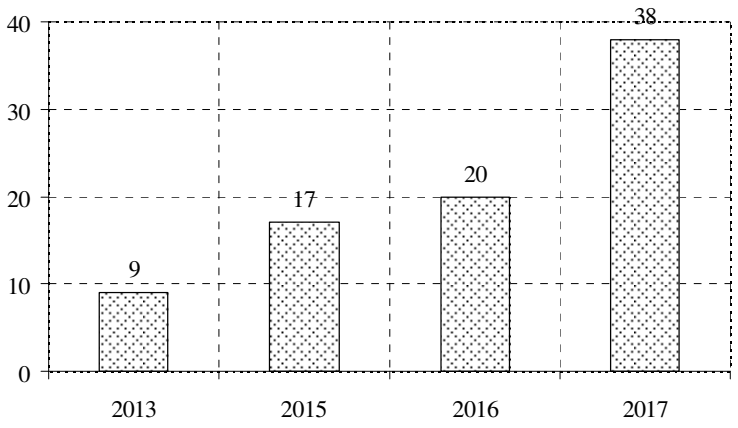

Figure 2. Squares of organic land in Kherson region, Ukraine (ha)

The following organic products are exported to the agricultural producers of the Kherson region: rape, winter wheat and yarrow, winter wheat and spring wheat, corn for grain, soybean, buckwheat, corn, sunflower, lentil, lupine, beans, perennial grasses, sunflower oil and sunflower oil. There is a tendency to increase the area and volumes of organic products (Table 2).

Table 2. Volumes of organic products exported from the Kherson region, Ukraine

Agricultural products	Exports, ths. Tons
Cereals (wheat, barley)	17,7
Olive (rape, soybean, sunflower)	4,68
Fruit and vegetable processing	2,32
Corn, peas, lentils, beans, perennial grasses, sunflower oil, sunflower	1,30
Total	**26,0**

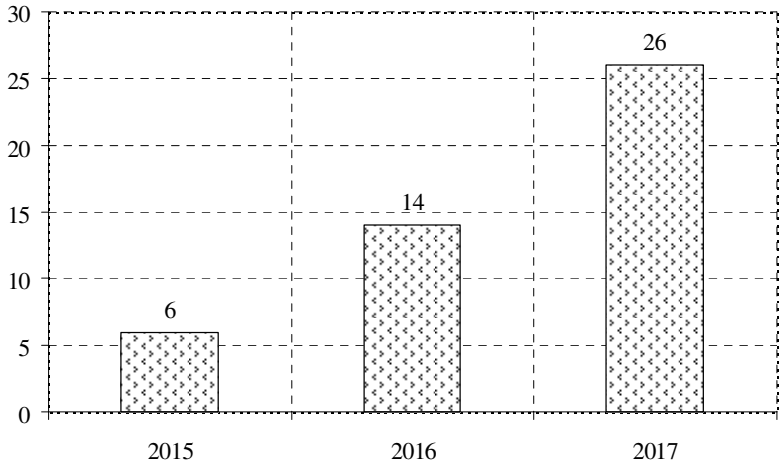

Figure 3. Export volumes of organic products from the Kherson region, Ukraine (thousand tons)

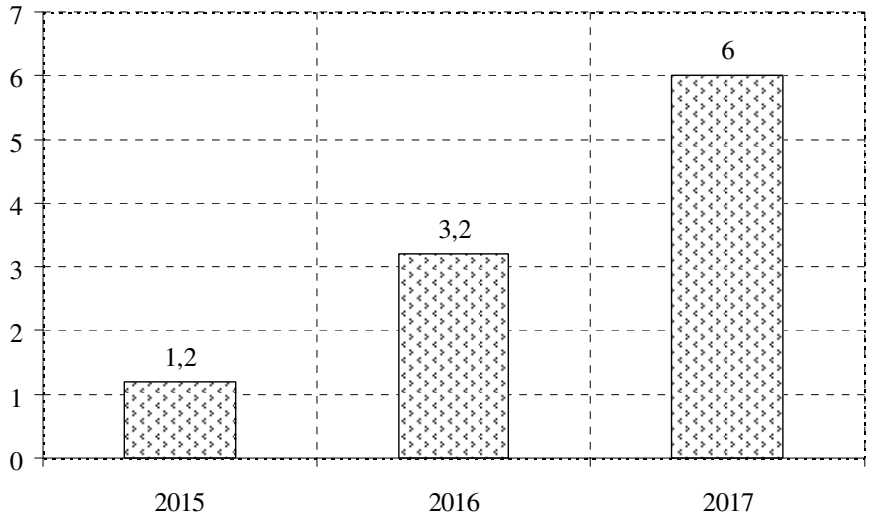

Figure 4. Exports from the Kherson region, Ukraine (million €)

The Cabinet of Ministers of Ukraine adopted the Strategy for the development of the agrarian sector, one of the key areas of which is the development of organic production. The Verkhovna Rada of Ukraine adopted the Law of Ukraine "On Basic Principles and Requirements for Organic Production, Circulation and Marking of Organic Products" on July 10, 2018, the Cabinet of Ministers of Ukraine adopted the "Detailed Rules for the Production of Organic Products (Raw Materials) of Plant Origin", which provides for legal regulation, labeling of products and adaptation of requirements to normative acts of the European Union (EU) [1, 2, 3].

In legislative provision, particular attention is paid to the suitability of land for the production of organic products and raw materials. The assessment of the suitability of land (soils) for the production of organic products and raw materials is carried out by the central executive authority, which implements the state policy for the implementation of state supervision (control) in the field of environmental protection, in the field of land protection. The procedure for assessing the suitability of land (soils) for the production of organic products shall be approved by the Cabinet of Ministers of Ukraine.

The soil cover of the Kherson region is heterogeneous. The complexity of the soil cover of the area is primarily due to zonal factors of soil formation due to the heterogeneity of hydrothermal conditions, the second - the development of gluten processes in the soils of the peat due to their sporadic water logging with thalli and rainwater, in the third - the intensity of the appearance of solonettinosity and salinity, depending on the depth of occurrence of groundwater .

Territorially, the Kherson region is located within two climatic zones: the Steppe southern moderately dry zone and Sukostepova zone (Table 3).

Table 3. Basic types of soils in the Kherson region

№	The name of the soil	Total area, in thousand hectares	Including arable land	
			thousand ha	% of the total surveyed area of arable land
1	Chernozems are ordinary	49,3	44,1	2,84
2	Black earths southern	828,3	764,6	49,20
3	Dark chestnut	582,9	525	33,78
4	Chestnut Salt	111,6	84,4	5,43
5	Almond-chestnut solonettes	60,6	9,1	0,59
6	Salt chunks	48,5	12,7	0,82
7	Alone-chernozem surface-gleyed	77,6	63,4	4,08
8	Beam-chestnut surface-gleyed	66,9	45,8	2,95
9	Sandy sand	58,0	3,2	0,20
10	Deep surface gley	6,8	0,4	0,03
11	Sodium surface-gley solodils	6,6	1,3	0,08
Total:		1897,0	1554,0	100

Table 4. Assessment of the suitability of the main types of soils in the Kherson region (Ukraine) regarding the suitability of organic farming

Soil type	Humus content,%	The content of trace elements, mg / kg			The content of salts of heavy metals, mg/kg							Residual amount of pesticides		
		$N\text{-}NO_3$	P_2O_5	K_2O	Cu	Limit-permissible concentration	Zh	Limit-permissible concentration	Pb	Limit-permissible concentration	Cd	Limit-permissible concentration	DDT and its metabolites	sum of isomers	2,4-D
Chernozem south right bank	2.80	21.7	44.8	403	0.37		0.77		1.41		0.12		not found	not found	not found
Chernozem south southwest	2.69	22.4	41.9	382	0.35	3.0	0.69	23	1.11	6.0	0.10	0.7	not found	not found	not found
Dark chestnut-hard-loamy	2.45	22.4	45.2	507	0.36		0.98		1.68		0.18		not found	not found	not found
Dark chestnut light-dense	1.27	10.5	48.4	248	0.41		0.79		2.05		0.18		not found	not found	not found

Conclusions

The researches have determined that the bulk of the Kherson region's lands is suitable for the organization and management of organic agriculture and has the status of an ecologically clean area.

For the development of organic production in the Kherson oblast, it is necessary to develop and

approve the Regional Program for the Development of Organic Production, which will provide the following tasks:

- stimulate the development of organic production by providing financial support to organic producers, as well as a number of incentives and preferences;
- to form and consolidate the trust of consumers in organic production and certified and properly marked organic products;
- to develop the market of organic products and increase, together with the growing market, the efficiency of production and processing of organic products;
- by promoting properly trained organic products, protect consumers' interests.

Implementation of the Program will be a significant contribution to the implementation of the state policy in the field of organic production and will contribute to:

- to increase the number of enterprises engaged in the production of organic products and production of high quality and safe, and therefore - competitive food;
- increase in the volume of consumption and export of certified organic food;
- protection and reproduction of soil fertility;
- improvement of the welfare of the rural population by obtaining a stable profit from producers of organic agricultural products and strengthening their financial and economic condition;
- to increase the level of investments in the agrarian sector of the country's economy;
- will contribute to the creation of eco-centers and will allow the development of ecotourism, education and educational activities, due to the availability of organic produce that attracts domestic and foreign agrarians and scientists, provides environmentally safe and high-quality food products.

Conflicts of Interest: The authors declare no conflict of interest.

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Review

Ecological Culture and Media

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Abstract

Mass media may have an influence and can enrich ecological culture and build up an ecological awareness. Some theoreticians (Giddens) believe that ecological culture could be determinants as a more or less of solid values and opinions about the environment, the state of resources, the dangers to which the environment is exposed and the concepts of its protection. When it suitable to concern the development of social and cultural factors are neglected, culture plays an important role in defining attitudes, values, behaviour and developing a framework for assessing the impact of culture on the environment. The role of the media is in the creation of an ecological culture consisting in the constant actualization of environmental threats. It is necessary constant redefining the concepts adapted for environmental protection, to regularly report on new scientific, theoretical and practical knowledge in the field of ecology. The paper also focuses on ecological culture through the prism of school programs as well as the educational function of the media that has shortcomings contained in this much text information, lack of necessary selection, the imperative of being first in the broadcast besides, the quality and content of the broadcasted information.

Key words: *ecology, media, ecology protection, environment*

Introduction

The role of specialized programs on television and radio dealing with environmental problems and the contribution that give print media publishing analytical shaped articles on the topic of environmental protection, environmental accidents, the concepts of environmental protection, and generally the global state of the environment and the threat to Earth is of great importance. Realization of informative and educational functions in the field of ecological culture derives from the very nature of the medium.

We should not drop of the sight that it as the most important factors in media-mediated information. In addition, the media should be guided by the program concept and editorial policy, but also that their own interests however the media putting certain professional and ethical obligations to deal with the contents in the field of ecology. "Ecology, as a term, first used in 1866 by the German biologist Ernst Haeckel (Ernest Haeckel) in his book" Natural History of Creation. "By then, ecology was considered as a sub-discipline of zoology that deals with research of the relationship between animals and their organic and inorganic environment. in 1869 the environmental Haeckel defined as the science of the household (economy) animal organizations.

According to his view of ecology for its object to analysis of mutual relations and function of living organisms and their natural environment, but also the relationship between the natural, organic and inorganic, and the existence of factors organisms, which classifies Haeckel as a man, as a full member of nature, and wildlife "(Marković, 2005: 10-11). "Ecology as an independent science is constantly in developing. In particular, we mention the social ecology and definition given by Professor Danilo Ž. Markovic, according to which the "social ecology is the specific sociological science which for its subject matter has a specific bonds between man and his environment,

examining the influence of the environment, and, the totality of the natural and social factors in human, as well as the rate of human a path" (Đukanović, 1996: 21). However, just at the links that exist between man and his environment is given insufficient attention.

Ecological knowledge to which they achieved a number of scientific workers, even those which are the subject of study and subjects matter that are part of the social ecology and unfortunately not sufficiently presented to the public. This contributes to almost all countries in the Balkans notes the lack of social action when it comes to environmental issues. "To knowledge that provides social ecology became the basis for initiating social action in the field of environmental protection, it is built on the existence of social consciousness.

It is expressed as a set of knowledge about the threat to the balance of nature, the impact of this distortion of the balance of the position and existence of man "(Marković, 2001: 71). Undoubtedly the impact of mass media is multifaceted, especially the preservation and improvement of the environment. The media should take advantage of the development of ecology as a separate scientific discipline, and to contribute to the preservation and improvement of the environment. In particular, as every human action has its beginning in theory that, knows the necessary facts, value determination towards them and making decisions about the need to change the situation, improve or preserve. "In doing so, it is important to have always in mind the theory of Gramsci's ideological hegemony, that is, the target value of the owners of monopoly (capital, ideology, force...) in the modern world are not imposed by brute force, but landing recipient or public, to adopt values that are spread via the communication channel or medium of mass communication. According to Gramsci's concept of "hegemony landing", about which he wrote in 1979, each relevant social group, class,, caste, at the same time has its own particular view of the world. "(Jakovljevic, 2015: 32).

Ecology and environmental media culture

Media are "swamped" by information. It's hard to admit, but we can say that the readers, listeners and viewers "poisoned" all sorts of content on social networks. Medians and other researchers are involved in most important phenomenon today and as that are mentioned, social networks still have no solutions on how to overcome the negative impacts of all that is happening in the field of (dis) respect the basic laws of media literacy "A long time ago already announced ecology media shows how is now crucial for the establishment of the human world. Pollution of virtual media space, so that no decontamination of media space has to establish "pure world." Commercial media must develop the most important programs founded on free research, truth, and free of any pressure "(Alić, 2016: 185).

Certainly the ecological culture of a society is not beyond the influence of "waves" of new informative era. Despite the accepted definitions which specifies the very ecological culture by Giddens would Ecological Culture could mark as a more or less strong value system of thinking about the environment, the state of its resources, the dangers of which the environment is exposed up to the concepts of its protection (Giddens, 2007: 24). When it comes to sustainable social development, mainly to highlight the economic, social and environmental, on the other hand, the cultural factors are ignored, despite the fact that it is culture that contributes to improving the quality of life and fostering partnerships in society is a key of cultural sustainability.

The culture of the 20th century, taken in the broadest sense as a set of spiritual and material creations, characterized by the triumph of the human spirit and a whole range of technological change, which is due to the speed and intensity that have been made can be called revolutionary. However, Human civilization is faced with the consequences of technological progress. He was not accompanied by adequate development of consciousness, nor was the value models created at a rate to critically examine the totality of the current changes. Built ecological culture, it was proved to be unable to compensate for the lack of adequate critical attitude towards the holders of technical and technological changes and their effects. Home to point to problems in the ecological range of the

human community, mostly arriving from mostly part of society. As the environmental situation moved to a state that could be described as a collection of constant danger to the environment, with a tendency to increase these threats and in number and in power, began the creation of value concept that can be defined as an ecological culture. In creating the concept of ecological culture, The important is in of the media, educational, and other institutions and organizations dealing with intellectual and spiritual side of human existence. Also, the arts, in all its aspects, in content contributed to forming of ecological culture.

The educational function of the media

One of the primary functions of the media is their educational and attainment function. Administration "in understanding the role of media in creation, the design and construction of environmental culture, we must point out the educational function of the mass media" (Radojkovic and Miletic, 2005: 103-104). The media, in fact, except its inform and entertain, must by recipients of messages transfers a valuable content higher levels of complexity. These are facilities that shape opinion about an issue, and the phenomenon occurs. Here we must mention the school, as an educational institution that has lost its monopoly on the educational role for young people. Perhaps the cause of this legal regime of the school system, as the institutional organization, which has made school insufficiently flexible to offer young people the knowledge that will be complete, and timely.

The media, on the contrary, thanks to the educational function, in that are much faster, and the area viewership, audience and transcends national boundaries, let only alone a limited number of students attending a school. Deficiencies in the educational function, when the role of the media in the creation of ecological culture in question are certainly contained in the overabundance of information, lack of the necessary selection, the imperative to be the first in a broadcast, rather than broadcast quality contenting information. Shows on television and radio dealing with environmental problems and the contribution that give print media publishing analytical shaped articles on the topic of environmental protection, environmental accidents, environmental protection concepts, and general state of the global environment and the threat to the Earth of immeasurable importance.

In practice, the mass media as part of the information and communication system, is mediate between those who shape and emits the message and the recipient of the message. Realization of informative and educational functions, when shaping the ecological culture, or to address environmental issues, arising from the very nature of the medium. In addition, the media are guided by their own interests and to deal contest of ecology. These interests included the concepts of programming and editorial policy of the media before putting certain professional and ethical obligations. Environmental issues are, because they concern health, living conditions and even the survival, at the top of the content that are the public expects and users accepted. The downside of this commitment is the emergence of media sensationalism.

The holders of public information touching on ethical issues: Media maximum "exploit" specific environmental incidents, not only because they want to inform the public about all the facts related to a specific event, but also because of the sensationalist tendency to point to the risks and possible new damage, targeting the fear that is inherent in every person, especially if he announces apocalyptic incident, increasing their viewers, listeners or readers. Yet, There is no doubt that environmental culture did not exist, or to the system adopted values existed, with the far smaller number of devotees, nature lovers and those who have above-average knowledge of the natural environment, that there was a huge dissemination of messages about environmental issues. Those were able to do only the mass media. The media are the ones who should show the dangers caused by the irresponsible behaviour of big companies that destroy the environment. and then, to report on endangered animal and plant species, the state of the sea, glaciers warming, ozone holes, pollution of cities, the use of unsafe food or feed for which genetic modification causes problems in humans... If we pay due attention to such issues, mass media to have justify the function that nature should be

performed by by auch a way remain part of a special segment of culture, i.e ecological culture that shapes attitudes on values, knowledge, ideas and concepts about the environment.

Conclusion

The dynamic role of the media is in creating ecological culture, as well as the attitude of the media towards the environment. All is reflected in constant actualization of environmental threats, redefining the concepts for the protection of this environment and almost daily reminder of the dangers of by environmental pollution, and regular reporting on new research, theoretical and practical knowledge in the field of ecology. This media as one of the pillars of ecological culture contributions, adoption of effective environmental policies, in order to preserve the environment, natural resources, protection of rivers, air, sea or shoreline. If a single ecological culture conditioned to certain information, the degree of the adopted environmental attitudes on important environmental issues, timely and meaningful information and continuous review of procedures and concepts of environmental protection, then the environmental policy is determined not only in national framework, but also the state of ecological culture in specific areas. Where the environmental culture is developed, and where a broader insight into the state of the environment and environmental hazards, there environmental policy will be determined at the time, and will deal with prevention, not just repairing the consequences of irresponsible acts that threaten the environment and cause ecological incidents. The media in all this must play an important role, timely and accurate information to the public.

Conflicts of Interest: The authors declare no conflict of interest.

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Review paper

Monitoring and Analysis of the Geodiversity and Biodiversity Status of the Skadar Lake

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Abstract

Both minor and wider area of the Skadar Lake is known for its natural value. The rich biodiversity of the greatest wetlands on the Balkans, is not only important for Montenegro and the neighbouring Albania, as a Lake is on their territories, but has a much greater significance for the region, entire Europe, and even wider. Precisely for this reasons, the Lake was the subject of great number of research and other activities when it comes to researching and monitoring geodiversity, and especially biodiversity of this area. The fact that this Lake is under protection of Ramsar Convention since 1995 speaks to significance of Skadar Lake, and Montenegro declared this are a National park earlier in 1983, while the Albanian Government gave this area the status of Nature Reserve in 2005, as a wetland of international significance.

Key words: *Skadar Lake monitoring, status of geodiversity and biodiversity, preservation, Skadar Lake basin*

Introduction

Skadar Lake with an area varying between 370 and 540 km² represents the largest freshwater surface in Southeast Europe. The largest measured depth is 60m, while the average depth varies between 4m and 7m. The lake is actually the widest area of scandalous depression, it is surrounded by Zeta plain, slopes of the Lovcen branch (1759m), the Sutorman mountains (1181m), Rumija (1593m), Tarabos (1188m), Sozina (922m), the Skadar plain and the branches of Prokletije.

Skadar Lake is cryptodepression, its bottom is below the sea level. The direction of delivery is as well as the direction of providing Dinaride, the northwest - southeast. The lake is famous for its numerous limestone islands. The most famous islands are: Grmozur, Starcevo, Beška, Moračnik, Gradac, Tophala, Gorica, Gljat and others. In the northeast, the lake is deep into the mainland, where the bay is Licheni and Hoti, and from the capes the Petrova ponta stands out.

The lake is kept mostly by the Morača River, which participates in the lake water balance with 62% (B. Radojičić, Geography of Montenegro - Natural Basis, Podgorica 2008). Water is lost by evaporation and the Bojana River that flows into the Adriatic Sea. Since the terrain between the lake and the Adriatic Sea is mainly limestone, a large part of the lake's water through these terrains goes to the Adriatic Sea.

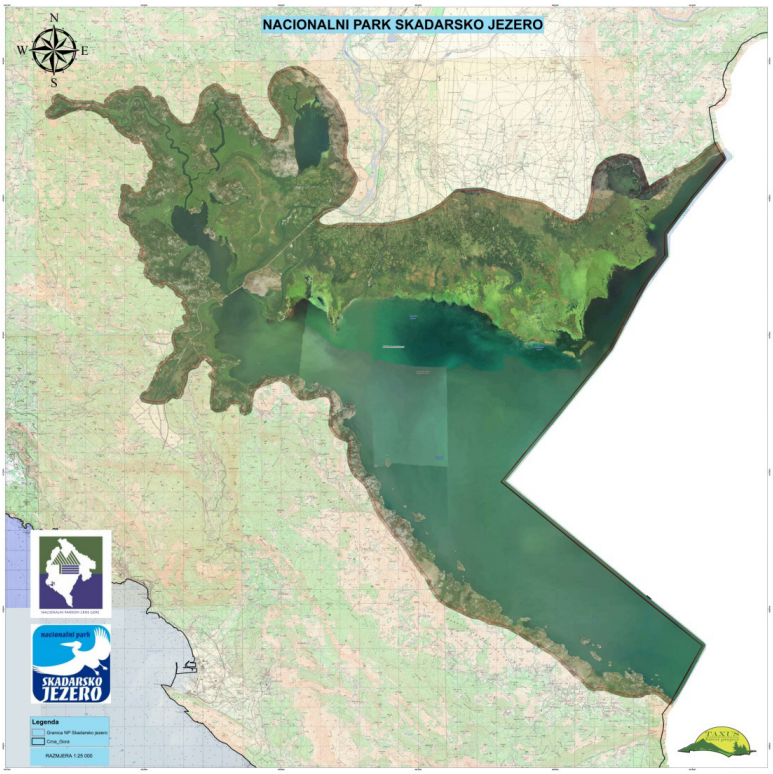

Figure 1. Map of Skadar Lake 1:25000
(Source NP of Montenegro)

We do not have accurate data about the formation of the lake itself. From the time of the Roman Empire, the lake is not mentioned at all, and it is assumed that it did not exist or there was none at all. According to documents from the 15th century, the surface of the lake is much smaller than today.

Biodiversity of the Skadar Lake

The flora of the Skadar Lake

The flora of the Skadar Lake is represented by many species, whose distribution is quite limited. Limestone and grab shrubs dominate on limestone terrains, while in the surrounding mountainous regions of Prokletije, Sutorman and Rumija, we have areas with locust forests. The forests are quite rare. Because of the relatively high altitude difference we have different climatic factors, which also affect the types of forests that occur in these areas. So at the higher altitudes of Rumija and Prokletije we have coniferous forests, and under these zones there are deciduous forests. The largest areas of the lower parts of the mountainous sides up to 1,300 m mainly cover oak forests. Forests located in the immediate vicinity of the settlement, anthropogenic impacts are devastated, those parts of the forest which are somewhat protected and preserved can be found. On cushy and sunny surfaces, which are protected from winds, plants and herbs, as well as grapevine, tobacco and olives, are successful. On the next map we see the display of vegetation, not the area of Skadar Lake.

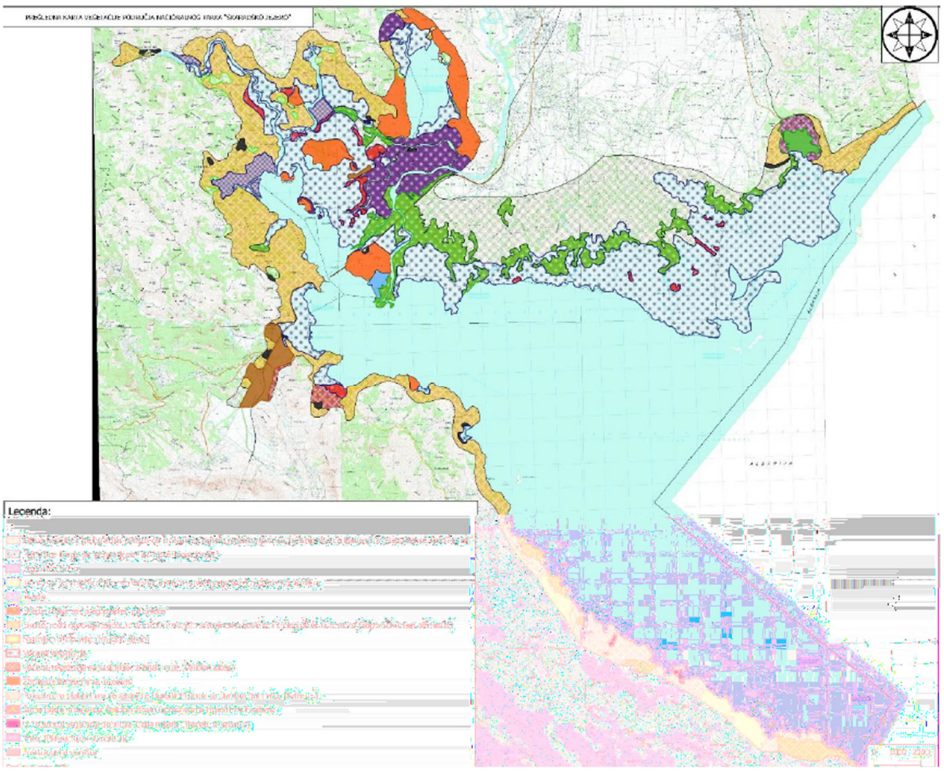

Figure 2. Map of the vegetation of the Skadar Lake
(source: Ministry of Sustainable Development and Tourism, PP Skadarsko j.)

Animal World of Skadar Lake

More recently, more and more attention is paid to the importance and protection of the environment and ecosystems. The area of Skadar Lake is known for its diversity of vegetal and animal life. In the lake we do not have a large number of endemics, however, in the ecosystem of the surrounding mountains of Prokletije, Rumija and Lovćen, about 50% of the total number of endemic Balkan is found.

There are about 45 species of fish in the lake, of which the most numerous are carp and bark. Eagles, as rare species, occasionally stay in the lake. Hunting fish in the lake is allowed and is based on the principle that it may be hunted as much as reproductive can be compensated. However, the curl has, however, and the national parks are those who deal with issuing permits and sanctioning criminals. The lake is also known as the general migration centre of migratory birds.

According to the National Parks of Montenegro, around 279 species of ornithofauna are temporarily or permanently resident on Skadar Lake. From autumn and winter to the lake are sailing various species of sailors. Especially in winter, along the lake you can see flocks of ducks, besides them we also have other representatives of birds, such as buns, ducks, heads, and geese, seagulls, herons, roe…

The game is less widespread, and the lake shore lives otters and rabbits, feeding fish, and in the higher regions live a rabbit, a fox and a wolf. In addition to water in the summer period, snakes live.

Monitoring the area of the Skadar Lake

When it comes to monitoring the area of Skadar Lake, Montenegro and Albania do not have a joint monitoring program. In Montenegro, water quality monitoring and monitoring of biodiversity is being carried out.

Monitoring of water quality

Regular activities of water quality monitoring in the Skadar Lake began in 1990. Previous monitoring was carried out at the locations of Vranjina, Virpazar and Plavnica. In the period 1990-1991, monitoring was mainly performed at locations near the Aluminum Plant in Podgorica, where the soil and groundwater were examined, then at the mouth of the Morača river, as well as the monitoring of fish and vegetation in the Skadar Lake. During the two following phases 1992-1993 and 1993-1996, in the Zeta Plain area, water, river, land, air quality were analysed, and emphasis was placed on areas under the direct influence of the Aluminum Plant. Different institutions dealt with sampling and analysis of water quality in the lake.

The National Monitoring Program in Montenegro is regulated by the Regulation and classification and categorization of surface and groundwater (official Journal of Montenegro, No. 02/07) (Annex two reports). These results cannot be compared with the results of other countries because the Regulation is not fully in line with the Water Framework Directive.

The institution that monitors the quantitative and qualitative characteristics of water is the Hydro meteorological Institute of Montenegro (HMZ), defined in Article 58 of the Water Act (official Journal of Montenegro, No. 27/07). The Ministry of Agriculture and Rural Development (Water Directorate) makes a decision on the monitoring of Skadar Lake, according to the annual Program for systematic testing of the quality and quantity of surface and groundwater. This program envisages sampling locations as well as sampling and sampling frequencies. The program lacks an integral approach to the catchment area, and for future activities on the Lake does not provide sufficient data. Sediment analysis, although not included in the monitoring program, was carried out by various institutions (Centre for Eco toxicological Testing, University of Shkoder, and University of Tirana).

According to the program of systemic analysis of surface and groundwater quality in Montenegro, there are 9 sampling sites on the lake: Kamenik, Vranjina, Virpazar, Plavnica, Podhum, middle of the lake, Starčeva gorica, Moračnik and Ckla, according to which 55 qualitative parameters should be analysed in leads.

On the lake there are two automatic hydrological stations (Plavnica and Ckla), on which the water level, flow and water temperature are measured. Data from these stations are available online, on the **Hydro meteorological** Institute website. There are hydrological stations on the Morača river, Rijeka Crnojevic and Orahovstica in the Jezera basin.

Monitoring of biodiversity

The Institute for Nature Protection of Montenegro, within the National Environmental Monitoring Program, has been conducting a biodiversity monitoring in Montenegro since 2000. Given that the monitoring program had limited resources, the data collected do not provide the possibility for complex trend analysis in the conditions of indicator species, species populations or changes in specific habitats and the overall environment.

In 2009, the Environmental Protection Agency (EPA) was established, as the main institution that regulates environmental monitoring, under the authority of the Ministry of Sustainable Development and Tourism. The EPA is in charge of preparing the Biodiversity Monitoring Program, which includes the Skadar Lake Biomonitoring Program, and then the tender for the selection of a qualified legal entity for the implementation of monitoring.

The biodiversity monitoring is regulated by the Law on Environment (official Journal of Montenegro, No. 48/08) and the Nature Protection Act (official Journal of Montenegro, No. 51/08). The program includes the monitoring of birds and some special plant and animal species, but some other indicators are missing that should be monitored. In 2010, EPA Montenegro adopted a new proposal for the Scandinavian Biodiversity Program for 2011, but due to the lack of financial resources, implementation of these activities was not possible.

Legislation and legal framework for the implementation of monitoring programs in Montenegro

Water monitoring in Montenegro is based on the Decree on the classification and categorization of surface and groundwater (official Journal of Montenegro, No. 02/07). The institution responsible for monitoring the quantitative and qualitative characteristics of water is the Hydro meteorological Institute of Montenegro, pursuant to Article 58 of the Water Act (official Journal of Montenegro, No. 27/07).

Montenegrin legislation in the area of monitoring Skadar Lake:

- Water Law (official Journal of Montenegro, No. 27/07)
- Law on the Environment (official Journal of Montenegro, No. 48/08)
- Law on Nature Protection (official Journal of Montenegro, No. 51/08)
- Law on National Parks (official Journal of Montenegro, No. 56/09).

Institutions in charge of implementing the monitoring program

In Montenegro, there are a large number of institutions and organizations dealing with environmental protection, and among other things by conducting monitoring on Skadar Lake.

- Ministry of Agriculture and Rural Development,
- Ministry of Sustainable Development and Tourism,
- Institute of hydrometeorology and seismology of Montenegro,
- Local government,
- National Parks,
- Institute of Nature Protection and Natural History Museum,
- Centre for Eco toxicological Testing,
- NGO Sector,
- International organizations,
- Private and local companies (hotels, restaurants, catering establishments, tourist organizations).

Sampling sites and their tracking

Water quality

Monitoring points were selected according to WFD. 8 points on the Montenegrin side are recommended (Table 1). In these locations, monitoring of all general chemical and physical - chemical elements (transparency, thermal conditions, oxygen conditions, salinity, nutrients, and acidification status) are predicted. Analysing specific pollutants (specific synthetic substances or specific non-synthetic substances) will be done at sites that are defined as sensitive areas. Depending on the pollution in the catchment area, parameters will be analysed.

Table 1. Recommended sampling points for the monitoring of physical-chemical and hydro meteorological quality elements in the Skadar Lake, on the Montenegrin side

Place of sampling	Coordinates	Criteria for selection
Vranjina	42° 16′ 32.79″N 19° 07′ 12.12″E	Flood waters of the right bank of the river Morača
Virpazar	42° 15′ 22.12″N 19° 06′ 19.85″E	Flood of water from the Virpazar Channel
Plavnica	42° 15′ 40.26″N 19° 11′ 54.02″E	Discharge of waste and the influence of groundwater
Kamenik	42° 17′ 32.63″N 19° 06′ 3.10″E	Flood water from Rijeka Crnojevića
Podhum	42° 16′ 2.58″N 19° 21′ 44.53″E	Impact of Aluminium Plant Podgorica, surface water and ground water
Starčevo	42° 11′ 19.82″N 19° 12′ 29.42″E	Dilution of pollutants in places away from sources of pollution
Ckla	42° 05′ 8.20″N 19° 22′ 46.30″E	Important cross-border output profile
The Lake	42° 10′ 47.50″N 19° 16′ 26.67″E	Water quality of the pelvis

Monitoring of particularly sensitive areas

Anthropogenic impact on the area of Skadar Lake is very pronounced. Urban and other settlements, industrial facilities, wastewater, agriculture are just one of the factors that influence the pollution of Skadar Lake. Monitoring of particularly sensitive areas includes the monitoring of Skadar Lake water, sediments and biota, for the presence of metals and organic pollutants.

Skadar Lake is contaminated by numerous pollutants, and most pollutants in water, air and soil originate from Podgorica. The largest quantities of pollutants in the lake are brought by the Morača river, and the main polluters are the Aluminium, Podgorica (KAP), the agricultural complex of the Plantaže, the Podgorica landfill, etc. In addition to the aforementioned, the main polluters of the Skadar Lake on the Montenegrin side are: Ironworks Nikšić, waste water from settlements and towns in the catchment area (Podgorica, Nikšić, Cetinje, Danilovgrad, Virpazar, Rijeka Crnojevića).

Predicted locations of fish monitoring

Fish

When it comes to monitoring the fish population it is necessary to follow the entire lake. Monitoring is mainly done during the period of spawning, when fish are collected at already known locations.

Birds

The Montenegrin part of the Skadar Lake is by D. Saveljić (NGO Centre for the Protection and Study of Birds), significantly more important than the Albanian part. In the following table we have marked species that will be monitored in the areas of their nesting and feeding.

Table 2. Sampling sites for monitoring

Place of sampling	**Coordinates**	**Criteria for selection**
Rijeka Crnojevića - cave "Obod"	42° 21′ 16,00″ N 19° 01′ 38,00″ E	Discharge of all waste water from the Cetinje Valley
Rijeka Crnojevića - sublacustic source "Ploče"	42° 17′ 32,00″N 19° 06′ 03,10″ E	Discharge of all waste water from the Cetinje Valley
A little mud - the source of the Biseva river	42° 20′ 36,00″ N 19° 10′ 28,00″ E	Drainage of groundwater, originating from the Morača River
The mouth of the right bank of the river Moraca - (Vranjina)	42° 16′ 32,00″ N 19° 07′ 12,12″ E	The Morača River Falls
The mouth of Virpazar Channel (Virpazar)	42° 15′ 22,12″ N 19° 11′ 54,02″ E	Discharge of Wastewater from Virpazar
Rijeka Plavnica - sublacustic source "Plavnica oko" (Plavnica)	42° 15′ 32,00″ N 19° 11′ 54,02″ E	The groundwater flow from the Zetian Straits, RSVs and RANs, points out harmful concentrations of biota (analysis 2005)
Gostinjska riva - a source	42° 15′ 32,00″ N 19° 14′ 28,00″ E	Flood of groundwater from the Zetian Plain and the impact of Aluminium Plant pollution
Hum (Podhum)	42° 16′ 02,58″ N 19° 21′ 44,53″ E	Trace elements have been found in the past (source Aluminium Plant Podgorica)

Table 3. Location and area for the monitoring of indicator species of birds

Indicator	Sampling area on Skadar Lake
Winter counting of birds	The whole lake
Spring-fall bird counting	The whole lake
Pelecanus crispus Dalmatian Cormorant	Oritological reserves Previous areas of nesting and feeding; Pančeva oka, Crni žar
Aythya nyroca Ferruginous Duck	Vegetation zones in the northern part of Jezera
Phalacrocorax pygmeus Pygmy Cormorant	Ornithological reserves (nesting area); Pančeva oka, Crni Žar, Monastery, Ckla.

Reservations are in accordance with the Nature Protection Act and the Law on National Parks of Montenegro, the area of littoral and sea, with unchanged and slightly changed overall nature, intended only for the conservation of the original nature, scientific research without changing the basic characteristics and biological diversity, monitoring the state of nature, which does not jeopardize the free implementation of natural processes. Skadar Lake is recognized as a zone of interspecific protection, especially when it comes to bird nesting. In the Montenegrin part, there are two reserves: Pančeva oka and the Manastirska tapija and the birds nesting areas Crni žar, Omerova gorica and Grmožur.

Pančeva oka is the nesting place of pelican, herons, cormorants and tigers of global significance. The area around Pančevo eye represents the area of willow vegetation, as well as the peat islands with colonies of pelican. In this area there are also colonies of cormorants, small white herons and yellow herons.

Manastirksa tapija, occasionally is the habitat of colonies of herons, cormorants, pelican and terns.

Manastirska tapija, occasionally the colony's habitat is a heron, a cormorant, a pelican.

The Crni Žar is a nest of cormorants, herons, pelican and terns. The area is located south of the Pančevo Eye site. On the surrounding smaller islands, formed from dying vegetation, there are colonies of pelican, common terns, whitening terns, etc.

Omerova Gorica, is an area known as one of the largest gray colonnade colonies.

Grubbing was a pelican nestling, and now the gulls are mostly chubby.

Monitoring otter

There is not enough information about the presence of an otter in the lake and its surroundings. A monitoring program for the otter and the locations to be performed will be determined during the first year of monitoring for the next year.

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Review paper

Control measures in the protected areas of Montenegro and their impact on the state of natural values

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Abstract

Nature conservation in Montenegro has a complex chronology which has varied in accordance with changes in social achievements during the 20th century. From the spontaneous actions of nature protection to organized planning activities, Montenegro has passed an important path that enables it to adequately manage its protected areas according to the current trends. This paper analyses the control measures in the protected areas of Montenegro on four different levels: legal framework, institutional framework, the existing control models and spatial planning. In addition to the present state of the control measures, the paper provides a list of advantages in the management and disadvantaged which should be eliminated.

Key words: *nature conservation, Montenegro, National Parks, spatial planning.*

Introduction

Historical overview of nature conservation is very complex and testifies to the rich civilization chronology of this important initiative. Initially, the conservation was linked to members of the high and ruling class. In the Roman Empire and medieval Europe, protected areas were reserved for exclusive use of the ruling classes (Prato, Fagre, 2005). In 1499 Poland passed the Act on the protection of moose and wild horse (Vidaković, 1997). In those early initiatives some of the movements and individuals, although they were not led by ideas that are promoted by nature conservation managers today, gave immeasurable contribution to the development of ideas for nature conservation.

Avant-garde move in the action of nature conservation occurred in the second half of the 19th century in the United States. Researches of the Western territory of the United States have finally resulted in the proclamation of the Yellowstone for the first National Park (Gray, 2004; Prato, Fagre, 2005).European case of the establishment of national parks was quite different. The first national park in the old continent was found in Sweden in 1909 – Abisko. Engadin in Switzerland, probably the most famous European national park, was found five year after that. The proclamation of national parks here was preceded by the protection of each isolated individual small territories (Stojanović et al., 2014).

At the time of these early initiatives for organized protection of nature and national parks Montenegro was still far from the concept of systematic nature conservation. Although some of actions have been taken in the 19th century, the organized nature protection and the organization of the first national parks appeared in the fifties of the 20th century. Only recently, in the last twenty years, Montenegro has shown an open activity in the field of nature protection with the adoption of a

number of relevant laws, spatial plans and with the nomination for the international status of nature protection. This process is, like in many other countries of South East Europe, full of challenges.

Nature conservation and protected areas in Montenegro

Nature conservation in Montenegro has a complex chronology. Although first national parks have been declared only at the time of Yugoslavia, after the Second World War, some forms of protection recorded their origins much earlier. For example, the protection of Biogradska forest as its origin back to 1878 when Biogradska forest with Biogradska River and Jezerstica got the status of the Prince's preserve (Tomić et al., 2000). This and similar activities are evidence of partial vision of nature conservation which was presented until the mid-20th century. The first institutional forms of protection of natural areas in Montenegro can be found only in 1952 when, on August 5, the Decree of the Presidium of the National Parliament of Montenegro adopted the Law on declaration of forest areas of Lovcen, Biogradska forest and Durmitor for national parks due to their particular natural beauties, cultural, historic and scientific importance, which constitute a single entity in the forest-breeding, hunting and tourist respect. At the same time, Biogradska forest and Durmitor were the first declared national parks in Montenegro (Internal documentation of the Public Enterprise National Parks of Montenegro).

The modern concept of protection is represented by the provisions of the Law on Nature Protection (Official Gazette of Montenegro, No. 50/09). The following categories of protected areas were established in Montenegro: Strict and Special Nature Reserve, national parks, Regional Park and Nature Park, Natural Monument, Protected Habitats, Landscapes with Outstanding Features. In addition to protected areas with a national prefix, the following areas in Montenegro are under the protection at the international level: Tara River Basin (1976) as a Biosphere Reserve within the Program Man and Biosphere, Durmitor as a World Heritage Site(1980), Kotor-Risan Bay as a Natural and Cultural World Heritage Site (1979) (Internet 1). Two Ramsar sites are protected by the same convention: Skadar lake (1995) and Tivat Saltpans (2013), as areas of characteristic wetlands (Internet 2). Based on the review of lists and maps of selected protected areas it is concluded that Montenegro has a wide network of protected areas, equally represented in the northern and southern parts of the country

The total area under the functional protection in Montenegro is about 10% of the national territory (internal documentation of the Public Enterprise for National Parks of Montenegro).Analysis of the percentage of the national territory that is under protection always leads to the dilemma whether it is enough and satisfactory, and also how the current protected territory meets the modern criteria of nature conservation.

The legislative framework of nature conservation

The protection of nature and environment is one of the priority tasks of socio-economic development of Montenegro. This attitude was originally expressed by the project, Montenegro Ecological State" and the first Article of the Constitution of Montenegro, which reads: *Montenegro is democratic, social and ecological state* (Internet 3). On 20 September1991, the Parliament of Montenegro adopted the Declaration on the Ecological State of Montenegro and thus expressed its intention to become the first ecological state in the world. This declaration was internationally verified next year at the United Nation Conference on Environment and Sustainable Development in Rio de Janeiro by inclusion of the Project in the Agenda of the Millennium Goals defined in the strategic messages of this conference.

The institutional framework for the protection of nature and natural areas in Montenegro is regulated by the adoption and implementation of two systematic laws: Lawon Nature Protection and Law on National Parks.

(1) **Nature Protection Law.** This law states that protected areas can be of: international importance; national importance and local importance. Their classification and categorisation

according to this law shall be based on the study of protection (expert basis) created by a legal person. The classification of protected natural areas of international and national importance is conducted by the Ministry of Tourism and Sustainable Development, and those of local importance by local government, based on the study of protection. The classification of protected natural areas is performed in accordance with this law. It is in line with the IUNC categorisation which is the most referent one in the world. According to this classification there are or there is a possibility to declare the following forms of protected areas in Montenegro: Strict and Special Nature Reserves; National Park; Regional Parks and Nature Parks; Monuments of Nature; Protected Habitats; Landscapes with Outstanding Feature (Official Gazette of Montenegro, No. 50/09);

(2) **The Law on National Parks**. This law represents *Lex specialis* of the law establishing the operation and organization of national parks in Montenegro. The law defines the term and concept of a national park that is compatible with the definition prescribed by the IUCN, with small corrections that corresponds to the specificity in which national parks in Montenegro operate. The law stipulates that national parks are managed by the Public Enterprise for national parks of Montenegro. The Parliament of Montenegro has the right and obligation as the founder of Public Enterprise (Official Gazette of Montenegro, No. 56/09).

Both laws have their advantages and disadvantages. The elimination of these disadvantages would improve the nature conservation system.

The Law on Nature Protection provides zoning of protected areas but only in its poor definition and it needs a detail description of zones–protection regimes for protected natural areas. We should in this section consider concrete solutions of similar laws in surrounding countries, some European laws taking into account the specificities of Montenegro and the constellations of existing protection zones in national parks. Then, the precision defining the content of management plans for the protected natural areas is not specified. There is no consistency of this law with other legal act that indirectly affect the protected natural areas (Law on Forests, Law on Freshwater Fisheries, Law on Roads, Law on Concessions, etc.). Certain provisions of this law have proven to be inapplicable in practice. As an example, a part of the law relating to the assessment of environmental impact where, among other things, it is stated that "the evaluation of acceptability" is done for the construction of specific facilities in national parks, and then it is stated that the competent Ministry prescribes detailed conditions for its development. Since in practice there are no such provisions and detail instructions regulating the matter, this article becomes the subject of disputes between the various entities, and is often subject to certain abuses and different interpretation of the relevant state and institutions within the system, which leads to the perception of insufficient harmonization and directly affect the inter-sectoral cooperation in the general interest as the ultimate goal.

Deficiencies of the law on national parks are terminological imprecision of certain terms (e.g. definition of forests and forests ecosystems in national parks). The content of the annual management programs is cumbersome and in some content elements quite confusing and should be adapted to the content of the management plans that prescribe a set of strategic measures for their implementation in practice. The possibilities of financing national parks are not fully exhausted by this law and it should be adapted to dynamic processes that follow the content of new activities in parks. This law defines very poorly compensatory measures for violation of authenticity of certain parts of protected area. In accordance with the best practice of the developed countries, it is not enough just to enumerate the compensatory measures, but this area should be regulated by adopting new by-laws (regulations, decisions, etc.), which will make this matter applicable in practice. This law is also not consistent with other laws that may be applied to national parks (Law on Forests, Law on Waters, Law on the Organization of Facilities and Construction, Law on Freshwater Fisheries, Law on Roads, Law on Concessions, etc.).

Institutional framework of the protection

In the system of functioning of protected areas in Montenegro a crucial role have two institutions, the Ministry of Tourism and Sustainable Development and the Environmental Protection Agency. The Ministry of Tourism and Sustainable Development, among other things, is in charge of the system of environmental protection whose subsystem includes National parks of Montenegro. As of parks the Ministry has administrative competences and the following delegating responsibilities: (1) the adoption and approval of the annual management program; (2) the adoption of the report on the work of the company competent for national parks; (3) the provision of approval for projects of interest for the development of tourism in national parks (building and use permit); (4) the control of work in other operating segments of national parks.

The Environmental Protection Agency was established by the Decree on amendments to the Decree on the organization of the work in the state administration (Official Gazette of Montenegro, No. 68/08). The Agency performs the following professional and related administrative activities in the field of environmental protection: (1) environmental monitoring; (2) draws up reports and issues permissions for scientific and research activities; (3) communicates with relevant national and international organizations, and the public; (4) performs other activities stipulated by the law on Environment.

The most important interaction between the Environmental Protection Agency and the Public Enterprise National Parks is reflected in granting permissions to work in national parks that relate to research and other projects, giving opinions, analyses and expertise, and common information exchange in connection with monitoring of individual species of population in order to create a special database for biodiversity. In this context, the inevitable problem for the professional and overall public in Montenegro is the elimination of the State Institute for Nature Conservation, which formally ceased to operate in 2012. A remarkable jubilee, the fiftieth anniversary of this institution was celebrated only a year before this action. This precedent is a huge mistake and a failure of those who have made such a decision. As a result, the nature conservation system has suffered serious damage and the competences have been assigned to the jurisdiction of a state body (Agency). Now this institution monitors both, the environment and nature, provides an opinion concerning projects of importance and impact on the environment, and within different sectors issues licenses and develops professional proposals to justify the declaration of protected area. All this, no doubt, creates certain confusion in the system, which causes serious cross-sectoral conflicts in terms of matters concerning the nature conservation and environment.

Control models of protected areas in Montenegro

The control of protected areas is a complex activity that depends on several factors. This primarily relates to the protection criteria such as: personality, vulnerability and usefulness of a natural resource (Primack, 1993). One of the most important issues in the organization of protected natural resources is their primarily goal. This may be conservation of species, habitats and entire communities or biodiversity in whole. The latest stance highlights that protected areas should protect and preserve processes, primarily those related to the functioning of the ecosystem (Vujić, 2008). The formation and size of protected natural areas depend on many issues, and the key ones are: schedule of the human population, natural resources, political support, developed awareness of citizens. The largest protected areas are located in areas that have low population density and which are not determined for agriculture, forestry, urban development and other human activities. Some of the key issues in the formation of protected area are the following: how big a space of protection must be, whether it is better to have one big protected area or several smaller ones, which is the best form of that area, what is the allowed distance of separated enclaves, whether it is necessary to connect corridors (Diamond, 1975).

Today, in Montenegro only national parks have institutional and legally formalized form of control over protected areas. In order to have more unique, efficient and functional organization and

management over national parks, the single Public Enterprise National Parks of Montenegro was established in 1993 with a head office in Podgorica. Its establishment ceased to be valid a single legal personality of parks and their competences are incorporated and transferred to the level of the new Public enterprise. This control model has remained in force with the adoption of the new Law on National Parks of 2009, which is normatively defined by a new Decision establishing the public enterprise for national parks. Generally speaking, in the Montenegrin public the current centralised approach in the functioning and organization of national parks still causes a number of controversies, polemics and different perceptions of their functioning in the future. Such management, generally, has its advantages and disadvantages.

Based on past experiences in the centralized national park management system, certain advantages in the way of functioning and organization of national parks in Montenegro may be defined. The advantages are based on the following parameters: (1) the specificity of territorial diffusion of national parks and size of the total area of the country that allows fast and efficient operation of common services in the organization units (national parks); (2) qualified personnel in one place which allows the writing and implementation of program documents; (3) lower costs in the organization of bureaucracy (in the case of individual management each park could separately organize special services for the operation); (4) concentration of institutions and organizations dealing with the environmental protection and nature conservation in a single centre (Ministry, Agency for environmental protection, expert and scientific organizations, most university units, key NGO organizations and associations, hiking, cycling, etc.); (5) more efficient implementation of strategic documents and guidelines of relevance to protected areas; (6) more balanced and fairer allocation of profit necessary for the operation of parks (this specially applies to parks which are not yet or are far from the level of financial and any other kind of self-sustainability); (7) ability to create uniform development policy and protection measures from the single level of operation; (8) joint promotion of natural and touristic potentials of national parks (joint and unique appearance on the touristic and ecological fairs and exchanges, networking of products of all parks into a single tourist package, etc.).

Some of weaknesses that were induced by the current model of operation of national parks within a single system can be exhaustively enumerate on the basis of external and internal parameters. Those are: (1) lack of competitiveness in the work of parks; (2) often insufficiently clear and coherent strategic guidelines in defining the vision, the implementation of certain strategic areas and some specific goals that are later shown in practice as unsustainable or poorly-thought out by central services at the level of national parks; (3) subjective sense of local managers that they do not participate sufficiently in policy-making and implementation of strategic plans in their national parks; (4) no clearly established system of coordination in the implementation of certain strategic areas of management plans of the joint affairs services and services which are concentrated in individual organizational units; (5) the bureaucratic approach in solving certain issues, which are much more difficult to apply to premises in national parks, than it is applied to other systems (enterprises, companies, state bodies, etc.); (6) impression of less political and total power in local communities, and their dependence on the central governance body; (7) the under-representation of local structures in defining plans and their inadequate representation in the management; (8) impression of the local population of considerable alienation and lack of transparency in all phases of operation relating to individual parks, this perception is especially present in national parks Durmitor and Skadar lake which in relative categories have reached the level of financial sustainability; (9) the feeling of excommunication, extra-territoriality and isolation of local actors and the structure of the decision making process and way of creating business policies of parks; (10) impression of the public about the lack of clear vision, clear strategic directions and reliable decision making process; short-term planning of their financial security (often depend on external factors) etc.; (11) lack of a process of participation of public, cooperation and coordination plans and activities performed with a special emphasis on the participation of local structures in these processes; (12) the centralization of decision-making has never, in any society been popular and generally acceptable category.

In the context of the previous analysis, which presented the advantages but also notified certain weaknesses in the centralised approach and organization of national parks, and in order to achieve more efficient control model and encourage adherence of organization units and local structures of the present control system, according to certain legal and practical solutions, certain recommendations that will preserve the current organizational form can be given. It would be the following measures and activities: (1) permanent presence of joint service in the areas of parks and their close cooperation with the management structure in the field; (2) strengthen the absorption capacity of employees in the joint structures of management which will continuously create innovative practices and increase the level of proactive and adaptive management and initiative; (3) clearly visible and measurable results in the implementation of plans and management programs (construction of new tourist facilities, technical protection, monitoring, education, etc.); (4) legal and normatively built-in mechanisms for the participation of local structure in the management bodies of national parks (equal participation in the management boards, formation of the Council for National Parks which would predominately consist of the representative of local governments, local and regional tourist organizations, real estate owners in national parks, etc.); (5) encouraging local entrepreneurship and the development of local businesses based on the nature (logistical support is provided by jointly formed departments at the central level); (6) restoration of old crafts and traditional values in the space and contact zone of national parks; (7) introduction of other important measured to reduce the gap between controller – local structure through stronger interaction of management structure of national parks in relation to local communities and raise awareness of local communities about the importance of nature conservation; (8) introduction of measures and mechanisms for new directions in strategic and business planning; (9) introduction of measures for performance management, risk management, participative management, knowledge management; (10) introduction of measures for improvement of tourism management, monitoring and evaluation, as well as the introduction of local structures for the management of natural resources, etc.

The importance of spatial planning in the control of protected area of Montenegro

The importance of spatial planning can be found in the statement that the space is a basic general resource of existence, survival and development of human society in whole. The spatial planning is developed from the regional planning, i.e. an attempt to optimize the use and integral approach to the use at regional level (Perišić, 1985). The spatial plans, in Montenegro and in the world, were created from the regional plans (Bakić, 1995).

According to the Law on Spatial Development and Construction of Facilities(Official Gazette of Montenegro, No. 47/11) the possibility and need to develop a spatial plan for special purpose (PPPN) are defined for the territory or territories of one or more municipalities which have on its area special significant and identifiable natural areas that can be treated as special in character or protection mode. Pursuant to this definition, PPPN is adopted for areas of national, regional, parks of nature, the Coastal, tourist and recreation areas, cultural and historical areas, as well the exploitation field where surface excavation of mineral resources is carried out.

In the previous period, Montenegro has adopted the PPPNs for areas of four national parks (except NP Prokletije), as well as PPPN for the coastal area. Thus, the plans of special purpose were adopted in 1997 for areas of national parks Durmitor, Biogradska forest, Lovcen. PPPN for NP Skadar Lake was adopted in 2001, but drafting the new is in progress. In the meantime, there is a change in circumstance within the series or in the contact zone of individual parks, as well as under the new imperatives of the time, so that new PPPN for the individual protected areas were developed.

In the next part of the paper we will stress the main structural units that make their content. In the conceptual and methodological sense PPPN are defined by the law, which forms the basis in defining the terms of references. Their key structural units according to the law are as follows: (1) **the textual part of the plan** which includes: a) analysis and assessment of the existing documentations and the current situation, b) planning part (concepts) which identifies key determinants and

development concept for the space that is subject in all segments, c) a statement of the strategic assessment of environmental impact which shortly sublimates the description of the current state of the environment with a set of accepted measures and activities that will operate in the process of implementation of the plan with the main objective of minimizing the negative consequences for the state of environment; (2) **the graphical part of the plan** which, as the textual one, contains the following: a) analysis and assessment of existing documents and presentation of the current situation with cartography, b)planning part (concepts) with a key determinant of cartographic presentation of the regime and level of protection by zones.

In particular, an elaboration for particular zones of protected areas stands out as important. This type of elaboration is carried out in the areas where the largest planned construction and/or permanent changes in the environment are anticipated. By analysing and researching the detail elaboration of specific sites in national parks, with planning and management aspect, it is important to point out the following facts: (1) zones of detail elaboration are the most practical and the only clearly directing concept plan; (2) defining the terms of references must precisely established and clearly define the number of zones for the detail elaboration for the processor; (3) for any plan their interpreter must precisely well developed; (4) all the conditions and assumptions from all management instances must be created, in order for the zones of detailed elaboration, or the concept that is placed in them must be implemented in accordance with the timetables of the plan and in an integrated manner; (5) the adoption of the new plan may not be welcomed with a slight or low level of implementation achieved (often incorrect) or completely unfulfilled, as it was the case with the previous (or still valid) planning documents for national parks and other protected categories of natural areas in Montenegro; (6) for future protected areas before making plans and their conception, with the planners point of view, it is important to consider all possible options and zones for the elaboration, but which will be fully compatible with the basic postulates – the protection function as a primary objective; (7) it is essential to provide the conditions for multidisciplinary approach in their development (consensus and the presence of a large number of professional and academic profiles).

Conclusion

The total area of Montenegro, its physical and geographical features are the most important components that directly affect the planning and establishment of institutional protection of natural areas that are managed or there are clear intentions for organizational management in the near or distant future. In that context, it is very difficult to expect that in one relatively small area, such as the territory of Montenegro (with a total area of 13.842 km^2) the need for its economic development and social needs of the society can be perfectly balanced on the one hand, and maximize the conceptions of protection and establishment of protected natural areas, on the other hand.

Therefore, the establishment of new and strengthening sustainable forms of current planning and management solutions for protected area in the country is imperative for all components of society with particular emphasis on individuals and organized groups that, with their sophisticated, scientifically based and societal acceptable measured, work on further promotion of the values that paved the way to the premises of sustainable development. This path has become a synonym for a fairer, more ethical and generally acceptable form which is tended by modern humankind, which will utilize natural resources to a lesser extent that it has been until now and thus significantly reduce the chances of the coming generations for a safer future and a healthy natural environment. And such a healthy natural environment in the sphere of dominant expansion of industrial-technical-information system (expressed in recent decades throughout the world) has become an essential need of the present generation, who have a historical mission to project its future based on ecological principles and shape the development in the broadest sense of the word.

Conflicts of Interest: The authors declare no conflict of interest.

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Review paper

The War Crime of Anticipatory Collateral Damage to the Natural Environment - Critical Inquiries into its Anthropocentric Incongruence

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Identifying the background condition(s) of the issue to be explored and demarcating the parameters of their implications are essential for any analytical investigations in the disciplines of social science. The law as social construct is 'a cultural medium of expressive form', [6] which should be comprehended as profoundly rooted in a specific spatiotemporal context. [7]

It might be commented that one of the seminal aspects of the 1998 Rome Statute of the International Criminal Court (ICC Statute or Rome Statute) is its explicit inclusion of a war crime relating to the protection of natural environment[8]. Yet, as critics quickly notice[9], it is not the environmental damage per se that constitutes an offence under international criminal law. Instead, the Rome Statute's achievement in this regard is limited only to criminalizing the attack against a military objective that causes collateral damage to the natural environment. As paradoxical as it may seem, it is not the damage to the natural environment by a direct attack but the damage incidental to the attack against a military objective that features as a specific crime in the ICC Statute. This is prescribed in the text of Article 8(2)(b)(iv) of the Rome Statute, which is (so far) the only provision that expressly refers to the natural environment. Indeed, Article 8(2)(b)(iv) of the ICC Statute is the conceptually incoherent admixture of two different genres of war crimes that only share the common

[6] Desmond Manderson, *Songs Without Words – Aesthetic Dimension of Law and Justice*, (Berkeley: Univ. of California Press, 2000), at 201.

[7] Costas Douzinas and Adam Gearey, *Critical Jurisprudence – The Political Philosophy of Justice,* (Oxford: Hart, 2005), at 248.

[8] Surely, under Article 8(2)(b)(ii) ICC Statute, any intentional attack against civilian objects, which can encompass those found in the natural environment, is a war crime based on '[o]her serious violations of the laws and customs applicable in international armed conflict'. For environmental protection during warfare in general, see Karen Hulme, *War Torn Environment: Interpreting the Legal Threshold* (Leiden: Martinus Nijhoff Publishers, 2004); Richard A. Falk, 'The Inadequacy of the Existing Legal Approach to Environmental Protection in Wartime' in J.E. Austin and C.E. Bruch (eds), *The Environmental Consequences of War: Legal, Scientific and Economic Perspectives* (Cambridge: Cambridge University Press, 2000) 137; MA Drumbl, 'Waging War against the World: The Need to Move from War Crimes to Environmental Crimes' in Austin and Bruch (eds), *ibid*., 620; and Michael N. Schmitt, 'War and the Environment: Fault Lines in the Prescriptive Landscape' in Austin and Bruch (eds), *ibid*., at 87.

[9] See, for instance, Tara Weinstein, 'Prosecuting Attacks That Destroy the Environment: Environmental Crimes or Humanitarian Atrocities?' (2004–2005) 17(4) *Georgetown International Environmental Law Review* 697; and Tara Smith, *Creating a Framework for the Prosecution of Environmental Crimes in International Criminal Law*, (Ashgate, 2013).

denominator of anticipated 'collateral damage': The one relating to civilian casualties (incidental loss of lives of civilians, injury to civilians, or damage to civilian objects); and the other on damage against the natural environment. [10] Under this provision, it is a war crime to launch intentionally an attack 'in the knowledge that such attack will cause…widespread, long-term and severe damage to the natural environment which would be clearly excessive in relation to the concrete and direct overall military advantage anticipated'. This provision includes the (near) identical terms in the relevant treaties of international humanitarian law (IHL), namely, Articles 35(3) and 55 of the 1977 First Additional Protocol to the Geneva Conventions (API), and in Article 1 of the 1976 Convention on the Prohibition of Military or Any Other Hostile Use of Environmental Modification Techniques (ENMOD Convention). Such a modest place occupied by the protection of the natural environment in the ICC Statute should be criticized as evidencing our humanity's continuing failure to address squarely vital environmental concern in the system of international criminal justice. Further, as will be seen below, the concern for the natural environment is undercut by the essentially subjective value-judgment involved in the proportionality appraisal.

There is every reason to criticize that the war crime of collateral damage to the natural environment can be established only in highly limited circumstances. This can be done only where the perpetrators have proceeded with the attack against a particular military objective even though they were aware that such collateral environmental damage would be expected to upset a proportionate balance that ought to be struck between the injury to the natural environment and the speculative military advantage. [11] More specifically, under the Rome Statute the individual war crimes responsibility may arise only where the 'widespread, long-term and severe' damage to the natural environment would be 'clearly excessive' in proportion to the military advantage anticipated.[12]

For the ICC Statute, a violation of the relevant IHL rule based on 'widespread, long-term and severe damage' to natural environment alone is not sufficient, however grave such damage may be. Perpetrators, though having executed an attack that is likely to cause such potentially catastrophic environmental damage (or even has actually caused the damage), [13] may be exonerated if presenting the counter-argument based on the overriding military advantage. By subordinating environmental damage to such a balancing process, the ICC Statute manifests how the ecological consciousness of the international lawyers and diplomats lagged far behind the consciousness of the natural scientific community. In contrast, under the API (Articles 35(3) and 55) or the ENMOD Convention, the state responsibility (as opposed to individual war crime responsibility) for the environmental damage can be established solely on the basis of the degree of damage. For this purpose, there is no need for balancing inquiries into countervailing military interests. In that sense, the ICC Statute is a clear setback.

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[10] As a result of such a hodgepodge, the war crime based on incidental damage to the natural environment suffers from the incongruence of the elements of crimes. As will be discussed below, this is caused by the introduction of the superfluous qualifying words, which was originally relating solely to the collateral civilian casualties.

[11] Put differently, Article 8(2)(b)(iv) ICC Statute can be invoked only where the injury to the environment is found to outweigh the military gains accruing from that attack.

[12] By comparison, as noted above, for the intentional attack against the natural environment to constitute a violation of IHL and hence to engage the state responsibility, there is no need to appraise the proportionality of the countervailing interests.

[13] The Preparatory Commission of the ICC Statute decided that this war crime is not based on the actual 'result', but its 'conduct' as such is considered so blameworthy to be criminalised: Knut Dörmann, *Elements of War Crimes under the Rome Statute of the International Criminal Court – Sources and Commentary*, (Cambridge: Cambridge University Press, 2003), at 162.

Moreover, the bar for such a balancing is set so high that a judge must be convinced of an exceptional scenario: the perpetrators have proceeded with an attack despite their cognizance that such damage is expected to be 'clearly excessive' in relation to 'the concrete and direct overall military advantage anticipated'. In practice, such a threshold of damage makes the possibility of prosecuting the war crime of collateral environmental damage unrealistic. [14] The drafting history that explains why such a high bar is placed is also indicative of the scant regard paid to the protection of the natural environment. In the process of drafting the Rome Statute, some powerful Western states led the 'battle' over the choice of the words in relation to the war crime of collateral civilian casualties. They succeeded in having the qualifying words 'clearly' and 'overall' inserted for the very purpose of lessening the risk that their military personnel might be charged for this war crime. [15] The problem is that the war crime of collateral damage to the natural environment is tethered to that of collateral casualties of civilian persons and to that of collateral damage to 'civilian objects'. As a consequence, those controversial words purported to make it harder for prosecuting the latter war crimes end up being 'grafted' onto the element of the former. Such drafting manoeuvring has yielded 'collateral damage' to the ecological movement. Apart from adding uncertainty through the conceptually incongruent concept of 'concrete and direct overall military advantage', it has resulted in the diminished chance of identifying an individual criminal responsibility relating to the incidental damage to the natural environment.

As discussed above, in order for the war crime of collateral damage to the natural environment to materialize, a perpetrator must evaluate ex ante the factual circumstances and come to the conclusion that the damage to the environment would be excessive. Subsequently, ex post facto, the prosecutor and the judges must evaluate the perpetrator's value-judgment based on 'the requisite information available to the perpetrator at the time'. [16] While the first process is predicated on a subjective value-judgment, international criminal lawyers argue that the second process is supposed to be 'objective' evaluations by the judiciary (while taking into account the circumstances at the material time) [17]. Yet, such a supposedly 'objective' evaluation itself is a suspicious and contestable concept. International judges are inculcated with diverse a priori values. [18] This may result in their different value-judgments in evaluating the meaning and implications of the key elements of the two overall opposing interests (namely, the collateral injury to the environment; and the military advantage).[19]

Further, the judges' effort to acquire the perpetrator's 'knowledge' of anticipated environmental damage at the material time is marred by 'legal technical' issues. To the dismay of

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[14] See Ines Perterson, 'The Natural Environment in Times of Armed Conflict: A Concern for International War Crimes Law? (2009) 22(2) *Leiden Journal of International Law* 325-343.

[15] It is the United States that led the initiative in the drafting process to insert the qualifying adverb 'clearly' before the adjective 'excessive' and the qualifying adverb 'overall' to the phrase 'concrete and direct military advantage anticipated'.

[16] Footnote 36 attached to the Elements of Crimes concerning Article 8(2)(b)(iv) of the ICC Statute.

[17] Dörmann, *supra* n. 6, at 165.

[18] In this regard, to what extent can such judge's *ex post facto* evaluation can be approximated to 'objectivity' of the kind that the post-Enlightenment natural scientists have assumed as their basis for their inquiries?

[19] One may include knock-on effect of the collateral damage that may materialise in the long-term while others may be ready to take into account geographically remote and long-term military advantage in the wording 'overall'. For more in-depth inquiries, see ICRC, *The Principle of Proportionality in the Rules Governing the Conduct of Hostilities under International Humanitarian Law* (Geneva: ICRC, 2018).

legal advisors (and no doubt to the natural scientists!), the drafting records show that different meanings are ascribed to three elements that are nevertheless almost identical in the two relevant treaties: 'wide-spread, long-term and severe' in the API, as compared with 'widespread, long-lasting or severe' in the ENMOC Convention. For instance, the temporal scope implied by the adjective 'long-term' in the API is decades-length. Yet, the comparable adjective 'long-lasting' under the ENMOC Convention is understood as equivalent to 'a period of months or approximately a season'.20 Faced with such incoherence in the meaning attached to the near identical terms, how can a perpetrator (above all, a commander) be judged to harbour a requisite degree of knowledge about the temporal ambit, geographical scope and material degree of the anticipated collateral damage to the natural environment, and even about the relative weight of such damage in proportion to the gain in military advantage?

This paper will engage in critical appraisal of how the anthropocentric military rationales that underlay the drafting of the ICC Statute have resulted in undermining the vital environmental concern. Needless to say, to avoid normative disorder of international criminal justice,21 it is essential that there be clear guidance for ascertaining the perpetrator's awareness of the degree of environmental damage that is speculated to arise incidentally. Yet, such an endeavour to obtain as 'objective' a standard as possible proves to be, if not fruitless, all the more elusive. It would be stuck in the conundrum of how to extrapolate 'objective knowledge' that is constructed and systematized by our subjective sense-data.22

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[20] ICRC, *Commentary on the Additional Protocols of 8 June 1977 to the Geneva Conventions of 12 August 1949* (Geneva: ICRC, 1987), at 416, para. 1452.

[21] Hume, a sceptical philosopher, is said to warn that '[d]isorder arises from the intolerable impossibility of certainty in questions of knowledge and justice, an impossibility whose intolerability seems soluble by the imposition of authority'. See Ian Duncanson, 'Law as Conversation', in Anne Orford (ed.), *International Law and its Others,* (Cambridge: Cambridge University Press, 2009), 57-84, at 79.

[22] To recall, just before his death in 1938, Edmund Husserl wrote that 'the objective-scientific method rests upon a never questioned, deeply concealed subjective ground whose philosophical elucidation will…reveal…the true ontic meaning of the objective world – precisely as a transcendental-subjective meaning': Edmund Husserl, *The Crisis of European Sciences and Transcendental Phenomenology – An Introduction to Phenomenological Philosophy* (1954); translated by David Carr, (Evanston: Northwestern University Press, 1970), at 100, para. 27.

Review paper

The current development level of sustainable rural tourism in Montenegro

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Abstract

The notion of sustainable rural tourism gains importance towards the end of the 20th century, as it advocates the type of tourism that minimally influences the environment and local culture. Its main goal is to enable tourists to gain knowledge on the natural, historical and cultural characteristics of a unique environment while simultaneously preserving the local integrity and encouraging economic growth and benefits of local community. When it comes to Montenegro, as a small European country with features of both coastal and mountainous regions, it must be first seen in the context of the very concept of sustainability and the idea of energy efficiency, the usage of renewable resources and the preservation of the overall natural environment. However, in spite of its preserved environment and socio-cultural and historical diversity, Montenegro's rural areas are still highly underdeveloped. The main objective of this research was to define key factors of development level of sustainable rural tourism in Montenegro, based on a PESTEL analysis and on overview of domestic and foreign literature. Considering natural, geographical, historical and socio-cultural characteristics of Montenegro, the conclusion indicates that Montenegro, despite of existing strategies and legal documents, has not yet successfully implemented the concept of sustainable rural tourism. The success of development of sustainable rural tourism depends largely on the level of collaboration between the key players in the rural areas, and especially among the all stakeholders on regional and national level.

Keywords: sustainable rural tourism, rural development, Montenegro**,** PESTEL analysis

Introduction

In the context of wider interpretation of rural tourism, the available domestic and foreign literature offers numerous definitions of rural tourism. From the standpoint of European Union, "rural tourism" encompasses a wide spectrum of touristic activities in rural areas, majority of which could be roughly divided into separate rural tourism branches such as rural households tourism, hunting tourism, fishing tourism, ecotourism, sports-recreational tourism, residential tourism (holiday houses), educational tourism, gastronomical tourism and ethno-gastronomical tourism, natural reserves tourism, cultural tourism, and other specific types of tourism. According to the wider definition, rural tourism is a part of the wider notion of tourism focused on rural areas, outside the city (Seoskiturizam, 2005).

To better understand the specificities and the features of this term, it is necessary to remember that rural tourism unites the complexities of all activities and aspects of all-encompassing tourism products (to exemplify: countryside leisure activities, enjoying pastime and the tranquility of rural areas, enjoying nature and landscapes, national parks, nature parks, extraordinary areas, cultural tourism, countryside households tourism) (Cawley and Gillmor, 2008, pp. 316-320). On the

other hand, in other definitions, the notion of rural tourism entails the set of different services and experiences which descriptively define a specific area. Some of such experiences combine natural environment and countryside lifestyle with the opportunity to participate in activities and traditions of local population. Rural touristic destinations can practically be observed as separately identified areas presented to tourists as selected for visiting, with the primary motive of enjoying countryside, its ambient and activities.

Having in mind that the literature on rural tourism offers numerous definitions based on different approaches, for this study paper we will understand the concept of rural tourism as amerged context of nature and countryside life, its art, culture, history and heritage, which plays an important role in restructuring and positioning of agriculture as the main economy branch (Živković, 2018, 2017).

One of the main goals of sustainable rural tourism is the conservation of natural resources and the support to maintaining village arts and crafts, features, specificities, history and landmarks. However, it must be pointed out that the crucial stage of sustainability lies in the necessity to provide additional income to countryside population so as to improve their life standards, infrastructure, educational level and social norms. In that sense, it is clear that the development of tourism in rural areas could significantly decrease or prevent depopulation. Indirectly, this not only takes us to the problem of depopulation, but also to birth rate, sustainability, innovation implementation, as well as to continuous development. In such a way, sustainable rural tourism permanently contributes to the improvement and preservation of environment, cultural values, natural and social resources and the integrity of local community (Vujić, 2012, p. 476). This is achieved by minimising the negative and maximising the positive tourism effects on environment and the available resources (Maksin-Mićić, 2007, p. 128). To summarise, sustainable rural tourism aspires to satisfy the need of tourists, but not at the expense of culture, ecological processes and natural resources – it is rather based on the conservation of these values. In this context, it is stated that the conceptualisation of sustainable development necessarily aims at connecting and uniting all four pillars of rural development: people, economy, environment and industry. That is why sustainable rural tourism prevents vulnerability of natural and social processes, and undisturbedly directs the development of domestic environment and its population (Tomka, 2010; Lun, Pechlaner and Volgger, 2016).

Current level of development of sustainable rural tourism in Montenegro

Montenegro can be divided into two main bio-geographical regions as Mediterranean and Alpine. The continental region of Montenegro is divided into 5 sub-regions given the status of national parks: Lovćen, Lake Skadar, Durmitor, Biogradska gora and Prokletije. Montenegro also has three regional parks: RP Komovi (municipilities of Podgorica, Kolašin and Andrijevica), RP Piva (Plužine) and RP Orjen (Herceg Novi). Montenegro has two Ramsar protected areas, the IBA protected status, two UNESCO sites and more than a dozen Emerald zones.

By taking a wider overview of the level ofrural tourism in general, we made a selection of theoretical approach to the topic, based on the normative scales aligned with sustainability indicators. According to it, there are three theoretical approaches (Đorđević Milošević and Milovanović, 2012, p. 20):

1. rural areas are facing immense challenges undermining the territorial cohesion within countries;
2. rural areas often have an economic potential, mostly unused, or could be used better for the benefit of rural population and the overall national development; and
3. no sector policy nor market forces are able to fully respond to the heterogeneity of the challenges and potentials of rural areas, nor to deal with all positive and negative external factors.

All these three above mentioned theoretical approaches can be applied to Montenegro and its adopted laws and current strategies. The Ministry of agriculture and rural development has adopted

several crucial systematic documents, the most important of which is the following "Strategy for the development of agriculture and rural areas 2015-2020". However, to encourage, develop and improve the current policy of rural development and align it with the EU standards, the most important is the IPARD II program for the period 2014-2020. It defines the support measures for investment into primary agriculture and processing industry, and the support to widening the scope of economic activities in rural areas of Montenegro (Government of Montenegro: Ministry of Agriculture and Rural Development). Another important document in the development of rural areas is the LEADER program, based on the objectives of the European Agricultural Fund for Rural Development, which has a unique set of rules for rural development and propagates tasks aimed at strengthening the finances of rural areas through activities of strengthening the competitiveness of the agriculture and forestry sector, improvement of the environment and agricultural areas, and improving the quality of life in rural areas and achieving the diversity of the rural economy (Rahoveanu and Rahoveanu, 2013).

The most functional document concerning sustainable rural development is the "Strategy for regional development of Montenegro 2014-2020" and enlists the following as essential measures (Ministry of Economy, 2014):

– achieving balanced social inclusion and economic development of all units of local self-government and the region, especially of the continental part of the country, based on competitiveness, innovation level and employability;

– improving the system of monitoring the implementation of "Strategy of regional development" and statistical indicators;

– encouraging measures for underdeveloped municipalities, so as to mark the investment areas; and

– raising the level and the quality of education to align it with the needs and the demands of the work market.

The "Law on agriculture and rural development" (Law on Agriculture and Rural Development, 2017) is the basic legal document for the agrarian policy, and it regulates the following: planned development of agriculture and rural areas, measures of agrarian policy, agricultural and rural development encouragements, pre-conditions for their realization, encouragement users, producers organization, quality and demarcation of agricultural produce, trade and commerce of agricultural produce, additional activities, public interest work, registers and evidences, as well as other matters concerning the development of agriculture and rural areas. More in-detail explanation on the agrarian policy guidelines are given in the four main groups: market-value policy, rural development policy, public interest work and social transfer to family agricultural households (Law on Agriculture and Rural Development, 2017).

This Law is the only law in Montenegro regarding rural tourism and activities related to rural development and rural regions. Its main clauses, however, do not even precisely define neither the notion of rural development nor rural areas. It rather defines general goals and measures of agrarian policy, related to the market prices value and the measures for market stability, with a wide overview of the external and internal trade market. The same law mentions, as a significant item, the importance of encouragement of rural producers via subventions and additional funds, as well as via international projects, donations and other sources. Having in mind that Montenegro aims to establish deeper connections with the EU, this law also defines special advice for agriculture and rural development which enlist authority, measures and activities of agricultural inspectors, food inspectors and phytosanitary inspectors (Law on Agriculture and Rural Development, 2017).

This wide area of rural topics in mostly regulated by the "Law on tourism," adopted in 2010. However, "Law on tourism" also does not define the notion of rural tourism, while on the other hand the notion of ecotourism is clearly defined. This allows for a conclusion that rural tourism in the legal system on Montenegro is not clearly defined yet. When it comes to rural tourism and rural regions,

the "Strategy for development of agriculture and rural areas 2015-2020" and the "Strategy for development of tourism in Montenegro until 2020" are two most important strategic documents.

"Strategy for development of agriculture and rural areas 2015-2020" enlists, together with the analysis of the current state, the development vision, as well as measures for development of rural tourism compliant with the EU policies. This document points out to the authorities the importance of reforms in rural-touristic sector and demands for adopting of numerous strategic documents. What is necessary for such documents to be even possible to devise and adopt is to devise a thorough and a detailed master plan of measures and activities to be realized so as to align the entire scope of documents with the political, legal and administrative stipulations by the EU (Government of Montenegro: Ministry of Agriculture and Rural Development, 2015).

"Strategy for development of tourism in Montenegro until 2020" enlists as achieved the following goals (Ministry of Tourism and Environmental Protection, 2008):

– focusing market economy to sustainability of resources and attracting domestic as well as foreign investors;
– improving the quality of touristic offer and arrangements;
– a positive trend of increased demand for touristic offers in the entire Montenegro is evident from 2007 onward;
– the need for constructing new touristic attractions has been justified;
– inclusion of parts of central and northern region of the country in touristi coffers is on the increase;
– launching the website of the NTO of Montenegro, designed to be innovative and interactive; and
– increased interest of investors for investments in tourism of Montenegro.

To be able to turn the aforementioned tendency to reality of, the Strategy suggests the following (Ministry of Tourism and Environmental Protection, 2008):

– emergence on the market and the development of lifestyle products;
– emergence on the market and the development of quality products;
– devising touristic offers based on the mixture of sports and nature activities, which are both healthy and relaxing;
– devising touristic offers to satisfy the demands of tourists with academic background; and
– developing more touristic offers with all-inclusive arrangements.

PESTEL analysis of Montenegro

The improvement of sustainable rural tourism in any rural region implies integration through rural touristic spectrum respecting ecological, socio-cultural and economic possibilities of the environment. We must emphasize here that the notion of sustainability does not imply strictly ecological norms, but sustainable approach, principles and the system which promotes, defines the concept and guides rural tourism. Since we live in a society characterized by prompt changes, it is evident that those changes must be transferred to touristic social segment as well, with a solid foundation to maintain social stratification and the mobility of tourists (Oh and Schuett, 2010). Having in mind all these facts, the first stage in establishing and accepting new norms in rural areas is to raise awareness in rural population that social-professional structure must be changed in such a way to maintain its genuine, traditional aspects of the region, while on the other hand the society must not neglect the acceptable progress. However, rural regions tend to perceive the notion of modernization not solely as the acceptance of contemporary technological advancements but also as the ability to discover or devise new ideas to improve productivity, economic status, life standard, and the like (Šimić, 2016).

PESTEL is an acronym for Political, Economic, Social, Technological, Environmental and Legal factors. According to Gregorić (2014) this tool is helpful to evaluate companies and industries that are engaged in exporting and depend on global markets or to "recognize the external forces that could impact their market and analyze how they could directly impact their business"[23]. In tourism, it might be useful tool to analyze a specific tourist destination as a market and impact on business/ tourism industry and tourism demand itself. In this case we will conduct a PESTEL analysis as a framework to categorize diverse influences that might affect development of sustainable rural tourism and tourism in general in Montenegro. This analysis applied to Montenegro as the development tourist destination and can be represent "as a collection of tourism supply of an area, region or country offered to tourists on the international markets" (Gregorić, 2014).

The PESTEL analysis of Montenegro has been made of various strategic documents, (inter)national reports and studies. The below analytical study includes data sources from the European Commission reports – Transport Development Strategy Report for Montenegro, 2017; Economic Reform Program of Montenegro for the period 2018-2020, 2018; Montenegro 2018 Report, 2018; UNDP Human Development Report, 2016; national strategies and official reports – National Strategy for Sustainable Development of Montenegro until 2030, 2016; Strategy for the Information Society Development 2020, 2016; National Innovation System Study, PESTLE&SWOT Analysis-Montenegro, 2012; studies from the Statistical Office of Montenegro MONSAT, 2018; the waste management study in SEE by a group of authors led by Hogg (Hogg at all, 2017); a research study of Western Balkan countries, 2015, by Radović-Marković et al. 2015; and a study of the development of rural tourism in underdeveloped regions of Montenegro by Živković, 2017, 2018.

Political factors:

1. Montenegro is member of the UN, EBRD, EIB, IFAD, WTO[24] and the World Bank Group. The country acquired the official status of a candidate for the EU membership in December 2010;
2. Since 2002 Montenegro uses euro as the official currency;
3. In 2007 Montenegro joined CEFTA and 2011 EFTA organizations;[25]
4. Montenegro became 29th NATO's member in June 2017;
5. One of Montenegro's main goals is to join the European Union;
6. Montenegro has long-term institutional stability with no changes in the structure of political party leadership;
7. Montenegro is a relatively low ranked country in 77th place to the value of Global Competitiveness Index;[26]
8. Montenegro has made significant political progress in several spheres, including the rule of law, the fight against corruption and the fight against organized crime;
9. During the past few years, Montenegro has liberalized foreign trade and low and attractive tax policies for investors;
10. Limited political progress has been made in the process of decentralization on a local and regional level;
11. Montenegro is following the Europe 2020 strategy[27] related to energy efficiency, increase of investments in RTD and innovations, but the budget contributions to the RTD overall remains at the

[23]https://blog.oxfordcollegeofmarketing.com/2016/06/30/pestel-analysis/.

[24] United Nations (UN), The European Bank for Reconstruction and Development (EBRD), The European Investment Bank (EIB), The International Fund for Agricultural Development (IFAD), The World Trade Organisation (WTO).

[25] The Central European Free Trade Agreement (CEFTA), The European Free Trade Association (EFTA).

[26]http://reports.weforum.org/pdf/gci-2017-2018/WEF_GCI_2017_2018_Profile_MNE.pdf.

[27]The Europe 2020 is a 10-year strategy with the main priorities of smart, sustainable and inclusive growth as a way to improve the Europe's economy competitiveness and productivity of a sustainable social market economy (EUROPE 2020. A European strategy for smart, sustainable and inclusive growth, 2010, pp. 3-4).

low level. National research, technological and educational structure system is mostly dependent on state budget.

Economic factors:

1. The entire economy of Montenegro expanded by 4.2% 2017 with projection of economic growth from about 2.8% in 2018 to 2.6% in 2020;
2. Gross domestic product per capita in Montenegro in 2016 was 45% of the EU average[28] or 6,354 Euros[29] and real growth is noticeable compared to previous years;
3. Economic growth of Montenegro is based on tourism, energy, agriculture and rural development and industry;
4. The official credit rating for Montenegro stands at B1[30] or B+[31] with stable outlook;
5. Montenegro has a relatively fragile economy supported by investment mostly in the tourism and energy sector. The economic situation remains macroeconomic vulnerables because of high level of illiquidity in real sector, high claims from the business sector, growth of public debt,[32] high unemployment rate,[33] inflation and fiscal deficit;
6. The Fiscal Strategy of Montenegro, has the main goal to turn the 5% of GDP budget deficit into a 4.5% of GDP surplus for period 2017-2020;
7. Economic development of Montenegro presented in strategic document "Montenegro in 21st century – era of competitiveness" is oriented towards open economy, strengthening of economic freedom[34] in order to develop the entrepreneurial environment for all three regions;
8. In the last few years Montenegro has implemented many structural reforms aligned with the EU regulatives in order to facilitate entrepreneurial environment and to remove business barriers. However, those structural reforms still need to focus on the labour market performance in general, together with human and physical capital;
9. The economy of Montenegro has an unequal balance between import and export of goods;
10. Montenegro has shown a high potential in the sector of tourism year by year, especially on the coast and provides one-quarter of the national GDP and employs over three-quarters of the workforce. Agriculture and Industry employ around 25% of total workforce, while service sector represents the rest;
11. Montenegro is working on the improvement of its agricultural sector following the EU regulations, which at the moment has low productivity and insufficient mechanization. For instance about 30% of the total population works in agriculture as a primary economic activity;
12. Recently the tourism infrastructure on the coast has been improved, but the development of rural infrastructure and living conditions of the rural population still represent a big improvement challenge for the Government;
13. Lack of investment activity in the rural tourism industry and lack of transport links within the continental regions and with other destinations "in terms of quality, security, capacity and management efficiency".[35]

Social factors:

1. Montenegro is a multicultural democratic country of about 620,000 habitants;
2. Montenegro is rich in natural, historical and unique socio-cultural heritage;

[28] https://www.monstat.org/cg/novosti.php?id=2559.
[29] GDP report for 2016, Statistical Office of Montenegro, 2017.
[30] Moody's credit rating (https://tradingeconomics.com/montenegro/rating).
[31] Standard & Poor's credit rating (https://tradingeconomics.com/montenegro/rating).
[32] For 2017 was about 65% of GDP.
[33] The unemployment rate in the northern part of Montenegro is about 35%, the total unemployment rate for Montenegro is about 17% up to date.
[34] The economic freedom score of Montenegro is 68th freest economy in the world in the 2018 Index (https://www.heritage.org/index/country/montenegro).
[35] Transport Development Strategy Report, 2017, p. 10.

3. About 37% of the total population is inhabited in rural areas. Most of the population lives in the urban areas and in the coastal region;
4. Average gross income was about 765 Euros and average net income was about 510 Euros in 2017. The highest wage income was in financial sector, whilst the lowest one was in administration services. By regions, the highest average wage was in the coastal region, the lowest one in the central (except the capital of Podgorica) and northern region;
5. Poverty rate is higher for minorities, the long-term unemployed and people with disabilities. The poverty is higher in the north, compared to other regions of Montenegro. The newly established UNDP Social Welfare Information System or Social Card aims to improve the proficiency and effectiveness of the administration, and providing of social services;[36]
6. The educational and management skill sets are insufficiently developed. The cooperation between educational system and labour market needs still remains weak. The most noticeable progress has been achieved in improving the vocational education and training system from primary school and secondary education. Regarding the higher education Montenegro has one public university[37] and two private universities.[38]Montenegro has low number of researchers per capita, with a current rate of 0.38% of GDP invested in research and innovations with the goal up to 0.6% to be reached by 2020. The national level of literacy rate is about 99%;
7. Montenegro has the potential of human resources even with a lack of skilled tourism and hospitality workers and short-term/ seasonal employment in the tourist industry;[39]
8. With life expectancy at birth of 76.4 years Montenegro is ranked on 48thplace with Very High Human Development, according to the UN's Human Development Index for 2016.

Technological factors:
1. ICT remains an important factor in contribution to the creation of a digital Montenegro and his economy in the "Strategy for the Information Society Development 2020";
2. Montenegro is ranked 45th in the world in the field of the development of the electronic government;
3. Legislation in the field of the information society is largely harmonized with the EU laws. Nowadays, Montenegro strives to achieve digital integration of the ICT in all sectors;[40]
4. According to the statistical official data for 2017, 70.6% of households have access to the Internet. The territorial representation of the Internet in households is the smallest in the northern region about 57% and largest in the coastal region about 85%. In rural areas the Internet access is 60%, whilst in urban regions is 76%. About 73% of households in Montenegro use broadband connection. The use of computers in the business environment is about 98% with 99% of the Internet access;
5. The Strategy sets one of the main goals to achieve 100% household coverage with speeds above 30 Mbit/s by 2020, whereas the current coverage is about 26%;
6. The telecommunications sector in Montenegro is 100% privately owned and the market penetration rate for mobile telephone users is one of the highest per capita in the world;[41]

[36]The UNDP Social Welfare Information System is the project that enables enforcement of social welfare reform ensuring more effective approach of social protection for quality service for the poor and vulnerable. Social Card is an electronic system for processing, approval, audit of social benefits/ transfers, record-keeping, calculation and payments reporting (www.me.undp.org/content/montenegro/en/home/operations/projects/socialinclusion/SWIS.html).
[37]University of Montenegro.
[38]University Mediteran and University Donja Gorica.
[39]Montenegro has one public Faculty of Tourism and Hotel Management in Kotor and one private Faculty of Tourism (Montenegro Tourism School) in Podgorica.
[40]Development of electronic communications: e-learning, e-government services, e-health, e-payment, etc.

[41]https://www.export.gov/article?id=Montenegro-telecommunications.

7. Montenegro faced with lack of business structure, as well as service culture specifically in rural areas. The country provides insufficient budget support for development of technological and research centres.

Environmental factors:
1. Montenegro was established as a first ecological country in the world 1991 when the Parliament adopted the Declaration on the Ecological State of Montenegro;
2. One of the requirements in the process of joining the EU is the establishment of the Natura 2000 network[42]and Montenegro is currently in the field of study research and implementation of projects for the establishment of a network of areas important for the conservation of endangered species and habitats. Montenegro is part of the Bern Convention on the Conservation of European Wildlife and Natural Habitats since 2008. The network of protected areas currently covers between 13% and 17%[43]of the territory of Montenegro: national and regional parks, Ramsar protected areas, Emerald zones and other special natural protected areas;
3. Montenegro civil and government organizations are aware of environmental issues[44] but they are not fully involved in processes such as waste recycling and waste management program. Also the mentioned program are not completely aligned with National Waste Management Plan in all municipalities;
4. The wastewater treatment plant program is currently under development. In terms of landfill infrastructure, the sanitary landfills are also under development. According to the Report on the Implementation of the National Waste Management Plan, of the total amount of waste generated, about 30% ends up in unregulated landfills, and about 30% in uncontrolled landfills, especially in the northern and central region;
5. In general, some limited progress has been made in terms of waste legislation, however none of the 23 municipalities is ready to implement waste management properly;
6. Over the past few years Montenegro has implemented many environmental policies and created the necessary institutional framework aligned with the EU ecological sustainable standards;
7. The last few years, the infrastructure of central and northern region of Montenegro is focused on the development of eco and ethno rural tourism;
8. In 2013 Montenegro adopted the "Montenegro Development Directions 2013-2016", as a model of the "Europe 2020" strategy with three key factors: smart, sustainable and inclusive growth;
9. Quality and detailed planning is one of the most important prerequisites for long-term sustainable development of tourism. Montenegro still has a problem with Illegal construction which is strictly prohibited by law especially in the natural reserved areas, but in recent years can be visible progress in that area;

[42]The Regional Environmental Center for Central and Eastern Europe (REC) is an international organisation with a mission to assist in solving environmental issues of Central and Eastern European Countries. REC Montenegro is an essential institution in the country since 2001 whose mission is to provide support in the process of public participation in the final decision of environmental related topics, as well as to promote sustainable development education, water resource management, cooperation between NGO's, government institutions, business sectors and other stakeholders (http://montenegro.rec.org).
[43]http://www.vijesti.me/vijesti/za-naturu-ce-trebati-tri-godine-900847.
[44]Natura 2000 is the EU network of core breeding and resting sites for rare and threatened species, and some rare natural habitat types which are protected in their own right. Natura 2000 is established 1992 to ensure the long-term survival of Europe's most valuable and threatened species and habitats, listed under both the Birds Directive and the Habitats Directive and this network actively involves people in the process. Stretching over 18 % of the EU's land area and almost 6 % of its marine territory, it is the largest coordinated network of protected areas in the world (http://ec.europa.eu/environment/nature/natura2000/index_en.htm). Natura 2000 consists of two central areas: SAC - Special Areas of Conservation and SPA - Special Protection Areas (Ministarstvo održivog razvoja i turizma, 2015, p. 16).

10. The first National Strategy for Sustainable Development of Montenegro adopted 2007 was developed in cooperation with the Mediterranean Commission for Sustainable Development and the Mediterranean Action Plan of the United Nations Environment Program. After a five-year period of implementation of the Action Plan within NSSD 2007, 53% of strategic measures were achieved or half of 24 priority areas. Followed the previous one, the new NSSD of Montenegro until 2030[45] has been implemented (Ministry of Sustainable Development and Tourism, 2016).

Legal factors:
1. Montenegro has been made a good progress with the reform of its public administration at national, regional and local level. Also some progress has been made in the judicial system, the fight against organized crime, war crimes, terrorism and money laundering and fight against corruption at all levels. Despite that of all efforts have been made, the corruption remains a big issue;
2. On fundamental rights and freedom of expression slightly progress has been made, especially on protection of general human rights;
3. In the legal framework, Montenegro has been made a significant progress in the areas of food safety, agriculture and rural development, company law, veterinary and phytosanitary policy followed by the European standards;
4. The involvement of partnerships, associations and stakeholders at national, regional and local level in Montenegro is insufficiently developed;
5. Montenegro has insufficiently implemented local action groups, "bottom to top" decision-making system and the entrepreneurship development in rural areas;
6. The Regional Development Agency for Bjelasica, Komovi and Prokletije are the only regional agency in Montenegro with the goal to enhance the development of northern municipalities of Bjelasicamountain (NP Biogradskagora), RP Komovi and NP Prokletije.[46] Through cooperation with all stakeholders, diverse EU funding project programs and new employment opportunities, the Agency strives to achieve the economic development of region, in alignment with the EU and domestic strategic development plans.

Conclusion

The PESTEL analysis gives a clear picture of the current developmental level of Montenegro and it can be a very helpful tool to understand the financial impact of investments on tourism in Montenegro in general. This analysis and various legal documents have shown that Montenegro as a tourist destination has a lot of room to improve different kind of tourism, especially in rural areas. Besides the material and non-material infrastructural monuments, rural regions of Montenegro, unlike the coastal region, thrive on a completely different lifestyle – a lifestyle that could turn quite attractive to tourists who come to familiarize themselves with the local culture and its traits. Likewise, even tourists who visit rural region yet whose main goal is not just to learn about the local culture, could enjoy from various sports-recreational/ leisure activities (Dašić and Jovičić, 2011, pp. 136-137; Krajnović, Čičin-Šain and Predovan, 2011, Bećagol, 2014; Kaluđerović, 2015).

Namely, as stated by Radović, Pejanović and Radosavac (2013), the main limitations to development of rural tourism include the following:
— lack of finances;
— underdeveloped infrastructure;
— lack of accompanying touristic activities;

[45] This follows the UN Agenda 2030 as a commitment to eliminate poverty in all its forms and achieve 17 pre-set global goals for sustainable development by 2030 (http://ec.europa.eu/environment/sustainabledevelopment/SDGs/index_en.htm).
[46]Municipalities of Mojkovac, Kolašin, Bijelo Polje, Berane, Andrijevica, Plav, Rožaje, Petnjica and Gusinje (www.bjelasica-komovi.me).

— insufficient awareness of local population on the importance of cultural-historical heritage;
— manifestations held are not linked to other touristic activities;
— underdeveloped promotional activities; and
— difficult access to destinations in winter.

Simultaneously, it is necessary to enrich the touristic offer with numerous arrangements interesting to tourists of various age. To make sure that the offer could satisfy all demands and criteria, it must also be balanced and combine both the traditional and contemporary principles of tourism. It must not be forgotten that the direction of development does not necessary include the absolute modernization of rural areas, primarily because there is a great affinity of urban tourists for visiting industrially underdeveloped regions. At the same time, we must emphasize that the system of implementation of innovative approaches does not always refer to modifying the traits of natural areas – because what may happen is the deterioration of the area's natural beauty and the loss of that special notion of un spoilt nature (Bećagol, 2014).

To conclude, we believe that, to enable further development and realization of new possibilities, it is necessary for the country to fund projects in rural areas through domestic and foreign investments. Such projects should be decided on the principle of attractiveness, on the planned expenses, the rationale behind them, necessary funds and demands enlisted in a detailed financial plan, aligned with the legal regulation of Montenegro accompanied with the EU regulation and norms of ecological/sustainable living. Although, the collaboration between all stakeholders is also a key factor of implementation of sustainable rural tourism. Montenegro though must turn to sustainability instead of the massiveness of tourism fostered only in the coastal region. The current capacities of the infrastructure do not support the development of sustainable tourism in general, bearing in mind the overcrowding and inadequate expansion of the infrastructure on the coast in relation to the continental part of Montenegro. If Montenegro wants to be an ecological country, as it is written in the Constitution, and to develop sustainable (rural) tourism, it has to prove in practice its own readiness to take concrete steps, not copying other countries, but to be a good example to others.

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Review paper

Establishment and development of credit cooperatives in Bulgaria

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Abstract

The article analyses the experience of credit cooperatives in agriculture and forestry in Bulgaria and the development of legislation in this area. It was found that due to the small size of individual plots and their dispersion in the region of the lands of the settlements it is not possible an effective management of these properties. These factors appear as a reason for difficulty in financing from the programs for rural development by the European Union.

Credit conditions by commercial banks are unprofitable and difficult to implement, and interest rates are too high, which is why for small and medium-sized forest and agricultural landowners one of the possibilities for short-term loans at preferential conditions is credit cooperatives.

A well-developed system of credit cooperatives is in the countries of Western Europe and the US. Founders of this type of cooperatives are the activists of the German cooperative bank - Raiffeisen – for local credit banks and Schulze – for popular regional banks. For more than century-old history credit cooperatives in many countries hold a significant portion of their financial-credit system, directly serving private agricultural and forestry producers.

Bulgaria was a country with a well-developed market-oriented cooperative sector. The credit cooperatives have been prevalent in this sector. Over the years the importance of cooperatives, including credit cooperatives, not reduced, but changed conditions in the period of planned economy led to the decreasing of their number and to the disappearing of credit cooperatives in Bulgaria. After the changes occurred in the country (restoration of property on the lands and forests and the emergence of many and small owners without the necessary financial resources and skills to manage them) arises the need to restore the credit cooperatives.

The purpose of the creation of credit cooperatives is to assist private forest and agricultural landowners to have care for them, to assist in the expansion and strengthening of their farms, to increase the quality of life and to improve the local economy.

There are made proposals to create conditions for development of the credit cooperatives in the country and are referred the positive results expected from the creation of credit cooperatives.

Keywords: credit cooperatives, agriculture, forestry, cooperative legislation.

Introduction

The core of the credit cooperative is the economic prosperity of its members. The members of credit cooperatives are both its owners and clients. Credit cooperatives are built locally, with accumulated savings being invested in the same location and thus improving the economic environment in the region. Credit cooperatives are two types:

- Credit cooperatives whose members are employed in different spheres of the economy and different professions, and
- Credit cooperatives with a specific professional, social, etc. composition.

The credit cooperative is a credit institution for the collection and reallocation of monetary capital. Its own funds consist of equity and reserve funds.

The collection of share capital and the formation of reserve funds take place in the course of the expansion of the business of the credit cooperative.

In credit cooperatives there are usually no limitations on the number of units which the co-operator may hold but in the general meeting of the credit cooperative, each co-operator, regardless of the size of his / her share capital, has one vote.

The main source of deposits of credit co-operatives is the free cash of citizens and legal entities that it receives in the form of indefinite, term and saving deposits. The collection of these funds by the credit cooperatives takes place through their branch network.

For credit cooperatives, deposits are the cheapest credit resource and therefore they pay extraordinary attention to their recruiting. In this regard, the interest rate is of particular importance.

Modern credit cooperatives strive by offering the full range of financial products and services at competitive prices to attract the free money of citizens and businesses without being able to pay higher interest rates, which is not only possible due to the need to generate sufficient profit but and due to the existing legal framework in a number of countries that treats the payment of higher interest rates as a form of unfair competition (1).

Materials and Methods

Objective and Tasks

The objectives of the present study are to study the current conditions for establishing and development of credit cooperatives in Bulgaria and to show the positive effect of their use and development.

Methods used

According to the objectives of the report, which aims to give a comprehensive overview of the problems and positive aspects of credit cooperatives in the country, a combination of methods is applied. These include a review of literature, secondary data, expert interviews, and expert knowledge of the author.

The data includes quantitative data from official statistics and scientific research (National Statistical Institute, National and Regional Forest Statistics - Annual Forestry Reports by the Forestry Executive Agency and the Ministry of Agriculture and Food and Research and quality data - expert knowledge of the author of the report and the experts from the Forestry Executive Agency, the Ministry of Agriculture and Food, the State Forest Enterprises, the State Hunting Enterprises and the different owners of the agriculture and forest lands).

There have been numerous meetings and consultations with representatives of different property owners in the country in order to answer different questions, to provide review assessments and to give examples of individual cases.

Results and Discussions

By 1887, the country has only 13, and after ten years it reached 180 urban and rural credit cooperatives. Until the adoption of the Law for Cooperative Associations in 1907, co-operative societies were registered under the Commercial Act, adopted in 1897. In 1907 it was passed the first law for cooperative association, which governs the creation and activity of cooperatives.

In 1910 it was established the Central Cooperative Bank. So far were registered 981 cooperatives: agrarian, credit, consumers and special. The main part of these cooperatives (811), were rural consumer cooperatives that protect economic and social interests of the rural population (Zlatev, 1999). The members of these cooperatives provided mutual assistance in combating poverty and usury, cheaper credit and cheaper goods and care for improving agricultural production.

In the period 1914-1918 in the United Union members there were 586 cooperatives, including 573 credits, 7 consumers', 3 sheep-breeding, 1 tobacco growing, 1 winemaking and 1 agricultural syndicate.

During the government of Bulgarian Agricultural People's Union(BAPU) (1920-1923) was created the Agricultural Cooperative Bank. Efforts were being made for lending tobacco growing and rose-production cooperatives. With the Law for amending the Law on the Bulgarian Agricultural Bank the controls and lending of all types of agricultural cooperatives are concentrated in it. It was accepted The Forestry Act (1922) (State Gazette, 24/3.05.1922), which provides for the operation of public forests and processing of timber to be carried out by forestry cooperatives, which at that time were 88 in number. The members of these cooperatives are obliged to participate with personal labour in logging, transportation and processing of logs.

In 1925 in Bulgaria there were 26 general and regional cooperative organizations.

In 1945 the number of members of all forest cooperatives was 31 451 and 205 forest cooperatives were members of the Forest Cooperative Union. In the period 1945-1946, it was founded 64 new forest cooperatives with 15 000 new members.

Since the beginning of the twentieth century until World War II (WWII) cooperative entered widely in the life and business of different layers of Bulgarian society, especially the peasantry. It constantly increased its share and influences the economic life of the country despite the lack of capital and insufficient economic efficiency. Totally in 1944 there were 4114 cooperative associations. Of these, 3156 were in rural areas. The members of cooperatives were 625 000.

The cooperative movement in Bulgaria was characterized by a wide variety of forms, types of cooperatives and their penetration in various sectors of economic, social and cultural life of the country. Too large was the number of credit cooperatives - more than 76%.

In 1945 is published the Ordinance-Law on Labor Cooperative Farms (State Gazette 95 /25.04.1945), introducing order and organization in the agricultural cooperative movement. The law provides for the retention of private ownership of land and the right of every co-operator to receive rent paid for land in the cooperative, which initially reached 40% of the annual income of the cooperative sector. Then the rent decreased to be abandoned in the late 50s. It acquired later inventory and working livestock, farm buildings, various funds and additionally purchased or received state land are owned by cooperative. Each co-operator retains personal property productive livestock, perennial crops and small plot of land. The envisaged assistance from the state and municipalities with staff, resources, techniques, releasing the cooperative from all direct taxes for three years, gratuitous transfer of state and municipal land, use free veterinary and agronomic assistance, obtaining loans on favourable terms, etc. The law established the principle of voluntariness in cooperation.

In 1948 was accepted a new Law on Cooperatives (State Gazette 282/01.12.1948), and in 1953 - the next Cooperatives Act (Izvestiya 13/02.13.1953). In 1954 are published The Rules of cooperative organizations (Izvestiya 27/2.04.1954).

In 1956-1958, it is completed the process of cooperation in agriculture. The Labor Cooperative Farms(LCF) has already been placed under the full direction and control of the state, losing the remnants of self-government and autonomy as businesses (enterprises). They are subject to centralized state planning. All their production activities are regulated. At the same time they must be self-financed and delivered to the state at low prices most of its production. Although the land is not nationalized, the Local Cooperative Farms did not have it free and cooperative in fact no longer owners paid in land.

In the 70s was created so-called Agro-Industrial Complexes (AIC), which turned into large centralized agricultural enterprises. The LCF flowed into these complexes losing their legal independence. Their land was actually nationalized. They recovered again as LCF since 1987, when was started a new restructuring of agriculture and liquidation of AIC.

Central Cooperative Union (CCU) was developing in accordance with the Act on cooperative organizations (1983) as a public, commercial organization built on voluntary and cooperative ownership of the means of production. Cooperative unions and associations, members of the CCU retained their legal and economic independence and governed their relationships with it on the statute and contract basis.

The first Cooperatives Act after the changes of 1989 was passed in 1991. In 1992, the cooperative farms were disbanded and replaced by liquidation boards. Later they began to form a so-called private agricultural cooperative in which land has already been restored by a privately-owned cooperative.

Other regulations have been adopted, governing the formation and operation of cooperatives. With the financial support of PHARE program of European Union was created a small number of credit cooperatives that served private farms. They operated from 1995. Most of these cooperatives are in the regions Thrace and the middle Rhodopes. The last Cooperatives Act was passed in 1999.

The creation and functioning of cooperatives (including credit) in Bulgaria is regulated by the Cooperatives Act (CA).

The Cooperatives Act clearly defines the legal steps for establishing a credit cooperative. Unlike the legislation in many European countries, however, in our country there is a legal obstacle for cooperative banking organizations to develop their business in the deposit and credit sphere. The sting comes from Art. 36, para 3, where it is stated that: (3) The cooperative may, by decision of the General Meeting and by a resolution of the Bulgarian National Bank, under conditions and procedures regulated by a special law, perform deposit-credit activities.

In para. 3, a legal opportunity is provided for the cooperatives to carry out deposit-credit activities subject to a special law and a decision of the Bulgarian National Bank.

The problem in this situation is that the law, which can be treated as "special" and which actually regulates credit activity in the country, the Law on Credit Institutions (LCI), does not define or discuss the terms and conditions for the creation of credit cooperatives. Exceptions are made again by the mutual credit cooperatives of private farmers established under the Agricultural Capital Fund Scheme in accordance with the contracts concluded between the Government of the Republic of Bulgaria and the European Commission for the utilization of gratuitous funds, which are regulated in the "predecessor" credit institutions, namely the Banking Act.

In Art. 1, para 3 of the Law on Credit Institutions states that "The provisions of this Act shall also apply accordingly to the banks established by a separate law, insofar as it does not provide otherwise", but a separate law regulating the cooperative banking in Bulgaria there is no real thing.

There is only a draft Law on Credit Cooperatives and Associations (LCCA), dictated by:

- The requirements of Art. 36, para. 3 of the Cooperatives Act,
- the existence of credit cooperatives established under the PHARE program with the project "Establishment of an agricultural capital fund", regulated by § 17 of the Transitional and Final Provisions of the Law on Banks;
- Bulgaria's obligations under the European Association Agreement and the call for membership to ensure gradual alignment of its legislation with that of the Community, where a number of countries such as Germany, France, Austria, the Netherlands, Denmark, have highly developed financial institutions based on the cooperative;
- Established traditions in the Bulgarian cooperative work until 1951

This draft was submitted on 18 July 2001 by the member of the 39th National Assembly, Yordan Nihrizov, to the Budget and Finance Commission, but was not put to the vote by National Assembly, which terminated this sole attempt to legislate for credit cooperatives in Bulgaria.

On the basis of this, we can conclude that in Bulgaria there is a gap in the legislation, which prevents any initiatives to create a credit cooperative.

This type of activity, although not strictly forbidden for cooperatives, could take place under certain conditions provided for in the special law - Art. 36, paragraph 3 of the CCA.

It is necessary to decide the general meeting of the respective cooperative. However, it is also necessary for the specialized body, as in the case of the Bulgarian National Bank (BNB), to take a decision to allow the respective cooperative to do so. However, the special law on the terms and procedures on which the BNB will take such decisions has not yet been adopted.

The indications over the years lead to the conclusion that measures to regulate legislation in this area may not be undertaken in the near future. These doubts are further reinforced by the declared BNB resistance to regulating co-operative banking in Bulgaria.

Already in 2007 in a document titled "Basic Principles of the BNB Licensing Policy", the Bank's Management Board stated that "The creation of low-quality" quasi-banks "would be an adventure with extremely severe consequences that the BNB will not support." from BNB clarify that it is mostly about cooperative banks.

The BNB is an institution which is legally responsible for ensuring the stability of the banking system and hence the macroeconomic stability of the country. From this position, it may raise concerns about the existence of certain financial organizations.

However, as it has been clear from the data so far, the existence of cooperative banks is a well-established European practice that continues to this day. On the basis of the financial information, the opinion of some experts has also been confirmed that this type of institutions is even more stable and enjoys greater trust than commercial banks. Especially in crisis situations, it is noted that cooperative banks are the key to the survival of small and medium-sized businesses and so they are a means of implementing anti-crisis policy.

The BNB's considerations for co-operative banking in terms of creating "low-quality" quasi-banks are not very small, as opposed to the policy it is pursuing with regard to the lending institutions in our country. It is mainly about the penetration by granting a license as a financial institution, namely by the BNB, on the Bulgarian market for the so-called "quick loans" companies. Until the latest proposals for amendments to the Consumer Credit Act (from the beginning of July 2012), some of the activities of these organizations, such as loans up to 400 / four hundred / levs, did not even fall into it, thus creating an opaque environment in the formation of interest rates and uncertainties about the functioning of part of the credit market in Bulgaria. The consumer never understood how much he would return until he had to pay the repayments. This is a danger that the BNB should indicate and take the appropriate actions according to its inherent responsibilities. As an institution, the BNB has no legislative functions, but it can initiate discussions on many lending issues in Bulgaria.

The BNB should really be concerned about the risks to the banks and the stability of the system, but on the other hand it should also be an arbitrator for bad practices and banks (part of which came out, especially during the crisis period 2008-2011). The problem of backward liquidation of companies and the concern of the BNB is just one example of how the bank responds very quickly when the interests of commercial banks are affected and is not so active when it comes to consumers. This can be confirmed by the information in the BNB press releases from 2000 to 2011, which shows that the BNB reacted very strongly against certain statements concerning the banking system and the incorrect practices and did not give opinions whether again and where European practice does not apply in the course of lending.

On the basis of the financial results of cooperative banks in Europe, the reluctance of the BNB to support their creation seems even stranger. As it has become clear, banking cooperatives maintain very good levels of capital adequacy, even above the minimum required. High credit ratings also prove their financial stability.

From this point of view, the BNB should initiate the discussion on the legal settlement of cooperative banking in Bulgaria, as the work of both the guardian and the guarantor of financial stability will be greatly facilitated.

It is clear from the above that the BNB may have to rethink whether resistance to cooperative banks is justified since it allows certain bad practices to exist on the Bulgarian credit market, and proven successful institutions such as bank cooperatives are not desirable. [1]

Conclusions

Cooperative banking in Bulgaria can be the key to establishing a new stage of credit market development based on the principles of fairness, subsidiarity, solidarity, proximity to the client.

Moreover, Bulgaria has an established tradition in this field and the success of the cooperative banks, both in managerial and in financial terms, can be assumed with a certain amount of security.

The transition to a market economy has shown that without the support of small and medium private business, it is not possible to restructure the economy and build a national entrepreneurial class.

In Western Europe and the United States, these cooperatives carry out significant volume of deposit-credit operations. In the countries of the European Union, they account for about 19 per cent of all banking operations.

Particularly developed in this respect are countries such as Germany, France, Austria, Italy, the Netherlands and others.

In this sense, the creation and development of deposit-credit cooperatives are socially necessary, especially under the current conditions.

The full transparency of financial affairs, the equality of each member of the cooperative, regardless of the amount of his / her share capital, interest on deposits / levies, easily accessible and cheap loans and the flexible management of the deposit-credit cooperatives make them the most sought average financial institutions.

Conflicts of Interest: The authors declare no conflict of interest.

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Original Scientific paper

Plant species diversity and structural characteristics of the old-growth spruce-fir-beech forests in Biogradska Gora

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Abstract

Species richness is a fundamental parameter of the site biodiversity potential. Quantification and comparing the taxon richness of mixed forests of spruce, fir and beech (*Piceo-Abieti-Fagetum* Čol. 1965.) were conducted according to the phytosociological studies followed by the Braun-Blanquet method. We recorded 41 vascular plant species in four sample plots of the total area of 1 ha. Concerning the plant species composition, we found differences in species richness between mixed forests of spruce, fir and beech and fir-beech forests in Biogradska Gora. Number of species was lower than in the forest community of fir-beech in the same preserved area, but higher than in the managed forests of spruce-fir-beech.

The overview of structural characteristics was developed on the basis of data from four clusters. The number of trees per unit area varied from 300 to 445 trees per ha. Estimated values of timber volume were from 558.9 m^3/ha to 749.0 m^3/ha.

The results presented in this paper show that mixed forests of spruce, fir and beech on Biogradska Gora belong to forest ecosystems of high degree of preservation, with high values of growing stock and with a structure that is characteristic of the old-growth forests.

Keywords: old growth forest, spruce, fir, beech, Biogradska Gora

Introduction

Biogradska Gora, on the mountain Bjelasica in Montenegro, is one of the largest long-term preserved forests in the south-east Europe. The history of this forest protection dates back to 1878 when the local people, after the liberation of Kolasin area from the Turks, had offered the Biogradska Gora forests to the duke Nikola Petrovic and he protected it as the hunting preservation area. Since 1952 Biogradska Gora has been the National Park with strong preservation forest area of 1600 ha.

The term old-growth forests include such forests developed during the long periods without relevant human impact and natural catastrophic disturbances (Peterken 1996). Extensive forest exploitation in Europe caused their structural and compositional homogenization and simplification, leaving only remnants of old-growth temperate forest ecosystems. (Axelsson and Oestlund 2001). The mountainous regions of the Bavarian Limestone Alps were primarily covered by the mixed Norway spruce (*Picea abies* [L.] Karst.) - European Beech (*Fagus sylvatica* L.) - Silver fir (*Abies alba* Mill.) forests, but currently, many initially mixed forests have been replaced by spruce dominated or pure spruce stands (Baier et al 2005).

Forest ecosystems of Biogradska Gora are characterized by a high degree of preservation. The Biogradska Gora mixed *Abies-Picea-Fagus* forest is one of the largest old-growth forest remaining (1230 ha) in the central-southern Europe (Motta et al 2015).

According to the First National Forests Inventory of Montenegro (MARD, 2013),the forests of fir, spruce and beech (*Piceo-Abieti-Fagetum* Čol. 1965.) constitute 4.7% of the surface area covered by forest in Montenegro. For a long time it has been considered that mixed forests of spruce, fir and beech (*Piceo-Abieti-Fagetum* Čol. 1965.) are just sub-associations of beech and fir forest association

(*Abieti Fagetum moesiacae* B. Jov. 1976.), but the prevailing opinion is that it is a separate association (Mišić and Jovanović 1983).

Understanding the development process in intact forests is essential for learning the most effective way to manage forests in a manner that would retain sufficiently high level of biodiversity (Medarevic et al 2004). Structure and species composition of old-growth forests should be base for management approaches according to the future climate changes (Millar et al 2007).

The ecological value of unevenly aged, old growth forests can be seen also through stability in relation to erosion processes. So the basin of the Biogradska River, according to the erosion processes is classified as the 4th category of destructiveness and, regarding the classification of erosion intensity, belongs to the areas of weak mixed erosion (Spalevic et al 2004). In some other parts of Bjelasica Mt. with not stabile forest ecosystems, erosion processes are classified as more dangerous (Spalevic et al 2013; Spalevic et al 2015; Spalevic et al 2016; Vujacic et al 2017).

The significance of this research is also resembled in the fact that there is modest knowledge about competitive and facilitative interactions between trees and species in the mixed fir–beech–spruce forests (Bosela et al 2015).

Materials and Methods

The basic information for plant species richness was collected by the Braun-Blanquet method (1964) of phytosociological study. In total, 4 phytosociological relevés were taken in tree dominant mixed forests of spruce, fir and beech. The surface of each experimental plot was 0.25 ha.

Structural characteristics were researched on the basis of data from 4 clusters (Figure 1). Each cluster consisted of four sample plots, placed on the top of a square whose leg length was 200 m. The sample plot consisted of four concentric circles, with the radius of the first circle r1=5.64 m (p1=1 acre on plain terrain), of the second circle r2=7.98 m (p2=2 acres), of the third circle r3=12.62 m (p3=5 acres) and the radius of the fourth circle was r4=17.84 m (p4=10 acres). Diameter measurement was performed on the first concentric circle, on all trees above 10 cm. On the other concentric circle, diameters were measured on all trees above 15 cm, on the third circle diameters on all trees above 30 cm and on the fourth circle diameters of trees above 50 cm. Measurement was performed during 2017.

Growing stock – timber volume was calculated using timber volume tables for Montenegrin forests. (Marković 2004).

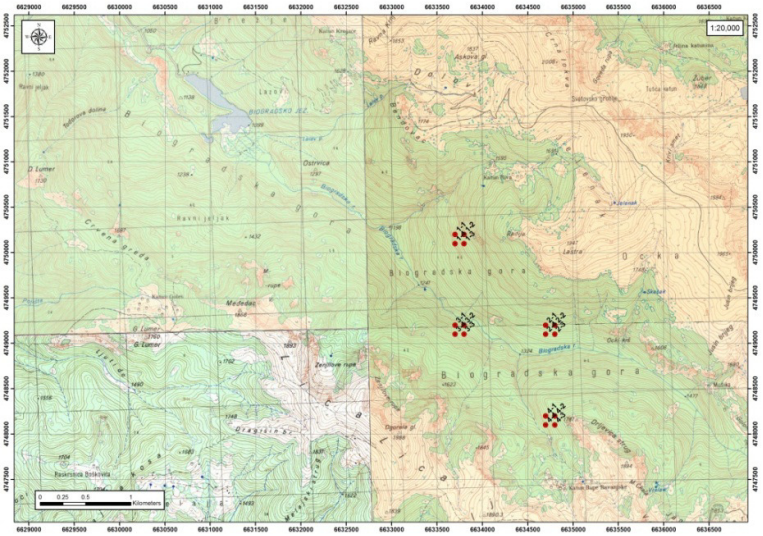

Figure 1. Position of clusters

Results and discussion

Vegetation

Total number of vascular plant species recorded in four sample plots of the total area of 1 ha was 41 (Table 1).

Table 1. Phytosociological releve´s performed in the studied sites

Association	***Piceeto- Abieti- Fagetum*** s. lat.				
Sub-association	*Typicum*				Presence degree
Locality	Biogradska gora				
Nº of releve´	F1	F2	F3	F4	
Altitude (m)	1200	1200	1200	1200	
Exposition	SSW	SW	N	NE	
Slope (°)	15-25	30	25	35	
Flor I					
Canopy	0,6	0,5	0,6	0,6	
Fagus moesiaca	2.1	1.2	2.2	3.2	V
Abies alba	3.2	3.2	3.2	1.2	V
Picea abies	1.2	1.2		1.2	V
Acer pseudoplatanus			+		II
Floor II					
Canopy	0,2	0,2	0,1	0,2	
Average height (m)	5	3	4	3	
Abies alba	1.2	1.2	1.2	1.2	V
Fagus moesiaca	1.2	+	1.2	+	IV
Picea abies		+	+		II
Sorbus aucuparia				+	I
Floor III					
Canopy	0,9	0,7	0,7	0,8	
Festuca drymeia	4.5	2.3	+.2	1.2	V
Athyrium filix-femina	+	+	2.2	1.2	V
Oxalis acetosella	1.2	1.2	1.2	1.2	V
Asperula odorata	1.2		+.2	+.2	V
Glechoma hirsute	+		+.2	1.2	V
Dryopteris dilatata	+	+	+	+	V
Asarum europaeum			+	+.2	IV
Dryopteris filix- mas	+	+	2.2	2.2	IV
Rubus hirtus		+.2	1.2	1.2	IV
Euphorbia amygdaloides	+				IV
Cardamine bulbifera	+				IV
Viola sylvestris	+				IV
Lonicera nigra		+			IV
Polygonatum verticillatum				+	IV
Geranium robertianum	+		+		IV
Mycelis muralis	+		+	+.2	IV
Prenanthes purpurea	+		+	+	IV
Vaccinium myrtillus		+			III
Lonicera alpigena				+	III

Saxifraga rotundifolia			+		III
Polystichum aculeatum			1.2	+	III
Epilobium montanum	+		+	+	III
Actaea spicata			+	+	III
Symphytum tuberosum			+		II
Neottia nidus-avis				+	II
Abies alba				+.2	II
Luzula sylvatica		3.3			II
Sorbus aucuparia			+		II
Lilium martagon			+		II
Paris quadrifolia	+			+	II
Veronica montana		+			II
Circea luteciana	+			+	II
Allium ursinum	+				I
Galium rotundifolium		+			I
Lunaria rediviva			2.2		I
Salvia glutinosa			+		I
Polypodium vulgare			+.2		I
Euphorbia carniolica				+	I

In the studied stands, spruce, fir and beech are dominating species in tree and shrub layers. Beside the basic floristic elements of those floors, we recorded also *Acer pseudoplatanus* and *Sorbus aucuparia*, as the secondary tree species in the researched stands.

The floor of the ground flora (III Floor) is characterized by larger number of acidophilic species. Among them, the highest abundance is of fescue (*Festuca drymeia*), followed by *Oxalis acetosella, Athyrium filix-femina* and numerous bryophytes.

Concerning the plant species composition, we found differences in species richness between mixed forests of spruce, fir and beech and fir-beech forests in Biogradska Gora. Species richness was a bit lower than in the forest community of fir-beech (*Abieti-Fagetum moesiacae* B. Jov. 1976), where 58 species were found (Curovic et al. 2011).

Also the number of recorded species (41) proved to be higher compared to the managed forests of the same mountain. In the managed forests of Bjelasica (Gazdic et al 2016), significantly higher number of plant species(60 species) in the beech and fir association were found, if compared to the association of beech, fir and spruce (30 species).

Structural characteristics

Basic insight into the structural characteristics of forests of spruce, fir and beech on Biogradska Gora can be obtained by analyzing the two basic structural elements - number of trees and wood volume per unit area (Table 2).

Present structure of Biogradska Gora forest is just the result of spontaneous development processes over a long time period because those ecosystems are developed without human activities influence. The average quantity of standing volume in forests of Biogradska gora indicates that these are very valuable and productive forest ecosystems. Estimated number of trees per unit area on the experimental plots is 300 to 445 trees per ha. Timber volume values varied,from 558.9 m^3/ha to 749.0 m^3/ha.

In the best spruce-fir-beech stands on the Mt. Ljubišnja in Montenegro at the Vukodol site, 501 trees/ha with 404.3 m^3/hawere measured. The average number of trees per unit area at the Sula site was 644 trees/ha and determined standing wood volume was 584.4 m^3/ha (Curović et al 2011a).

The average values of those two structure elements within the stands of mixed forests of spruce, fir and beech type in the "Tara" National Park, in Serbia, according to Medarevic et al.(2007), were 456 trees/ha with the average volume of 709 m^3/ha. The mixture per volume for fir: spruce : beech = 50% : 2% : 48%, was measured in the virgin Pecka forest in Kosevski rog (Slovenia), with an average standing volume of 647 m^3/ha (Boncina et al 2003).

Table 2. Basic structural elements

Species	N/ha	N%	V/ha	V%	G/ha	Ds	Hs	Hs/Ds
Cluster 1	Pcs/ha		m^3/ha		m^2/ha	cm	m	
Fir		51.7		76.8		54.6	26.6	0.5
Spruce		4.2		3.3		43.6	24.6	0.6
Beech		44.2		19.9		37.4	20.7	0.6
Total Cluster 1	**300**	100.0	**692.0**	100.0	44.7	50.1	25.1	0.5
Cluster 2								
Fir		48.5		71.6		53.9	26.8	0.5
Spruce		13.1		14.8		59.4	32.7	0.6
Beech		38.5		13.6		34.9	19.4	0.6
Total Cluster 2	**325**	100.0	**749.0**	100.0	44.1	51.2	26.3	0.5
Cluster 3								
Fir		16.9		55.9		60.1	32.2	0.5
Spruce		19.7		16.7		37.2	24.7	0.7
Beech		58.5		24.6		23.9	18.1	0.8
Sycamore maple		4.9		2.8		29.7	19.9	0.7
Total Cluster 3	**355**	100.0	**558.9**	100.0	31.6	44.0	26.2	0.6
Cluster 4								
Fir		25.3		35.7		44.4	19.9	0.4
Spruce		2.8		15.7		80.5	34.4	0.4
Beech		71.9		48.5		32.5	19.8	0.6
Total Cluster 4	**445**	100.0	**613.8**	100.0	44.9	42.7	21.8	0.5
Average values for all 4 Clusters								
Fir		34.1		61.2		53.7	26.5	0.5
Spruce		9.6		12.4		57.3	30.0	0.6
Beech		55.1		25.8		32.4	19.7	0.5
Sycamore maple		1.2		0.6		29.7	19.9	0.7
Average	**356**	100.0	**653.4**	100.0	41.3	47.2	24.7	0.5

Compared to the stands of the presented research, although there are smaller number of trees per unit of area, the presence of remaining trees of extremely large dimensions in Biogradska Gora resulted in a substantially higher standing volume.

Mixed forests of spruce, fir and beech on the studied sites, according to the structural data, represent the forests of high site classes, in which all three main species reached high dimensions of average diameter (Ds) and height (Hs). According to the achieved dimensions in the studied areas, spruce and fir are dominant, while beech is rather in a subordinate position.

There are numerous studies showing that the share of fir in European blending forests is decreasing (Jaworski et al 2002; Diaci et al 2010; Vacek et al 2014). In mixed forests in the Lom forest reserve in Bosnia and Herzegovina Bottero et al. (2011) found a sharp decrease in the share of fir. A decrease in fir representation in mixed forests was also observed in Slovakia (Štefančik 2006). In the studied stands on Biogradska Gora, the share of fir is quite good. Primeval forest structure and full canopy is especially suited for fir. Natural regeneration of fir in the fenced parts of the mixed forests is much higher than in the unfenced parts (Klopčić et al 2010). Vrska et al (2006) concluded that completely fencing virgin forests is currently the only way of ensuring relatively natural spontaneous development of mixed spruce-fir beech forests.

Conclusions

Presented data show that forest ecosystem of spruce, fir and beech (Piceo-Abieti-Fagetum Čol. 1965) in the Biogradska Gora preservation area is predominantly characterized by structurally irregular forests of primeval character. It shows the typical structural characteristics of old-growth forests: presence of large and old trees with relevant growing stock.

Biodiversity is significant and larger than in managed forests of the same forest communities in Montenegro.

All three main species reached high dimensions of average diameter (Ds) and height (Hs). Spruce and fir are dominant according to the achieved dimensions in the studied areas, while beech is rather in a subordinate position.

There are numerous studies showing that the share of fir in the European blending forests is decreasing. Natural regeneration of fir in the fenced parts of the mixed forests is much higher than in the unfenced parts. Completely fencing virgin forests is currently the only way of ensuring relatively natural spontaneous development of mixed spruce-fir beech forests.

By comparing the determined wood volume with the presented results of studies from the best quality forests in the South-East Europe, it can be concluded that mixed forests of spruce, fir and beech on Biogradska Gora are forests of the high site class with large standing volume and with high biodiversity level.

Conflicts of Interest: The authors declare no conflict of interest.

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Original Scientific paper

The Automatization of Forest Management Works in Romania using GIS and UAV Photogrammetry

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Abstract

Developing forestry management maps, in Romania, is essential especially in the context of illegal logging. Specialized law requires elaboration of forest management maps to regulate the work of cutting, sanitation, conservation, harvest optimization etc. The area taken into study is around Lake Nistru, Maramureș county, Romania. This paper aimed to develop a workflow in order to automate the forest management mapsrealization and also to highlight the benefits of using digital photogrammetry and GIS techniques. We acquired highresolution data (0.0589 m spatial resolution) using a consumer-grade Unmanned Aerial Vehicle (UAV) a having on board a 24.3 megapixels digital camera. By processing the photogrammetric data we obtained a point cloud with a density of 18 points/m2 and a digital elevation model with a resolution of 23.6 cm/pixel. By using some GIS spatial analyst tools, we obtained the cartographic support for forest management. Based on the high accuracy point cloud we analyzed the possibility of computing the wood mass, applying methods like the regression equations. We also determined the necessary forest management elements: surface, consistency, exposure, medium altitude. For referencing the photogrammetric measurements 8 ground control points (GCP) were used for which we computed the root mean square error (RMSE) related to the spatial coordinates (X, Y, Z): 6.059 cm on X, 3.761 cm on Y and 4.596 cm on Z.

Keywords: Forest management map, GIS database, point cloud, UAV photogrammetry

Introduction

The role, importance and methods for forest management maps

The importance of forests for our planet and for humanity is well known, the forests offering materials and shelter for satisfying the needs of living (Giliam, 2014).

For a durable management of the forests, knowing them both from a quantitative perspective, as geospatial extent, as well as from a qualitative perspective, as structure and condition is necessary. Taking into consideration the high costs of forestry arrangements by classical means, new methods have to be implemented. The forest is a complex, whose exploration under the two aforementioned aspects was realized in the beginnings of the management by classical methods, involving movements and measurements, often in rough and barely accessible terrain(Vauhkonen *et al.* 2014; Clark *et al.* 2000).

The investigation methods of the standing crop evolved, so that for the aid of foresters, new techniques and modern remote sensing methods have evolved, offering a superior efficiency that

allow investigation and long range input of data regarding the forest vegetation. The terrestrial determinations have maintained their importance, even though they imply high efforts, usually being more accurate, but these have also benefitted from a technological boost in productivity and even precision (Clark *et al.*, 2000; Tomaštík *et al.*, 2017).

The forest geomatics, which represent a complex of techniques and modern technologies based on IT that are fused into a system with the scope of quantitative and qualitative findings of the land surface, the soil casing and biometrical characteristics of the forest(Tomaštík *et al*., 2017). The geomatic technologies include, mainly the satellite remote sensing, digital photogrammetry, geo – topographical land measurements with modern and highly efficient equipment, namely GNSS systems, laser equipments and total stations, as well as softwares that allow the input, fusion and integrated processing of digital data resulting in geographic information systems (GIS) in the shape of a mathematical model that contains input and processing algorithms (Baltsavias *et al.,* 2008).

The areas of interest in which these systems are integrated are usually those regarding climate data and vegetation, soil and subsoil, the covering and use of the terrain, as well as the human population and infrastructure. They actually refer to the environment, agriculture and forestry, climate, pedology, geology, hydrology, risk of calamities, but also include detailed information on the forest vegetation, forestry arrangements data, monitoring and inventorying forest agriculture, forestry and agricultural cadastre, protected areas, biodiversity, ecology, genetic resources etc. (Kolström *et al.*, 2011).

All this information is geographically positioned in a spatial reference system, being connected by coordinates to the real surface of the Earth and has a digital geographic fundament (a GIS database) that includes both vectorial data (data layers on different types of borders, contour lines, digital models of the terrain, GPS or total station measurements), as well as data in raster format (scanned maps and plans, aerial and satellite digital orto-adjusted imaging, LIDAR data). Furthermore, this data is integrated, interoperable, allows for interrogation and analysis and has a periodical and occasional update system, a hierarchically structured access and validation system and a high level of physical and informational reliability (Krista Merry *et al. 2016).*

Under these conditions, in order to overcome the delay, namely to rapidly and efficiently reach this technological level, but also for avoiding the beginner mistakes of the other countries that evolved during the process, a standardization of the input, processing and storage methods of the data is necessary. For this reason, in our country the problem is not so much insuring the necessary technological platform, but the unitary creation and correlation of the databases, which are mostly inexistent or incomplete.

Legal grounds regulating the forestry arrangements in Romania

The regulation is provided by the Forestry Code, Law no. 26/1996, while the cadastre of the forest fund is elaborated as per the methodology for introducing the forestry cadastre. The performance of the forestry management maps is based on the Technical Norms on forest arrangements, namely Norms no. 3814/2012. Art. 20 of the Forestry Code project provides that "the performance of the forestry management maps is mandatory for the properties of forest funds larger than 10 ha", meaning that, as opposed to current regulations, where the arrangement is mandatory for any forest, in the future regulation the arrangement is no longer mandatory for the forest smaller than 10 hectares. As per the Ministry for Environment, Waters and Forests, Romania currently owns approximately 700.000 ha of forests that would fall under the incidence of art. 20 of the Forestry Code. Out of these, approximately 420.000 ha are lands owned by small owners that do not have the necessary means to associate in order to obtain land surfaces of at least 10 ha, for the elaboration of the forest management maps, as the law currently provides.

Based on the current Forestry Code, because the wood mass cannot be exploited, these areas are unguarded and practically unmanaged.

Compliance with the forestry regulations by the small owners involves very high costs of arrangement.

Motivation of the research and objectives

Because of a lack of consistent subsidies for forest lands aiding the owners (as in the case of agricultural lands) and the economical benefits of the forest being obtained in 25 – 100 years, depending on the specie, the small owners only bear maintenance costs. In Romania, the exploitation of lands with forestry management plans is permitted, but only in the maximum limit of $3m^3$/year/ha, a small quantity form a forestry perspective (approximately half of the average annual growth which is of $5,6m^3$/year/ha), but which helps small owners a lot, guaranteeing fire wood for heating. Any cutting of more than $3m^3$/year/ha is prohibited without a forestry arrangement. Taking into consideration this circumstance, a better management of forest lands is necessary and implicitly the realization of forestry management plans.

In order to obtain forestry management plans, with low costs and high precision, within this article the use of the UAV techniques was chosen for obtaining spatial data and implicitly the forestry arrangement.

Along with the use of the UAV photogrammetric method for determining the cartographic database of the forestry management map, the integration of the data in a GIS data base for spatial analysis was performed. All the analysis on the characteristics of the arboretum were performed on the point cloud. Also, the automatic verification of the results obtained from the point cloud was chosen, by comparison with the data taken on site.

Most of the arranged land plots are bordered by valleys, ridges or different types of forests. The natural borders may be obtained with high precision and efficiency from the perspective of costs by the photogrammetric UAV method (Wallace *et al.* 2016; Tang and Shao, 2015). The area studied is subject to forest and shepherd arrangements, being a partially forested pasture, so that it incorporates both forest, as well as pasture specific vegetation.

Materials and Methods

Studied area

The area taken into study in this article is around Lake Nistru, located in Tăuții Măgherăuș, Maramureș County, Romania. This area is presented in Figure 1.

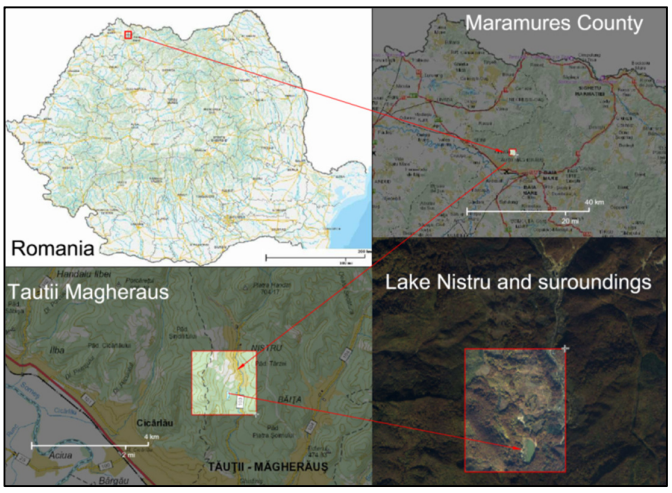


Figure 1. Position of the studied area

The town of Tăuții Măgherăuș consists of 7 neighborhoods, namely Tăuții Măgherăuș, Băița, Bozânta Mare, Nistru, Ulmoasa, Bușag and Merișor. The study was performed in Nistru, the Nistru Lake area, which is under the administration of the Tăuții Măgherăuș forest range.

From the Tăuții Măgherăușforest range, a surface of about 80 ha was studied, surface in the public ownership of the town of Tăuții Măgherăuș.

Preliminary operations and the UAV flight

The initial stages in performing a point cloud by the UAV photogrammetric method are the following: terrain recognition, identification, marking and determination by measurements of possible ground control points, but also of certain verification points in order to obtaine the desired precisions. The equal distribution of the control points on the charted surface will be taken into consideration, as well as insuring their optimal dimension for identification and precision pointing of them on photogramms (Whitehead *et al. 2014)*.

The marking and determination of the control points was traced at the beginning of every series of photography and, when possible, at the middle and end of the series. With a large lateral overlap of the photos, more than 80% of the control points can be found in more series.

For obtaining a better precision at editing and processing of the photogrammetric data, pre-tracing of ground control points was necessary. The position of these points can be seen in Figure 2. These control points were made of canvas of about 60/60 cm (Figure 3b), placed on 5 wooden rods. On the center wooden rod a screw corresponding to the center of the trace was placed (Figure 3a), whose position was determined by GNSS measurements.

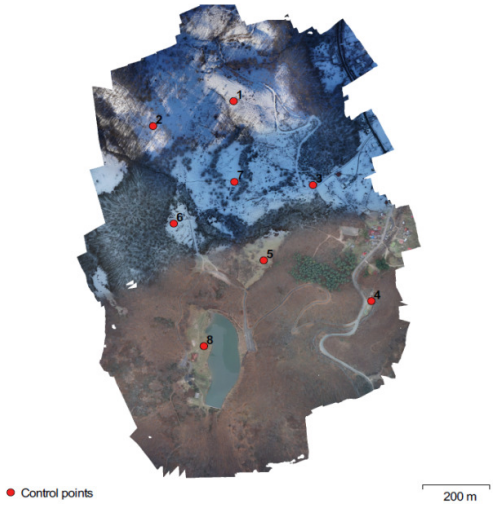
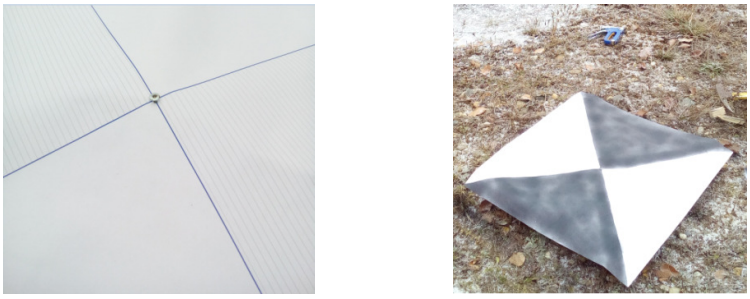


Figure 2. Ground control points location



(**a**) (**b**)

Figure 3. (**a**) Center of the Ground Control Point; (**b**) Final overview of a Ground Control Point

The control points were determined by GNSS methods, using two South S82V GNSS receivers with dual frequencies, set on base – rover radio mode. This method offers a higher precision than the measurements made by a single GNSS receiver using the RTK method, several studies confirming this theory (Dragomir, 2013; Paziewski and Sieradzki, 2017; Li *et.al.* 2017; Brack, 2017; Selmira *et al.* 2017).

This method was chosen given that the area does not provide a good GSM coverage, thus connecting and obtaining corrections from a permanent ROMPOS or EUREF/IGS station being difficult. A point was marked and determined using GNSS static measurements, using the corrections from the permanent station from the network EUREF – IGS BAIA00ROU. The standard deviation for determining the base point was of 0.05 m, given the short base from the permanent station to it, and the determined control points in relation to this station were within +/- 0.007 m on the horizontal axis and +/- 0.04 m on the vertical axis, values expressed as Route Mean Square Error (RMSE).

For image acquisition, a hexacopter drone was used. The drone was attached with a mirrorless 24.3 megapixels Sony Alpha 6000 digital camera, with ultra fast autofocus Fast Hybrid AF System (0.06 seconds). A flight plan was determined that insured an 80% overlap of the images, for obtaining the most accurate and complete 3D model. For the geolocation of the images, the GPS coordinates provided by the drone's GPS were used, but later they were precisely located taking into consideration the coordinates of the control point on the ground. The flight strips can be seen in Figure 4, namely the covering and overlap between the images. Also, it can be observed that the studied area is covered, in average, by 8 -9 images in each area.

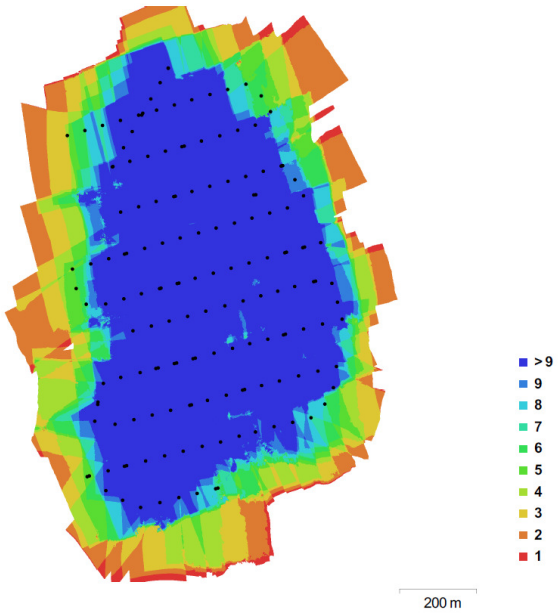


Figure 4. Camera locations and image overlap

Given the forest vegetation in the studied area, but also due to a significant altitude difference on the terrain (220 m between the highest and lowest point), the flight altitude was set for 260 m. The approximation relationships of the connection between the flight altitude, the ground coverage and the characteristics of the camera used are implemented in the flight plan software, in our case Mission Planner 1.2.87.

The area studied was of 1.2km^2, being covered by 156 images, which after being processed resulted in an ortophotomap with a resolution of 5.89 cm/pixel and the average re-projection error resulted was of 1.85pixels. The obtained precisions are more than satisfactory, taking into

consideration that the forest management plans in Romania are elaborated at the scale of 1:10000 or 1:20000. The errors on the control points are presented in Table 1.

Table 1. Errors of the Ground Control Points

Point ID	X error (cm)	Y error (cm)	Z error (cm)	Total (cm)	Image (pix)
8	-5.20066	2.37973	0.300367	5.72715	0.204 (17)
4	-2.21518	0.274554	-0.915712	2.41266	0.077 (8)
5	13.4092	3.15887	6.94507	15.4278	0.187 (7)
1	-1.0845	-3.42334	1.24482	3.80066	0.316 (15)
2	1.66981	3.59033	1.93091	4.40536	0.241 (8)
3	-0.723386	3.22033	2.92185	4.40806	0.247 (19)
7	3.54467	-3.29696	-10.2781	11.361	0.253 (13)
6	-8.05644	-7.18699	0.661443	10.8165	0.241 (14)
RMSE	**6.05927**	**3.76198**	**4.59693**	**8.48522**	**0.239**

The point cloud obtained has a point density of 18 points/m^2 and the digital elevation model (DEM) has a resolution of 23.6cm/pixel, which, for the chosen purpose is within the necessary precision. The errors of the ground control points were between the limits of 4.596 cm on elevation, 6.059cm on the X axis and 3.761cm on the Y axis. Point 5 was identified on only 7 images, points 4 and 2 on 8 images and the rest of the control point were identified on 13 – 19 images, as per Table 1. The biggest identification error on images was for point 4, which has a deviation of 0.316 pixels. The evaluation of the obtained precisions was made using RMSE (Mokroš *et al.*, 2017).

The next stage, after editing the images, processing and obtaining the point cloud, respectively the ortophotomap, is the interpretation and modeling in order to obtain the necessary information for elaborating of the forest management map.

For determining the vegetation, knowing the height of the ground is necessary first, the data referring to vegetation being obtained subject to this. It was opted for two flights over the studied area, one performed late in the autumn, before snow fell and the other a few days after the first snowfall, but on a small snow (2-3 cm) so that the height of the ground was not influenced too much. During both flights, the same control points were used, as they remained marked.

Comparing the date of the two flights, several advantages/disadvantages of the two may be identified. From the point of view of the visual identification of the hydrography (creeks, valleys), the ortophotomap obtained in snow (Figure 5a) is very advantageous. Also, from the point of view of identifying trees, they are very well distinguished, but on the snowy image the communication paths, namely the artificial arrangements (dams, platforms etc.), cannot be well identified, these being covered in snow. On the images obtained in autumn (Figure 5b) the trees in the broadleaf class are hard to distinguish, but the coniferous class can be distinguished well. Also, on the image obtained in autumn, the roads can be well distinguished, respectively the art works on the roads. The major creeks can also be identified, but the small ones are hard to distinguish.

(**a**) (**b**)

Figure 5. Ortophotomap: (**a**) early winter; (**b**) late autumn

If we are to evaluate the results and to automatically classify the point cloud, the conditions of photogram acquisition do not affect the results so much. What is hard for the human eye to distinguish, because of the similar colors, the specialized software can do it without a problem. This is why it was opted for interpreting the results on the point cloud and not on the ortophotomap.

The first stage in performing the forest management map is charting the place, the water flows, the valleys, ridges etc.

For modeling in ArcGIS, the database organization has to be considered, both form the point of view of the inputs, as well as the outputs. The precision of the output is in direct connection to the input. If the input database has a poor precision, this will influence the analysis and the surface modeling.

The performance of a toolbox for the hydrological modeling was opted. This toolbox uses an elevation digital model as input. The advantage of the toolbox is exactly the fact that, at any given time, the input data can be modified and by a simple rendering the results for the new DEM are generated. The toolbox may be exported and used for other projects, on other studied areas and may even be edited, integrated in other strings of operations. The conceptual model is presented in Figure 6. For the performance of the DEM the classified points corresponding to the ground were used as general input data.

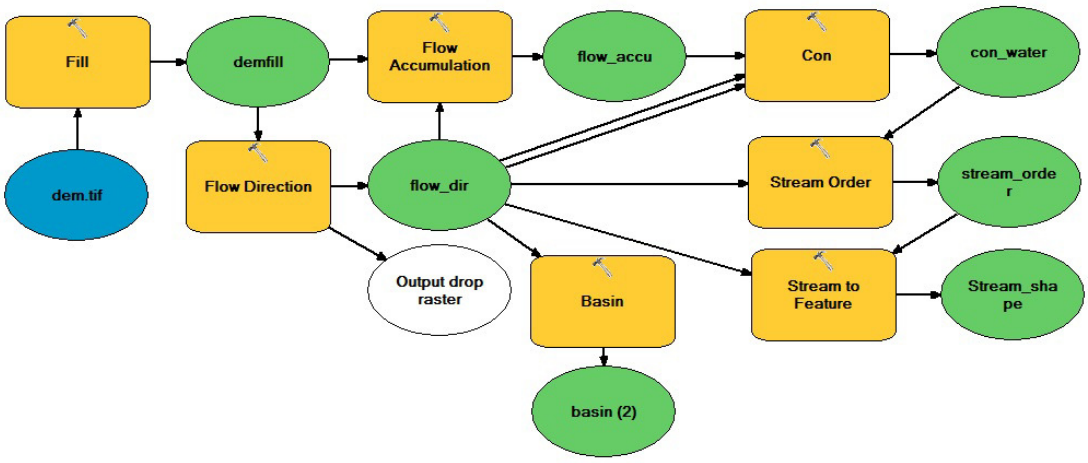


Figure 6. Model Builder GIS

The main outputs of the model presented in Figure 6 are the water streams in vectorial format. Their vectorisation usually takes time and most of the time may be performed erroneously. Based on the point cloud, they can be generated with a satisfactory precision. In Figure 7, one can notice a creek automatically generated based in the DEM created from the filtered point cloud.

Another important element in elaborating the forest management plan is the determination of the elements of forest dendrometry. The most important element is the wood mass. For determining

the wood mass, several methods can be used, among which: method with trial trees: the Urich method II (Mederski *et al., 2015)*, the Hartig method (Fernow, 2017) or the Draudt method; cubage table methods: with two entrances, on series of volumes, methods using regression equations: the double logarithmic volume equation, based on the mathematical model of the relative heights series etc.

For this research, it was opted for determining the volumes using cubage table methods with two entries, namely the trunk diameter and the height for the trees extracted automatically by filtering and analyzing the point cloud, as per Figure 10. The height of the tree can be determined directly from the point cloud, whereas for determining the diameter of the trunk one can use the calculation method using the regression equations. By filtering the point cloud and obtaining the map of the ground and afterwards obtaining the points corresponding to vegetation, a few characteristic aspects of the area were determined, such as: bin size, minimum height, maximum height, delta, expected terrain slope obtained from the DEM. Based on this individual characteristic specific to each arranging unit, we were able to filter the point cloud (Holopainen *et al.*, 2014; Yu *et al.*, 2011).

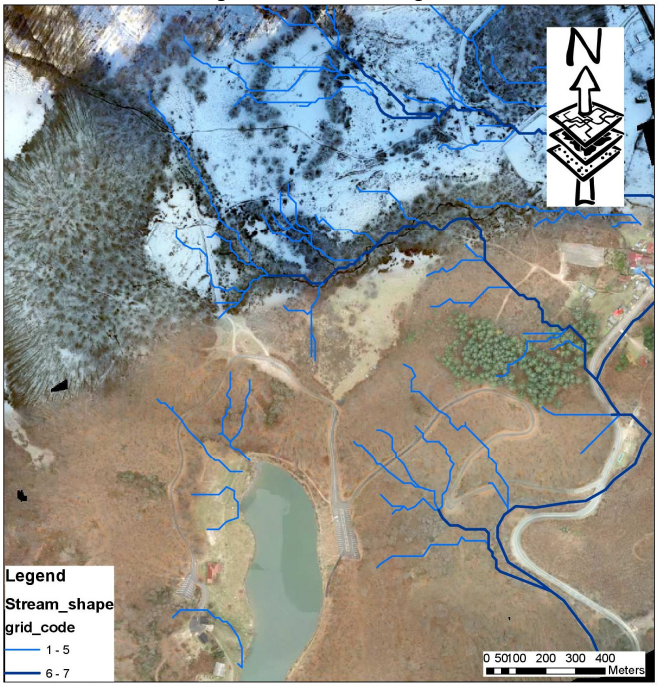


Figure 7. Automatically generated stream using DEM

The specialized doctrine provides more regression equations that determine the correlative relation between the base diameter and the canopy diameter or the base diameter, the canopy diameter and height (Giurgiu, 1979; Giurgiu, 1999).

Taking into consideration that, on site, the surface of the canopy projection was measured, the diameter of the canopy (d_{cro}) is calculated considering the surface of the projection (Pr_{cro}) approx. as a circle:

$$d_{cro} = \sqrt{\frac{4Pr_{cro}}{\pi}} \quad (1)$$

The regression coefficients can be calculated for different expressions of the base diameter subject to the canopy diameter or the canopy diameter and height. $d = a_0 + a_1 d_{cro}$

$$d = a_0 + a_1 d_{cro} + a_1 d_{cro}^2 \quad (2)$$

$$d = a_0 + a_1 h + a_2 d_{cro} + a_3 h^2 + a_4 h d_{cro}$$

Calculating the regression coefficients for different expressions of the base diameter subject to the canopy diameter or the canopy diameter and the height, the best model that estimates the relationship between the base and the canopy diameter can be determined for the studied model.

After determining the regression equations, the adjusted base diameter calculation is done, using as variables the characteristics measured stereo-photogram metrically.

The regression equation used for calculating the volume of the tree is the following:

$$\lg v = a_0 + a_1 \lg d + a_2 \lg^2 d + a_3 \lg h + a_4 \lg^2 h \quad (3)$$

where:

d – base diameter of the tree [cm]; h – height of the tree, [m]; v – volume of the tree [m^3];

$a_{0\text{-}4}$ –regression coefficients.

The regression coefficients by species extracted from the dendrometric tables were used (Table 2).

Table 2. The regression coefficients by species (Giurgiu *et al*., 2004)

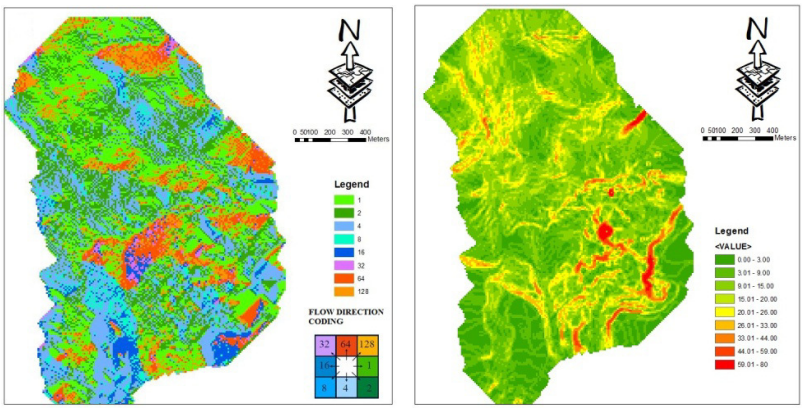
Species/coefficient	a_0	a_1	a_2	a_3	a_4
beech	-4.11122	1.30216	0.23636	1.26562	-0.079660
oak	-4.13329	1.88001	0.04880	0.95371	-0.063638
hornbeam	-4.23139	2.15204	-0.00988	0.59652	0.112810
cherry	-3.59371	1.95047	0.04086	-0.12835	0.374948
spruce	-4.18161	2.08131	-0.11819	0.70119	0.148181
fir	-4.46414	2.19479	-0.12498	1.04645	-0.016848
larch	-4.59667	2.26066	-0.13256	1.02582	0.007491
pine silvester	-3.84672	1.82103	-0.04107	0.35677	0.334910
pine strobe	-4.36966	1.55475	0.14981	1.40295	-0.157352
black pine	-4.01698	1.96342	0.01241	0.57848	0.094783
birch	-4.16999	2.27038	-0.21540	0.30765	0.368258
linden	-4.80605	1.92424	0.02214	1.96408	-0.452969

Results

Thematic maps

Based on the point cloud and the digital elevation model (DEM), several thematic maps could be drawn, in order to complete the data of the forest management map. This data can be regarded as much more accurate than those in the old arrangements, for several reasons: the old arrangements were performed analogically, without a detailed verification; no individual measurements were performed etc.

An important factor in the forest arrangement is the terrain slope. Also by spatial modeling, the terrain slope can be easily obtained; it is presented in Figure 8. In the studied area, the majority terrain slope is between 0 and 20 degrees and in some places, especially in the areas with creeks, it can reach 30 – 40 degrees. Slopes of more than 40 degrees and close to 80 degrees can be found in abrupt areas, mainly in those occupied by cliffs. In order to perform the optimal arrangement, but also for choosing the adequate trees for the forest arrangement, the specialists shall take into consideration the slopes.

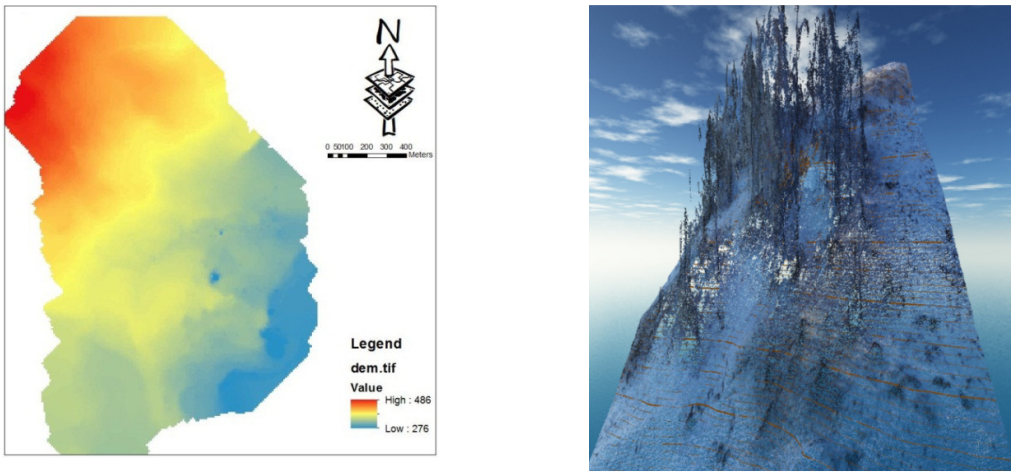


(**a**) (**b**)

Figure 8. Terrain configuration: (**a**) slopes in degrees; (**b**) flow direction

Another important element in choosing the adequate tree species is the terrain orientation, namely the sun/shade degree. Subject to the flow direction of the water on the descents, the exposition of the terrain can be determined and therefore whether it is sunny/shaded. The interpretation of Figure 8b is done according to the legend attached, obtaining the water flow direction and implicitly the exposure of the terrain. Over these maps, the vectorial data representing the forest management map can be overlapped so that one can determine an average orientation and then an average slope of the arranged parcel and, given these conditions, one can choose the best adapting species to the surface.

Also for determining the optimal species of arboretum, the altitude of the arranged unit shall be taken into consideration. The altitude can be extracted from the digital elevation model (DEM), as well as it can be noticed in Figure 9a. As an alternative to the use of the digital elevation model, contour lines can be generated directly on the point cloud, as seen in Figure 9b. An advantage of visualizing the contour lines on the point cloud is the fact that the vegetation is easily identified, so that decision in this regard may be taken. Also, the point cloud takes the colors of the photogrammes, respectively the RGB code for each element, generating a virtual reality of what is on site. This way, the time spent on site is considerably reduced, and the possible measurement omissions made by classical methods may be completed from this model, no additional on site travels being necessary.



a b

Figure 9. Altitudes extraction: (**a**) Terrain elevation; (**b**) Contour lines and vegetation on pointcloud

Another automatic process of the point cloud interpretation is the self classification of points in categories: land, buildings, low vegetation, high vegetation. For this study, we were interested in the classification of the points representing high vegetation, which in case of forest areas represents the majority of the wood mass. The low vegetation may, most of the times, be neglected as volume of wood mass, representing a small percentage of the total volume of wood mass.

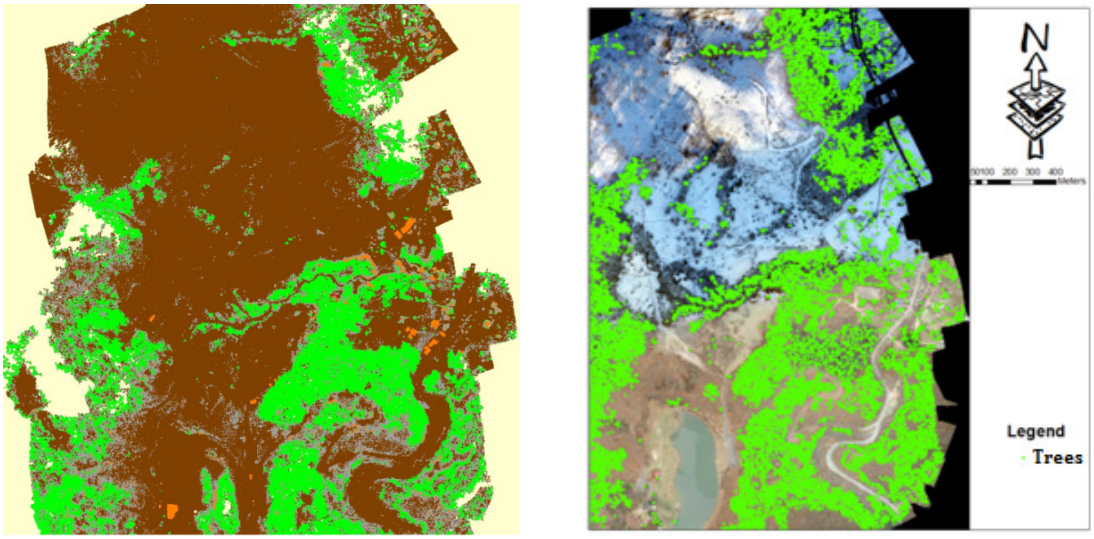
Filtering, classifying and analizyng of the point cloud

Modeling the classified point cloud (Figure 10a), the central points of the tree can be extracted under the shape of a vector (Figure 10b). This vector data, by mathematical modeling and automated interpretation of the geometries, respectively of the connections between the surface of the ground and of the trees will be accompanied by the main biometrical characteristics of the arboretum (height, canopy surface). For the sample studied, it was opted for the automatic generation of the tree height and the average spread. Statistically, in the studied area, the heights of the trees are between 3 and 42 meters.

By using the relations (1) – (3), respectively the regression coefficients (Table 2), the dendrological characteristics of the arboretum can be determined. Initially canopy diameter was determined by tabular calculation directly in the GIS database according to relations (1) and (2).

Next, the volume of wood was determined according to relation (3), subject to the species regression coefficients.

Having this data determined, the map of the forest arrangement could be generated, as well as its geo data base. Figure 11 shows the map of the forest arrangement overlapped with the ortophoto map of the studied area, while Figure 12 shows the attributes of the elements that constitute the forest management plan, obtained by the GIS spatial analysis.



a b

Figure 10. (**a**) Classified point cloud; (**b**) Extracting trees and their characteristics as vector data

For the forest management maps performed in Romania, the parcels outlined by natural borders are numbered using figures, while the sub parcels with letters (Figure 11). There are a few standard codifications for sub parcels with special functions, such as: parcels used as meadow that provide food for the animals are marked with "V", high voltage lines with letter "R", water with "T", working roads with letters "DE" etc.

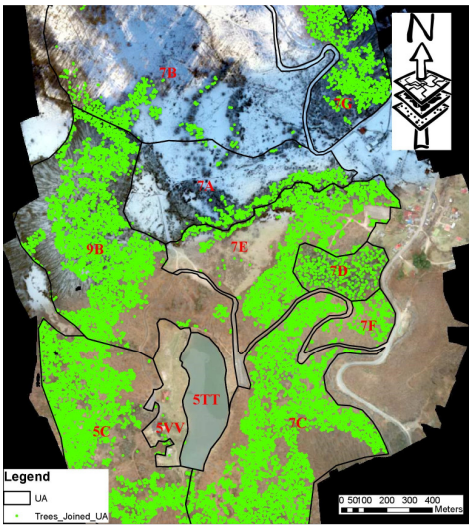


Figure 11. Forestry management map

As one can notice in Figure 12, representing one of the attribute tables included in the geo data base of the forestry arrangement, all the characteristics of the arboretum were determined. The surface of the forestry arrangement, the total volume of wood mass, the wood mass per hectare, number of trees, the surface covered by the canopy, as well as the consistency of the forest were all determined. The tree species was identified by examining the trees on site, but also by examining of the orthophotomap.

UA

FID	Shape	UA	Area[ha]	Volume[m3/ha	Total_Volume[	No_Trees	Trees_Area	Consistency	Species
3	Polygon	5C	10.4921	17.2409	180.893	2917	5.9741	0.6	FA
1	Polygon	5TT	2.28773	0.0139	0.0318	2	0.0038	0	FA
0	Polygon	5VV	1.99559	0.00209	0.00417	9	0.015	0	FA
8	Polygon	7A	6.80475	0.606341	4.126	440	0.8865	0.1	FA
10	Polygon	7B	17.5393	1.01646	17.828	639	1.2063	0.1	FA
4	Polygon	7C	12.8125	46.9408	601.429	4336	8.9652	0.7	FA
5	Polygon	7D	1.78737	27.1897	48.598	394	0.8635	0.5	MO
7	Polygon	7E	7.84992	40.434	317.404	1842	4.1094	0.5	FA
6	Polygon	7F	1.40297	19.736	27.689	273	0.5559	0.4	FA
9	Polygon	7G	7.06363	12.2671	86.65	1571	3.1208	0.4	FA
2	Polygon	9B	12.9978	5.91246	76.849	3306	6.3223	0.5	FA

Figure 12. Atribute table of the forestry management map

From the forest consistency, one can notice that there are forestry management units under the consistency bar of 0.4, therefore these shall be recorded as pasture lands. Taking into consideration that the area analyzed represents a pasture that was later forested, we have forestry management units at the borderline of the minimum consistency for being regarded as woods, and thus the volume of the wood mass is relatively small.

Discussion

Taking into consideration the slopes map, tilts, heights, but also the general map of the forestry management plans, respectively the characteristics of the existing vegetation, we considered several types of arrangement procedures, such as forestations, controlled cuts etc. These works are essential for the good administration of the forests, for avoiding excessive deforestations, landslides, but also for a good management of both the state and private owned forests. By analyzing the maps presented in Figure 8, methods for combating the excessive erosion effects that water has on some areas on

terrains may be adopted. Also, more efficient and suitable hydrological methods of arrangement can be adopted.

Taking into consideration that 41% of the total forests in Romania are privately owned and only 59% is state owned, alternative ways, cheaper and faster for obtaining the forestry management plans are necessary.

The vast majority of the private owned forests are scattered, in very small surfaces, so that an arrangement unit of 1-2 hectares may have several owners, fact that impedes the arrangement, because it is very hard for all the owners to agree in order to meet the condition to have the minimum surface that can be considered for performing a forestry arrangement.

This way of performing the forestry arrangement offers precisions comparable to the performance of the arrangement by classical means, currently in use.

Another advantage of the proposed method lies in determining the areas affected by calamities, possible attacks of pests, wind blow damages, illegal deforestation etc. By this method, aside from performing the forestry arrangement, we can monitor the forest.

For determining the trees, as it was presented in the present paper, in Figure 5, the best period for performing the point cloud, respectively the ortophotomap is the winter, when there is a thin layer of snow and the trees in the broadleaf class reflect well on the white surface of snow because of a lack of leaves. Of course, for a better determination of the species using the ortophotomap, the performance of a set of ortophotomaps during the summer would be necessary, when the leaves and the species of tree may be noticed. Nevertheless, the onsite verification of the results and possible correction of interpretation errors from an expert cannot be neglected, on small control parcels.

Conclusions

It can be said that the UAV photogrammetric method of obtaining the point cloud, respectively the GIS methods presented are a very efficient method from the point of view of the time spent on site. Using this method, large areas can be covered, but mostly hard accessible areas can be charted, and the extraction of the characteristics of the forest, as well as of the land, respectively the update of the forestry management maps becoming a much more accessible task.

The extraction of the arboretum characteristic, using the equation and the regression coefficients for calculating the arboretum volume offers a satisfactory precision, comparable to the precisions obtained by classical methods of species cubage tables.

Although the UAV photogrammetric method offers a great advantage from the perspective of charting time, this method must be thoruoghly verified and confronted with measurements, at least on samples. This method, combined with classical on site determinations of the characteristics of the trees offer a far superior arranagement precision, taking into consideration the fact that the current map database of the forestry management map in Romania is obtained by scaning and vectoring the old analogical maps, which are less accurate.

6. Author Contributions: All authors contributed extensively to the work. T. Sălăgean and E.E. Șuba performed the processing and wrote the manuscript. I. Pop, and F. Matei supervised the research work, reviewed the manuscript, and provided comments and suggestions to improve the manuscript. J. Deak and S. Chiorean contributed materials.

Conflicts of Interest: The authors declare no conflict of interest.

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